Exploring mutualistic interactions between microalgae and bacteria in the omics age

Matthew B. Cooper[1,2] and Alison G. Smith[1,3]

[1]Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, UK

[2]Present address: Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PL, UK (matthew.cooper-4@manchester.ac.uk)

[3]Author for correspondence: Smith, Alison G. (as25@cam.ac.uk)

Abstract

Microalgae undertake a wide range of mutualistic interactions with bacteria. Here we consider how transcriptomic, metagenomic and metabolomic approaches have been combined with microbiological and biochemical analyses to expand our understanding of algal-bacterial interactions. Identification of the major bacterial species associated with algae indicates that specific bacterial groups, particularly the alpha-Proteobacteria, are found more frequently, suggesting that these may have the means to initiate and maintain symbiotic relationships. Nutrient exchange is frequently the basis of algal-bacterial mutualism, and as the compounds involved are characterised, evidence is accumulating that these are complex and specific molecules, offering opportunities for signalling processes and regulation rather than merely passive diffusion. At the same time, it is clear that the interactions are not static, but can be initiated and broken in response to environmental and developmental cues.

Main text

Introduction

Algae are a large group of eukaryotic, mostly photosynthetic, organisms. There are two broad groups, the multicellular macroalgae (or seaweeds) and the microalgae, which are generally unicellular. Phylogenetically, however, they are incredibly diverse, with the only unifying feature being the presence of plastids, derived initially from an endosymbiotic event between an early eukaryotic cell with a photosynthetic cyanobacterium (Figure 1). This gave rise to the basal groups of algae: green, red and glaucophytes. Subsequent secondary and tertiary endosymbioses of red or green algae by other heterotrophic eukaryotes resulted in the range of extant algal groups. The distribution of algae is similarly broad. They are found in all aquatic habitats on the planet, and the microalgae make up a significant proportion of oceanic phytoplankton, which has been estimated to be responsible for up to 50% of global carbon fixation [1]. These organisms thus play key roles in many biogeochemical cycles.

As primary producers, algae are the basis of the food web, but in addition, they often engage in symbiosis with other organisms. The term symbiosis is generally considered to be beneficial, although in fact it was first coined to mean 'living together' [2] and so strictly also includes pathogenic or parasitic relationships. These are beginning to be recognised and studied at the molecular level [3], such as that between the bacterium *Nautella* sp. R11, which causes bleaching disease in the rhodophyte marine macroalga *Delisea pulchra* [4]. Nonetheless, many interactions are mutualistic or commensal. Perhaps the best known mutualism is in lichens, where algae provide

photosynthate to their fungal partners in return for a habitat that protects them from desiccation or light stress [5]. Similarly, species of dinoflagellate algae are important symbionts of corals [6,7] and other cnidarians such as *Hydra viridissima* [8]. Other more specialist interactions exist, such as that between embryos of the salamander *Ambystoma maculatum,* whose development is enhanced by the presence of a mutualistic green alga *Oophila amblystomatis*. It has been proposed that the algae ensures increased provision of oxygen, and in turn receives nitrogenous waste from the developing embryo [9].

Interactions between algae and bacteria, particularly for the provision of fixed nitrogen or micronutrients such as vitamins and Fe, are increasingly being recognised. As well as providing insight into important biogeochemical cycling in the aquatic world, studies of these relationships have begun to address the molecular mechanisms underlying the exchange of materials. However, in the microbial world where the distinction between host and symbiont is less clear, it is challenging to identify partners in more complex communities, and to establish the benefit(s) that is gained by each. The advent of metagenomics and other omics methodologies, combined with extensive sampling of biomes dominated by microorganisms and advances in community modelling, are revolutionising our perspective on microbial interactions [10]. These approaches are now being applied to understand better those between algae and bacteria. In this review, we outline some of the highlights of recent studies, and consider how this is shedding light on the evolution of these mutualisms.

Bacterial partners – are some better than others?

Large, multi-cellular macroalgae, or seaweeds, such as kelps and sea-lettuce, which inhabit the littoral zone (close to the shore) present an obvious surface for bacterial colonisation, and symbiotic interactions between seaweeds and their bacterial community are well-documented [11]. As well as simply providing a habitat for the prokaryotic organisms, there are also examples of direct benefit, such as to the green macroalga *Codium decorticatum,* whose cyanobacterial endosymbionts *Calothrix* sp., *Anabaena* sp., and *Phormidium* sp., have been shown to fix nitrogen for their hosts [12]. In nutrient poor waters in the ocean, even single algal cells can provide a vital source of nutrients to bacteria, acting in a similar way to the surface of particulate organic matter such as faecal pellets or other types of marine snow. Single algal cells produce significant amounts of dissolved organic matter (DOM), sometimes enough to create a noticeable concentration gradient of nutrients from the cell surface to the outside environment [13]. This region has been termed the

phycosphere [14], and often contains populations of bacteria distinct from the surrounding open habitat.

Analysis of bacteria associated with particular algae has provided evidence for some degree of co-evolution between the alga and its associated bacterial community. Pyrosequencing of 16S rRNA from bacteria found with three species of the diatom genus *Pseudo-nitzschia* revealed different community composition, and transplantation of one community into a non-native diatom species sometimes resulted in a deleterious effect for the alga [15]. In a meta-analysis of bacteria associated with over 40 different macro- and microalgae, it was found that the majority of the 101 isolates were from six bacterial phyla: Bacteroidetes (42 genera), Proteobacteria (32), Firmicutes (7), Actinobacteria (8), Verrucomicrobia (3) and Planctomycetes (1) [16]. Similarly, a wide-ranging analysis of diatom-bacterial interactions revealed a preponderance of species from the Bacteroidetes and Proteobacteria, particularly the alpha- and gamma-Proteobacteria [13]. Intriguingly, an analysis of the microbiome associated with the maize rhizosphere also found that Proteobacteria were enriched in this region compared to bulk soil [17].

One group within the alpha-Proteobacteria, the Rhodobacterales family, appear to be particular well-adapted to close association with phytoplankton in general [18]. They are commonly found in natural assemblages with marine algae, and have been shown to increase during phytoplankton blooms [19,20]. One member of this family, *Dinoroseobacter shibae*, is a successful mutualist to at least 3 distinct species of marine microalgae *Isochrysis galbana*, *Amphidinium operculatum* and *Thalassiosira pseudonana* [21,22], proving itself to be a rather generalised symbiont. In fresh-water systems, another alpha-proteobacterial family, the Rhizobiales, are common contaminants of green algal cultures, contributing 50% of the bacterial load in xenic *Chlamydomonas reinhardtii, Chlorella vulgaris* and *Scenedesmus* sp. cultures [23]. They also appear to be common growth promoters of algae: for example, a novel *Rhizobium* species from *C. vulgaris* was found to increase significantly the growth of several algae [23]. Rhizobia appear to be a rarer occurrence growing in the marine environment, but a recent paper reports detection of rhizobial DNA in cultures of 13 strains of the marine picoeukaryote *Ostreococcus tauri* [24].

Nutrient exchange cements the relationship

The delivery of fixed carbon via photosynthate is the major factor contributed by the algal partner in algal-bacterial interactions. In many cases, bacteria may simply be able to access DOM in the phycosphere, but mutualistic interactions in which bacteria provide factors that enhance algal

growth may help initiate and/or maintain the relationship, and offer them a selective advantage. Although the underlying reason(s) for the growth stimulating effect of the bacteria on algae are not always known [25], for many interactions it has been demonstrated that the mutualism is mediated by provision of nutrients by the bacteria. The diazotrophic cyanobacterium *Richelia intracellularis* has long been studied for its ability to engage in symbiosis with various genera of diatoms, fixing nitrogen in exchange for carbon [13,26], and the uncultured cyanobacterium *Candidatus* a*telocyanobacterium thalassa* has been demonstrated to perform the same role with certain types of prymnesiophyte algae [27]. A metagenomics analysis using 16S and 18S rRNA abundance showed that similar cyanobacteria associate with a range of unidentified unicellular haptophytes throughout the North Atlantic ocean, indicating this symbiosis may play a significant role in nitrogen fixation in those waters [29]. Such interactions may eventually evolve into much more specific and developed mutualisms, as is found in the Rhopalodiaceae family of diatoms, many of which contain organelle-like structures called spheroid bodies. The genome of these bodies has been sequenced, revealing them to be the remnants of a non-photosynthetic cyanobacterium that co-evolved with these diatoms. As with other endosymbionts this co-evolutionary process resulted in a highly reduced genome that, regardless, is still apparently capable of fixing gaseous nitrogen for their hosts to utilise [30].

Many examples of specific algal-bacterial interactions are based on the exchange of micronutrients. For example, iron is often limiting in the marine environment, and iron-fertilisation experiments in high-nutrient, low chlorophyll (HNLC) regions has been shown to cause extensive algal blooms [31]. Although bacteria and algae will in many cases be competing for this micronutrient [32], several examples of mutualism are known, including the release of light-labile iron chelating siderophores by algal-associated bacteria of the *Marinobacter* genus (gamma-Proteobacterium), but not their free-living relatives [33]. Another important group of micronutrients are the B-vitamins, water-soluble organic micronutrients that act as co-factors for enzymes of central cellular metabolism. Despite their photosynthetic lifestyle, over 50% of microalgal species surveyed have an obligate requirement for an exogenous source of vitamin B_{12} (cobalamin) for growth, and 22% require vitamin B_1 (thiamine) [34]. The fact that the trait is found in all algal phyla (Figure 1), with no phylogenetic relationship between vitamin-requirers [35,36], indicates that environmental factors have been the drivers in its evolution throughout the algal lineages. This point is underlined by the fact that artificial supplementation of marine ecosystems with either vitamin B_{12} or B_1 led to an increase in algal productivity and altered the shape of the microalgal community [36,38]. It is not just eukaryotes that require B-vitamins, however, and a bioinformatics investigation

into the vitamin requirements of bacterial families commonly associated with algae surprisingly revealed many were auxotrophic for various B-vitamins themselves [39*].

In the case of vitamin B_{12}, since this is made only by prokaryotes, these must be the ultimate source of the vitamin for algal B_{12}-auxotrophs, and indeed several specific algal-bacterial mutualisms have been reported where cobalamin is exchanged for photosynthate [21,35]. In contrast to environmental samples, where interactions in the complex communities can be weak or transient, and where the identity of key mutualistic partners is not always distinguishable, model systems have been particularly useful in studying the molecular basis for mutualisms based on vitamin B_{12} provision. One such system developed between the freshwater green alga *Lobomonas rostrata* and *Mesorhizobium loti* (Rhizobiales) [40], has proved amenable to mathematical modelling, the result of which indicated that there was an active and regulated exchange of nutrients between the partners [41]. Metabolomic analysis of a model system between the diatom *T. pseudonana* and *D. shibae*, also initially developed based on a mutualism for vitamin B_{12}, found that several amino acids, fatty acids and C4 sugars were up-regulated in co-cultured *T. pseudonana* cells in comparison to mono-cultures [22]. *D. shibae* also had a small but significant growth enhancing effect on *T. pseudonana* at the early stage of the co-culture even when vitamin B_{12} was not limiting.

Analysis of sequenced genomes and transcriptomes (including metagenome/transcriptome datasets) have also provided insights into algal B-vitamin physiology. Often, only portions of vitamin biosynthesis pathways are present [42], indicating that the organism is not capable of *de novo* synthesis. Some algal species have been shown to be able to utilise precursor molecules, which they may scavenge from their environment. *Pavlova lutheri* for example can synthesise thiamine (vitamin B_1) when supplemented with the pyrimidine moiety 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP), which it is unable to produce itself [43]. The same study also found that the bacterium *Pseudoalteromonas* sp. TW7 made an unusable form of thiamine more bioavailable to *Ostreococcus tauri* when grown in co-culture.

Insights beyond symbiosis

Closer investigation of interacting algae and bacteria can lead to unexpected and novel findings. A transcriptomic and metabolomic evaluation of *Ruegeria pomeroyi* (Rhodobacterales) in co-culture with *T. pseudonana*, again initially developed for the provision of vitamin B_{12}, revealed that in exchange for cobalamin the alga produced the sulphur-containing compound C3-sulfonate 2,3-dihydroxypropane-1-sulfonate (DHPS), which in turn could be metabolised by *R. pomeroyi* [44], and

indeed Rhodobacterales generally appear to encode a suite of genes specifically capable of utilising DHPS. These observations thus provide a possible explanation for the previously cryptic role for this molecule, which, like dimethylsulfoniopropionate (DMSP), is produced in large amounts by many marine algae.

A combination of transcriptomic analysis with microbiological and biochemical experiments was used to study the mutualistic interactions that occur between the coastal diatom *Pseudo-nitzschia multiseries* and its associated bacterial community [45**]. Certain members of the algal-associated bacteria, in particular a *Sulfitobacter* species (Rhodobacterales), had mutualistic effects on specific *P. multiseries* strains only. Transcriptomic analysis revealed an up-regulation of the *P. multiseries* genes for tryptophan biosynthesis, whilst in *Sulfitobacter* genes associated with biosynthesis of the auxin, indole-3-acetic acid (IAA), from tryptophan were upregulated, suggesting that the bacteria use the amino acid supplied from the diatom to synthesise this compound. The authors were able to demonstrate levels of IAA in coastal water samples equivalent to that found in laboratory co-cultures. Since IAA has no known metabolic role in bacteria, they propose that it may facilitate signalling with diatoms.

A last example shows how algal-bacterial mutualism can be alter over time. In the model system involving the widespread haptophyte *Emiliana huxleyi* and *Phaeobacter gallaeciensis* (Rhodobacterales), the bacterium initially provides a growth enhancing effect when in co-culture with the alga by synthesising an antibiotic and auxin, but when the algal cultures reach stationary phase increasing amounts of *p*-coumaric acid, a degradation product of the cell wall, are released [46]. This is taken as a senescence signal by *P. gallaeciensis*, inducing it to produce algicidal compounds that increase cell death of *E. huxleyi*. The bacteria are then able to out-compete the alga for remaining nutrients in the media while still being able to utilise fixed carbon from the lysed *E. huxleyi* cells [46]. Interestingly, the algicidal compounds are synthesised from the growth promoting compounds provided by the alga in the mutualistic phase of the interaction [47]. A similar effect was recently noted in another model system featuring *T. pseudonana* and *D. shibae*, indicating this shift from mutualism to parasitism may be a common feature in Rhodobacterales-based symbiosis [48].

Concluding remarks

The rapid explosion in studies of algal-bacterial interactions in recent years has been driven by developments in omics methodology on the one hand, and the identification of specific systems that are amenable to manipulation in the laboratory on the other (Figure 2). Several common themes are beginning to emerge. Clearly there are intimate and complex exchanges of nutrients

between the partners, implying regulation of the mutualism. Vitamins or amino acids such as tryptophan are readily detectable by partner organisms so may act as signals as much as nutrients [41, 44]. Secondly, the fact that many of the mutualistic bacteria found with algae are frequently from the Rhodobacteriales and Rhizobiales families of Proteobacteria may indicate that these organisms are particularly suitable for initiating and maintaining microbial communities associated with photosynthetic partners. Indeed, it has been speculated that these simpler interactions between microalgae and rhizobial bacteria may have acted to "prime" the more intimate and involved legume-rhizobial symbioses [23].

Finally, as a result of the increasing interest in exploiting microalgae as biotechnological platforms for the production of high value products and biofuel molecules [49,50] understanding algal-bacterial interactions has taken on a singular importance. Utilising mutualistic interactions during mass algal cultivation has been proposed as a way to improve yields and reduce costs [51*, 52] and, indeed, an increase in lipid productivity has been noted in at least one co-cultivation system [53]. The rapidly diminishing costs of performing next generation sequencing experiments and the wealth of freely available data are already having dramatic effects on current work, allowing interactions between algae and bacteria to be characterised in exquisite detail. It is likely that further insight into the evolution and establishment of mutualisms will be forthcoming and the combined use of the various techniques described above should allow us to understand how to exploit algae efficiently to solve some of the problems the world faces now and in the future.

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Figure legends

Figure 1 Schematic of algal phylogeny indicating origin of the major groups.

All algal species are thought be derived from an initial endosymbiotic event between an ancient heterotrophic eukaryote with a cyanobacterial partner, giving rise to three basal algal groups, which together with all land plants form the archaeplastida. Secondary and tertiary endosymbiotic events led to further radiation of the algal lineages. Indicated are certain well known examples of species in each of the groups, but the total number of extant algae is considered to be over 300 000 [54,55].

 Figure 2

Different approaches used to characterise algal-bacterial interactions.

The phycosphere surrounding algal cells contains DOM, including fixed carbon that can be accessed by bacteria. In return provision of micronutrients such as vitamin B1 or B12 have been demonstrated in laboratory co-cultures with standard microbiological approaches [21, 34•]. Transcriptomics of a coculture between *P. multiseries* and *Sulfitobacter*-related bacteria revealed up-regulation of tryptophan biosynthesis genes in the alga and indole 3-acetic acid production in the bacterium, an auxin that promoted cell division in the diatom [45••]. A metagenomics approach (green shaded) based on 16S and 18S rRNA gene pyrosequencing was used to elucidate the range of algal-–bacterial species engaged in nitrogen symbioses in the Atlantic Ocean [28••]. Metabolomic analysis (yellow shaded) of the algal partner, *T. pseudonana*, in symbiosis with *D. shibae* revealed the increased production of C3/C4 sugars and amino acids in comparison to an axenic alga [22••]. Finally, classical/computational approaches (red shaded) are still being used to identify unique aspects of symbioses in individual model systems have been revealed by detailed biochemical analysis, such as the mutualistic/parasitic relationship between *Emiliana huxleyi* and *Phaeobacter gallaeciensis* [46].