MINIREVIEW

What we can learn from sushi: a review on seaweed–bacterial associations

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Abstract

Many eukaryotes are closely associated with bacteria which enable them to expand their physiological capacities. Associations between algae (photosynthetic eukaryotes) and bacteria have been described for over a hundred years. A wide range of beneficial and detrimental interactions exists between macroalgae (seaweeds) and epi- and endosymbiotic bacteria that reside either on the surface or within the algal cells. While it has been shown that these chemically mediated interactions are based on the exchange of nutrients, minerals, and secondary metabolites, the diversity and specificity of macroalgal–bacterial relationships have not been thoroughly investigated. Some of these alliances have been found to be algal or bacterial species-specific, whereas others are widespread among different symbiotic partners. Reviewing 161 macroalgal–bacterial studies from the last 55 years, a definite bacterial core community, consisting of Gammaproteobacteria, CFB group, Alphaproteobacteria, Firmicutes, and Actinobacteria species, seems to exist which is specifically (functionally) adapted to an algal host–associated lifestyle. Because seaweed–bacterial associations are appealing from evolutionary and applied perspectives, future studies should integrate the aspects of diverse biological fields.

If there is one thing we can learn from sushi, it is that seaweed-associated bacteria can have unexpected beneficial effects. The carbohydrate active enzyme porphyranase from the marine Bacteroidetes bacterium *Zobellia galactanivorans* breaks down the sulphated polysaccharide porphyran from the red alga *Porphyra* (nori) traditionally used to prepare sushi. Moreover, the genes coding for this porphyranase have been horizontally transferred through dietary seaweed from *Z. galactanivorans* to the gut microbe Bacteroides plebeius from particularly Japanese people, allowing them to digest the algae that wrap sushi rolls and other delicacies (Hehemann *et al.*, 2010). This not only indicates that the human gut microbiota may become proficient at using dietary polysaccharides by horizontal gene transfer; it also highlights the significance of macroalgal–bacterial associations.

Like sushi, algae come in many forms and flavors ranging from microscopic unicells to gigantic kelps inhabiting oceans, freshwater habitats, soils, rocks, and even trees (van den Hoek *et al.*, 1995). Consequently, this review needed some delimitation and is restricted to the studies of bacteria associated with marine macroalgae (seaweed) belonging to the Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyceae (brown algae). Seaweed and bacteria have come a long way because algal plastids originated from endosymbiotic cyanobacteria (Margulis, 1998). Like their unicellular ancestors, marine macroalgae form the modern-day playground for a wide diversity of bacterial associations ranging from beneficial (mutualistic), harmful (parasitic), and neutral (commensal), over obligate and facultative, to endo- and ectophytic interactions (Relman, 2008). This, along with applied aspects of current algal–bacterial symbioses, makes their associations appealing for evolutionary, ecological, and biochemical studies. Nevertheless, investigations of macroalgal–bacterial associations lag behind these of other marine eukaryotes (Goecke *et al.*, 2010). Whereas the full cycle 16S rRNA approach (Olsen *et al.*, 1986) is well established to characterize the microbial associates of unicellular algae, corals, and sponges (Geng & Belas, 2010; Olson &

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Kellogg, 2010), these molecular techniques are just beginning to be applied to macroalgae (Goecke *et al.*, 2010 and references therein).

From a kitchen secret to molecular microbiology: a historical overview

Foundations

The first report of a seaweed–bacterium alliance – although artificial – is one that altered bacteriology forever. In 1881, Walther Hesse, a German physician, joined Robert Koch's laboratory to study the bacteria responsible for his patients' illnesses. But, like his colleagues, Hesse encountered major technical problems attaining pure bacterial cultures on solid gelatin-based media. The gelatin often liquefied because of bacterial enzymes or because of the incubation temperature. When he vented his frustrations to his wife Fanny, she suggested using a seaweed extract, agar–agar, which she had used to thicken her jellies and puddings for years (Hesse & Gröschel, 1992). The practical application of this kitchen secret accelerated bacteriological research greatly, opening the way also for real-life macroalgal–bacterial studies. In fact, it was Walther Hesse himself who developed agar plate techniques to count bacteria in water samples. Techniques the ship's physician Bernard Fischer (1889) used to great success in the tropical waters of the Sargasso Sea during the Plankton Expedition of the Humboldt Foundation across the Atlantic Ocean (ZoBell, 1946). Throughout that trip, Fischer noted that the greatest abundance of culturable marine bacteria was associated with planktonic organisms and seaweeds. Hans Gazert (1906) who was in charge of the bacteriological investigations of the German South Polar Expedition made similar observations in the South Atlantic and Antarctic Ocean where some of the largest bacterial populations were found in the vicinity of seaweeds (ZoBell, 1946). Although these observations are mainly founded on a high influx of organic matter from the remains of dead seaweeds (ZoBell, 1946), also symbiotic (here defined as mutualistic) associations with living macroalgae might have contributed. Simultaneously with these initial notes of seaweed–bacterial alliances at sea, scientists in the laboratory deduced similar conclusions from their preliminary late 19th century macroalgal culture work. The German botanist Georg Klebs (1896) was aware of the presence of bacteria in his seaweed cultures and tried to set up pure, axenic cultures of filamentous and siphonous algae. While he was successful in growing the algae, he was not able to keep his cultures bacteria-free (Andersen, 2006). Even though Klebs was a former assistant of Anton de Bary who first introduced the term 'symbiosis' in biology, it was Johannes Reinke (1903) who was the first to suggest a true symbiotic marcoalgal–bacterial partnership. The occurrence of *Azotobacter* as an epiphyte on marine algae led him to propose that a symbiosis may exist in which the algae supply *Azotobacter* with carbohydrates and use the nitrogen fixed by the bacteria (Waksman *et al.*, 1933; ZoBell, 1946). Also Edgar Johnson Allen (1910), Director of the Marine Biological Association of the United Kingdom, and his collaborator E.W. Nelson recognized a symbiotic aspect in xenic marcoalgal cultures (Andersen, 2006). As they laid the foundations for seaweed culture, they noticed good growth of algae only when small quantities of natural seawater were added to the artificial culture media. Allen remarked that these effects may be caused by products of the metabolism of bacteria (Andersen, 2006).

First cultivation and microscopy studies

It took until after World War II for Luigi Provasoli and colleagues to establish the first bacteria-free cultures of the green foliaceous seaweed *Ulva* using newly discovered antibiotics (Andersen, 2006). Provasoli, however, observed that the typical foliose morphology of *Ulva lactuca* was lost in the absence of bacteria and – even more interesting – that the normal thallus morphology was restored when certain bacteria previously isolated from the algal surface were re-added to the culture medium (Provasoli, 1958; Provasoli & Pintner, 1980). In 1955, Harold and Stanier were the first to exhaustively describe the bacterium *Leucothrix mucor* that was found consistently as an algal epiphyte, showing macroalgae not only to interact with bacteria but also to represent a distinct source of new microbial taxa. With the introduction of electron microscopy to study the macroalgal ultrastructure in the 1970s, an intriguing new form of seaweed–bacterial interactions was discovered. In addition to epiphytic bacteria, various siphonous seaweeds such as *Bryopsis*, *Caulerpa*, *Chlorodesmis*, *Halimeda*, *Penicillus*, and *Udotea* were also shown to harbor intracellular bacteria within their cytoplasm and/or vacuolar systems (Burr & West, 1970; Burr & Evert, 1972; Turner & Friedmann, 1974; Colombo, 1978; Dawes & Lohr, 1978; Menzel, 1987). Simultaneously with these early microscopic observations, the first cultivation studies aiming to examine the total diversity of bacteria associated with macroalgae arose. Although the bacteria were initially identified only by morphological and biochemical tests, the epiphytic flora on seaweeds was clearly very diverse, covering numerous bacterial taxa (Berland *et al.*, 1969; Chan & McManus, 1969; Tsukidate, 1971; Laycock, 1974; Kong & Chan, 1979; Mazure & Field, 1980; Shiba & Taga, 1980; Lakshmanaperumalsamy & Purushothaman, 1982; Lemos *et al.*, 1985; Lewis *et al.*, 1985). Not only were these macroalgal-associated bacteria

distinct from the surrounding seawater communities, they also appeared host-specific with clear differences in occurrence among green, red, and brown seaweeds (Kong & Chan, 1979; Shiba & Taga, 1980; Lakshmanaperumalsamy & Purushothaman, 1982; Lewis *et al.*, 1985). A stable association between algal hosts and bacteria was observed (Kong & Chan, 1979; Shiba & Taga, 1980; Lewis *et al.*, 1985), even though the bacterial flora may vary between seasons and/or between different parts of the algal thallus (Chan & McManus, 1969; Laycock, 1974; Mazure & Field, 1980). From these and other studies in the 1970s and 1980s, Bolinches *et al.* (1988) concluded the existence of both positive and negative macroalgal–bacterial interactions based on the algal capacity to produce organic compounds and oxygen that are utilized by bacteria. In turn, bacteria produce morphogenic factors, fixed nitrogen, enzymes, and vitamins which promote algal growth (Head & Carpenter, 1975; Provasoli & Pintner, 1980; Rosenberg & Paerl, 1981; Lakshmanaperumalsamy & Purushothaman, 1982; Croft *et al.*, 2005, 2006). In addition, epiphytic bacteria as well as the seaweed hosts themselves produce antibiotic substances that prevent colonization of the algal surface by bacterial competitors and pathogens (Sieburth, 1968; Lemos *et al.*, 1985).

Emergence of molecular techniques

Although the number of macroalgal–bacterial studies has risen steadily during the last two decades, these have not significantly increased our understanding of macroalgal–bacterial interactions as postulated above. Thanks to the improvement of analysis techniques, both symbiotic partners can be characterized biochemically and phylogenetically in more detail. However, many questions remain (Goecke *et al.*, 2010). In the following sections, we review the current knowledge on the diversity and functional ecology of bacterial communities associated with green, red, and brown marine macroalgae.

Chemical interactions between seaweeds and bacteria

The relationship between macroalgae and bacteria in which seaweeds provide nutrients, while the bacterial community promotes algal growth and protects the host against pathogens, has been elaborated over the last 20 years. Figure 1 depicts the complex, chemically mediated interplay of beneficial and detrimental relations that exists between macroalgae and bacteria. The variety and nature of these chemical interactions have been exhaustively reviewed by Goecke *et al.* (2010) and are summarized in the remainder of this section.

Seaweed partner

From the algal host perspective, macroalgal–bacterial interactions are not unexpected. Seaweed surfaces provide a protected and nutrient-rich 'hot spot' for opportunistic bacteria that are abound wherever organic material is available (Armstrong *et al.*, 2001). In most cases, molecular investigations have confirmed the outcome of initial cultivation studies, that is, that the attraction of bacteria by seaweeds turns out to be highly specific. While the composition of the bacterial flora can change over seasons, life span and different thallus parts as a result of biotic and abiotic factors (Staufenberger *et al.*, 2008; Bengtsson *et al.*, 2010; Tujula *et al.*, 2010), marine macroalgae generally associate with specific bacterial communities that differ significantly from those occurring in the surrounding seawater (Longford *et al.*, 2007; Lachnit *et al.*, 2009). Recently, however, Burke *et al.* (2011b) found highly variable bacterial species compositions among local individuals of *Ulva australis* by means of in-depth 16S rRNA screening, suggesting each *U. australis* plant hosts a unique assemblage of bacterial species. Moreover, using a metagenomic approach, they subsequently showed that the bacterial community composition on *U. australis* is driven by functional genes rather than the taxonomic or phylogenetic composition of its species (Burke *et al.*, 2011a). This implies that functional groupings (or 'guilds') of – not necessarily phylogenetically related – bacterial species exist of which the composition on a single algal individual is determined stochastically by recruitment from within those guilds. Even if the specificity of a seaweed-associated bacterial community may be based on functional genes rather than species, it is known that the physiological and biochemical properties of the algal host predetermine the composition of the adhering bacterial communities. For example, algal cell wall components and secondary metabolites can trigger specific interactions between seaweeds and beneficial bacteria (reviewed in Engel *et al.*, 2002; Lachnit *et al.*, 2010). Algal bioactive compounds also have antimicrobial properties – with interesting biomedical and industrial applications – which protect the seaweed surface from bacterial pathogens, grazers, and biofouling, that is, the undesirable accumulation of micro- and macroorganisms as biofilms on the seaweed surface (Steinberg *et al.*, 1997; Engel *et al.*, 2002; Bhadury & Wright, 2004; Paul *et al.*, 2006; Lam *et al.*, 2008; Goecke *et al.*, 2010, table 5). Besides these bioactive compounds, macroalgae control bacterial colonization by interfering with bacterial quorum sensing (QS) systems that regulate bacterial cell-to-cell communication (Kjelleberg *et al.*, 1997; Maximilien *et al.*, 1998; Steinberg & de Nys, 2002; Goecke *et al.*, 2010, table 6). In addition to these induced defense mechanisms, seaweeds

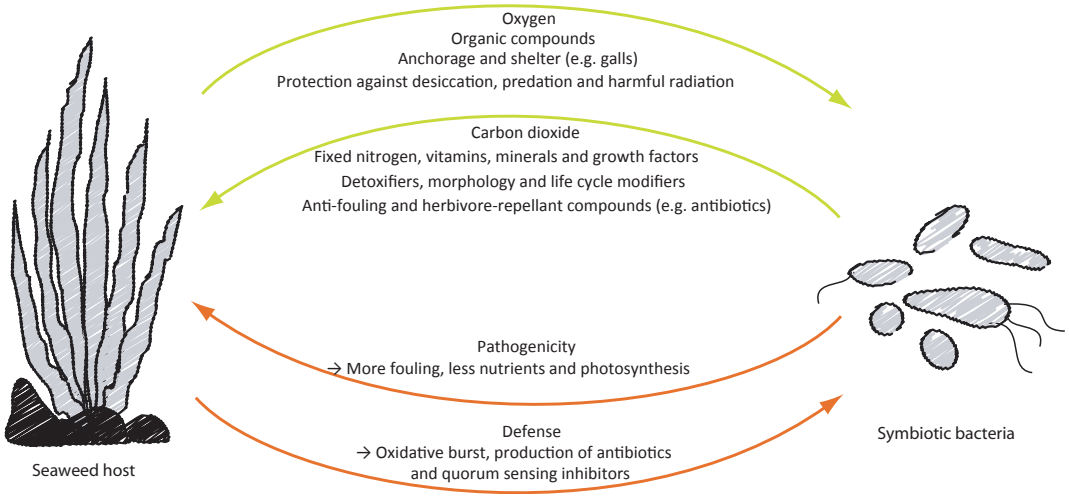

Fig. 1. Overview of beneficial (green) and detrimental (red) interactions between macroalgae and bacteria.

also possess nonspecific defense responses against bacterial pathogens similar to the 'oxidative burst' process of higher plants (Weinberger, 2007; Potin, 2008).

Bacterial partner

Many bacteria growing on seaweed surfaces are able to enzymatically decompose algal cell walls, making them key players in biotransformation and nutrient recycling in the oceans (Michel *et al.*, 2006; Goecke *et al.*, 2010, table 2). Also specific, beneficial bacterial–macroalgal interactions are based on the bacterial capacity to mineralize algal organic substrates and subsequently supply the seaweed host with carbon dioxide, minerals, vitamins, and growth factors (Armstrong *et al.*, 2001; Croft *et al.*, 2005, 2006; Dimitrieva *et al.*, 2006; Singh *et al.*, 2011b). Several studies also revealed that seaweed-associated bacteria are important sources of fixed nitrogen and detoxifying compounds (Chisholm *et al.*, 1996; Riquelme *et al.*, 1997; Goecke *et al.*, 2010 and references therein). Besides nutritional and growth-promoting effects, bacteria may shape the morphology and life cycle of their algal host. Bacterial effects on morphogenesis have been reported in foliaceous green macroalgae such as *Ulva* and *Monostroma* (Fries, 1975; Provasoli & Pintner, 1980; Tatewaki *et al.*, 1983; Nakanishi *et al.*, 1996; Matsuo *et al.*, 2003; Marshall *et al.*, 2006) and have been shown to be controlled by a highly potent differentiation inducer, thallusin, isolated from well-defined associated bacteria (Matsuo *et al.*, 2005; Goecke *et al.*, 2010, table 4). Thallusin and other secondary metabolites, including signaling and QS molecules, also play a role in the host's life cycle completion as well as in algal spore release and germination (Joint *et al.*, 2002; Patel *et al.*, 2003; Matsuo *et al.*, 2005; Joint *et al.*, 2007; Weinberger *et al.*, 2007; Goecke *et al.*, 2010, table 4; Wichard & Oertel, 2010). Furthermore, QS inhibitors and antimicrobial compounds produced by numerous epiphytic bacteria work in concert with seaweed-derived metabolites to protect the seaweed surface from pathogens, herbivores, and fouling organisms (Boyd *et al.*, 1999; Egan *et al.*, 2000; Zheng *et al.*, 2000; Armstrong *et al.*, 2001; Dobretsov & Qian, 2002; Rao *et al.*, 2007; Wiese *et al.*, 2009; Goecke *et al.*, 2010, table 4). Pathogenic bacteria can cause severe degradation of algal host cells or even lead to seaweed mortality, causing major financial losses to seaweed mariculture every year (Correa *et al.*, 1993; Vairappan *et al.*, 2008; Goecke *et al.*, 2010, table 4). Also biofouling forms a permanent threat to macroalgae as bacterial biofilms increase the hydrodynamic drag on their host and enhance the attachment of other fouling organisms and grazers. Biofilms may also compete for nutrients, inhibit gaseous exchange, or block light, essential for photosynthesis. Thus, both bacterial and algal secondary metabolites are essential chemical mediators in macroalgal–bacterial associations that jointly control the composition and density of bacterial biofilms thereby defending the seaweed surfaces against biofouling (Steinberg *et al.*, 1997; Goecke *et al.*, 2010 and references therein). In addition, bacterial bioactive compounds may represent a more promising – and easier to handle – source of natural products with biotechnological applications in comparison with seaweed-derived compounds (Burgess *et al.*, 1999; Zheng *et al.*, 2005; Penesyan *et al.*, 2009; Qian *et al.*, 2009).

Endophytic seaweed–bacterial relationships

Besides being epiphytic on algal surfaces, bacteria also live inside the thallus or cells. Seaweed grazers or epiphytic

bacteria capable of degrading algal cell walls can damage algal thalli and provide an entrance for pathogenic and opportunistic bacteria (Craigie *et al.*, 1992; Correa & McLachlan, 1994; Craigie & Correa, 1996; Wang *et al.*, 2008). These latter bacteria might become detrimental if they are able to enter the algal tissue and contribute to further disintegration of the host, finally leading to thallus rupture (Goecke *et al.*, 2010 and references therein). In addition to these pathogenic associations, also nondetrimental seaweed-associated endophytic bacteria are described. Bacteria are present inside algal galls (i.e. abnormal tissue growths of seaweeds) reported on more than 20 species of red and brown macroalgae (reviewed in Apt, 1988). In the red seaweed *Prionitis*, endophytic bacteria are responsible for gall formation by overproduction of the phytohormone indole-3-acetic acid (IAA), thereby creating a suitable microhabitat for their own proliferation (Ashen & Goff, 1998, 2000). Even though the benefits for the seaweed partner are not well understood, coevolution between *Prionitis* hosts and their gall-forming endobionts has been suggested (Ashen & Goff, 2000). Also in the red macroalga *Gracilaria dura* endophytic bacteria enhance the algal bud induction by the production of IAA and fixed nitrogen (Singh *et al.*, 2011b). In various siphonous (single celled, multinucleate) green seaweeds, endophytic bacteria have been reported over the past 40 years. Even though these endophytic bacteria have been associated with detoxification, nitrogen fixation, and photosynthetic functions (Chisholm *et al.*, 1996; Meusnier *et al.*, 2001; Delbridge *et al.*, 2004; Hollants *et al.*, 2011a, b), the true physiological nature of these endobiotic siphonous seaweed–bacterial symbioses remains unknown.

Bacterial diversity associated with seaweeds

Broad-spectrum seaweed–bacterial diversity studies identifying the total bacterial community are scarce. This is not surprising given that the number of seaweed-associated bacteria exceeds those in the surrounding seawater by 100–10 000 times (Chan & McManus, 1969). Total viable counts reach up to 10^7 bacterial cells per gram dry algal weight using the agar spread plate method, a number that even increases by two orders of magnitude when applying direct enumeration techniques (Chan & McManus, 1969; Mazure & Field, 1980; Largo *et al.*, 1997). Consequently, most macroalgal–bacterial studies focus on the identification and characterization of specific bacterial taxa, for example those with bioactive potential or pathogenic activity, rather than investigating the total bacterial diversity (Nakanishi *et al.*, 1996; Dobretsov & Qian, 2002; Wang *et al.*, 2008; Wiese *et al.*, 2009). Until recently, most of these investigations used traditional culture-based approaches, which are often considered insufficient because only 1% of all known bacteria are estimated to be culturable (Amann *et al.*, 1995). However, current molecular methods such as clone libraries, denaturing gradient gel electrophoresis, quantitative PCR, and fluorescent *in situ* hybridization also have their limitations for grasping the entire diversity of a microbial community, even in a single environmental sample, because they mainly reveal a snapshot in time of the dominant bacterial community members only (Philippot *et al.*, 2010).

In the following paragraphs, we review 161 studies from the last 55 years which dealt with bacteria associated with a total of 159 seaweed species (36 green, 72 red, and 51 brown marine macroalgae, see Supporting Information, Table S1). The bacterial diversity was compared between brown, green, and red seaweeds at all taxonomical levels. Wherever possible, the identity of the associated bacteria was linked to their ecological function.

Identity of bacteria associated with seaweeds: higher taxonomic ranks

Bacteria described from seaweed surfaces or within algal thalli belong to the (super)phyla Proteobacteria, Actinobacteria, Bacteroidetes (CFB group), Cyanobacteria, Firmicutes, Planctomycetes, Verrucomicrobia, Chloroflexi, Deinococcus-Thermus, Fusobacteria, Tenericutes, and the candidate division OP11. In all studies reviewed, Gammaproteobacteria were the most common bacterial clade associated with seaweeds (37% relative abundance, that is, percentage of published records), followed by the CFB group (20%), Alphaproteobacteria (13%), Firmicutes (10%), and Actinobacteria (9%) (Fig. 2a). On a lower taxonomic level, the orders Flavobacteriales (14% relative abundance), Alteromonadales (12%), Vibrionales (10%), Pseudomonadales (9%), Bacillales (9%), Actinomycetales (8%), and Rhodobacterales (7%) were most abundant in seaweed-associated bacterial communities (Fig. 2b). Comparing the relative abundance of bacterial taxa on brown, green, and red macroalgae, bacterial representatives of the major phylogenetic groups mentioned above were isolated from all three seaweed groups (Fig. 3a). Despite this similarity, green macroalgae associated more with the CFB group, and Alphaproteobacteria compared to brown and red seaweeds. Brown and red macroalgae, on the other hand, harbored more Firmicutes, Actinobacteria, and Planctomycetes species, respectively. Figure 3b shows that the discrepancy between brown, green, and red seaweed-associated bacteria at the order level can mainly be attributed to the differences in the number of reports of Rhizobiales, Rhodobacterales, Alteromonadales, Vibrionales, Cythophagales, Flavobacteriales, Bacillales, and Actinomycetales species.

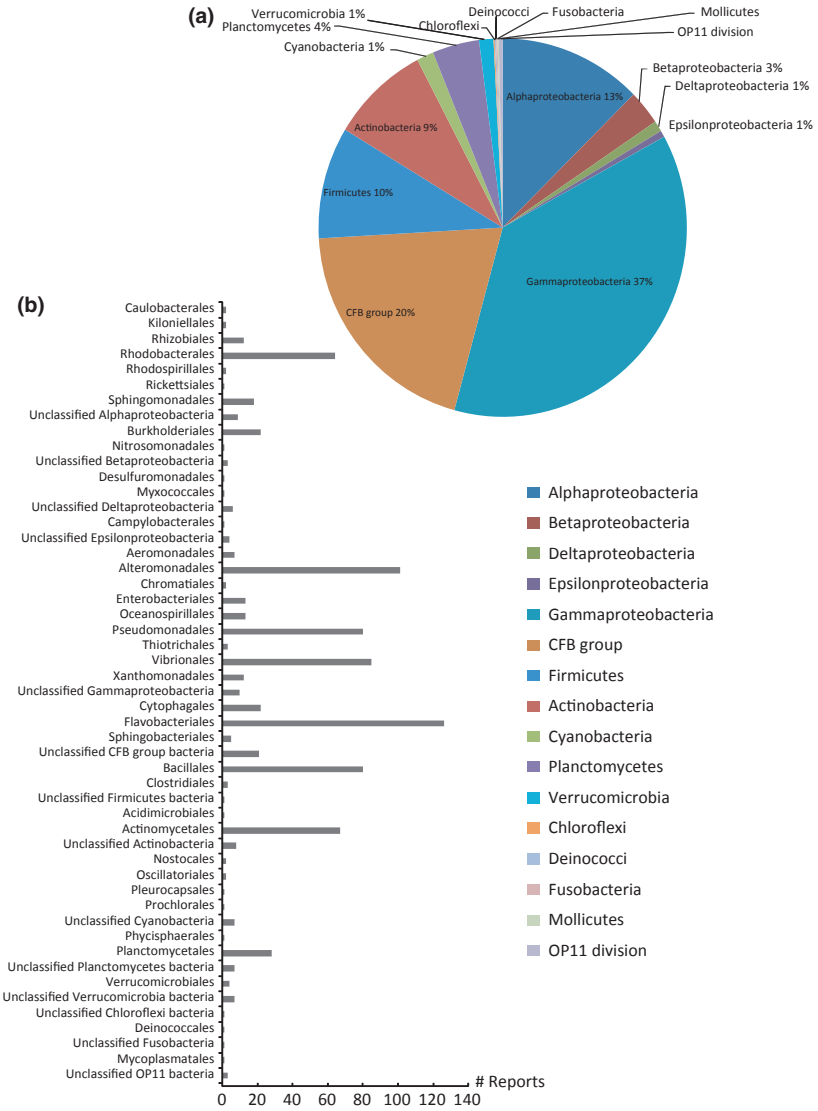

Fig. 2. Percentage of reports of bacterial classes or phyla (a) and number of reports of bacterial orders (b) associated with seaweeds.

Identity of bacteria associated with seaweeds: genus/species level

The similarities observed at high taxonomic ranks appear to decrease at lower ranks of both the host and bacterial partner. Even though a consistent bacterial core community at higher taxonomic levels (i.e. Alphaproteobacteria and Bacteroidetes) was observed on different *U. australis* and *Saccharina latissima* samples (Staufenberger *et al.*, 2008; Tujula *et al.*, 2010; Burke *et al.*, 2011b), closely related seaweeds do not necessarily harbor the same bacterial taxa (for example, different species in the genera *Fucus*, *Laminaria*, *Monostroma*, *Ulva*, *Gracilaria*, *Polysiphonia* and *Porphyra*, see Fig. S1 and Table S2). Likewise, only 33 bacterial genera including *Alteromonas*, *Bacillus*, *Flavobacterium*, *Pseudoalteromonas*, *Pseudomonas*, and *Vibrio* have, to a greater or lesser extent, been described from green, red, and brown seaweeds (Fig. 4). Genera like *Cytophaga*, *Planococcus* and *Tenacibaculum*, on the other hand, are regularly reported from green and red seaweeds, whereas they are virtually absent on brown macroalgal surfaces. Also specific bacterial species have rarely been isolated from different seaweed species, even within a single algal genus (see Table S2). Exceptions are outlined in Table 1 and include for example certain *Bacillus* and *Pseudoalteromonas* species that are present on or within a variety of brown, green, and red seaweeds. This table also illustrates that several of these bacterial species (*Cellulophaga fucicola*, *L. mucor*, *Pseudoalteromonas elyakovii*, *Tenacibaculum amylolyticum*, and *Zobellia galactanovorans*)

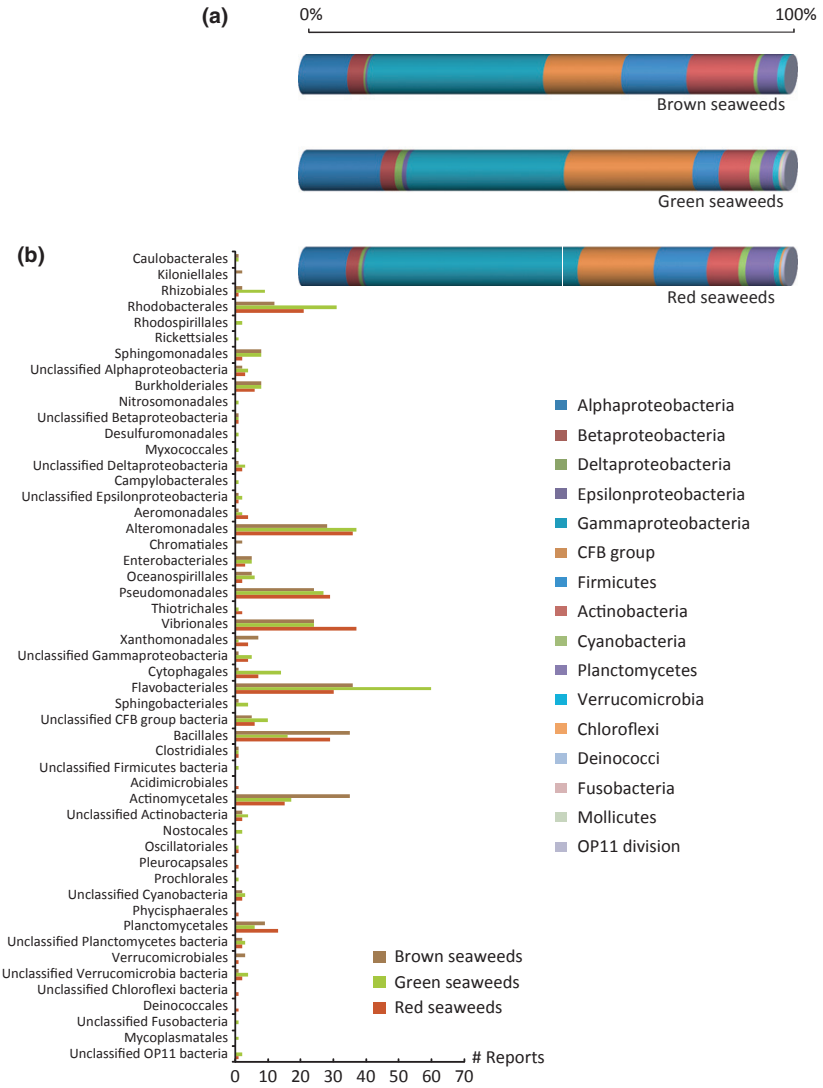

Fig. 3. Percentage of reports of bacterial classes (a) and number of reports of bacterial orders (b) associated with brown, green, and red seaweeds.

were newly described from their algal host, indicating marine macroalgae represent an important habitat for the discovery of novel bacterial diversity. To date, more than 50 new bacterial species initially isolated from seaweeds have been validly published (for an overview, see Goecke *et al.*, 2010, table 1). In contrast to the similarities in bacterial communities at higher taxonomic levels, almost no individual species was consistently found on the surface of different *U. australis* and *S. latissima* samples (Staufenberger *et al.*, 2008; Burke *et al.*, 2011b). Consequently, there does not appear to be a consistent core community of macroalgal-associated bacterial species, suggesting that a large number of bacterial species are able to colonize seaweed surfaces. This variability at the species level appears to be an emerging feature of host-associated microbial communities in general (Burke *et al.*, 2011b). Endobiotic associations, on the other hand, seem to be more uniform at lower taxonomic ranks compared with epiphytic bacteria. For example, different *Prionitis* species host similar bacteria of the *Roseobacter* group inside their galls (Ashen & Goff, 2000). Also, the siphonous seaweeds *Caulerpa* and *Bryopsis* harbor one and the same *Herbaspirillum* and Flavobacteriaceae species, respectively (Meusnier *et al.*, 2001; Hollants *et al.*, 2011b; Hollants *et al.*, submitted). These host-specific endophytes were found to be present in different *Caulerpa* or *Bryopsis* species as well as in geographical diverse algal samples from the same host species.

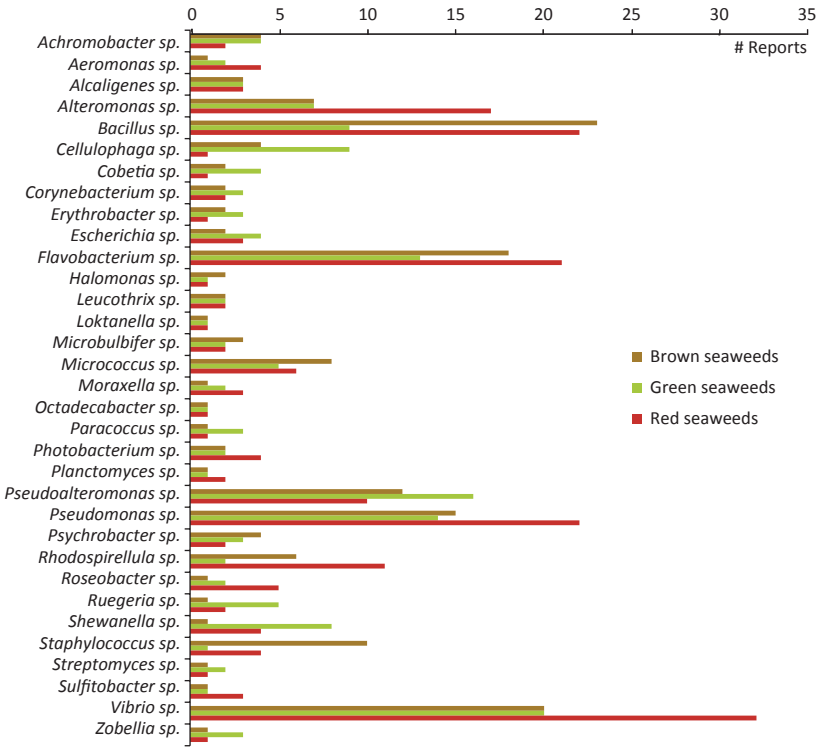

Fig. 4. Number of reports of bacterial genera isolated from all three macroalgal groups.

Linking identity to function

Although the ecological relevance of most bacterial associates on or within macroalgae remains unclear, a number of beneficial and detrimental functions have been postulated for particular bacterial species. For example, Alpha- and Gammaproteobacteria, Cyanobacteria, Actinobacteria, and CFB group species have been identified as the causative agent of various macroalgal diseases (for an overview of macroalgal diseases caused by bacteria, see Goecke *et al.*, 2010, table 3). The sushi-alga nori (*Porphyra*), for example, may be infected by species of *Flavobacterium* (Anaaki disease, Sunairi *et al.*, 1995), *Pseudomonas* (green spot rotting, Fujita *et al.*, 1972; Nakao *et al.*, 1972), and *Vibrio* (green spot rotting and white rot disease, Fujita *et al.*, 1972; Nakao *et al.*, 1972; Tsukidate, 1977, 1983). In addition, a wide variety of bacterial species isolated from seaweeds are capable of assimilating algal cell wall sugars. Besides key players in nutrient recycling processes, they are thus also potential pathogens as they can damage algal tissues and provide an entrance for opportunistic bacteria. These algal cell wall degrading bacteria mainly belong to the Alphaproteobacteria, Gammaproteobacteria, and the CFB group. Especially *Alteromonas*, *Flavobacterium*, *Pseudoalteromonas*, *Pseudomonas*, *Vibrio*, and *Zobellia* species possess sugar-degrading enzymes like agarases, carrageenases, and aliginases (for an overview of macroalgal cell wall–degrading bacteria, see reference Goecke *et al.*, 2010, table 2). Also antimicrobial, including anti-settlement and QS inhibiting, functions that protect the algal surface from pathogens, herbivores, and fouling organisms have been assigned to a broad range of seaweed-associated bacterial species. Not unexpectedly, nutrient-rich seaweed surfaces attract many opportunistic micro- and macroorganisms, thereby creating a highly competitive environment in which the production of defensive compounds can serve as a powerful tool for bacteria to outcompete other surface colonizers (Burgess *et al.*, 1999; Armstrong *et al.*, 2001; Penesyan *et al.*, 2009). As a result, the production of these antimicrobial compounds is not restricted to a certain bacterial group but appears to be widespread across alphaproteobacterial, betaproteobacterial, gammaproteobacterial, flavobacterial, actinobacterial, and bacilli clades (Fig. 5). In particular, *Micrococcus*, *Phaeobacter*, *Pseudoalteromonas*, *Shewanella*, *Vibrio*, and various *Bacillus* species are efficient producers of compounds with antimicrobial, antifouling, and QS inhibiting features, making them highly successful colonizers of seaweed surfaces (Kanagasabhapathy *et al.*, 2006; Goecke *et al.*, 2010). Besides these defense functions, bacteria also sustain the normal morphology and life cycle of their algal hosts. Morphogenesis and germination of

Table 1. Overview of bacterial species isolated from two or more host species/samples in independent macroalgal–bacterial studies

Bacterial species	Host (bacterial type/bacterial function)	References
Bacillus licheniformis	*Colpomenia sinuosa* (QSI), *Fucus serratus* (AB), *Palmaria palmate* (AM) and *Gracilaria dura* (EP/GF, NF)	Yan *et al.* (2002); Jamal *et al.* (2006); Kanagasabhapathy *et al.* (2009); Singh *et al.* (2011b)
Bacillus pumilus	*Ecklonia cava* (AM), *Sargassum fusiforme* (AM), *Porphyra yezoensis* (AM), *Lomentaria catenata* (AM), *Chondrus oncellatus* (AM), *Colpomenia sinuosa* (AM), *Gracilaria dura* (EP/GF, NF) and *Delisea pulchra* (AM)	Kanagasabhapathy *et al.* (2006, 2008, 2009); Penesyan *et al.* (2009); Singh *et al.* (2011c)
Cellulophaga fucicola	*Ulva australis* and *Fucus serratus* (SN)	Johansen *et al.* (1999); Rao *et al.* (2005, 2006, 2007)
Cobetia marina	*Antithamnion plumula*, *Cladophora rupestris*, *Ulva linza* (GF, MG), *Ulva compressa* (GF, MG) and *Ulva lactuca* (GF, MG)	Barbeyron & Berger (1989); Marshall *et al.* (2006)
Escherichia coli	*Monostroma undulatum* (FI), *Cladophora* mats (FI), *Kappaphycus alvarezii* (FI), *Laminaria religiosa* (FI) and *Ulva reticulate* (FI)	Vairappan & Suzuki (2000); Vairappan *et al.* (2001, 2008); Gallardo *et al.* (2004); Olapade *et al.* (2006)
Leucothrix mucor	*Ulva lactuca* (SN), *Clathromorphum* and *Sporolithon* sp.	Harold & Stanier (1955); Johnson *et al.* (1971); Bland & Brock (1973)
Phaeobacter gallaeciensis	*Ulva australis* (AF) and *Delisea pulchra* (AM)	Rao *et al.* (2005, 2006, 2007); Penesyan *et al.* (2009)
Pseudoalteromonas citrea	*Ulva* spp. (GF, MG)	Patel *et al.* (2003); Marshall *et al.* (2006)
Pseudoalteromonas elyakovii	'*Enteromorpha*' sp. (SZ) and *Laminaria japonica* (SN/D)	Sawabe *et al.* (1998, 2000); Patel *et al.* (2003)
Pseudoalteromonas gracilis	*Ulva australis* and *Gracilaria gracilis* (D)	Schroeder *et al.* (2003); Rao *et al.* (2005, 2006, 2007)
Pseudoalteromonas tunicata	*Ulva australis* (AF, AM) and *Ulva lactuca* (AF, AM, AS, SZ)	Egan *et al.* (2000); Rao *et al.* (2005, 2006, 2007); Penesyan *et al.* (2009)
Shewanella japonica	*Ulva australis* (AM)	Burmolle *et al.* (2006); Penesyan *et al.* (2009)
Tenacibaculum amylolyticum	*Ulva* sp. (GF, MG), *Monostroma* sp. (GF, MG) and *Avrainvillea riukiuensis* (SN)	Suzuki *et al.* (2001); Matsuo *et al.* (2003, 2005)
Vibrio tasmaniensis	*Laminaria japonica*, *Polysiphonia urceolata* and *Plocamium telfairiae* (AM)	Kanagasabhapathy *et al.* (2008); Wang *et al.* (2009)
Zobellia galactanovorans	*Ulva* sp. (GF, MG), *Monostroma* sp. (GF, MG), *Delesseria sanguine* (SN) and *Enteromorpha*' sp. (SZ)	Barbeyron *et al.* (2001); Matsuo *et al.* (2003, 2005); Patel *et al.* (2003)

Type: EP, endophyte; FI, fecal indicator bacteria; SN, new bacterial species (sp. nov.) originally described from the algal host.
Function: AB, antibacterial activity; AF, antifouling activity; AM, antimicrobial activity; AS, antisettlement of invertebrate larvae; D, disease; GF, growth-enhancing activity; MG, morphogenesis activity; NF, nitrogen fixation; SZ, settlement of zoospores; QSI, quorum sensing inhibitory activity.

foliaceous green macroalgae was linked to the production of thallusin by an epiphytic *Cytophaga* species isolated from *Monostroma* (Matsuo *et al.*, 2005). But also other bacterial species from the CFB group and members of the Alphaproteobacteria, Gammaproteobacteria, Actinomycetales, and Bacillales have been described as inducing morphogenic effects (Tatewaki *et al.*, 1983; Nakanishi *et al.*, 1996; Matsuo *et al.*, 2003; Marshall *et al.*, 2006; Singh *et al.*, 2011a). Likewise, *Cytophaga*, *Polaribacter*, *Pseudoalteromonas*, *Pseudomonas*, *Psychroserpens*, *Shewanella*, *Vibrio*, and *Zobellia* species have been shown to either stimulate or inhibit the zoospore settlement of *Ulva* seaweeds (Fig. 5) by the production of QS metabolites (Egan *et al.*, 2001; Patel *et al.*, 2003). Growth-promoting and nutritional effects, on the other hand, have been attributed to endophytic *Bacillus pumilus* and *Bacillus licheniformis* as well as to epiphytic *Exiguobacterium homiense*, *Pseudoalteromonas porphyrae*, *Azotobacter*, and various cyanobacterial species (Fig. 5) (Head & Carpenter, 1975; Rosenberg & Paerl, 1981; Dimitrieva *et al.*, 2006). These latter two bacterial taxa fix nitrogen and subsequently supply it to their *Codium* host. In other green siphonous seaweeds such as *Caulerpa* and *Bryopsis*, this nitrogen supply is provided by endosymbiotic bacteria from the order Rhizobiales (Chisholm *et al.*, 1996; Hollants *et al.*, submitted). Additionally, these macroalgae also host photosynthetic Alphaproteobacteria in their cytoplasm (Delbridge *et al.*, 2004; Hollants *et al.*, 2011b). These endosymbiotic associations may provide a physiological explanation for the successful – and sometimes invasive – spread of siphonous green algae in oligotrophic environments (Chisholm *et al.*, 1996).

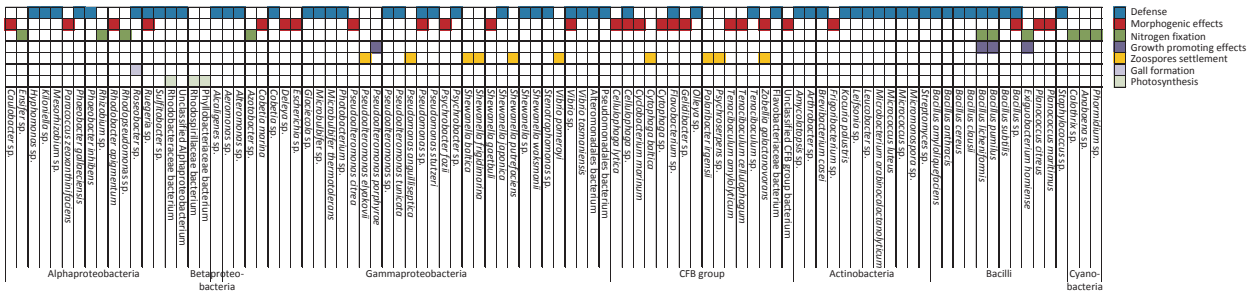

Fig. 5. Potential host-beneficial functions associated with certain bacterial genera.

Beyond sushi: the applied aspects of seaweeds and the role of bacteria therein

As key and engineering species, seaweeds play critical roles in the structuring and biodiversity of marine communities (Burke *et al.*, 2011b). Besides these significant natural functions, marine macroalgae also possess a wealth of applied aspects. First of all, seaweeds are a substantial part of the daily diet in Asian countries and are included in a great variety of dishes such as sushi, salads, and soups. In the west, seaweeds are largely regarded as health food, but in the last decades, there is a renewed interest in the Americas and Europe in their use as sea vegetables (Moore *et al.*, 2002; MacArtain *et al.*, 2007; Gupta & Abu-Ghannam, 2011). In addition, algal cell wall polysaccharides such as alginate, agar, and carrageenan have commercial significance as food additives with preservative, prebiotic, and gelling properties (O'Sullivan *et al.*, 2010; Gupta & Abu-Ghannam, 2011). Because of this latter feature, seaweed sugars are also used in a variety of industrial and laboratory applications with agar-based solid culture media as one of the best examples (Hesse & Gröschel, 1992; Michel *et al.*, 2006). Furthermore, marine macroalgae are one of nature's most rich resources of biologically active compounds. They form an important source of iodine and produce various metabolites with antimicrobial and antimacrofouling activities. As a result, seaweed-derived compounds have mayor therapeutic applications and can be used in cosmetics or antifouling paints (Bhadury & Wright, 2004; Smit, 2004; Qian *et al.*, 2009). Besides this, macroalgae can be used as animal feed additives, fertilizers and biofilters (Neori *et al.*, 2004; Hernández *et al.*, 2005; Gardiner *et al.*, 2008; Khan *et al.*, 2009) and are a potential source of bioethanol (Borines *et al.*, 2011). For most of the applications mentioned above, the algae need to be farmed on a large scale. Seaweed mariculture is a huge industry in Asian countries as recent cultivation figures suggest a harvest of tens of millions of tons per year (http://www.seaweed.ie/). However, as this success gradually promotes monocultures, bacterial diseases have started to surface (Vairappan *et al.*, 2001). Surface-associated pathogenic bacteria cause substantial financial losses and are a major threat to the mariculture industry (Steinberg *et al.*, 1997). From this point of view, there is an extensive need to characterize seaweed-associated pathogenic and decomposing bacteria (Goecke *et al.*, 2010). On the other hand, also an increasing interest in beneficial macroalgal–bacterial associations exists as many bacterial epiphytes represent a rich source of toxins, signaling compounds, and secondary metabolites with an array of biological activities (Armstrong *et al.*, 2001; Penesyan *et al.*, 2009). Moreover, it has been proven that seaweed-associated bacteria are involved in metabolite production originally attributed to the host (Penesyan *et al.*, 2009). Because seaweed mariculture for chemical compound production is technically challenging, epiphytic bacteria may represent a more promising and manageable source of bioactive metabolites. Therefore, it is anticipated that increasing numbers of natural product research teams will turn their focus to seaweed-associated bacteria instead of their hosts (Qian *et al.*, 2009).

Conclusion

Seaweed–bacterial associations have been studied from the end of the 19th century onwards and were shown to be highly diverse, covering a wide range of beneficial and detrimental interactions between various macroalgal and bacterial partners. A rather complex – chemically mediated – interplay exists among seaweeds and bacteria based on the exchange of nutrients, minerals and secondary metabolites (Fig. 1). Notwithstanding this diversity, all studies conducted so far have shown that seaweed-associated bacterial communities are highly specific as they differ significantly from those occurring in the surrounding seawater. This specificity is predetermined by physiological and biochemical properties of both the seaweed and bacterial partner; however, the taxonomic level at which to address this specificity remains unknown. Lower levels

seem not the answer as similar bacterial taxa are present on different algal hosts, and on the other hand, samples from the same seaweed species harbor distinct bacterial communities. Hence, it has been proposed that functional genes, rather than species may be the appropriate perspective from which to understand these specificity patterns (Burke *et al.*, 2011a). Macroalgal-associated bacterial communities appear to contain a consistent functional profile with features related to an algal host-associated lifestyle. Most of these functions can be performed by phylogenetically distinct bacterial taxa (Fig. 5). Nevertheless, a definite bacterial core community at higher taxonomic levels, mainly consisting of Gammaproteobacteria, CFB group, Alphaproteobacteria, Firmicutes, and Actinobacteria species, seems to exist which is specifically (functionally) adapted to life on brown, green, and/or red seaweed surfaces (Fig. 2). These three macroalgal groups, however, show some quantitative, rather than qualitative, differences as they harbor the same higher bacterial taxa at dissimilar (relative) abundances (Fig. 3). While such an ecological coherence at high bacterial taxonomic ranks has also been observed in other aquatic systems, intra- and intercellular bacterial communities generally show more specificity at lower taxonomic levels (Philippot *et al.*, 2010). Likewise, endobiotic macroalgal–bacterial relationships seem to be highly species specific.

As both epi- and endobiotic seaweed–bacterial associations are appealing from evolutionary, ecological, and applied perspectives, studies should be scaled up. Sequenced-based metagenomic analyses in combination with high-throughput next-generation sequencing technologies would be required to examine the macroalgal-associated bacterial diversity in a more effective way. Advances in molecular techniques have, however, revealed that obtaining an accurate picture of the composition of symbiotic bacterial communities presents an unusually difficult challenge (McFall-Ngai, 2008). Therefore, summarizing the immense bacterial diversity at the species level by integrating it into higher levels of organization (both phylogenetic and functional) would provide a framework to study (epiphytic) macroalgal-associated bacterial communities in a more practical way (Philippot *et al.*, 2010). Besides looking at 'who is (in) there', also the question 'what are they doing there?' should be tackled more profoundly in future research. Whole-genome sequencing and functional metagenomics could reveal insights into the role of bacteria associated with seaweed hosts. Sequence-based analyses of complete genome sequences may shed light on the metabolic potential of the bacterial epi- and endophytes (Medina & Sachs, 2010; Shi *et al.*, 2010; Hongoh, 2011), and functional screening of metagenome libraries may identify new genes and/or novel natural products of bacterial origin (Zaneveld *et al.*, 2008; Brady *et al.*, 2009). To fully elucidate symbiosis systems, however, it will be necessary to go beyond bacterial genome studies alone by integrating data at all levels (genes, transcripts, and proteins) from all symbiosis partners, including the seaweed host, as well as information on the interaction of these molecules at a systems biology level (Medina & Sachs, 2010; Knief *et al.*, 2011). Despite the potential of 'omics' technologies and high-throughput screening methods in generating data, the extraction of useful biological information from these data sets remains a significant (computational) challenge (Shi *et al.*, 2010). It has been suggested that the true 'omics' power will be realized when these technologies are integrated with 'classical' approaches that examine gene expression or functional activity *in vivo* (Riesenfeld *et al.*, 2004). Nevertheless, macroalgal–bacterial studies will always remain a difficult balancing act between examining the seaweed and bacterial partner on their own or studying them as a whole (i.e. as a holobiont). Either way, there is a strong need to integrate the aspects of different biological disciplines such as microbiology, phycology, ecology, and chemistry in future macroalgal–bacterial studies.

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Supporting Information

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

Fig. S1. Percentage of reports of bacterial classes associated with green (A), red (B) and brown (C) seaweeds.
Table S1. Overview of bacteria isolated from macroalgae in 161 studies from the last 55 years.
Table S2. Bacterial phylogenetic diversity (genus/species level) associated with brown, green and red seaweeds.

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