REVIEW ARTICLE

The seaweed holobiont: understanding seaweed–bacteria interactions

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Abstract

Seaweeds (macroalgae) form a diverse and ubiquitous group of photosynthetic organisms that play an essential role in aquatic ecosystems. These ecosystem engineers contribute significantly to global primary production and are the major habitat formers on rocky shores in temperate waters, providing food and shelter for aquatic life. Like other eukaryotic organisms, macroalgae harbor a rich diversity of associated microorganisms with functions related to host health and defense. In particular, epiphytic bacterial communities have been reported as essential for normal morphological development of the algal host, and bacteria with antifouling properties are thought to protect chemically undefended macroalgae from detrimental, secondary colonization by other microscopic and macroscopic epibiota. This tight relationship suggests that macroalgae and epiphytic bacteria interact as a unified functional entity or holobiont, analogous to the previously suggested relationship in corals. Moreover, given that the impact of diseases in marine ecosystems is apparently increasing, understanding the role of bacteria as saprophytes and pathogens in seaweed communities may have important implications for marine management strategies. This review reports on the recent advances in the understanding of macroalgal–bacterial interactions with reference to the diversity and functional role of epiphytic bacteria in maintaining algal health, highlighting the holobiont concept.

Introduction

The past decade has seen an increasing interest in the field of marine microbial ecology, in part driven by the technological advances that allow for a comprehensive and detailed description of bacterial diversity and function. As a result, it is now clear that the marine environment is home to an enormous diversity of bacteria (Giovannoni & Stingl, 2005; Zinger *et al.*, 2011). While marine diversity surveys were initially focused on planktonic communities, there is growing interest in characterizing microbial communities associated with eukaryotic hosts. It is becoming clear that many marine eukaryotes possess stable associations with bacterial partners and depend on them for growth, development, supply of nutrients as well as protection from colonization and predation (Dubilier *et al.*, 2008; Egan *et al.*, 2008; Crawford & Clardy, 2011; Wahl *et al.*, 2012).

Seaweeds or marine macroalgae are sessile multicellular photosynthetic eukaryotes that are differentiated from plants by their lack of specialized tissues (e.g. root system and vascular structures) (Graham & Wilcox, 1999). Fossil records of macroalgae date back more than 1200 million years, predating the evolution of land plants and in the

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case of the red algae *Bangiomorpha* sp. represent the oldest taxonomically resolved multicellular organism (Butterfield, 2000). Today macroalgae play important ecosystem engineering roles on rocky shores in coastal temperate marine environments. Here, they make a major contribution to primary productivity and determine the physical structure of the habitat (Schiel & Foster, 2006). They allow for the maintenance of local biodiversity (Schiel, 2006; Schiel & Lilley, 2007), act as nurseries and protective shelter for many invertebrate species and provide essential space for epibionts ranging from bacteria to macroinvertebrates (Wilson *et al.*, 1990; Bulleri *et al.*, 2002). In a commercial context, macroalgal aquaculture has increased over the last few years, in particular for the Asian food market and as feed stocks in biofuel production (Neori, 2009; Borines *et al.*, 2011).

The assertion at the core of this review is that macroalgae functioning in both ecological and industrial settings cannot be understood without considering interactions with their associated microbiome. There is substantial laboratory-based evidence that macroalgal health, performance and resilience are functionally regulated and assisted in part by epiphytic bacteria. This functional assistance implies that macroalgae and all their associated microbiota form a singular entity or holobiont (Fig. 2), in line with what has been suggested for the coral holobiont (Rosenberg *et al.*, 2007; Bourne *et al.*, 2009). In fact Barott *et al.* (2011) have recently suggested this interaction may be so important in tropical reef algae that they have similarly proposed an algal-holobiont concept for these systems.

The holobiont concept proposes the need for a collective view of all interactions and activities within and between a host and all its associated organisms. Knowledge of many individual aspects of these interactions has rapidly expanded in the last few years (for recent reviews see Gachon *et al.*, 2010; Goecke *et al.*, 2010; Hollants *et al.*, 2012; Wahl *et al.*, 2012), including the chemical interactions between bacteria and seaweed hosts (Goecke *et al.*, 2010; Wahl *et al.*, 2012), bacterial diversity (Hollants *et al.*, 2012), and microbial diseases of algae (Gachon *et al.*, 2010). Here, we will focus on the current knowledge of diversity and interactions displayed by bacteria associated with marine macroalgae. Specifically, we will address which bacteria are likely to contribute to the 'holobiont' and what environmental factors influence the maintenance, stability and establishment of such interactions. We will then discuss functional outcomes of these interactions and how environmental stress may result in a loss of holobiont function. Finally, we address the potential role of nonbacterial members in the seaweed holobiont and discuss the future directions and research opportunities.

Bacterial communities associated with macroalgal hosts – who is there?

Surface colonization is ubiquitous in the marine environment and macroalgal surfaces are no exception. Indeed marine macroalgae are typically home to a diverse group of bacteria with densities varying from 10^2 to 10^7 cells cm^{-2} depending on the macroalgal species, thallus section and season (Armstrong *et al.*, 2000; Bengtsson *et al.*, 2010). Image analysis of the microbial community associated with the surface of *Ulva australis* indicates that bacterial density increases by an order of magnitude from the thallus tips (10^6 cells cm^{-2}) to the algal base (10^7 cells cm^{-2}) (Tujula, 2006; Fig. 1). As early as the 1970s, culturing- and microscopy-based studies indicated clear differences between the microbial composition associated with macroalgae and that of the surrounding seawater, between different algal species, across different seasons as well as between different sections of a macroalgal thallus (Cundell *et al.*, 1977; Bolinches *et al.*, 1988). These observations of host specificity as well as temporal and spatial variation were further refined by a number of recent culture-independent studies (see Supporting Information, Table S1).

Host specificity refers to the occurrence of a specific set of bacterial epiphytes on one type of alga that are absent (or only found in very low numbers) on other algal species. In support of host specificity, bacterial community fingerprinting (denaturing gradient gel electrophoresis – DGGE) of various macroalgae at different locations showed that community patterns are more similar to those of conspecific macroalgae from different geographic origins than to other macroalgal species or the seawater from the same environment (Lachnit *et al.*, 2009). Similar patterns were observed for the active communities associated with the red alga *Laurencia dendroidea*, where transcriptomic profiling found little differences in the taxonomic composition of the community across different sample sites (de Oliveira *et al.*, 2012). Such host specificity may also apply to bacteria living within algal cells. Despite being described more than 40 years ago (Colombo, 1978) the endophytic communities of siphonous green algae, such as *Caulerpa* sp. and *Bryopsis* sp., have only recently been shown to be stable over time (Meusnier *et al.*, 2001; Hollants *et al.*, 2011b) and truly distinct from the epiphytic community of the same alga (Hollants *et al.*, 2011a).

Contrasting with this specificity on some hosts is the possibility that there are generalist epiphytes common to all or many macroalgae, or alternatively that some macroalgae may not harbor strongly host-specific communities (Burke *et al.*, 2011a, b). Indeed, common taxa have been identified on macroalgal surfaces albeit mostly at the

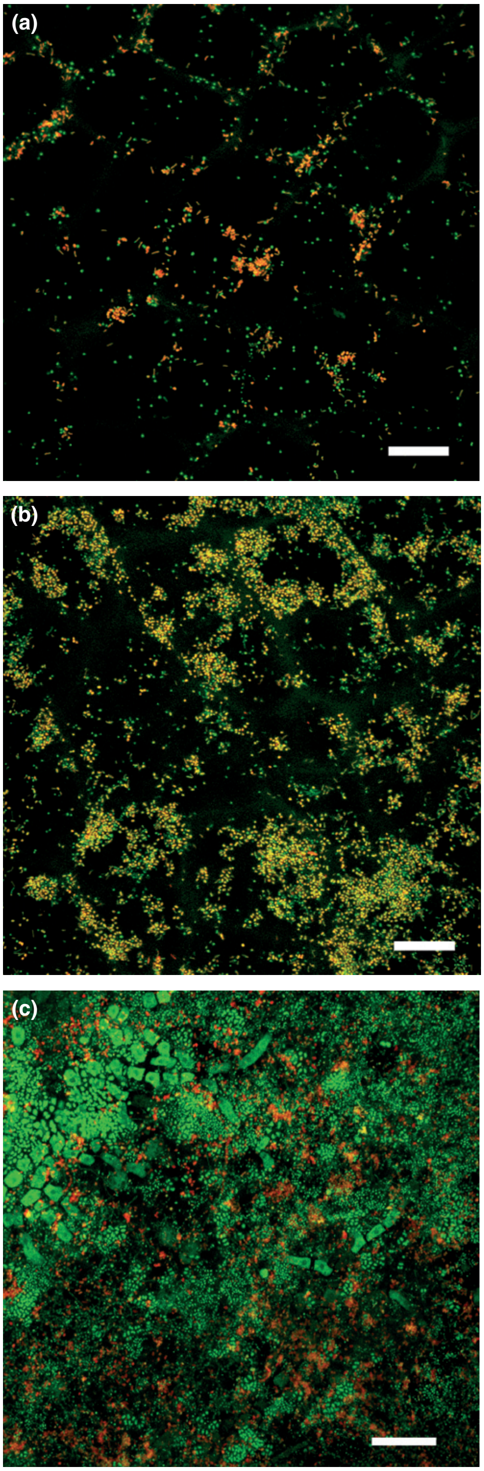

Fig. 1. Bacterial surface community on a macroalga host. Microscopic images showing the bacterial community on the distal tips (a), mid thallus (b), and base (c) sections of the green alga *Ulva australis*. Bacteria were detected with confocal microscopy using CARD-FISH. All bacteria (green), *Alphaproteobacteria* (red). Scale bars represent 10 μm length. These images were taken in the Centre for Marine Bio-Innovation, UNSW, Australia by Dr Niina Tujula.

phylum level. For example, bacteria belonging to the phyla *Planctomycetes* and *Verrucomicrobia* are abundant on Norwegian kelp (*Laminaria hyperborea*) (Bengtsson & Øvreås, 2010; Bengtsson *et al.*, 2010) and on *Fucus vesiculosus* from the Baltic Sea (Lachnit *et al.*, 2011). However, these phyla were notably absent from a related species of kelp [*Saccharina latissima* (previously *Laminaria saccharina*)] from both the Baltic and North Sea (Staufenberger *et al.*, 2008), as a result of either host specificity for these phyla or biogeography. For other macroalgae, common epiphytic bacteria include members of the *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, and *Cyanobacteria* with little distinction at these higher taxonomic ranks between the communities associated with different algal groups (i.e. red, green, and brown algae) (see also Table S1 and Hollants *et al.*, 2012). Interestingly in many cases, the similarities at higher taxonomic ranks (i.e. phylum or class) are not observed at lower ranks (i.e. genera or species). It is possible that limitations of the data sets currently available (as discussed below) preclude identification of genera, species, or even strains that are common to all macroalgal surfaces, and hence, it may be too early to suggest specific bacterial groups that can be considered 'typical' or 'core', and potentially unique macroalgal epiphytes.

Macroalgal communities also experience spatial and temporal shifts, which may be a reflection of the changing local conditions, host physiology, or chemical and physical parameters. For example, Lachnit *et al.* (2011) found reproducible seasonal shifts in the bacterial communities of three different co-occurring seaweed hosts, with a specific winter and summer bacterial community composition recurring over consecutive years. The observed variations and similarities can also be impacted by methodological limitations. These limitations are exemplified by studies on the cosmopolitan green alga *Ulva lactuca* (also referred to as *U. australis*), where DGGE-based analysis suggested the existence of a core community that is stable over space and time (Longford *et al.*, 2007; Tujula *et al.*, 2010). In contrast, extensive 16S rRNA gene sequencing of the bacterial community of *U. australis* was unable to detect a core community with only six bacterial species of a total of 528 being common between six individual algae (Burke *et al.*, 2011a). These seemingly contradictory results are likely to be a reflection of the higher resolution techniques used by Burke *et al.* (2011a) nevertheless, results from these more advanced techniques stand in contrast to the more specific communities described above.

Differences in the specificity of microbial communities on different host seaweeds may be reconciled by consideration of microbial functioning rather than phylogeny, as recently demonstrated for the bacterial community of

U. australis (Burke *et al.*, 2011b). Through shotgun metagenome sequencing of the alga's epiphytic community a set of core functions could be identified that was consistently present on *U. australis* individuals, despite a lack of commonality in taxonomic composition at lower levels (i.e. below family). These core functions were consistent with the conceptual understanding of the ecology of an algal- or surface-associated bacterial community. For example, functions associated with the detection and movement toward the host surface and attachment and biofilm formation were more abundant in the *U. australis* community then compared to planktonic community. Other overrepresented functions related to the response to the algal host environment, defense, and lateral gene transfer. The latter function represents one possible mechanism generating functional similarity in phylogenetically distinct bacteria on the surface of *U. australis* (Burke *et al.*, 2011b).

The data from the *U. australis* metagenome implies that community composition is largely determined by function, rather than taxonomic identity. Macroalgal surfaces are often freshly colonized by bacteria from the plankton, which likely contain many species with equivalent functionality that would allow them to become part of a surface-associated community. If initial colonization is by chance (a 'lottery') from a set of functionally equivalent planktonic bacteria (a 'guild'), then final community composition will have no recognizable taxonomic pattern, yet contain consistently all the traits that are necessary for an epiphytic community to function (Burke *et al.*, 2011b). Such a scenario might not only be restricted to macroalgal surfaces, but also apply to marine invertebrates or even a series of other microbiomes, such as those from the human gastro-intestinal tract, were bacterial community associates are recruited from the environment (i.e. horizontal acquisition). In this model, phylogenetic specificity (or lack thereof) is determined by the extent to which phylogeny maps onto function, which in the case of *U. australis*, was poor.

Future studies of taxonomic and phylogenetic community composition using high-resolution methods are required to shed light on the possibility of a core seaweed-associated bacterial community. Here, we would argue that functional studies (e.g. metagenomics, transcriptomic, proteomics, metabolomics, etc.) should be carried out in parallel with standard phylogenetic analyses if at all possible. Notwithstanding, however, given the diversity of macroalgal hosts and the variability of the environment in which they live, it is likely that macroalgal–bacterial interactions will be equally diverse and range from specialist to generalist. Therefore, it is important to gain an understanding of the biological, physical, and chemical factors that influence the epiphytic community on individual macroalgal species.

Factors that influence the assembly and maintenance of bacterial communities on seaweed hosts

A range of biological, physical, and chemical properties on the macroalgal surface is likely to play a role in structuring both qualitatively and quantitatively the associated microbial community and its metabolic activity. Parameters that define the macroalgal surface environment include algal metabolites, the existing resident microbial community with its pool of microbially derived secondary metabolites, and physico-chemical conditions on the thallus surface such as oxygen and carbon dioxide that can further modulate surface pH (Fig. 2). Many of these parameters are subject to daily (Spilling *et al.*, 2010), Fischer *et al.*, unpublished) or seasonal modulations (Hellio *et al.*, 2004). Bacteria entering into a stable association with a macroalgal host thus have to possess adaptive traits that reflect these niche conditions.

Oxygen

Macroalgal surfaces, unlike nonphotosynthetic or abiotic marine surfaces, generate oxygen via photosynthesis. Host photosynthesis would thus allow aerobic processes to occur in situations where oxygen might otherwise be limited. Trias *et al.* (2012) specifically tested this idea by hypothesizing that the surface of deep-sea macroalgae could represent a selective habitat for the oxygen-demanding process of ammonium oxidation. Using qPCR, it was found that ammonium-oxidizing bacteria were of relatively high abundance (1% of total bacteria) on the surface on the algae compared to that previously demonstrated for other marine habitats [e.g. 0.1% for marine sponges (Bayer *et al.*, 2008)]. Oxygen, however, can also become detrimental to bacterial epiphytes, especially if it results in the production of harmful reactive oxygen species (ROS). In fact, macroalgae can rapidly release large amounts of ROS such as superoxide ions and hydrogen peroxides (so called 'oxidative bursts') to defend themselves against bacterial attack [reviewed in (Weinberger, 2007)]. In turn, to protect themselves resident bacteria can express peroxidase, catalase and other oxidases that degrade ROS and hence minimize damage. While the importance of these defenses has not yet been directly established, it is noteworthy that the genomes of several macroalgal-associated bacteria, the microbial metagenome of *U. australis,* and the transcriptome of the microbial community associated with *L. dendroidea,* all contain an abundance of genes related to oxidative stress response (Thomas *et al.*, 2008; Burke *et al.*, 2011b; Fernandes *et al.*, 2011; de Oliveira *et al.*, 2012).

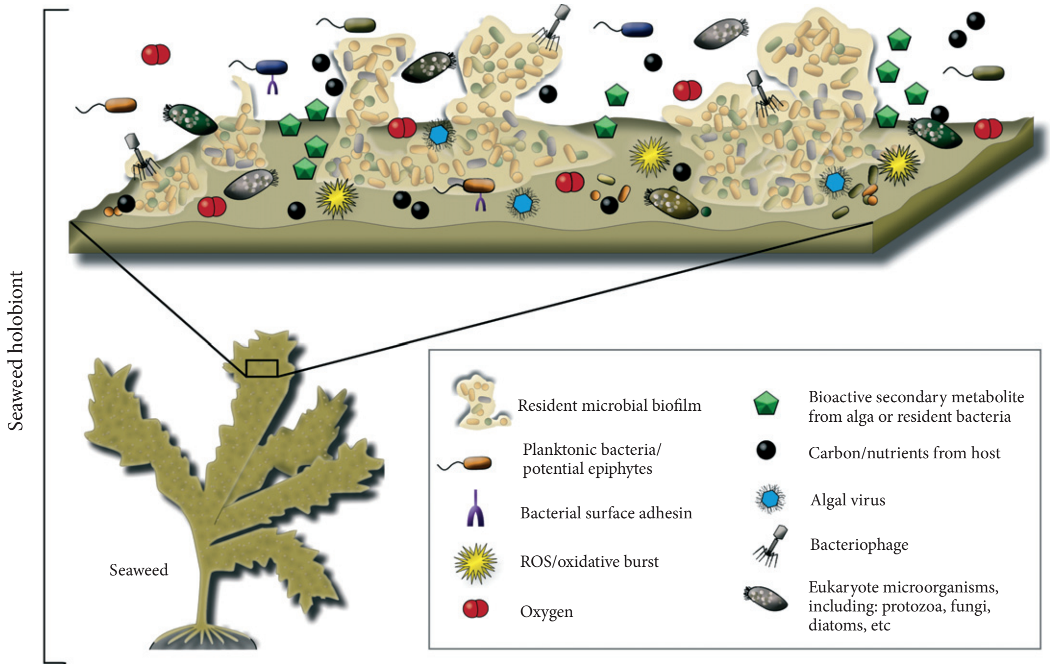

Fig. 2. The seaweed holobiont and the factors predicted to influence bacterial colonization on macroalgal hosts.

Polymers and nutrients

The presence of carbon-rich constituents of macroalgal cell walls (e.g. agar, carrageenan, alginate, fucan, laminarin, cellulose, and pectin) represents another factor that is likely to be important for bacterial colonization. Macroalgal cell wall components may constitute a nutrient source for bacteria capable of utilizing these biopolymers. In support of this are several studies demonstrating the ability of specific marine bacteria to degrade various macroalgal polymers. An overview of the specific enzymatic activities detected in relevant marine bacteria that degrade macroalgal cell walls is given in Goecke *et al.* (2010).

Polymer (e.g. cell walls or storage materials) degradation can obviously have a detrimental impact on the host, if not controlled. Stable or long-term bacterial associates of macroalgae might therefore lack the capacity for the initial polymer degradation (or have to tightly control it). This is supported by the observation that the common macroalgal bacterial epiphyte *Pseudoalteromonas tunicata* lacks the enzymes required to hydrolyse macroalgal cell wall polymers, but still contains the structures involved in polymer binding (e.g. a partial cellulosome) (Thomas *et al.*, 2008). Bacteria with polymer-degrading traits may thus represent opportunistic pathogens or saprophytes, rather than commensal or mutualistic macroalgal symbionts. Once damage to the host occurs, harmless associates might, however, contribute to the degradation of the host or take full advantage of nutrients released. For example, *P. tunicata* maintains the capability to utilize monomers derived from the degradation of typical macroalgal polymers, such as cellulose and xylan (Thomas *et al.*, 2008) and this will benefit the organism once its host is compromised. Such a shift in behavior was also recently observed for a bacterial symbiont of the microalga *Emiliania huxleyi*. Here, the symbiont *Phaeobacter gallaeciensis* produced a potent algaecide in response to an algal break-down product (p-coumaric acid), thus contributing to the further destruction of its aging host (Seyedsayamdost *et al.*, 2011).

Recent genomic and metagenomic data of macroalgal associates have revealed further evidence of nutrient scavenging, such as phosphorous, nitrogen, and iron utilization (Thomas *et al.*, 2008; Burke *et al.*, 2011b; Fernandes *et al.*, 2011). Members of the *Roseobacter* clade are commonly isolated from macroalgae (Brinkhoff *et al.*, 2008) and their genomes encode for functions allowing for the utilization of algal osmolytes, such as putrescine, taurine (Kalhoefer *et al.*, 2011), creatine, sarcosine (Thole *et al.*, 2012) and dimethylsulfoniopropionate (DMSP). In addition to being common in the phytoplankton (Curson *et al.*, 2011), DMSP is often produced by macroalgae (e.g. *Polysiphonia and Fucus*) (Malin & Erst, 1997; Saha *et al.*, 2012) and algal-associated metabolism of this compound may play a yet-unexplored role in global sulfur cycling.

As a final comment on this topic, while bacterial degradation of macroalgal tissue is detrimental to the host, this process is critical to global carbon and nutrient cycling.

Moreover, if managed correctly, such degradation could potentially be used to facilitate effective decomposition relevant for converting macroalgal polymers into biofuels (Wargacki *et al.*, 2012), an area of increasing commercial interest.

Defense and secondary metabolite chemistry

Numerous macroalgal species have been postulated to rely on secondary chemical defenses against fouling and potentially pathogenic microorganisms (reviewed in Goecke *et al.*, 2010) and this could clearly be a strong selective factor for epiphytic bacterial colonizers. Recent studies directly investigating the influence of secondary metabolites on bacterial surface colonization have demonstrated how specific macroalgal extracts have a marked effect on bacterial biofilm formation and community composition under both laboratory and field conditions (Lachnit *et al.*, 2010; Sneed & Pohnert, 2011). An experimental system designed to simulate the release of macroalgal metabolites from an artificial surface was used to measure the impact of macroalgal metabolites on bacterial colonization under ecologically realistic concentrations. Based on community fingerprinting analysis, the composition of test samples was distinct from control samples, yet similar to that of the natural macroalgal surface (Lachnit *et al.*, 2010), showing that algal metabolites alone are a strong selective force for community composition. The impact of macroalgal metabolites can also extend beyond that of the host surface with several studies demonstrating how macroalgae can affect bacterial community structure of the plankton (Lam & Harder, 2007; Lam *et al.*, 2008; Sneed & Pohnert, 2011).

In addition to crude macroalgal extracts, specific metabolites have also been identified and shown to influence bacterial community composition and/or growth on macroalgal hosts (Table 1). With knowledge of the localization, delivery rates and the effects of specific macroalgal metabolites on colonizing bacteria, several studies have begun to address their ecological relevance (Dworjanyn *et al.*, 1999; Nylund *et al.*, 2010; Persson *et al.*, 2011; Saha *et al.*, 2011). One example is the red alga *Delisea pulchra,* which produces a range of halogenated furanones that interfere with surface fouling of micro- and macroorganisms and maintain health and reproductive performance of this macroalga (Campbell *et al.*, 2011). Furanones are localized in the central vesicle of gland cells and continuously released to the surface, where they reach surface concentrations of approximately 100 ng cm^{-2} (Dworjanyn *et al.*, 1999). Furanone concentration decreases away from the distal tips of the macroalga; however, these lower concentrations remain sufficient to deter ecologically relevant epibiota and alter bacterial community composition (Maximilien *et al.*, 1998; Campbell *et al.*, 2011).

Table 1. Macroalgal metabolites that influence bacterial and fungal colonization under ecologically relevant conditions

Macroalga	Algal metabolite	References
Delisea pulchra	Halogenated furanones	Maximilien *et al.* (1998) and Dworjanyn *et al.* (1999)
Lobophora variegata	Cyclic lactone – lobophorolide	Kubanek *et al.* (2003)
Asparagopsis armata	Bromoform	Paul *et al.* (2006)
Asparagopsis armata	Dibromoacetic acid	Paul *et al.* (2006)
Bonnemaisonia hamifera	Polyhalogenated 2-heptanone	Nylund *et al.* (2008)
Callophycus serratus	Bromophycollides	Lane *et al.* (2009)
Fucus vesiculosus	Fucoxanthin	Saha *et al.* (2011)

Another example is the red alga *Bonnemaisonia hamifera*, whose main bioactive metabolite – 1,1,3,3-tetrabromo-2-heptanone – is stored in surface-localized gland cells and can reach concentrations of up to 4 μg cm^{-2} (Nylund *et al.*, 2008). This metabolite, when coated in a relevant concentration on field panels, alters the bacterial community density, diversity, and composition (Persson *et al.*, 2011). Moreover, the brown alga *Fucus vesiculosus* produces the pigment fucoxanthin, which at ecologically realistic concentrations (0.7–0.9 μg cm^{-2}) prevents the attachment of bacterial isolates from co-occurring macroalgae (Saha *et al.*, 2011). In contrast, bacteria isolated from the alga itself remain relatively insensitive to the effect of fucoxanthin (Saha *et al.*, 2011). A subsequent study showed that while a significant reduction of bacterial colonizers was observed, fucoxanthin had little impact on the overall bacterial community composition (Lachnit *et al.,* unpublished). Therefore, unlike furanones from *D. pulchra* or the polyhalogenated 2-heptanones from *B. hamifera,* fucoxanthin appears less selective, acting as a general inhibitor of bacterial attachment, rather than a specific inhibitor of bacterial growth that impacts on community composition. Nevertheless, variable sensitivity of individual bacteria to specific macroalgal metabolites is likely to be a common theme influencing the composition of epiphytic bacterial communities. For example, Saha *et al.* (2012) have recently shown that common macroalgal metabolites such as DMSP and the amino acids proline and alanine inhibit surface attachment of specific bacteria (e.g. *Cytophaga* sp), while promoting the attachment of others (e.g. *Rheinheimera baltica*).

The fact that macroalgal secondary metabolites are often produced and released by specific cells is likely to result in strong local effects on the bacterial epiphytes. Recent advances in analytical chemistry techniques now allow for fine-scale direct evaluation of metabolites on native surfaces under ambient conditions. Lane *et al.* (2009) were the first to apply an imaging mass spectrometry technique (desorption electrospray ionization mass spectrometry) on the native surface of the red alga *Callophycus serratus* to visualize and measure a group of antifungal algal metabolites – bromophycolides. This approach revealed a patchy distribution of the antifungal metabolites across the surface of the macroalga, suggesting that macroalgal surfaces are not homogenous with respect to bioactive metabolites. Correlating these fine-scale gradients of metabolite composition with high spatial resolution analysis of bacterial community composition (e.g. fluorescent *in situ* hybridization) is a powerful tool to assess the direct influence of macroalgal surface chemistry on the host-associated microbial diversity. In fact, it is likely that steep local gradients of macroalgal metabolites would create many specific microniches (analogous to a soil environment), thus potentially influencing the overall microbial diversity and composition of the macroalgal host.

Attachment mechanisms and surface competition

Bacteria use a range of appendages to attach to a surface that can mediate host specificity (Klemm & Schembri, 2000). For example, lectins are sugar-binding proteins that can mediate bacterial attachment to many biological surfaces (Rudiger & Gabius, 2001; Ogawa *et al.*, 2011). However, the role for lectin-mediated binding to macroalgal surfaces remains unexplored. In fact, there are very few studies that have experimentally tested the role of specific adhesins for the attachment to macroalgal surfaces. Mannose-sensitive hemagglutinin (MSHA)-pili are involved in the attachment of *P. tunicata* to *U. lactuca*. However, this organism might complement MSHA-mediated binding with multiple other adhesion mechanisms, including curli-proteinaceous fibers known to be important for plant colonization in *E. coli* (Thomas *et al.*, 2008), a lipoprotein (LipL32) – involved in adhesion to common extracellular matrix (ECM) fibers (Hoke *et al.*, 2008) and several divergent pili proteins (Thomas *et al.*, 2008). The importance of surface attachment is also reflected in the genomes of other bacterial epiphytes including *Nautella sp.* R11 and *P. gallaeciensis,* all of which encode for a number of known and hypothetical adhesins and extracellular polymers involved in biofilm formation (Fernandes *et al.*, 2011; Thole *et al.*, 2012). Furthermore, transcripts corresponding to genes involved in bacterial extracellular polysaccharide production were overrepresented in the microbiome of the red alga *L. dendroidea* (de Oliveira *et al.*, 2012).

Once attached, bacteria must compete with other microbial epiphytes for nutrients and space within the macroalgal surface biofilm. In such a situation, the production of antagonistic chemical metabolites (e.g. antibiotics) would be advantageous. The ecological importance of this is suggested by the frequent isolation of bacterial strains that produce bioactive substances from macroalgal surface [reviewed in (Egan *et al.*, 2008) and discussed below]. In the bacterial community associated with *U. australis,* nonribosomal peptide synthetases, which often produce bioactive substance, and multidrug-efflux pumps are generally abundant, further supporting the role of chemically mediated antagonism and counteractive defense processes in such environments (Burke *et al.*, 2011b). Increased expression of the antimicrobial metabolites within a biofilm has also been observed and may further improve the ability of these bacteria to compete on host surfaces (Matz *et al.*, 2008).

The ecological importance of chemical antagonism implied by the observations above has also been supported by experimental studies in the laboratory. For example, *P. tunicata* and *P. gallaeciensis* are superior competitors to other co-occurring epiphytic bacteria for settlement on *U. australis*, yet mutant strains lacking antibiotic production [AlpP and tropodithietic acid (TDA), respectively] are significantly less competitive (Rao *et al.*, 2005). Interestingly, while this shows the advantage of the production of antagonistic metabolites during the early establishment of a natural epiphytic community, the importance for subsequent bacterial colonization remains to be determined. In fact, pre-established natural epiphytic communities might be resilient to the introduction of new members, as *P. tunicata* and *P. gallaeciensis* were recently shown to be poor invaders of pre-established biofilms on both artificial and macroalgal surfaces (Rao *et al.*, 2010).

Overall, a multitude of host factors, microbial associates and environmental conditions are likely to play a role in shaping microbial community composition on marine macroalgae. An improved understanding of the extent to which these various factors influence the surface-associated microbiome *in situ* will be critical for predicting the potential impact of microbial symbionts on their host in terms of health and function, as discussed in the following section.

Functional outcomes of seaweed–bacteria interactions

While macroalgae represent niches with unique and selective properties, they also experience a range of beneficial and detrimental interactions with their bacterial symbiotic community. Given the ecological and applied importance of macroalgae, there has been an increasing interest in defining the outcome of these interactions.

Bacteria supply key nutrients and are required for normal morphological development of marine macroalgae

Epiphytic heterotrophic bacteria not only provide CO_2 for macroalgal photoautotrophy, but in some cases also provide fixed nitrogen (Penhale & Capone, 1981; Phlips & Zeman, 1990). Indeed nitrogen-fixing cyanobacteria were recently observed to be among the dominant active members of the microbial community associated with *L. dendroidea* (de Oliveira *et al.*, 2012). Epiphytic bacteria may also assist in or complement the macroalgal host's primary production as autotrophic cyanobacteria are often abundant on benthic macroalgal species (Barott *et al.*, 2011).

In addition, bacteria have a positive impact on the morphological development of several macroalgal species. Arguably, the best-studied example comes from early observations that certain green macroalgae do not develop normal morphology in the absence of native bacterial communities (Provasoli & Pintner, 1980). Specifically, axenically grown *U. lactuca* developed an abnormal 'pincushion'- like morphology, which could be restored to the typical foliose thallus upon reinoculation with bacterial strains isolated from the alga. Similar effects have been reported for other species of green algae, including, *Ulva linza*, *Ulva compressa* (formally *Enteromorpha linza and Enteromorpha compressa*) (Fries, 1975), *Ulva pertusa* (Nakanishi *et al.*, 1999), *Ulva fasciata* (Singh *et al.*, 2011), and *Monostroma oxyspermum* (Matsuo *et al.*, 2003). While in each case, normal morphology could be restored by 'reinoculation' with appropriate bacteria, the mechanisms of this interaction appear to vary between macroalgal hosts. Both Nakanishi *et al.* (1996) and Marshall *et al.* (2006) have provided evidence that bacteria from a range of phyla including members of the Proteobacteria, Bacteroidetes, and Firmicutes are able to induce normal morphogenesis in *Ulva* species and that bacterial attachment to the host may be required for restoration of normal macroalgal morphology (Nakanishi *et al.*, 1999). In contrast in a screen of over 50 isolates, Singh *et al.* (2011) found only five strains belonging to either *Marinomonas* sp. or *Bacillus* sp. that were able to induce normal development in axenic *U. fasciata.* Moreover, studies with *M. oxyspermum* also suggest that morphogenic induction is restricted to certain bacterial groups (*Cytophaga – Flavobacterium – Bacteroides*) (Matsuo *et al.*, 2003) and occurs in response to a secreted morphogenesis factor, called thallusin (Matsuo *et al.*, 2005). Thallusin is effective in low concentrations (fg mL^{-1} range), but activity is lost over time suggesting that the macroalga may rely on a continual supply of the inducer from the epiphytic bacterium. Interestingly, both the producing bacterium and pure thallusin were able to restore the normal morphology of other green algae, suggesting it to be a universal cue for morphogenesis in green algae (Matsuo *et al.*, 2003, 2005).

Macroalgal-associated bacteria contribute to host defense against unwanted colonization and biofouling

There are numerous laboratory studies demonstrating that epiphytic bacteria have inhibitory activity against common biofouling organisms (as reviewed in Holmström *et al.*, 2002; Dobretsov *et al.*, 2006; Egan *et al.*, 2008). For example, aqueous extracts and biofilms of a macroalgal-derived *Vibrio* sp. and a *Pseudoalteromonas* sp. inhibit the settlement and metamorphosis of the polychaete *Hydroides elegans* (Dobretsov & Qian, 2002). Also *Pseudoalteromonas* strains from *U. lactuca* in both temperate (Egan *et al.*, 2001) and tropical waters (Kumar *et al.*, 2010) possess activities against various fouling organisms (bacteria, diatoms, fungi etc.). In fact, *Pseudoalteromonas* species are commonly isolated from algal surfaces and have regularly displayed antifouling properties. Specifically, *P. tunicata* has become a model organism for antifouling as it possesses activities against a range of target organisms, including algal spores, invertebrate larvae, benthic diatoms, various bacteria, fungi (Bowman, 2007; Egan *et al.*, 2008), protists (Matz *et al.*, 2008), and nematodes (Ballestriero *et al.*, 2010). Remarkably, while *P. tunicata* and the closely related species *P. ulvae,* appear in relative low densities (10^3 cells cm^{-2}) on macroalgal hosts (*U. lactuca* and *Ulvaria fusca)* in the field (Skovhus *et al.*, 2007), these densities are still sufficient to inhibit fouling by macroalgal spores, marine fungi, and invertebrate larvae (Rao *et al.*, 2007). Similar observations were recorded for the antifouling properties of *P. gallaeciensis* 2.10 (Rao *et al.*, 2007).

Antifouling and antimicrobial activities are found in a wide range of bacterial taxonomic groups. For example, the brown kelp *S. latissima* (previously *Laminaria saccharina*) harbors more than 100 different antimicrobial strains covering the phyla *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Wiese *et al.*, 2009). In another study, 30 strains with antimicrobial activity were identified from *D. pulchra* and *U. lactuca.* While these shared the same broad taxonomic classification, there was little overlap at the species or genus level between the two macroalgal hosts (Penesyan *et al.*, 2009). The majority of studies aimed at assessing antifouling/antimicrobial properties of epiphytic bacteria has focused on cultured strains and hence are likely to have missed the potential of the uncultured fraction of the community. Indeed this notion, was recently supported by the outcome of a functional metagenomics screen of microbial communities associated with marine sponges and macroalgae, in which new classes of antibacterial proteins were discovered (Yung *et al.*, 2011).

Disturbance of the macroalgal holobiont by bacterial pathogens

Microorganisms are increasingly recognized for their etiologic role as agents of disease of marine animals, plants, and algae. This interest in microbial disease in marine ecosystems is, in part, driven by concerns that climate change-related stress on marine habitat formers (corals, macroalgae, etc.) and their associated microbiome will render them more susceptible to potential opportunistic pathogens (Harvell *et al.*, 1999). Macroalgal pathogens are diverse and include viruses, eukaryotic parasites and bacteria. Here, we focus on the current knowledge of macroalgal interactions with bacterial pathogens. For details of other microbial pathogens readers are referred to a recent review by Gachon *et al.* (2010), which predominately discusses eukaryotic and viral pathogens and their role as drivers of ecosystem function and macroalgal evolution.

The study of bacterial macroalgal pathogens is still in its infancy. Distinguishing the causative agents from other opportunistic bacteria remains one of the main obstacles to delineating virulence mechanisms from saprophytic processes. However, one example where substantial progress has been made in understanding these aspects is that of bleaching disease in *D. pulchra*. Here, two pathogens (*Nautella italic* sp. R11 and *P. galleciensis* LSS9) have been shown to colonize and infect *D. pulchra* under laboratory conditions, resulting in thallus bleaching, similar to that observed in the field (Case *et al.*, 2011; Fernandes *et al.*, 2011). These two bacteria belong taxonomically to the marine *Roseobacter* clade, and other members of this group cause gall-like tumors in the red alga *Prionitis* (Ashen & Goff, 2000).

The availability of cultured pathogens for the *D. pulchra* bleaching disease opened the way to define the molecular mechanisms of pathogenicity. Comparative genomics of *N. italic* sp. R11 and *P. galleciensis* LSS9 with 18 closely related nonpathogenic bacteria revealed the presence of several putative virulence genes in these strains (Fernandes *et al.*, 2011). One gene unique to both pathogens was found to encode a Lux-R type transcriptional activator, similar to those involved in AHL-mediated quorum sensing (QS), which is known in several well-characterized pathogens to regulate colonization and virulence (Venturi, 2006; Barnard *et al.*, 2007; Charkowski, 2009).

Pathogenicity based on an AHL-type QS system provides an ecological link to the chemical defense of *D. pulchra* (see above), which is based on furanones that act as QS blockers (Givskov *et al.*, 1996). A healthy chemically defended *D. pulchra* could thus have the capacity to repress virulence gene expression (and consequently disease) by *N. italic* sp. R11 and *P. galleciensis* LSS9. Interestingly, during summer months when *D. pulchra* loses its furanones, a higher incidence of bleaching is observed (Campbell *et al.*, 2011). These observations agree with a model that *N. italic* sp. R11 and *P. galleciensis* LSS9 transition from commensal to pathogenic traits via the QS-based activation of virulence mechanisms.

If such a model is correct then macroalgal surfaces may host other bacterial pathogens that only express virulence genes under certain conditions or when the host is compromised (i.e. opportunistic pathogens). Indeed community fingerprinting (t-RFLP and DGGE) and 16S rRNA gene clone libraries have confirmed that many bacterial members differ in both abundance and presence/ absence between healthy and diseased *D. pulchra* (Campbell *et al.*, 2011, Fernandes *et al.,* unpublished). For example, bacteria belonging to the taxa *Colwelliaceae, Thalassomonas, Rhodobacteraceae,* and *Celluophaga* were abundant in bleached tissue and absent or reduced in abundance in healthy tissue. In addition, metagenomic analysis revealed changes in functionality, with the community of diseased tissue being enriched in secondary metabolite production, transport systems, chemotaxis, and gene regulation.

Enrichment of certain bacteria has also been observed in rotting disease of kelp (see Gachon *et al.*, 2010). For example, Wang *et al.* (2008) cultured a large number of bacteria from *Laminaria japonica* thalli that displayed symptoms of hole-rotten disease and found a striking abundance of *Pseudoalteromonas* sp. and *Vibrio* sp. While reinfection of kelp tissue with these strains did result in observable symptoms, no attempt was made to reisolate the potential pathogen (i.e. demonstrate Koch's postulates), and thus, it is unclear if these strains are in fact the true causative agents of the disease. Indeed, it is likely that some of the bacteria found on diseased macroalgal tissue (including the ones on *D. pulchra*) are secondary colonizers that act potentially as saprophytes or decomposers. Both culture-based and more recent genomic studies have shown that many macroalgal-associated bacteria harbor enzymes for the degradation of complex polysaccharides components of the macroalgal cell (Sakai *et al.*, 2003; Kalhoefer *et al.*, 2011). It is thus likely that particular bacterial epiphytes may be otherwise commensal, but under conditions of infection or stress of the macroalgal host, they become predominantly saprophytic.

Disease in marine macroalgae has been noted for many years; however, the observation and models derived from *D. pulchra* and other macroalgae now indicate a complex interplay between the host and the microbial community, not previously appreciated. Moreover, the work on *D. pulchra* has shown that genome sequencing of macroalgal pathogens and comparative metagenomic analysis of disease and healthy macroalgae can rapidly provide insights into disease ecology and function. This knowledge when applied in the framework of existing marine and chemical ecology provides a powerful systems biology tool to generate and subsequently test new hypotheses.

Other microbial members of the seaweed holobiont

The vast majority of studies related to the microbiome of macroalgae have to date focused on bacteria. Interestingly, the largely historical focus on bacteria is in agreement with recent metagenome and transcriptome analysis, which indicates that bacteria indeed dominate these communities (Burke *et al.*, 2011b; de Oliveira *et al.*, 2012). Nevertheless, with the holobiont concept in mind, it is important to also consider the role of other host-associated microbes (e.g. archaea, eukaryotic protist, and viruses).

Mesophilic *Crenarchaeota* have been observed in many marine habitats, including sessile invertebrates such as sponges, where they are thought to play a key role in the oxidation of ammonia (Taylor *et al.*, 2007; Turque *et al.*, 2010). Ammonium-oxidizing archaea have also been detected on some macroalgal host, however, unlike other marine habitats, they appear underrepresented compared with their bacterial counterparts (Trias *et al.*, 2012). Moreover, archaea constitute only minor proportion of the epiphytic microbiome of *U. lactuca* (Burke *et al.*, 2011b). While this observations imply that archaea play a minor role in a seaweed holobiont, more research is required to define their potential as macroalgal epiphytes.

A number of studies have reported on the abundance and diversity of various groups of eukaryotic microbes, including dinoflagellates (Armstrong *et al.*, 2000; Porto *et al.*, 2008), ciliates (Armstrong *et al.*, 2000), diatoms (Armstrong *et al.*, 2000), amoebae (Rogerson, 1991; Armstrong *et al.*, 2000), and fungi (Zuccaro *et al.*, 2008). Analysis of 18S rRNA gene sequences from the microbiome of *U. lactuca* further revealed the presence of protist, including the ciliate *Ephelota* sp., fungus *Tremisus helvelloides,* and the diatom *Asterionellopsus glacialis* (Burke *et al.*, 2009). Epiphyitc eukaryotes can have pathogenic or saprophytic interactions with their host, such as fungal invasion and necrosis of algal tissue (Kawamura *et al.*, 2005), infection of algal tissue by oomycete (water molds) (Grenville-Briggs *et al.*, 2011) and tumor formation in large kelp (Goecke *et al.*, 2012). Marine epiphytic and endophytic fungi are also a source of natural defensive compounds that can be exploited as novel therapeutics (Rateb & Ebel, 2011), which could suggest a positive or protective role for fungi within the holobiont. However for the most part, the ecological role of eukaryotic microorganisms in the health and function of the algal host is speculative and remains largely unknown.

Viruses are abundant in the marine environment and have been extensively studied in the plankton and for their role in ocean nutrient cycling (see (Suttle, 2005) and references there in). With respect to viruses on macroalgae, those from the filamentous brown alga *Ectocarpus* sp. are arguably the best studied and most diverse (Van Etten *et al.*, 2002; Dunigan *et al.*, 2006). These large DNA viruses infect free-living gametes or spores, then integrate into the host genome, where they remain latent in the vegetative parts of the alga, but become active in the reproductive algal cells (Van Etten *et al.*, 2002). Recent analysis of the *Ectocarpus siliculosus* genome revealed that up to 50% of natural algal population are infected (Cock *et al.*, 2010), suggesting that viruses have the potential to strongly influence the evolution and ecology of macroalga.

Perspective

Marine macroalgae are important ecosystem engineers, yet until recently little was understood with respect to the diversity and function of their associated bacterial community. Epiphytic bacterial communities are likely to consist of both generalist and specialist populations and are quite dependent on the algal host species as well as the geographical location. While diversity studies have indicated core phyla (*Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*) that are common members of algal communities, there is little evidence to support the idea that individual bacterial species are host specific. Rather, it is possible that recruitment of bacteria to an algal surface (and hence host specificity) is based on the selection of specific functional traits such as those discussed above.

Irrespective of the mechanism, the maintenance of specific bacterial groups and/or their functional traits is likely to reflect their benefit to the host. Ultimately, this interaction would result in the development of an intimate relationship between the alga and its associated microbiome, thus giving support for seaweed holobiont concept. Moreover, evidence that bacteria and their secondary metabolites (e.g. AHLs) are important cues for algal spore release (Weinberger *et al.*, 2007) and settlement [reviewed in (Joint *et al.*, 2007)] highlights a role of bacteria in the early life-history stages of macroalgae that extends beyond a holobiont concept and toward the colonization of new surfaces.

While the field has moved a long way from the first observations that native bacteria are essential for the normal morphological development of macroalgae, there is clearly more work to be carried out, probably most critically is relating functional studies from the laboratory to outcomes in natural communities. A detailed understanding of the mechanism and functional role of all microbial members, whether bacterial, archaeal, viral, or eukaryotic, in a seaweed holobiont and their ecological role in the alga's life cycle would be valuable to the management of seaweeds in both natural and man-made aquaculture settings. The role of microorganisms in algal disease is of growing interest, and future work in this area should shed light not only on specific algal pathogens but also on the potential probiotic effect of the host microbiome. In each case, the challenge remains to obtain information not only on the mechanisms of these specific interactions but also on their ecological significance. To achieve this, future studies should move away from predominately laboratory-based experimentation and focus on obtaining sound data from manipulative studies conducted in the field. Finally, while technological advances will continue to provide the tools to progress this research, it will be the interaction of scientists with complementary skills, including chemists, ecologists, and microbiologists that will ensure that these opportunities are maximized.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Diversity studies of bacterial epiphytes from marine macroalgae from 2007-2012.