Chapter 16
Signal and Nutrient Exchange in the Interactions Between Soil Algae and Bacteria

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16.1 Introduction

In aquatic and terrestrial environments, microorganisms are typically found in multicellular consortia, which often include prokaryotic and eukaryotic organisms. Such consortia have important roles in aquatic and terrestrial ecosystems. For example, in many arid environments consortia of cyanobacteria, algae, and lichens (as well as biological polymers and small molecules released by them) form biological soil crusts (BSC). The crusts contribute to preventing erosion, improving soil structure and texture; they serve as sources of fixed carbon and nitrogen in ecosystems that otherwise have limited productivity. Aquatic microbial consortia ("biofilms") have similarly important functions in nutrient cycling, surface conditioning, etc. Precise gene regulation and exchange of chemical cues between the members of the consortium contribute to structuring and function of these communities. The goal of this chapter is to discuss signal and nutrient exchange that may contribute to the interactions between bacteria and soil algae within multispecies communities.

Even though soil is sometimes considered an inhospitable environment for photosynthetic microorganisms (like cyanobacteria and algae), they have been identified in samples collected from terrestrial ecosystems on all continents (Flechtner 1998; Otsuka et al. 2008; Langhans et al. 2009). Furthermore, over one hundred species of photosynthetic organisms representing 70 different genera have been recovered

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from surfaces or interior of rocks (van Thielen and Garbary 1998 and references therein). These observations indicate that soils and rocks can sustain diverse populations of eukaryotic and prokaryotic photosynthetic microorganisms. The interpretation of earlier studies on the diversity of soil algae may be complicated by the recent changes in the classification of algae and related organisms. For example, cyanobacteria were traditionally grouped with algae under the name "blue-green algae." However, cytological and genetic evidence places cyanobacteria within Negibacteria of the kingdom Bacteria (Cavalier-Smith 2004). Within the Six Kingdoms of Life, organisms that have been traditionally referred to as eukaryotic algae are now placed into two kingdoms, Plantae and Chromista (Cavalier-Smith 2004). The latter also contains oomycetes (including plant pathogens like *Pythium* and *Phytophtora*), which have been earlier classified as fungi. In this review, we will mostly consider interactions of proteobacteria and posibacteria with algae from the phyla Glaucophyta, Rhodophyta, Chlorophyta (kingdom Plantae), and members of the kindgom Chromysta. Even though our main goal is to focus on terrestrial algae, this review will be incomplete without comparing terrestrial prokaryote–algal interactions with the interactions that take place in aquatic environments. As appropriate, such comparisons will be introduced and discussed in this chapter.

16.2 Phylogenetic Diversity of Algal-Associated Prokaryotic Microbiota

Soil surfaces and subsurface environments not only harbor but also support growth of an impressive diversity of algae, their close relatives oomycetes, as well as cyanobacteria. For example, BSC contain up to 69 different chlorolichens, 68 representatives of algae, 62 bryophytes, 35 different species of cyanobacteria, and 13 cyanolichens [(Langhans et al. 2009) and references therein]. A sample of BCS harvested in sand dunes of the upper Rhine Valley (Germany) contained 26 algal species (including *Bracteacoccus* cf. *minor*, cf. *chlorosarcinopsis*, *Chlamydomonas*, *Chlorella*, *Chlorococcum* cf. *infusionum*, *Cocomyxa* cf. *confluens*, *Cylindrocystis*) and 13 species of cyanobacteria (including *Nostoc*, *Lyngbya*, *Microcoleus*) (Langhans et al. 2009). Interestingly, studies in other arid and semiarid areas similarly identified microalgae *Chlorella vulgaris* and *Bracteacoccus* (as well as *Stichococcus* and *Diplosphaera*) as dominant Chlorophytes consistently found in seven different locations in North American deserts (Flechtner 1998). *Chlorella* and *Stichococcus* were also commonly isolated from surfaces or in the interior of rocks (van Thielen and Garbary 1998). In addition to these common algae, soil samples collected in California, Arizona, and New Mexico contained 14–35 different species of Chlorophytes, 1–8 species of Xanthophytes and some samples also contained Eustigmatophytes (Flechtner 1998). It should be noted, however, that the dominant algal species identified in different studies may vary (Flechtner 1998),

and some of this uncertainty could be due to the techniques used to recover and enumerate algae (e.g., direct counts vs. culture-based techniques) (Langhans et al. 2009).

In soil as well as aquatic environments, algae are found in association with bacteria. To characterize bacteria associated with soil isolates of *Chlorella* spp., Otsuka et al. carried out denaturing gradient gel electrophoresis (DGGE) profiling of DNA isolated from seven independent algal cultures and also sequenced 16S ribosomal RNA (rRNA) genes of the bacterial associates of the alga. Prior to DNA extraction, bacteria and algae were cocultured in a liquid medium. PCR-DGGE profiles revealed banding patterns that were unique to each *Chlorella* isolate, although several common bands were also present (Otsuka et al. 2008). Sequencing of the 16S rRNA genes of the culturable bacteria isolated from *Chlorella* revealed that previously unculturable planctomycetes and flavobacteria, as well as *Sphingomonas melonis*, were associated with multiple *Chlorella* cultures. At least six novel[1] bacteria were common to multiple cultures of *Chlorella* (Otsuka et al. 2008). A comparison of 16S rRNA gene sequence profiles of bacteria associated with the alga after 1 month and 1 year of nonauxenic *Chlorella* cultures revealed that temporally separated samples contained flavobacteria, sphingobacteria, and α-proteobacteria (Otsuka et al. 2008). In a 1-year-old nonauxenic culture of *Chlorella*, Otsuka et al. also identified 16S rRNA gene sequences belonging to α-proteobacteria (*Afipia massiliensis*, *Caulobacter vibriodes*, *Phyllobacterium leguminum*, *Azospirillum* spp.), β−proteobacteria (*Ralstonia* spp.), γ-proteobacteria (*Lysobacter koreensis*, *Pseudomonas fragi*, *P. migulae*), and actinobacteria (Otsuka et al. 2008). Interestingly, *Sphingomonas* spp. and *Ralstonia* spp. were isolated from nonauxenic laboratory cultures of *Chlorella* propagated by another group (Watanabe et al. 2005). Even though bacterial profiles of soils from which the alga were harvested were not determined, and despite the fact that less than only 50 16S rRNA sequences were characterized in the two studies, it is still tempting to suggest that some soil bacteria may have evolved to interact with the soil algae.

A hypothesis that the phycosphere microbiota is specific to a particular species of microalgae was suggested by studies of bacteria associated with phytoplankton blooms (Hasegawa et al. 2007; Sapp et al. 2007). Ribosomal Intergenic Spacer Analysis (RISA) fingerprints of bacterial communities associated with six phytoplankton species in Helgoland Roads (Germany) were clearly distinct (Sapp et al. 2007). Sequencing of the most prominent DGGE bands suggested α- and γ-proteobacteria, as well *Flavobacteria-Sphingobacteria*, were most commonly found associated with the six algae. The majority (89%) of α-proteobacteria were either *Roseobacter* or *Sulfitobacter*; approximately 6% were *Sphingomonads*. Alteromonads and oceanosprilliae were most common γ-proteobacteria isolated from phytoplankton (Sapp et al. 2007). Phylogenetically similar bacteria were isolated from a toxic dinoflagellate *Alexandrium fundyense* in Canada (Hasegawa et al. 2007).

[1]"Novel" was defined by the authors as having less than 94% similarity in the V3 region of the 16S rRNA gene to the closest known relative (Otsuka et al. 2008).

Microbiota associated with *A. fundyense* was distinct from free-living bacteria in the water column and those isolated from particles; however, there was also a significant overlap in the microbial species composition in these three habitats (Hasegawa et al. 2007), which makes it difficult to establish unequivocally that a particular bacterium is an obligate symbiont associated with phytoplankton.

Collectively, the results of these studies suggest that some bacterial species enter into commensal or mutualistic interactions with algae in soil and aquatic environments. It is far from clear, however, whether these interactions are truly coevolved. Studies in other eukaryote-bacterial symbioses have revealed intricate signal and nutrient exchange between the partners, their ability to alter gene expression and effect organogenesis (Hirsch et al. 2003; Gil et al. 2004; Nyholm and Mcfall-Ngai 2004). Below, we will analyze recent discoveries of the signal and nutrient exchange between bacteria and algae to test the hypothesis that it contributes to the establishment of algae-associated microbial communities.

16.3 Nutrient and Signal Exchange in Algal–Bacterial Interactions

16.3.1 Carbon and Nitrogen Exchange in the Phycosphere

Mucus released by the algae is the main source of fixed carbon in the phycosphere. The mucus sheath of *Chlorella sorokiana*, for example, consists of carbohydrates (3.6 mg g^{-1} of dry cell weight), proteins (0.8 mg g^{-1} of dry cell weight), and metals (mostly Mg^{2+}, Fe^{2+}, Mn^{2+}, at 1168, 4.7, and 3.3 mg g^{-1} of dry cell weight), respectively (Watanabe et al. 2006). Sucrose and ribose were the most abundant sugars in mucus of *C. sorokiana* (1718 mg g^{-1} of dry cell weight and 216 mg g^{-1} of dry cell weight, respectively); it also contained galacturonic acid (750 mg g^{-1} of dry cell weight), xylitol (435 mg g^{-1}), innositol (317 mg g^{-1}), as well as smaller amounts of mannose, galactose, arabinose, rhamnose, and fructose (Watanabe et al. 2006). The composition of *Chlorella* mucus is clearly distinct from plant root mucus (mucilage) secreted by the vascular plants in their rhizosphere; the latter mostly consists of arabinose and galactose, with smaller amounts of uronic acids and other sugars [(Knee et al. 2001) and references therein].

The ability to efficiently utilize mucus polymers from the host is usually an important trait of coevolved symbionts. For example, supplementation of a mineral salts medium with high molecular weight mucilage from pea promoted growth of the plant symbiotic bacterium *Rhizobium leguminosarum* by ~50–100-fold. The utilization of pea mucus by *R. leguminosarum* was further increased by the addition of naringenin, a plant flavonoid symbiotic signal (Knee et al. 2001). Other soil bacteria were also capable of utilizing pea mucus, although to a lesser degree: the supplementation of mineral medium with pea mucilage increased their growth by 10–50-fold (compared with mineral salts). The addition of naringenin did not

increase pea mucilage utilization by nonsymbiotic bacteria (Knee et al. 2001). A similar study with bacteria isolated from surfaces of microalgae revealed that the addition of *Chlorella* extracellular organic carbon increased growth of its bacterial commensals in a pure culture by at least fivefold (Watanabe et al. 2005). However, there is no published evidence that mucus secreted by *Chlorella* is utilized differently by its commensals or free-living soil bacteria.

In addition to using organic carbon released by the algae, bacteria isolated from surfaces of microalgae can in turn promote growth of algae. A coculture of *Chlorella sorokiana* with bacteria increased growth of the alga by 10–20% (Watanabe et al. 2005). Auxenic cultures of *C. sorokiana* that were maintained in light for 5 months on agar slants lost ~40% of their chlorophyll, compared with cocultures with a bacterial consortium under the same conditions (Watanabe et al. 2005). Similarly, growth (and/or chlorophyll content) of pure cultures of *Chlamydomonas reinhardtii* was increased by ~sixfold in the presence of native bacteria (Nikolaev et al. 2008). The supplementation of *C. reinhardtii* cultures with *Bacillus* spp. or *Rhodococcus terrea* had a more modest effect on growth and/or chlorophyll production (Nikolaev et al. 2008). These results clearly indicate that both the microalgae and their bacterial commensals derive benefits from the association.

To better understand the mechanisms of algal growth promotion by bacteria, artificial "symbioses" between *Chlorella*, *Chlamydomonas*, and various well-characterized plant growth promoting bacteria were started under laboratory conditions. In earlier studies, cultures of *Chlamydomonas reinhardtii* were mixed with nitrogen-fixing *Azotobacter* on agar plates lacking carbon and/or nitrogen (Gyurjan et al. 1984, 1986). Under these conditions, a monoculture of *C. reinhardtii* lost chlorophyll and died within the first 2 months of the study, while *Azotobacter–Chlamydomonas* consortia persisted for at least 2 years (Gyurjan et al. 1986). The growth promoting effects were at least in part due to the ability of the bacteria to fix nitrogen, as suggested by nitrogenase activity (measured as acetylene reduction) (Gyurjan et al. 1984, 1986). The ability of nitrogen-fixing *Bacillus pumilus* to promote growth of *Chlorella vulgaris* was recently demonstrated using coimmobilization of the two organisms in alginate beads (De-Bashan and Bashan 2008; Hernandez et al. 2009). In the presence of *B. pumilus* (originally isolated from arid soils), cell numbers of *C. vulgaris* increased by 3×10^6 compared with a culture that was not supplemented with the bacilli, reaching population densities that were essentially the same as in cultures supplemented with ammonium chloride (Hernandez et al. 2009). The growth promoting effects of *B. pumilus* on algae were abolished in the presence of ammonia, suggesting that nitrogen fixed by the bacilli is most likely responsible for the growth promoting effects (Hernandez et al. 2009).

Results of these studies demonstrate that microalgae and their associated microbiota can benefit from the interaction. Similar loose interactions between plants and free-living nitrogen-fixing bacteria (*Azotobacter* spp., *Azosprillum* spp among others) are now well documented (Baldani and Baldani 2005).

16.3.2 Bacterially Produced Plant Hormones Stimulate Algal Growth

Production of plant hormones (or their analogs) by bacteria plays an important role in many plant–bacterial interactions. Plant symbiotic bacteria (e.g., *Rhizobia* spp, *Azospirillum*) and plant pathogens (*Agrobacterium* spp., *Erwinia herbicola*) produce plant hormones, which are thought to contribute to the development of new plant organs occupied by the microorganisms (Lambrecht et al. 2000). Thus, it appears that the ability to manipulate gene expression and relevant physiological changes by the production of plant hormones is an important, coevolved trait in plant-associated bacterial symbionts and pathogens.

A hypothesis that plant hormones produced by bacteria would also stimulate growth of microalgae was tested (de-Bashan et al. 2008; De-Bashan and Bashan 2008) in the medium that already contained soluble nitrogen (25 mg/L NH_4Cl). In the presence of *A. brasiliense* and increasing concentrations of tryptophan (a precursor for an auxin plant hormone IAA), growth of *Chlorella vulgaris* was increased fourfold (de-Bashan et al. 2008; De-Bashan and Bashan 2008). A coculture of *C. vulgaris* and IAA-deficient mutants of *A. brasiliense* had either a reduced effect on algal growth or had no growth promoting effect at all (de-Bashan et al. 2008). Growth of the alga was promoted by the culture filtrate of the bacterial IAA mutant only if the culture filtrates were also supplemented with IAA. In control experiments, supplementation of the growth medium with 10 mg mL^{-1} IAA promoted growth of *C. vulgaris* (de-Bashan et al. 2008).

16.3.3 Bidirectional Vitamin Exchange and Vitamin-Mediated Signaling in Algal–Bacterial Interactions

Many algae require vitamin B_{12} (cobalamin), vitamin B_1 (thiamine), and vitamin H (biotin) for growth, although not all algae require all three of these vitamins (Croft et al. 2005; Grossman et al. 2007). Recent genomic sequencing of *Chlamydomonas reinhardtii* and parallel physiological studies indicate that this microalga does not require these vitamins for growth (Grossman et al. 2007). At least 171 species of algae (of 326 tested), however, required external cobalamin for methionine synthesis and growth, suggesting that many algae rely on their commensal or symbiotic bacteria for the supply of cobalamin (Croft et al. 2005). An isolate of *Halomonas* sp. was shown to provide cobalamin to a marine red alga *Porphyridium purpureum* (Croft et al. 2005). Interestingly, in a legume-*Sinorhizobium* symbiosis, a bacterial mutant that was unable to synthesize cobalamin was defective in forming symbiotic nodules (at least on some plant hosts) (Medina et al. 2009). However, because cobalamin is required for methionine synthesis, a cobalamin mutant is also a methionine auxotroph (Medina et al. 2009). It is not yet clear whether the symbiotic

defect of the cobalamin-deficient *Sinorhizobium* was due to the defect in the vitamin exchange with the plant host or whether it was a result of the inability to synthesize methionine.

In addition to their role as enzyme cofactors, vitamins appear to play important signaling roles. For example, vitamin riboflavin (vitamin B_2) and its derivative lumichrome (Fig. 16.1) were shown to affect plant growth and physiology. Treatment of plant seedlings with riboflavin promoted their resistance to viral, bacterial, and fungal pathogens (Dong and Beer 2000). The addition of lumichrome at nanomolar level increased plant shoot and root growth (Phillips et al. 1999; Matiru and Dakora 2005). Treatment of plant seeds and seedlings with lumichrome increased growth and stimulated stomatal conductance (Phillips et al. 1999). These studies suggested that both riboflavin and lumichrome, both self-produced and supplied by the commensal bacteria, have important roles in eukaryote–bacterial interactions. Intriguingly, lumichrome and riboflavin produced and secreted by *Chlamydomonas reinhardtii* were shown to alter population density-dependent gene expression in bacteria (Rajamani et al. 2008).

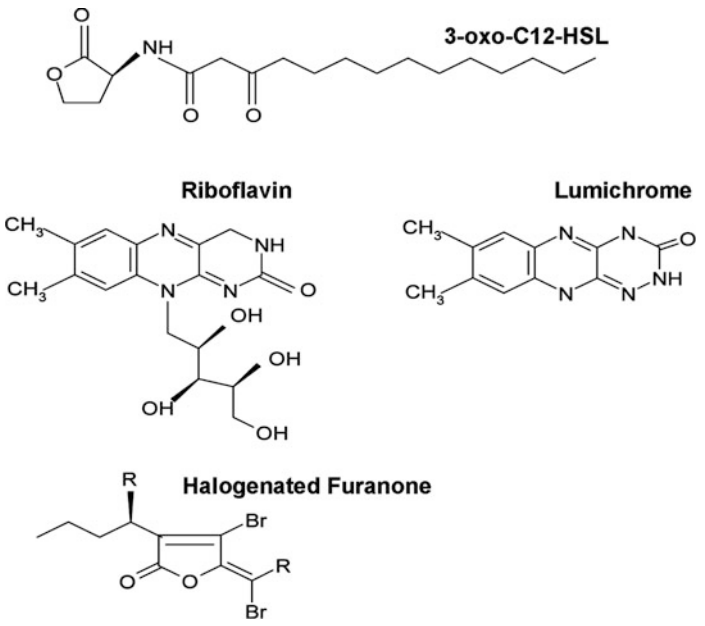

Fig. 16.1 Bacterial QS signals and algal QS signal-mimics. 3-oxo-dodecanoyl homoserine lactone (3-oxo-C_{12}-HSL) is a signal produced and perceived by the *Pseudomonas aeruginosa* Las QS system (Kiratisin et al. 2002). Vitamin signals and QS agonists capable of binding to LasR and stimulating LasR-mediate gene expression were identified in culture filtrates of a soil microalga *C. reinhardtii* (Rajamani et al. 2008), although these compounds are known to be produced by bacteria and by plants (Treadwell and Metzler 1972; Phillips et al. 1999; Joseph and Phillips 2003). Halogenated furanone produced by a red alga *Delisea pulchra* are the best characterized QS antagonists (Givskov et al. 1996). These compounds block bacterial QS by binding to the AHL receptor polypeptides and targeting them for degradation (Manefield et al. 2002; Koch et al. 2005)

Lumichrome and riboflavin were recently identified in a search for microalgal compounds capable of affecting cell-to-cell signaling in bacteria (Rajamani et al. 2008). Many bacteria rely on small diffusible signal molecules to effect changes in gene expression that parallel increases in bacterial population densities within diffusion-limited environments [rev. Dobretsov et al. (2009)]. This type of cell-to-cell signaling is known as "quorum sensing" (QS). Acyl homoserine lactones (AHLs, Fig. 16.1) are one of the best characterized bacterial QS signals. Inside bacterial cells, AHLs are bound by LuxR-like regulators, and the LuxR–AHL complex then binds within promoters of the genes subject to QS control (Zhang et al. 2002; Koch et al. 2005). Compounds that bind to LuxR proteins and thus inhibit bacterial QS have previously been characterized (see below); however, lumichrome and riboflavin are the first characterized biologically derived agonists that are structurally distinct from AHLs yet are capable of interacting with AHL receptors.

The ability of lumichrome and riboflavin to interact with AHL receptors was first detected using semisynthetic bacterial QS reporters (Rajamani et al. 2008). These reporters consist of a gene encoding an AHL receptor (*lasR*, a *luxR* homologue from *Pseudomonas aeruginosa*), a promoter controlled by LasR and a downstream promoterless *luxCDABE* cassette (Winson et al. 1998). To rigorously test the hypothesis that lumichrome and riboflavin interact with the LasR AHL receptor, additional reporters were constructed and their responses to synthetic lumichrome and riboflavin were tested. A study of Rajamani et al. (2008) demonstrated that the effect of lumichrome and riboflavin on the LasR-based tandem dimer RFP (tdTomato) reporter required the same amino acid residues that are also involved in the interactions of the receptor with the cognate AHL signals (Rajamani et al. 2008). In silico modeling and gel mobility shift assays using purified LasR further supported the hypothesis that lumichrome and riboflavin are the first characterized vitamin signals produced by microalga and capable of affecting QS in soil bacteria (Rajamani et al. 2008). The function of these compounds in structuring of the bacterial communities associated with algae remains to be elucidated.

16.3.4 The Role of Algal Signals in Modulating Bacterial Quorum Sensing

In terrestrial and aquatic environments, microorganisms are found within multicellular consortia. Microphotographs reveal that bacteria colonize surface of *Chlorella* and *Chlamydomonas* as microcolonies that are held by an extracellular matrix (Gyurjan et al. 1984, 1986; Watanabe et al. 2005; Imase et al. 2008). This is not uncommon: bacteria that colonize surfaces of plants are also found as microcolonies or biofilms encased in the extracellular matrix of plant and microbial origin [rev. Danhorn and Fuqua (2007)]. To form multicellular communities, to interact with other organisms within these communities, and to colonize biotic substrata, bacteria rely on a variety of self-produced signals and chemical cues.

Quorum Sensing is one of bacterial gene regulatory systems that contributes to structuring of biofilms [rev. (Pasmore and Costerton (2003); Wolfe et al. (2003); Stanley and Lazazzera (2004); Dobretsov et al. (2009)]. The presence of QS was not tested in the phycosphere of soil algae. However, bacterial AHL signal production and perception associated with QS is well-documented in the rhizosphere of plants (Pierson and Pierson 1996; Ramos et al. 2001; Gao and Teplitski 2008); therefore, it is reasonable to hypothesize that bacteria similarly use QS to control gene expression within their colonies on surfaces of algae.

Algae and vascular plants produce compounds that alter QS in the associated bacterial communities (Givskov et al. 1996; Teplitski et al. 2000, 2004; Manefield et al. 2001; Gao et al. 2003, 2007; Bjarnsholt et al. 2005; Koch et al. 2005; Skindersoe et al. 2008). Halogenated furanones produced by a marine red alga *Delisea pulchra* are the best characterized eukaryotic inhibitors of bacterial QS [(Givskov et al. 1996) and Fig. 16.1]. In vivo, these compounds bind to the nascent AHL receptor polypeptide and prevent its correct folding, thus targeting the misfolded peptide for degradation by proteases (Manefield et al. 2001; Koch et al. 2005). Under laboratory conditions, halogenated furanones inhibit QS-mediated behaviors in gram-negative bacteria (Givskov et al. 1996; Manefield et al. 2001; Arevalo-Ferro et al. 2003; Hentzer and Givskov 2003; Hentzer et al. 2003). In situ, vesicle-mediated release of halogenated furanones on the surfaces of algal thalli shifts population of associated bacteria from gram negative (common marine microorganisms) to gram positive, which are typically under-represented in marine environments (Dworjanyn et al. 1999; Dworjanyn et al. 2006).

The ability to produce inhibitors of QS was tested in four soil algae: *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *C. fusca*, and *C. mutabilis* (Teplitski et al. 2004). As shown in Fig. 16.2, overlays of algal colonies with bacteria in which QS contributes to the production of light suggest that some soil algae are capable of modulating bacterial light production, either by affecting AHL-mediated signaling or the cell-to-cell signal transduction cascade controlled by an AI-2 signal. In addition to affecting QS-mediated light production, colonies of *C. reinhardtii* secreted compounds that reduced QS-controlled production of antibiotic pigments violacein and phenazine in two soil bacteria (Fig. 16.3).

In addition to the compounds that inhibit bacterial QS, *C. reinhardtii* was shown to produce chemically separable activities that stimulate QS in the semisynthetic reporters and also in the wild-type soil bacteria (Teplitski et al. 2004). Further bioassay-guided purification of bioactive compounds identified lumichrome as a QS agonist (Rajamani et al. 2008) and also revealed at least two peaks of activity separable with reverse phase Si HPLC (Teplitski et al. 2004). Treatment of pre-quorate cultures of a wild-type soil bacterium *Sinorhizobium meliloti* with a purified QS signal mimic from *C. reinhardtii* affected accumulation of 25 polypeptides. Sixteen of the 25 polypeptides responsive to the algal mimic were also subject to regulation by bacterial AHLs (Teplitski et al. 2004). These results indicate that both aquatic and soil algae are capable of QS in the associated bacteria and thus alter bacterial behaviors that may be relevant to the algal–bacterial interactions.

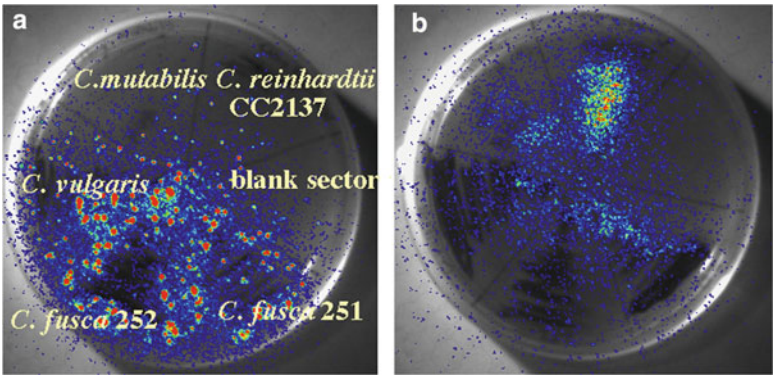

Fig. 16.2 Soil algae affect luminescence in *Vibrio harveyi*. *Chlorella mutabilis*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and two strains of *Chlorella fusca* were grown on TAP agar. Plates with algal streaks were then overlaid with a soft agar suspension of the wild-type *V. harveyi* 404 (PMH 2193 SK) (**a**) or *V. harveyi* BB170 (a reporter in which luminescence largely depends on the production of the AI-2 signal) (**b**). Luminescence was measured with a Hamamtsu C2400 intensified CCD camera. The false-color image of luminescence intensity was superimposed onto the black and white image of the plates

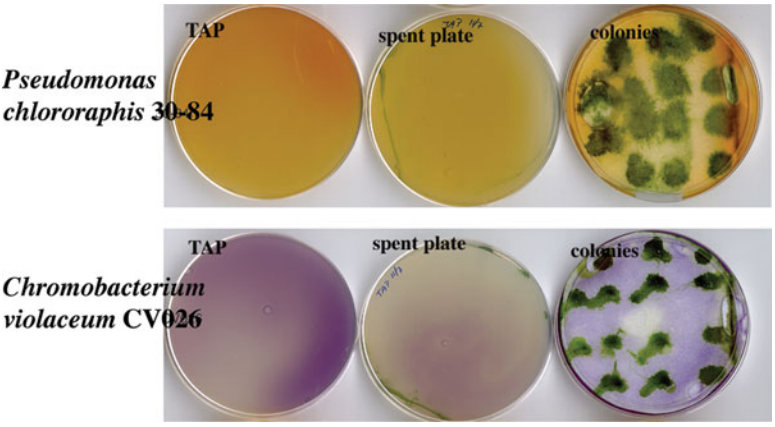

Fig. 16.3 QS-dependent pigment production in soil bacteria is affected by *Chlamydomonas reinhardtii*. In *Pseudomonas chlororaphis* 30–84 and in *Chromobacterium violaceum*, production of the antibiotic pigments requires functional QS circuitry (Pierson and Pierson 1996; McClean et al. 1997). As shown in the top panel, wild-type *P. chlororaphis* 30–84 produces bright *orange* pigment phenazine when seeded in LB agar overlaid on the TAP medium (Tris-acetate agar used to culture *Chlamydomonas*). Less phenazine was produced when bacterial suspension was overlaid on TAP agar, where *C. reinhardtii* was previously cultured (middle panel). Production of phenazine was further reduced in the bacterial lawn seeded on top of colonies of *C. reinhardtii* CC2137 (*right panel*). Similarly, less violacein was produced by *Chomobacterium violaceum* CV026 reporter when seeded onto spent plates or on top of algal colonies. Because CV026 does not produce own AHLs, top agar overlays were supplemented with C_4-HSL as in (Teplitski et al. 2000). *C. reinhardtii* CC2137 was grown on cellulose Whatman #1 filters, which were placed on top of TAP agar. For the assays, filter paper with algal colonies was lifted off the plates. Spent plates and filter paper with algal colonies were overlaid with suspensions of the bacteria in LB agar (0.3% wt/v)

In addition to manipulating bacterial QS, some algae appear to detect AHL signals produced by bacteria. For example, C_4-HSL (one of seven AHLs tested) promoted release and settlement of spores produced by a rhodophyte *Acrochaetium* (Weinberger et al. 2007). In these assays, C_4-HSL was active at 100 mM (Weinberger et al. 2007). Such high concentrations of AHLs are usually found within biofilms (Charlton et al. 2000; Dobretsov et al. 2009). Preferential settlement of spores from *Ulva intestinalis* on AHL-producing biofilms was also demonstrated (Joint et al. 2002). *Ulva* spores also exhibited chemokinesis along a gradient of AHLs (Wheeler et al. 2005). The ability to detect and respond to bacterial AHLs is not uncommon in eukaryotes and was reported in plants and animals (Smith et al. 2002; Joseph and Phillips 2003; Mathesius et al. 2003). However, the mechanism(s) by which eukaryotes detect these bacterial signals are not yet known.

16.4 Conclusions and Future Directions

Studies of the interactions of soil and aquatic algae with their associated microbiota suggest that some species of bacteria may be more commonly isolated from phycosphere of specific algae. This conclusion, however, is based on a limited number of studies. Further surveys are needed to rigorously establish bacterial diversity and species richness in phycosphere.

Several studies have demonstrated that algae may benefit from the association with bacteria. Algae may derive nitrogen, vitamins, and also plant hormones from their bacterial associates. Using defined and random mutants of bacteria, it will be important to learn whether other bacterial behaviors or metabolites are capable of modulating growth of the algae and contribute to structuring of algal-associated bacterial communities.

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