Algae–bacteria interactions: Evolution, ecology and emerging applications

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Algae and bacteria have coexisted ever since the early stages of evolution. This coevolution has revolutionized life on earth in many aspects. Algae and bacteria together influence ecosystems as varied as deep seas to lichens and represent all conceivable modes of interactions — from mutualism to parasitism. Several studies have shown that algae and bacteria synergistically affect each other’s physiology and metabolism, a classic case being alga–roseobacter interaction. These interactions are ubiquitous and define the primary productivity in most ecosystems. In recent years, algae have received much attention for industrial exploitation but their interaction with bacteria not only enhance algal growth but also help in flocculation, both essential processes in algal biotechnol-

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1. Introduction

Algae are the undisputed primary producers in the aquatic ecosystem and contribute approximately half of the global net primary productivity (Field et al., 1998). These photosynthetic organisms along with cyanobacteria live in the planktonic region of the aquatic habitat and are collectively called phytoplankton (Buchan et al., 2014). Phytoplankton and bacterioplankton numerically dominate the ocean and freshwater planktonic community (Sarmento and Gasol, 2012). These planktonic communities together influence the global carbon cycle and ultimately the climate. Therefore, the interactions between these two groups of plankton and the influence of their interaction on each other and on a global scale are areas of recent research interest (Amin et al., 2015; Landa et al., 2015). Several studies show that heterotrophic bacteria play a ubiquitous role in algal growth and survival (Amin et al., 2015; Gonzalez and Bashan, 2000; Kim et al., 2014a; Seyedsayamdost et al., 2011). Thus, it opens the possibility for revisiting the global carbon cycle and other biogeochemical processes (Amin et al., 2012, 2015; Landa et al., 2015). Similarly, decades earlier in terrestrial ecosystem, it was proven that heterotrophic bacteria not only decompose plant and animal organic matter, but also promote plant growth by complex communication mechanisms and nutrient exchange (Philippot et al., 2013). In this context, evidence of prominent rhizosphere bacteria associated with algae casts light on the possibility of coevolution (Cooper and Smith, 2015; Goecke et al., 2013; Kim et al., 2014a; Ramanan et al., 2015). Therefore, mass cultivation in algal biotechnology should integrate the essence of evolutionary and ecologically relevant relationship between algae and bacteria. Together they not only influence ecosystems but also could potentially influence the growth of future biotechnology industry (Subashchandrabose et al., 2011; Wang et al., 2015). Thus, this review attempts to articulate algal–bacterial interactions in totality, from ecology and evolution, to the use of this knowledge to invigorate their combined biotechnological potential.

Evolution of life was transitional where self-replicating molecules and chemicals formed the basis of prokaryotes. Subsequently, aggregation of prokaryotes led to eukaryotes. Cyanobacteria, a prokaryote, and their association with eukaryotes evolved into algae. A group of single-celled algae and other ancestors led to multicellular organisms (Herron and Michod, 2008). In this evolutionary hierarchy of life, a significant step is that of association of algae and bacteria. To completely understand the ecophysiology and symbiosis between algae and bacteria, thousands of years of time scale needs to be breached to reach their evolution. This evolutionary journey of algae and bacteria and their symbiosis taken together shall be a fair opening deliberation in this review.

2. Evolution of bacteria and algae

The evolution of life is one of the most intriguing research questions that is still in shade. But a prominent bright spot in the overarching shade is a general agreement on the role played by algae and bacteria in earth’s evolution. One of the most potential reasons for existence of human or multicellular organisms on earth is due to the presence of archaea, bacteria, cyanobacteria and subsequently eukaryotic algae. These prokaryotic organisms (bacteria and cyanobacteria), which are the linchpin in the formation of eukaryotic algae and their subsequent interaction with each other, are discussed vividly in the subsequent sections.

2.1. Bacterial evolution benefitted algae

Earth is 4500 million years old and Earth’s atmosphere was devoid of oxygen at origin. Oxygenic photosynthesis is the main reason for the present day atmosphere (Blankenship and Hartman, 1998). According to Earth scientists, life would have originated approximately 3800 million years ago (mya) in a hyperthermal environment as Earth and its oceans were boiling at about 100 °C. But whether life originated in oceans (Nisbet and Sleep, 2001), hydrothermal vents (Martin et al., 2008), rock environment or anoxic terrestrial geothermal fields (Mulkidjanian et al., 2012) is a question under serious debate. Nonetheless, it is clear that first organisms in Earth were perhaps prokaryotic thermophiles capable of living in a methane and sulfur atmosphere, crucially a life without oxygen (Gribaldo and Brochier-Armanet, 2006; Sleep, 2010). By 3500 mya, Earth has stabilized considerably from multiple explosions and bombardment resulting in photosynthesis, first anoxic and much later, oxygenic (Arndt and Nisbet, 2012; Sleep, 2010; Zahnle et al., 2010). This early phase in evolution could be understood from tracking the availability of atmospheric oxygen. Geological features suggestive of oxygen, such as red beds, lateritic paleosols, and sedimentary sulfate deposits, indirectly provide ample proof for atmospheric oxygen (Kopp et al., 2005; Rasmussen et al., 2008; Tomitani et al., 2006). The accumulation of oxygen occurred in two phases. Atmospheric oxygen increased gradually from void to 1–2% around 2400–2000 mya (Rasmussen et al., 2008). Scrutiny of oldest morphological fossils suggests that cyanobacteria originated around 2150 mya coinciding with the great oxygenation event (GOE). Although eukaryotes are known to have emerged 1780–1680 mya ago, levels of oxygen were stable perhaps due to trapping of oxygen by ferrous forming magnetite and other formations even by 850 mya (Holland, 2006). The second subsequent rise in oxygen to ~20% observed in today’s atmosphere is credited to the emergence of photosynthetic eukaryotes such as algae and increased photosynthetic productivity by lichens colonizing land masses. This eventually accelerated the degradation of rocks, thereby releasing fertilizing minerals around 800 mya which increased oxygen concentration in the Carboniferous era (360–300 mya) coinciding with the existence of vascular plants and increased carbon sink (Holland, 2006; Rasmussen et al., 2008). It is also widely accepted that cyanobacteria have played a major role in evolution of eukaryotic algae through the primary endosymbiosis (PE) event, in addition to their contribution to GOE. In PE, a heterotrophic eukaryotic ancestor engulfed a cyanobacterium and retained it as an organelle thereby enabling photosynthesis in eukaryotes (Curtis et al., 2012; Yoon et al., 2004). The host cell which received the cyanobacterium was earlier thought to have originated from bacteria, but increasing evidence suggests that, it was indeed an archaea.
Cyanobacteria were directly responsible for this oxygenic environment as well as for the birth of photosynthetic eukaryotes, and indirectly responsible for evolution of various aerobic organisms, including humans. Hence, from the perspective of this review, the paleo- and meso-proterozoic eras are of utmost relevance, where primary and secondary endosymbiosis (SE) is believed to have taken place (Yoon et al., 2004).

Cyanobacteria are considered to be algae by phycologists because of phenotypic similarities but the advent of molecular phylogeny and polyphasic studies proved their bacterial ancestry. Hence, the term cyanobacteria was previously used interchangeably with the term blue-green algae (Wilmotte, 2004), but the correct term is now accepted to be cyanobacteria. Apart from cyanobacteria, other prokaryotes which perform tetrapyrrole-based photosynthesis are proteobacteria (purple bacteria), heliobacteria, chloroflexi (green non-sulfur bacteria) and chlorobi (green sulfur bacteria). Cyanobacteria possess two types of photosystems (type I & II) and perform oxygenic photosynthesis as mentioned earlier. The other four groups have either of these two photosystems and perform anoxygenic photosynthesis. The defining moment in photosynthesis is the ability to use water as a source of hydrogen in the photosynthetic reaction, not the evolution of oxygen. Thus, photosynthesis originated in the anoxygenic form, perhaps in archaea, using a primitive photosystem I-like reaction center. Oxygenic photosynthesis is believed to have originated in the cyanobacterial lineage under ultra-violet light conditions prevailing then, in addition to depletion of electron donors. From this ancestor, photosynthesis possibly spread to other lineages through lateral gene transfer.

A simplistic view of algal–bacterial evolution and their role in endosymbiosis events is portrayed in Fig. 1. Cyanobacterium was retained as primary plastid over time in three distinct evolutionary lineages — red algae, green algae and glaucophytes. Study of plastid multi-gene phylogeny using molecular clock analyses placed the origin of first alga before 1558 mya (Parfrey et al., 2011; Yoon et al., 2004). Later, a series of secondary endosymbiosis events led to diversification of this ancestor (Curtis et al., 2012; Li et al., 2006). Therefore, the role of bacteria in this ancestral algal genesis is not questioned. But considering the fact that heterotrophic bacteria are always associated with algae in nature, the role of these bacteria during various secondary endosymbiosis events needs to be questioned. The interplay between cyanobacteria, algae, bacteria and protists in a series of endosymbiotic events has been discussed in several excellent reviews (Decker and Holde, 2011; Keeling, 2009; Prechtli et al., 2004; Thompson et al., 2012; Tomitan et al., 2006; Vaishnava and Striepen, 2006). Moreover, evidence of horizontal gene transfer from bacteria and archaea to algae to help adapt to extreme environments is also emerging (Schönknecht et al., 2013). Hence, the holistic role of endosymbiotic heterotrophic bacteria which surround the present-day algae in these endosymbiosis events is not well documented, apart from a few studies reviewed below.

2.2. Evolution of multicellularity in algae — do bacteria play a part?

After these endosymbiotic series, algae moved a level higher in complexity from cellular autonomy to cellular cooperation, i.e. division of labor within cells (Herron et al., 2009; Kirk, 2005). Although various studies were conducted to elucidate each step towards multicellularity in volvocine algae (Herron et al., 2009; Kirk, 2001), there are hardly any studies on the role of endosymbiotic bacteria which inhabit the cell wall and cell sheath in these algae. Fig. 2 shows the overwhelming presence of bacteria associated with various multicellular algae and...
their closest unicellular relative, *Chlamydomonas*. Recently, Kawafune and colleagues showed the presence of bacterial endosymbionts in two volvocine green algae: unicellular *Carteria cerasiformis* and multicellular *Pleodorina japonica*. Definitive evidence on the identity and molecular phylogenies of endosymbionts were unknown earlier although they were discovered within the cytoplasm of *Volvox* and other volvocales, as early as 1970 in case of *Volvox*. The endosymbionts belong to hydra group within the Rickettsiaceae family, the perennial endosymbionts of non-arthropod hosts (Kawafune et al., 2012, 2014). Members of Rickettsiaceae family also include the bacterial genus *Rickettsia* that are associates of invertebrates like blood-feeding arthropods and become pathogenic when transmitted to vertebrates (Andersson et al., 1998; Perlman et al., 2006). It is also well-known that the members of Rickettsiaceae family serve as the origin of mitochondria in eukaryotes, a defining event in their evolution (Andersson et al., 1998; Emelyanov, 2001; Gray et al., 1999).

Considering the increasing evidence of bacterial endosymbionts in multicellular algae, the role of bacteria in multicellularity can no longer be ignored. This leads us to question the theory of evolution of multicellularity. For example, one of the crucial steps in evolving to multicellularity is the conversion of cell wall structures to extracellular matrix (ECM), which constitutes 99% of cell volume in *Volvox*. This evolution not only gave an advantage of hosting large gonidia (reproductive cells) inside ECM, but also the competitive advantage over smaller algae for better access to nutrients (Kirk, 2005). Since bacteria are always associated with algae in the cell wall surface, corroborating with the evidence of bacterial endosymbionts, several questions arise. Were bacteria engulfed during the inversion process where cytoplasmic bridges play a critical role? Did cell-wall adhering bacteria help in creating specializations which keep individual *Gonium* cells together, that later transformed into ECM in *Pandorina* and *Volvox*? These are some of the unanswered questions in evolutionary biology of algal–bacterial interactions in algal multicellularity (Kirk, 2005; Nozaki, 1990).

These questions could be answered if the diversity of algae-associated bacterial community is ascertained in these lineages. Multicellular volvocine algae, red algae, brown algae, and plantae have independent origins for multicellularity, which occurred about 1000 mya. However, the bacteria associated with most green algae and plants consist of core group of genera called the Plant Growth Promoting Bacteria (PGPB). This term was first used by Kloepper and colleagues as Plant Growth Promoting Rhizobacteria (PGPR), which was subsequently expanded for other bacteria as well (Bashan and Holguin, 1998; Kloepper et al., 1980). Earlier studies show that specific bacterial genera are associated with each green alga vindicated by the diversity of secretory products. Our phylogenetic study demonstrates that an overarching clade of bacteria such as proteobacteria and bacteroidetes are more likely to be associated with green algae than other bacterial phylotypes. Moreover, these bacteria are also functionally equipped to be associated with green algae (Ramanan et al., 2015). This clade of bacteria associated with green algae (PGPR & Bacteroidetes) are similar and even in some cases identical, as in the case of *Rhizobium* (Kim et al., 2014a), and perform similar functions to that of plant–bacteria interactions. In the meantime, it is widely known that algae are ancestors of land plants, which originated relatively recently at approximately 450 to 470 million years ago (mya) (Bhattacharya and Medlin, 1998; Palmer et al., 2004). Algae have also played a defining role in animal evolution (Ni et al., 2012). Hence, another compelling query to be answered on coevolution is that whether some of the bacterial genera like *Rhizobium* and *Rickettsia*, for instance, continued to associate with the highly evolved descendant of algae and arthropods, respectively. Even though the role of algae and bacteria in evolution has never been questioned, their collective role is yet to be determined. In conclusion, both algae and bacteria conceivably coevolved from prokaryotes to form unicellular eukaryotes to multicellular higher plants and animals. In this context, studying both organisms and their interactions in unison from ecology to present day...
applications is more enriching and fulfilling than engaging in their own individual subsistence. As bacteria and algae have coevolved, along the way they have formed a diversity of interactions, some of which define their respective habitats (Ashen and Goff, 2000). For more detailed discussion on evolution of algae and bacteria, the readers are requested to refer on Refs. Bhattacharya and Medlin (1998), Blankenship and Hartman (1998), Herron and Michod (2008), Keeling (2009), Li et al. (2006), Ni et al. (2012), Rasmussen et al. (2008), Tomitani et al. (2006), Vaishnava and Striepen (2006), Williams et al. (2013), and Yoon et al. (2004).

3. Ecology of algal–bacterial interactions

3.1. Defining types of association

Algal–bacterial interactions cover the whole range of symbiotic relationships which are deemed possible. Algae, heterotrophic bacteria and archaea are the primary producers and decomposers, respectively, making them the structural pillars of the ecosystem and its foremost functional entities. However, most types of interactions between algae and bacteria in the planktonic zone are scantily studied and those studied often reflect the importance of taking up such studies. This is because of the onerous task of separating the partners which are naturally bound to each other. Azonal cultures of algae are difficult to isolate, maintain and study because of their completely different physiology and metabolism compared to their xenic counterparts, which are always laden with bacteria (Amin et al., 2015; Cho et al., 2013, 2015b).

Therefore, direct evidence on the amount of carbon and nitrogen exchange that takes place between a cyanobacterium and a nanoplankton was established only by 2012. This relationship is believed to be as important to vertical flux of carbon and nitrogen as their ancient, omnipresent mutualism (Thompson et al., 2012). Although the primary function of heterotrophic bacteria is decomposition, it is now accepted that some bacteria also play a part in algal growth promotion, establishing mutualistic interactions. This paradoxical dual function often complicate such studies on interactions, requiring a complex study on the complete set of ecological functions of each partner in the aquatic food chain. The emerging studies on the modes and factors influencing interactions also question conventional wisdom, with most studies pointing to relation continuum. Hence, this section will dwell on those emerging studies on algal–bacterial modes of interactions which would have enormous ecological significance in the future, as well as relevance for the algal biotechnology industry.

3.1.1. Mutualism

There are many examples of mutualism between algae and bacteria, the one mentioned before is the first study to conclusively prove single cell interactions. Other studies have also revealed the role of mutualism, in some cases obligate relationships, for each other’s subsistence. Croft et al. (2005), made a strong case for mutualism in Vitamin B_{12} auxotrophs, when they proved that bacteria supplied Vitamin B_{12} to algae in exchange for fixed carbon. Further studies by this group validated the evolutionary importance of this mutualism (Helliwell et al., 2011). Facultative relationship was also observed between Chlamydomonas reinhardtii, the model green alga encoding both Vitamin B_{12}-dependent (METH) and –independent (METE) methionine synthases, and heterotrophic bacteria which delivers Vitamin B_{12} whenever required, indicating a widespread distribution of such relationship (Kazamia et al., 2012b). The supply of Vitamin B_{12} by an associated bacterium results in repression of C. reinhardtii METE gene expression and subsequent utilization of Vitamin B_{12} supplied, indicating an opportunistic relationship. C. reinhardtii exuded photosynthetic carbon is not taken up by the bacterium, therefore the nature of the relationship is still unclear. But mutualism is not limited to micronutrient supply from bacteria alone (Droop, 2007).

Many studies have highlighted the role of Mesorhizobium and Azospirillum in algal growth promotion and vice-versa (Gonzalez and Bashan, 2000; Hernandez et al., 2009; Watanabe et al., 2005). One of the most important implications of these studies, especially by Bashan and colleagues, is that algae are dependent on macronutrients such as nitrogen (N) since they do not possess nitrogen fixing mechanism and that is supplemented by bacteria, especially in oligotrophic environment. A recent study conclusively proved the case of mutualism between a well-known PGPB, Rhizobium sp. and wastewater derived algae, Chlorrella vulgaris, highlighting the importance of this mutualism in freshwater (Kim et al., 2014a). However, considering the ecological structure and function, the major question which arises is the need for bacteria to benefit algal growth, while being a decomposer. Mutualism, as the word indicates is not a one way exchange, rather the bacteria stand largely benefitted by being associated with algae, a blessing in oligotrophic environment. We demonstrated that algal supply fixed organic carbon to an artificial consortium of mutualistic bacteria, mostly belonging to PGPB, and bacteria in return, supply dissolved inorganic carbon and low molecular organic carbon for algal consumption (Cho et al., 2015b). Mutualism is not limited to unicellular microalgae but also prevalent in macroalgae, in some cases they are endosymbiotic (Hollants et al., 2011). Such exchanges between biotic communities in aquatic ecosystems have a huge role in cycling of nitrogen, sulfur, carbon and phosphorus (Amin et al., 2009; Ask et al., 2009; Azam, 1998; Cho et al., 2015a; González et al., 2000; Grossart et al., 2006; Grover, 2000; Oh et al., 2001b). The mechanism of such mutualistic exchanges is covered extensively in Section 3.3.

3.1.2. Commensalism

Commensalism is a relationship in which only one partner benefits unlike mutualism. However, there is a very thin line that separates mutualism and commensalism. It is now understood with most cases of commensalism, mutualism, and parasitism that the thin line which not only delineates but also determines these relationships is environmental factors. And these interactions are a continual and not discrete interface (Ewald, 1987; Hu et al., 2010; Johnson et al., 1997; Karst et al., 2008; Neuhauser and Fargione, 2004; Valiente-Banuet and Verdú, 2008; van Ommeren and Whitham, 2002). From this perspective, most algal–bacterial associations studied till date are either mutualistic or parasitic; the intertwining relationship is almost deficient in literature. Although there are numerous studies which describe the change in community structure of algae and/or bacteria depending upon environmental factors (Bruckner et al., 2008; Carrillo et al., 2006; Cole, 1982; Interlandi and Kilham, 2001; Klepac-Ceraj et al., 2012; Mayali and Doucette, 2002; Sher et al., 2011), specific algal–bacterial interactions are viewed as discrete interactions and the continuum concept has not been applied. Only a handful of studies demonstrate, only partially, the role of nutrient availability in determining the relationship between algae and bacteria (Gurung et al., 1999; Kudela and Dugdale, 2000; Leung and Poulin, 2008; Liu et al., 2012; Sherr et al., 1988). An excellent early treatise on algal–bacterial competition and commensalism reveals the role of phosphorus (Bratbak and Thingstad, 1985). Other studies have indicated that phosphorus limited algae outcompete themselves allowing bacterial commensals to outnumber algae. Similarly, the role of nutrients, N:P ratio, carbon and light intensity in regulating the growth of these organisms in association have been partly discussed (Grover, 2000; Gurung et al., 1999; Currie and Kalff, 1984). However, mechanism or factors behind an apparent shift from mutualism to parasitism and vice versa via commensalism remains just a theory, although algae and bacteria serve as an excellent model system. Besides, there is a strong debate on whether mutualistic relationships become parasitic over time and later live autonomously, challenging the continuum concept (Sachs and Simms, 2006). In any case, commensalism has not been in the forefront in algal–bacterial interactions, which in itself raises the question of whether the moment is too fleeting. Or as an article pointed out, commensals could be
considered as non-interacting partners and therefore difficult to prove absence of interaction (Zapalski, 2011). Our latest study on the phycosphere bacterial diversity shows evidence of certain bacteria which might be commensals harboring the algal sheath for carbon and shelter (Cho et al., 2015b). From the studies available it can be speculated that commensalism though the partners are not highlighted while documenting algal–bacterial interactions (Hirsch, 2004; Zapalski, 2011). Therefore, studying algal–bacterial commensalism might as well shed light on true validity of the continuum concept.

3.1.3. Parasitism
Unlike mutualism and commensalism, parasitism is relatively well-studied. Many bacteria are known to negatively affect algae and hence very encouraging for scientists studying microalgae and cyanobacterial bloom control (Kim et al., 2008; Lee et al., 2010; Lovejoy et al., 1998; Wang et al., 2010). Moreover, algae also are parasitic, often to their higher taxa or their own counterparts (Goff and Coleman, 1984; Goff et al., 1996; Sachs and Wilcox, 2006). In fact, red algae are considered to be model parasites (Goff et al., 1996). About 10% of known red algae are parasitic and the mechanism of this parasitism is adequately established (Hancock et al., 2010). However, very few studies have been conducted on algal parasitism on bacteria and vice-versa, their mechanism of elimination and its ecological reasoning. These studies demonstrate that the algal cell lysis is achieved through a mechanism similar to plant–pathogen interaction. Glucosidases, chitinases, cellulyases and other enzymes that help degrade plant cell wall are also involved in the lysis of algal cells (Ali et al., 1996; Arora et al., 2012; Wang et al., 2010). In fact, this phenomenon of algal cell wall lysis is not only limited to bacteria, but also to fungal and mollusk enzymes (Nikolaeva et al., 1999). Apart from actual lysis of cells and utilizing the intracellular compounds as nutrients by bacteria and fungi, second form of parasitism is competition for existing nutrients with algae resulting in slower growth rates of algae, eventually after several generations, outcompeting algal existence in that environment. Yet another version of parasitism and competition is altruism, either self-driven or driven by the beneficiary, which would then closely resemble competition (Doncaster et al., 2013). Both parasitism and competition were manifested in a recent study on algal–associated fungi and bacterium, respectively (Cho et al., 2015b). Besides, such parasites are useful for many applications in algal and industrial biotechnology, which will be discussed in detail in following sections (Bhat, 2000; Dahiya et al., 2006). Hence, it is imperative to specifically learn the ecological and evolutionary significance of such degradation, and algal–bacterial parasitism, in general.

A general agreement for most associations is that they often happen in close proximities. For example, parasitic bacteria are usually present in the cell wall or its associated sheaths, to facilitate cell wall degradation (Wang et al., 2010). Besides, any association is a function of its microenvironment and the surrounding macroenvironment, which also strongly supports the continuum concept (Ewald, 1987; Johnson et al., 1997; Karst et al., 2008; Sachs and Wilcox, 2006). Hence the habitats play an important role in ecophysiology of these organisms in an association.

3.2. Habitats

It is widely known that algae, bacteria and cyanobacteria live in almost every niche of this Earth from Antarctic ice to hot springs (Castenholz, 1976; Sakai et al., 1995; Thomas and Dieckmann, 2002), from high altitudes to deep ocean sediments (Ask et al., 2009; Jørgensen et al., 1995; Schippers et al., 2005), from plant roots to leaves and branches (Cocking, 2003; Hoffmann, 1989; Madhaiyan et al., 2006; Neustupa and Skaloud, 2008; Pitman, 1982; Rovira, 1965) – and conceivably beyond. However, this section will deal with two levels of habitats – an intimate interaction of algae and bacteria in the ‘phycosphere’ and a collective interaction of algae and bacteria with organisms in some defining habitats. Algae and bacteria together play a wide range of roles with other organisms in every ecosystem (Grossart et al., 2006) and it would be beyond the scope of this review to deal with all major ecosystems. Hence, this section will define some interesting and well-known habitats in terrestrial, aquatic and extreme ecosystems.

3.2.1. Phycosphere – an intimate microenvironment
The term ‘Phycosphere’ used for the first time in 1972 (Bell and Mitchell, 1972), defined as “a zone that may exist extending outward from an algal cell or colony for an undefined distance, in which the bacterial growth is stimulated by the extracellular products of the alga” is used sparingly since. Studies from our laboratory (Cho et al., 2015b; Kim et al., 2014a; Lee et al., 2013; Ramanan et al., 2015) and elsewhere (Sapp et al., 2007) suggest that phycosphere is one of the most ignored and distinctive habitat for bacteria. Scanning Electron Microscopy (SEM) images show axenic C. vulgaris (Fig. 3A) and xenic C. vulgaris with bacteria on the cell wall and beneath the cell sheath (Fig. 3B). The phycosphere is equivalent to an oasis for heterotrophic bacteria, where high concentrations of fixed organic carbon is excreted for consumption, compared with the vast oligotrophic surroundings in ocean and freshwater. This demands a question of why precious fixed organic carbon is wasted by microalgae through excretion. Most

![Fig. 3](image-url)
plausible explanation is provided by studies which proved increased excretion as consequence of increased exposure to light, especially in the planktonic region as inorganic carbon is non-limiting and serves as a sink to prevent photoinhibition (Cherrier et al., 2014; Leboulanger et al., 1998). Other studies have also proved that algae abandon Redfield ratio under nitrogen depletion and increase organic carbon secretion, especially as RuBisco’s oxygenase activity fixed glycolate (Granum et al., 2002). Overall, precious fixed carbon is released for a reason by algae, but eventually benefitting bacteria. Recent studies have demonstrated that such benefits are not bestowed upon all invaders. Only bacteria which possess unique abilities are able to survive in this microscopic yet organic rich region.

Studies indicate that specific functional types of bacteria are associated with most algae, which exploit this unique habitat, in some cases also helping algal growth, in a classic case of mutualism (Kim et al., 2014a; Ramanan et al., 2015). These functions include ability to degrade complex polysaccharides, to stave off competition, and provide beneficial attributes to algae. And this requires enormous metabolic activity linked to a complex signaling network to maintain the relationship, backed by fluid genetic machinery. A detailed description of this mechanism is outlined in following sections. Since this close association involves sharing metabolic potential of each organism, in most cases, bacteria and algae define the survival characteristics of each other in that environment through specific interactions, viz., mutualism, commensalism or parasitism, as discussed earlier (Doucette, 1995; Wang et al., 2010). Therefore, a region which hosts such a vital relationship which influences nutrient cycling needs higher attention than a scant mention in few research studies.

3.2.2. Lichens

A classic example of algal symbiosis is considered to be lichens. Lichens and their importance have been extensively discussed (Lutzoni and Miadlikowska, 2009; Lutzoni et al., 2001). Studies suggest that lichen can be the determinants of ecosystem health by providing specific signatures on the habitat they live in (McCune, 2000; Stengel et al., 2004). However, a relationship which occurs in lichens has not warranted attention for reasons unknown (Hodkinson and Lutzoni, 2009). Only lately, the role of bacteria in lichens was recognized and has added a new dimension to its studies (Hodkinson and Lutzoni, 2009; Hodkinson et al., 2012). Bacterial diversity in lichens is predominately Rhizobiales but also encompass members of Acidobacteriaceae, Acetobacteraceae and Brucellaceae. As discussed elsewhere, Rhizobiales are also predominant in the algal phycosphere, help enhance algal growth and hence might have a major role to play in lichen physiology, in symbiosis with algal partner. Recently, microbiome studies on lichen lichen, Lobaria pulmonaria, show that the lichen hosts algal photobiont (Dictyochloropsis reticulata) and cyanobacterium Nostoc. Comparative omics analyses of L. pulmonaria elucidated that bacteria present in the lichen aid algae by supplying Vitamin B12, nutrients, growth hormones and conferring resistance to pathogens (Grube et al., 2015). Moreover, the lung lichen sampled from three different locations share a core fraction of microbiome, indicating that these symbiotic functions are not isolated (Aschenbrenner et al., 2014).

Bacterial symbionts in foliose lichen Peltigera membranacea are known to be involved in phosphate solubilization, possibly resulting in algal growth promotion (Sigurbjörnsdóttir et al., 2015). Finally, bacterial community structure is also influenced by the nature of the photobiont, thus proving that algae and bacteria share a symbiotic relationship affecting each other’s physiology and existence, therefore collectively affecting lichen survival. Thus, collective effect of algae and bacteria in lichen survival is unquestioned which indicates the ecological significance of these interactions, as lichens are known to be one of the oldest symbiotic relationships (Hodkinson et al., 2012; Lutzoni et al., 2001).

3.2.3. Corals

Corals are one of the most dynamic and aesthetic marine ecosystems. Corals resemble epiphytic lichens in many ways. Firstly, corals have a microbial community comprising algae, fungi, bacteria and archaea, similar to lichens. Secondly, coral is very sensitive to environmental changes and hence act as an indicator of ecosystem health (Gascuel et al., 2005; Hoegh-Guldberg et al., 2007; Hughes, 2002; Sheppard and Loughlough, 2002). Thirdly, algae have the pivotal role in lichens and corals, supplying photosynthetically derived carbon to sustain both ecosystems. Endosymbiosis between corals and algae is well-known, in fact algae incorporates the coral reefs with its vivid beauty (Brown et al., 1999; Douglas, 2003; Hughes et al., 2003). Algae in coral, popularly called as zooxanthellae, are unicellular dinoflagellates, mostly belonging to Symbiodinium genus. Corals help algae to fix carbon by acidifying the microenvironment and consume the photosynthetically derived carbon (Barott et al., 2015). Algal endosymbiosis also seems to play a major role in stress resistance to environmental changes among coral reef community (Wooldridge, 2009). Overall, it is believed that there are several modes of interactions between algae and coral (McCook et al., 2001), and hence highlight the role of algae in this ecosystem. Morphologically, dinoflagellates are housed inside the gastrodermis of coral animal cells, which together is known as symbiosome. The bacteria are located on the surface of the host cell (mucus layer), gastrodermis and calcium carbonate skeleton, but dominated by distinct populations (Rosenberg et al., 2007). Many studies on analysis of bacterial diversity in different corals from different geographic regions revealed that most dominant bacteria belonged to gammaproteobacteria followed by alphaproteobacteria, cyanobacteria, firmicutes and bacteroidetes (Bayer et al., 2013; Littman et al., 2009; Pantos et al., 2015). The overall bacterial diversity is quite similar to that of phycosphere bacterial communities in freshwater algae (Ramanan et al., 2015). Meanwhile, analysis of bacterial communities in corals under varying stages of bleaching demonstrated a subtle change at genus level, with Vibrio and Acidovorax dominating in the pre-bleached and bleached corals, respectively. The emergence of specific clades of Vibrio is an indicator of coral bleaching which results in possible breakdown in the symbiotic relationship between algae and the host (Bourne et al., 2007, 2009; Tout et al., 2015). Many studies also prove that algae and bacteria act in unison to maintain host health as any disturbance in either community leads to coral mortality (Barott et al., 2011, 2012; Reshef et al., 2006; Rosenberg et al., 2007).

The mechanism of symbiosis between algae and bacteria in coral was proved by one of the earlier studies which elucidated that algae supply oxygen at very high concentration, often super-saturating (200% saturation), aiding host and prokaryote growth in addition to preventing infectious organisms (Revsbech, 1995). The algae in the symbiosome help bacteria in the coral tissues, while cyanobacteria in the carbon skeleton aid their symbiotic bacterial partner (Rosenberg et al., 2007). Bacteria on the other hand may provide nutrients including nitrogen possibly to host and algae, as well as protecting them from pathogens by producing antibiotics and by competition, thereby safeguarding symbiosome function and eventually preventing coral bleaching (Lema et al., 2012; Pantos et al., 2015). Summarizing the algal–bacterial interaction in coral with recent evidence, it is concluded that algal exudates in coral are proven to be directly beneficial to bacteria; the bacteria are not yet proven to be directly beneficial to algae alone, rather to the host and algae taken together. Therefore, algal–bacterial interaction in coral is both special and complex. Although it can be safely concluded that type and health of algae–bacterial community would determine the survival of one the most magnificent ecosystems in the world, more research is needed on definitive roles of each of these partners.

3.2.4. Extreme environments

Algae, cyanobacteria and bacteria exhibit a strong resistance for a wide range of extreme habitats as mentioned before. An interesting study showcased the survivability of algae, bacteria and cyanobacteria in outer space environment and under UV radiation for a long time
(548 days) (Cockell et al., 2011). The study demonstrated that phototrophs survive in natural biofilms formed on the rocks when exposed to low-Earth orbit without known carbon and energy source, adding a new dimension to studies on growth of algae and bacteria in extreme environment. In the Antarctic sea-ice, it was proved that algae and bacteria coexist to fight high salinity, lower available free-water, extremely low temperature, low light and inorganic carbon conditions, and even high UV-radiation. Both bacteria and algae cope with such extreme conditions by secreting high levels of EPS, including organic carbon as seen earlier. Special ice-active substances like glycoproteins, which change the physicochemical surroundings of the immediate environment, are also secreted. Another possible mode of survival would be to alter each other’s metabolism to suit the partner needs, especially during winter trapping in ice sheets (Thomas and Dieckmann, 2002). In such extreme environments, mutualistic arrangement is the only viable survival strategy. Such arrangement is also seen in a vastly dissimilar habitat, the coastal range of Atacama Desert, the driest and oldest desert on Earth. Here, microalgae and cyanobacteria formed biofilms on spider-webs with coastal water evaporation being the sole source of nutrients (Azua-Bustos et al., 2012). Another study showed that algae and cyanobacteria adapted to the extreme environment in Atacama Desert by switching to endolithic habitats within gypsum deposits in organized succession. The lower cyanobacterial layer was in close association with sepiolite inclusions with little supply of water and an upper algal layer acted as buffer to heat and evaportranspiration. Carotenoids and scytonemin were produced by upper algal and lower cyanobacterial layers respectively to prevent photoinhibition and oxidative damage. The study also found heterotrophic bacterial communities such as proteobacteria and actinobacteria associated with the endolithic communities comprising dominant cyanobacterial lineages, Chroococcales and Synechococcales, and unclassified algae. In such an arrangement, the role of cyanobacteria might have been to supply nitrogen, and minerals through mineralization, while the algal–bacterial community would have buffered the photoinhibition and evaportranspiration by EPS production and recycling. Taken together, algal–bacterial interaction to produce EPS, packed and organized community structure within the endoliths, and production of anti-oxidants would have helped the community to sustain in such extreme habitat (Wierzchos et al., 2015). Genome of extremophilic red alga, Caldaria sulphuraria, which lives in hot, metal-rich, toxic and acidic environments, revealed that horizontal gene transfer from bacteria facilitated its survival over millions of years (Schönknecht et al., 2013). This pathbreaking study highlights both association and eventual evolutionary inheritance from bacteria to algae with long-term implications for understanding algal–bacterial interactions in extreme environments. These studies also give insights on the possibility of convergent evolution which might have paved way for land plants (Azua-Bustos et al., 2012; Wierzchos et al., 2015). Thus these extreme habitats warrant attention as natural mesocosms, providing evidence on organismal evolution and modes of survival.

3.2.5. Marine phytoplankton

One of the most ubiquitous interactions in the marine ecosystem is the Roseobacter–alga interaction. Members of the Roseobacter lineage play a major role in biogeochemical cycling, especially carbon and sulfur, by oxidizing greenhouse gas carbon monoxide and producing dimethylsulfide, both of which enormously influence global climate (Geng and Belas, 2010). The most interesting aspect from the pretext of this review is that they utilize algal osmolytes to produce these compounds. Roseobacter denitrificans is the first member of this lineage to be sequenced and they generate energy using anoxygenic photosynthesis process, and in the presence of oxygen (Wagner-Döbler and Biebl, 2006). Not only does this organism possess ancient photosynthetic machinery, but it is also known to be associated with algae ubiquitously, possibly throwing light on the age of such associations. Studies on the troponoids produced by marine Roseobacter lineage, coined Roseobacticides lately, show that these bacteria have a robust quorum-sensing based gene-regulation system which responds to environmental cues and dictates their relationship with algae (Geng and Belas, 2010). The mechanism of this ubiquitous interaction is discussed in detail in the following section.

3.3. Mechanism of interactions

The interaction between marine algae, Emiliania huxleyi, a single-celled phytoplankton ubiquitously distributed from tropic to sub-Arctic waters, possessing highly ornamental calcite shields called colloiths, and its associated bacterium of Roseobacter lineage is the most widely studied. Mechanism of their interaction is among the most complete and well-understood. The algae are subject to frequent upheaval and decline in population, succeeding every algal bloom. When algal population decline, members of the population release cell wall degradation products, such as lignin and its degradation-by-products, such as p-coumaric acid. It is established that members of Roseobacter lineage change from a mutualistic partner upon p-coumaric acid release, called an elicitor, to an opportunistic pathogen by releasing 11 types of troponoids, which eventually kill algae, and switch the Roseobacter member, from a sessile lifestyle on the algal cell wall to motile phase (Geng and Belas, 2010; Sule and Belas, 2013; Wagner-Döbler and Biebl, 2006). Moreover, it was demonstrated that in the brief period of high algal growth, Roseobacter not only serves as a perfect mutualistic partner by supplying vitamins and phytohormones to algae, but also provides antibacterial safeguards towards non-roseobacters, in cohabitation with algae (Sharifah and Eguchi, 2011). Besides, the precursors of troponoids are synthesized from algal derived carbon, which algae releases during their mutualistic relationship. Thus, algae synthesized molecules that serve Roseobacter member during mutualism are diverted for toxin synthesis during parasitism (Seyedsayamdost et al., 2011). This interaction is the closest example of classic continuum theory between algae and bacteria, not proven to be driven by environmental factors, but rather surprisingly determined by algal molecules and growth phase.

However, this mechanism is not the only possible strategy prevalent in all interactions. The mechanism of interaction is species specific as the microenvironment of each alga is different. Modes of interactions between algae and bacteria and their interrelation with the environment are depicted in Fig. 4. In the mechanisms proven so far, carbon and macro- and micro-nutrients seem to play a central role. Studies show micro-nutrients like Vitamins (Croft et al., 2005; Kuo and Lin, 2013; Teplitski and Rajamani, 2011), macronutrients like nitrogen and carbon (Bolch et al., 2011; Kazamia et al., 2012b; Kim et al., 2014a; Teplitski and Rajamani, 2011) and phytohormones (Teplitski and Rajamani, 2011) usually exchange between algae and bacterium. A recent study proved that Indole Acetic Acid (IAA) was transferred to algae in exchange for organosulfur compounds by Sulfitobacter, another member of Roseobacter clade. The study conclusively proved that both algae and bacteria altered their metabolism to suit each other’s needs, and this interaction is potentially very prevalent in the marine ecosystem (Amin et al., 2015).

Yet there are pertinent questions related to mechanism of such uptake. Most are based on the signal generation and recognition, transport and uptake processes. Some studies suggest that ‘quorum sensing’ plays a major part in these interactions, as in the case of the Roseobacter (Teplitski and Rajamani, 2011). It is also important to understand if algal genomic inflexibility to survive under certain conditions results in metabolic complementation and cooperative biosynthesis (Hom et al., 2015). Therefore, whether this chemical exchange and communication eventually leads to genome sharing via horizontal gene transfer is another element to this complex interaction. Most complex questions related to uptake, communication and overall mechanism in a diversity of algal–bacterial interaction are being addressed by single-cell
genomics and other omics approaches and this seems to be the way forward (Cooper and Smith, 2015; Thompson et al., 2012).

3.3.1. Omics approach to illuminate interactions

The omics approach to study microbial ecology has transformed our understanding of microbial communities and their environment (Jansson et al., 2012). Although community metagenomics and metatranscriptomics studies were performed in complex microbial communities predominated by algae and bacteria (Moran et al., 2013; Tringe et al., 2005), specific studies on microbiome of algal phycosphere in natural systems and artificial systems like photobioreactors using high-throughput sequencing were only recently constituted (Krohn-Molt et al., 2013; Ramanan et al., 2015). Recent study demonstrated power of using multi-omics data to illuminate the interaction and mechanism thereof in natural assemblages (Amin et al., 2015). Another study showed that the major phylotypes of bacteria from green algae isolated from different habitats are similar, using 454 pyrosequencing (Ramanan et al., 2015). The functional domain of the phylotypes also point to their similarity with PGPB. Unsurprisingly, the study also revealed that basic techniques like Denaturing Gradient Gel Electrophoresis (DGGE) show limited diversity whereas the same samples would have much higher diversity in reality. Several recent studies on the natural assemblages in aquatic ecosystems using next generation sequencing (NGS) technologies have shown unbiased results, as NGS eliminates cloning biases. Moreover, NGS would not only help in sequencing environmental DNA but also RNA as proved by studies on gene expression in oceans (Nowrousian, 2010). The depth and coverage of sequencing platforms could also help in using several genes for elucidating bacterial diversity as the widely used rRNA gene is known to be highly conserved. Besides, studies on specific bacterial community which are lesser-known or in other words not dominant in the association could be sequenced. A review on the role of omics in algal–bacterial interactions suggests the use of combination of methods including traditional approaches such as microbial and biochemical analyses in addition to metabolomics, metagenomics and transcriptomics. The review argues that a metabolomic analyses would be used to decipher range of compounds exchanged between the partners while metagenomic and transcriptomic approaches would corroborate the existing evidences with insights on cluster interactions, genomic machinery and regulatory pattern (Cooper and Smith, 2015). Taken together, the omics approach and NGS platforms would help answer some basic questions on algal–bacterial association. As microbiome is being explored as operational concept, such analyses using advanced tools inherited from highly studied communities like gut microbiota are being performed. And this knowledge would further unravel algae and dependent microbial communities, their active drivers, functionalities and implications, and the resulting applications.

4. Emerging applications

4.1. Environmental mitigation

4.1.1. Nutrient removal and wastewater treatment

Algae depend on nitrogen and phosphorus from the environment for growth as they are non-diazotrophic. Macronutrient (N, P, S and C) deprivation of algae for prolonged periods results in severe stress leading to stagnation and eventually death (Ramanan et al., 2013; Schmollinger et al., 2014). Heterotrophic bacteria require carbon and other nutrients for growth and are widely used for the treatment of wastewater. Naturally, algal–bacterial systems have been extensively used in the treatment of nutrient rich wastewaters since 1950s. One of the earliest descriptions of algal–bacterial interactions in wastewater treatment is presented by Oswald and Gotaas (1957). Early photosynthesis based systems were neither aerated nor mixed, therefore, the treatment efficiency achieved with these systems are a fraction of what could be achieved with ponds or systems developed later (Benemann et al., 1977; Hoffmann, 1998). As early as 1955, it was proposed that in oxidation ponds, algal–bacterial symbiosis results in sewage treatment with exchange of O2 and CO2 and NH4 ions. Thus it was proved technically that most nutrient rich, low
attached systems are complex, as systematic studies on the fundamental processes are less elucidated, and studies on the role of algal–bacterial interactions in such engineered biofilm systems treating wastewater are sparse (Kesaano and Sims, 2014).

### 4.1.2. Bioremediation

Many studies have dealt with algae–bacteria consortium for metal bioremediation (Boivin et al., 2007) and degradation of organic pollutants (Tang et al., 2010). The effective use of algal–bacterial interactions in degradation of organophosphate insecticides such as monocrotophos, quinalphos and methyl parathion was also demonstrated (Subashchandrabose et al., 2011). Besides, degradation of many other toxic pesticides including DDT, atrazine, α-endosulfan was reported (Subashchandrabose et al., 2013). Several studies have shown the involvement of bacteria/cyanobacteria and algae in the treatment of organic pollutants including black oil, acetonitrile, phenol, naphthalene, benzopyrene, dibenzofuran,azo compounds, among others (Mahdavi et al., 2015; Muñoz and Guieysse, 2006; Subashchandrabose et al., 2013). Recent study demonstrated the role of algal–bacterial interactions in the degradation of thiocyanate wastewater, which provides the glimpse of use of this interaction for degradation of toxic substrates (Ryu et al., 2015).

Algae also require several metals for normal growth and metabolism, albeit in small quantities and hence are considered micronutrients. Algae when deprived of these metals, exhibit systematic, specific stress responses, which are well documented (Glaesener et al., 2013; Kropat et al., 2015; Malasarn et al., 2013). On the other hand, higher levels of metal could lead to toxicity in algae, hence algal–bacterial community mutualistically detoxify and assimilate metals from metal rich environments. The process of accumulation of heavy metals would be through various means including physical adsorption, covalent bonding, ion exchange and chemisorption, surface precipitation, redox reactions or crystallization on the cell surface. Moreover, on a lesser scale, metals are quenched by active uptake into the cell interior for metabolism or as a defensive tool to avoid poisoning. Microalgal growth results in the release of metal chelators and increase in pH often also precipitates heavy metals which are taken-up by the associated bacteria. Few excellent reviews on detailed analysis of bioremediation of hazardous pollutants and heavy metals by algal–bacterial consortium are available (Muñoz and Guieysse, 2006; Subashchandrabose et al., 2013).

### 4.1.3. Bloom control

One of the first research studies on bacterial control of natural algal blooms was again on Roseductor lineage prevalent in algal blooms dominated by E. huxleyi and dinoflagellates (González et al., 2000). But from the context of application, it is important to seek solutions for harmful algal blooms (HAB) particularly in fresh waters of human use. For detailed understanding of HAB control by bacteria in marine environment, readers are referred to other studies (Bai et al., 2011; Buchan et al., 2014; Wagner-Döbler and Biebl, 2006). In freshwater ecosystems, several isolated studies have established the use of bacteria for HAB control. An early study on *Rhodococcus*, a Gram-positive bacterium prevalent in a eutrophic lake, shows the cyanobacterial activity of the bacterial filtrate (Lee et al., 2010). Studies on both marine and freshwater HAB revealed the resurgence of bacterial population corresponding to late or post bloom period, indicating that mechanism of action is different. Hence, monitoring of both algal and bacterial populations over time would not only help in understanding the dynamics in freshwater systems but also help in control of the blooms (Srivastava et al., 2014; Srivastava et al., 2013). On the other hand, bacterial growth after the algal bloom could cause grave damage, depriving oxygen leading to fish kills, symbolizing the overall collapse of the ecosystem. Therefore, bacterial role should be thoroughly studied to employ them at the right time and in the right environment. Moreover, the algal bloom is controlled by various positive and negative effectors, which interact in cohesion (Paerl and Otten, 2013). And finally, any treatment of algal or cyanobacterial blooms by heterotrophic, predatory bacteria should be preceded by or in combination with excess nutrient cutoff from the freshwater system.

### 4.2. Biotechnological potential

#### 4.2.1. Biorefineries — cultivation systems

As discussed in earlier sections, bacteria play a key role in providing phytohormones or macro- and micronutrients to algae which result in various physiological changes within algae most notably enhanced growth rate of algae. However, the role of bacteria in microalgal growth enhancement is often ignored. When the phyosphere bacterial communities, especially PGPB were eliminated, the algal growth rate under phototrophic condition was found to be very slow (Cho et al.,
alar algal harvesting

Another well-known application of algal–bacterial interactions is the role of bacteria in microalgal harvesting (Gardes et al., 2011 and Grossart et al., 2006). Until recently the role of bacteria in microalgal flocculation was speculative, unlike in yeasts (Bester et al., 2012). It is now established that bacteria increase the floc-size of algae thereby enabling settlement, even in lesser flocculation conducive condition. Even though axenic microalgal culture responds to flocculants, without bacterial surrounding its phycosphere, algae cannot form settleable flocs, resulting in non-settleable flocs, which are still in suspension (Lee et al., 2013; Powell and Hill, 2013, 2014). It is also thought that aggregation is made possible in part through charge neutralization by positively charged calcium ions binding to negatively charged tachic acid residues, especially for Gram-positive bacteria (Powell and Hill, 2014).

Although the role of bacteria is established, the complete mechanism is still not clear; especially the role played by Extracellular Polysaccharide Substances (EPS), and the cell wall and secretory proteins of algae. Bioflocculation was demonstrated more than a decade ago using EPS from *Porphyra pacifica*, yet the compounds or the class of compounds which induce such flocculation and their properties remain elusive (Oh et al., 2001a). A mechanism similar to yeast self-flocculation is also a possibility (Bester et al., 2012). There is also a possibility for a completely reverse mechanism given algal tendencies to shelter itself along the water column depending upon the intensity of light (Neale, 1987). Further research needs to be done to ascertain the function of EPS as bioflocculants, its composition and its interaction with algae. There are also questions over the role of genetic apparatus and quorum sensing in microalgal flocculation. This is important as microalgal harvesting is one of the major biomass production costs (20–30%) in biorefineries (Uduman et al., 2010). In a related development in the algal bioenergy research, algalidial bacteria have been shown to help in the lipid extraction process from microalgae, because of their role in algal cell lysis. This process could be used along with the bio-flocculation technique to enhance lipid productivity (Lenneman et al., 2014).

4.2.3. Advanced biorefineries

Algae are known to produce a variety of compounds, from fuels to cosmetics. The future biorefineries would not only look to capitalize on this enormous potential but also augment this approach to produce more compounds and enhance their respective amounts by using an ecological engineering approach (Cho et al., 2015b). Ecological engineering or synthetic ecology is a broad term used for artificial biometric systems which use multi-organism approach for present-day solutions (Cho et al., 2015b; Kazamia et al., 2012a). Any biorefinery system would benefit from the beneficial effects of bacteria for algal growth promotion and harvesting as discussed above. Yet, such interactions have potential to offer beyond what has been demonstrated so far. For instance, in the bioenergy sector, algae–bacteria interactions could be used to good effect, for biodiesel production, electricity generation, biogas, bioethanol and biohydrogen production. Several studies have demonstrated that electricity producing bacteria, such as *Geobacter*, can coexist with algae to synergistically produce electricity using light micro-bial solar/fuel cells (He et al., 2009; Nishio et al., 2013; Rosenbaum et al., 2005). Similarly, algal–bacterial wastewater treatment followed by the production of biogas from wet algal–bacterial biomass is also extensively documented (Prajapati et al., 2013). The role of algae and photosynthetic bacteria for biohydrogen production is being studied, but large-scale application has severe limitations (Das and Vezirgolu, 2001; Miura et al., 1992). A study demonstrated the use of symbiotic bacteria to enhance photo-fermentative hydrogen evolution of *Chlamydomonas*, by oxygen elimination and efficient bacterial respiration resulting in activation of algal Fe-hydrogenase (Lakatos et al., 2014). Similarly, two-stage process of coupled biohydrogen and biogas production was demonstrated using a natural mixed population of *Chlamydomonas* sp., *Scenedesmus* sp., and *Rhizobium* sp. Besides, hydrogen production was achieved without resorting to a sulfur–deprivation process and by mere elimination of oxygen by the symbionts, and the resulting biomass was used for the production of biogas (Wirth et al., 2015). Another two-stage production of value added chemicals from biogas was proved to be possible by using microalgae and methane–oxidizing bacteria. In the first stage, CO₂ in the biogas was fixed by *Scenedesmus* sp., producing oxygen, and lipids were produced by the N-depleted culture. In the second stage, the artificial resulting gas mixture of 60% methane and 40% oxygen was treated by a methane oxidizing bacterium to produce intracellular polyhydroxybutyrate (PHB) after repeated nitrogen limitation (van der Ha et al., 2012). This approach was also demonstrated in a single stage by co-cultivation. Such algal–bacterial PHB production process is also studied in laboratory photobioreactors using inoculum from natural environments (Fradinho et al., 2013).

Bio-ethanol production from algal–bacterial co-culture is an enriching advance, which is yet to be fully explored. Some algae can produce up to 38% of starch granules (dry cell weight basis), and various marine bacteria can utilize this starch to produce ethanol, which can be performed as a two-step or one-step process, based on the harmony between the species used (Matsumoto et al., 2003). Another promising approach which unites ecological and genetic engineering approach, is the use of photosynthetically fixed carbon from algae to produce a desired product, such as, succinate by a genetically engineered bacteria, such as *Corynebacterium glutamicum* (Lee et al., 2014a). This approach promises unlimited possibilities for the production of high-value compounds from light energy. In summary, the use of ecological engineering approaches opens a new era for exciting possibilities for algae-based biorefineries for sustainable production of fuels and chemicals (Smith et al., 2010). Finally, such multi-species approach would circumvent the restrictions imposed on using genetically engineered systems on a large scale (Ortiz-Marquez et al., 2013).

4.2.4. Sustainable aquaculture system

Until lately, algae were the only focus of aquaculture feed with scant attention to bacteria. Even within the aquaculture system, control of bacterial diseases was the only rationale, while the role that could be potentially played by beneficial bacteria was largely ignored. A healthy feed would comprise grazers, algae and their associated beneficial bacteria, and it has been used industrially for decades (Spolaore et al., 2006). Several algal species were also used for controlling pathogenic bacteria in aquaculture systems, like *Vibrio harveyi*, by disrupting the quorum sensing communication between the pathogenic bacteria (Natrah et al., 2013). It was also proved that co-ingestion of algae and bacteria results in healthier *Artemia* culture through better nitrogen assimilation (Toi et al., 2014). Many studies have dealt with algal–bacterial treatment of aquaculture wastewater, but the resounding demonstration that these flocs harvested after wastewater treatment would be used as feed again for Pacific white shrimps, *Litopenaeus vannamei*, throws light on the utility of such an integrated, sustainable and recyclable aquaculture system (Natrah et al., 2013; Van Den Hende et al., 2014).
5. Present & future prospects

As previously mentioned, there are more questions than answers in algal–bacterial interactions from its evolution to applications. The present day understanding of ecology and evolution of algal–bacterial interactions has not been exploited in algae based technologies (Shurin et al., 2013). Table 1 summarizes the need for such understanding and integration in algal biotechnology. Moreover, further studies in ecological engineering would help in the use of algae and bacteria as a production platform for host of industrially important chemicals and fuels in future biorefineries (Brenner et al., 2008; Cho et al., 2015b; Lee et al., 2014a). As prices of traditionally cultivated food products rise with burgeoning population and reduced land area, alternative food products such as algae based diet could gain prominence (Abreu et al., 2014; Tabarsa

Table 1
A summary of current understanding of evolutionary and ecological roles to harness biotechnological potential of algal–bacterial interactions.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Processes in algal biotechnology</th>
<th>Ecological/evolutionary role</th>
<th>Biotechnological applications</th>
<th>References</th>
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<tr>
<td>2.</td>
<td>Cultivation</td>
<td>Bacteria aid algal growth by supplementing various major and minor nutrients in oligotrophic environments. See Fig. 4 for details.</td>
<td>Enhanced growth rate and algal productivity. Reduced dependence on supplied nutrients.</td>
<td>Cho et al. (2015b), Gonzalez and Bashan (2000) and Kouzuma and Watanabe (2015)</td>
</tr>
<tr>
<td>3.</td>
<td>Harvesting</td>
<td>Bacteria initiate algal flocculation possibly for two reasons. Firstly, large algal–bacterial flocs help algae evade predators like zooplankton as large flocs are difficult to consume. Secondly, bacteria willingly settle algae resulting in algal death and subsequent bacterial degradation.</td>
<td>Harvesting accounts for 30% of overall costs in algal bio–product industry. Large algal–bacterial flocs settle readily, resulting in reduced use of flocculants and costs, and better yield.</td>
<td>Lee et al. (2013), Montemezzani et al. (2015), Powell and Hill (2013) and Wang et al. (2015)</td>
</tr>
<tr>
<td>4.</td>
<td>Extraction</td>
<td>Pathogenic bacteria weaken algal cell wall resulting in disruption and cell death, playing a leading role in decomposition.</td>
<td>A study showed enhanced lipid recovery from bacteria infested algae thereby reducing the cost of organic solvent extraction.</td>
<td>Bai et al. (2011), Halim et al. (2012) and Lenneman et al. (2014)</td>
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Fig. 5. Various applications of algal–bacterial interactions for biotechnology and environmental sectors.
et al., 2012; Wijesekara and Kim, 2015). Thus, there is a need to look at algal–bacterial interactions for both high-value products such as nutraceuticals & cosmetics, low value food products for aquaculture and animal feed as well as medium to high value chemicals such as fuels and PHBs. Secondly, algal–bacterial interactions are of potential use in environmental technologies. Algae offer photosynthetically produced oxygen which could be used for algae–bacteria based wastewater treatment (Praveen and Loh, 2015). It has been demonstrated adequately that algae and bacteria combine to remediate toxic chemicals and metals (Subashchandrabose et al., 2011). On the contrary, parasitic bacteria are known to help in the revival of HAB infested freshwater and marine environments (Fig. 5). Therefore, further studies on these interactions would not only help the commercialization stakeholders but also help in understanding some of basic but pertinent questions like involvement in — biogeochemical cycling, endosymbiosis, multicellularity and vital habitats. This makes for an exciting new era with a paradigm shift from single species based approach to community based integration. And this integration closely mimics the natural ecosystem, therefore driving us towards sustainable production and development.

Acknowledgments

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