Introduction to the Cyanobacteria

1

Brian A. Whitton and Malcolm Potts

Contents

Summary

Features of cyanobacteria are introduced for non-specialists by highlighting topics in the various chapters. Aspects where much more is known now than a decade ago are pointed out, such as the importance of cyanobacterial nitrogen fixation in the oceans. This is followed by an account of the recent molecular studies most relevant for ecologists, especially topics not mentioned elsewhere in the book. Several ecological subjects of current interest are discussed, including research which seems important, but has sometimes been overlooked. Topics mentioned include sensing the environment and other organisms and signalling between cyanobacterial cells and between cyanobacteria and other organisms, and methods for studying N and P. The authors air their views on past and present matters concerning cyanobacterial taxonomy, molecular biology and nomenclature. Finally, comments are made on practical topics such as the use of cyanobacteria for inoculating soils, barley straw to control blooms and the likely contribution of cyanobacteria to developments in algal biotechnology during the coming decade.

1.1 What Are Cyanobacteria?

The cyanobacteria are photosynthetic prokaryotes found in most, though not all, types of illuminated environment. They are also quantitatively among the most important organisms on Earth. A conservative estimate of their global biomass is 3×10^{14} g C or a thousand million tonnes (10^{15} g) wet biomass (Garcia-Pichel et al. 2003). They all synthesize chlorophyll *a* and typically water is the electron donor during photosynthesis, leading to the evolution of oxygen. Most produce the phycobilin pigment, phycocyanin, which gives the cells a bluish colour when present in sufficiently high concentration, and is responsible for the popular name, blue-green algae; in some cases the red accessory pigment, phycoerythrin, is formed as well. A few genera, however,

B.A. Whitton (✉)
School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK
e-mail: b.a.whitton@durham.ac.uk

M. Potts
Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, POB 2713, Doha, Qatar
e-mail: malcolm.p@qu.edu.qa

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produce neither, but form other accessory pigments. These include some ecologically very important members of the ocean plankton. Among these, *Prochlorococcus* was first reported as recently as 1988 by Chisholm et al., but is now realized to be of major importance in the oceans (Zwirglmaier et al. 2007). The fact that such a significant organism could be overlooked for so long should encourage readers to take a critical approach to the present literature on cyanobacteria. Zhang and Bryant (2011) did this for the notion that cyanobacteria have an incomplete tricarboxylic acid cycle, which persisted in the literature for more than four decades and even got into several prominent textbooks. In their account dispelling this, they note how the misinterpretation of negative results can have a powerful, long-lasting impact on a topic.

Although the cyanobacteria live in a diverse range of environments, a number of features often contribute to their success. The following short account indicates some of these, but more detailed information can be found in the Introduction by (Whitton and Potts in 2000) and the other chapters in the present book. The temperature optimum for many or most cyanobacteria is higher by at least several degrees than for most eukaryotic algae (Castenholz and Waterbury 1989), thus encouraging their success in warmer climates (Kosten et al. 2012). Tolerance of desiccation and water stress is widespread (Chaps. 12 and 18) and cyanobacteria are among the most successful organisms in highly saline environments (Chap. 15). Terrestrial forms often tolerate high levels of ultra-violet irradiation (Chap. 19), whereas the success of many planktonic forms is favoured by their ability to utilize light for photosynthesis efficiently at low photon flux densities (van Liere and Walsby 1982). Free sulphide is tolerated by some species at much higher levels than by most eukaryotic algae and H_2S is sometimes utilized as the electron donor during photosynthesis (Cohen et al. 1986). Photosynthetic CO_2 reduction can sometimes proceed efficiently at very low concentrations of inorganic carbon (Pierce and Omata 1988; Chap. 17). The ability to form gas vacuoles in some common freshwater plankton species and the marine *Trichodesmium*, and hence increase cell buoyancy, is an asset where the rate of vertical mixing of the water column is relatively low (Chaps. 6, 7 and 8).

The ability of many species to fix N_2 provides a competitive advantage when combined N concentrations are low (Chaps. 4 and 5). In most well-oxygenated terrestrial and freshwater environments this takes place inside the heterocyst (Wolk et al. 1994), a thick-walled cell often with a nodule of cyanophyin, a polymer of two amino-acids, at one or both ends of the cell. However, a number of cyanobacteria have physiological strategies which permit them to fix N_2 under well oxygenated conditions even without a heterocyst and this becomes more widespread under micro-oxic conditions (Chap. 4). This is of considerable importance in some ecosystems such as wetland rice fields. Although heterocystous cyanobacteria are important in the Baltic Sea, where salinity is much less than that of the oceans (typically about one-fifth), oceanic N_2 fixation mostly occurs without heterocysts. Because of the lack of heterocystous species it has only recently been realized that cyanobacteria are the main N_2 fixers in the oceans (Díez et al. 2008). In the filamentous *Trichodesmium* and *Katagnymene* this occurs in specialized cells called diazocytes (El-Shehawy et al. 2003), but it also occurs in a globally distributed unicellular cyanobacterium which does not form O_2 and can fix N_2 in the light (Zehr et al. 2008). Only a few unicellular cyanobacteria in the <1 μm cell size fraction were found to lack nitrogenase genes during a global survey of the oceans (Rusch et al. 2007).

There are many symbiotic associations which include cyanobacteria (Chap. 23) and in the majority of cases it is the ability of the cyanobacterium to fix N_2 and then transfer it to the partner which is a key factor in the relationship. Some of these symbiotic associations have a long geological record, whereas others depend on frequent reinfection by a compatible strain of a particular cyanobacterium, usually *Nostoc*. In some associations the cyanobacterium is intracellular and this includes two marine planktonic diatoms which have a heterocystous species (Chaps. 22, 23). However, the spheroid bodies inside another diatom, *Rhopalodia gibba*, are evolutionarily related to the free-living unicells mentioned above which fix N_2, but do not evolve O_2 (Bothe et al. 2011). Like several other blue-green structures inside eukaryotic cells, these are no longer capable of living independently.

Growth of cyanobacteria in many ecosystems is limited by the availability of P and the importance of P as a nutrient is considered in a number of chapters. Among various topics in Chap. 5 it is explained which parts of the oceans are mostly likely to be limited by N or by P and also the importance of considering the ratio between the two. N and P sources for picophytoplankton and their uptake are considered in Chap. 8 and the influence of N:P ratio on the occurrence of N_2-fixers in freshwaters in Chap. 9. Only a few data are available about what N and P concentrations are actually experienced by subaerial algae and virtually nothing about their periodicity (Chap. 10). However, Chap. 11 makes clear the importance of the P supply for biological soil crusts in semi-desert regions, which almost always include one or more N_2-fixers. Chapter 22 explains the changes in N:P supply during the growth cycle of Rivulariaceae and also how differing periodicities in P supply in various environments influence the success of particular genera and species. Although Chap. 23 focuses on the transfer of fixed N to the cyanobacterial partner in symbiotic associations, P transfer the other way is sometimes also important.

It has long been recognized that cyanobacteria in freshwaters and soils tend to be much more diverse and abundant at higher pH values. There are, however, a considerable number of records at lower pH values. For instance, heterocystous

forms (*Hapalosiphon* and/or *Tolypothrix*) are frequent in small pools at pH 4.1–4.5 in *Sphagnu*m-dominated regions of the Flow Country in N-E. Scotland (B.A.W., unpublished data). In general, heterocystous species seem to be the ones most successful at low pH values, so perhaps they can only compete effectively with eukaryotes in situations where nitrogen fixation gives a clear advantage. However, Steinberg et al. (1998) found populations of two filamentous cyanobacteria (*Oscillatoria*/*Limnothrix* and *Spirulina* spp.) at pH 2.9 in Lichtenuaer See, Lusatia, Germany; eukaryotic algae were almost absent at the time. The authors failed to find planktonic picocyanobacteria anywhere below pH 4.5. This is one of several reports of lakes in lignite-mining areas in Germany and Poland with pH values below 3.0, which mention narrow Oscillatoriaceae in their species lists, presumably based on preserved samples. *Lyngbya ochracea*, for instance, is listed by Koproskowa (1995). As some of the reports make only a brief mention of the cyanobacteria, more detailed studies are needed to confirm them.

Interactions with limestone are a feature of some cyanobacteria. One of the more intriguing aspects is the capacity of some strains (euendoliths) to bore directly into the carbonate substrate. Inhibition assays and gene expression analyses with *Mastigocoleus* BC008 showed that in the dissolution process the uptake and transport of Ca^{2+} is driven by P-type Ca^{2+} ATPases (Garcia-Pichel et al. 2010), a sophisticated mechanism unparalleled among bacteria. Much remains to be discovered about the extent to which nutrients are acquired by endolithic Stigonematales like *Brachytrichia* and *Mastigocoleus* from the surrounding rock or the outside environment (Sect. 10.3.2).

Chapter 21 describes how cyanophage are among the most abundant biological entities on the planet and how they influence community structure and biogeochemical cycling. Although the influence of some bacteria and protists on cyanobacteria was reported long before that of cyanophage, it is still difficult to generalize on their overall importance in cyanobacterial ecology compared with that of cyanophage.

Several ecological features of cyanobacteria have brought them to the attention of the general public. Some species form dense blooms and there are numerous accounts of the problems caused by these and the methods adopted to control the blooms. Worldwide there are fewer than 30 species which cause a real nuisance, yet it is still difficult to generalize about the ecological requirements of many of them. However, understanding about one genus, *Microcystis*, is increasing especially rapidly (van Gremberghe et al. 2011; Chap. 7). Research in recent years has been accelerated by concern about massive *Microcystis* blooms in several large lakes in China, especially L. Taihu (Fig. 6.11), which are the source of drinking water for millions of people. The most serious problem caused by these blooms is the presence of toxins, which are harmful to humans, other mammals and often other types of organism (Chap. 24). At least some populations of all freshwater bloom-forming species studied contain toxins, and toxins have also been reported from many other cyanobacteria, including the marine *Trichodesmium* (Kerbrat et al. 2011). It is fortunate that the strains of *Arthrospira* which are marketed as "Spirulina" are not toxic, since this organism is now grown on a large scale for incorporation into human and animal foodstuffs (Chaps. 25 and 26).

1.2 Past and Present

1.2.1 The Geological Record

The cyanobacterial record extends back to ~3,500 million years ago (Chap. 2). The considerable geological evidence for this comes from various sources, including microbially laminated structures known as stromatolites, cyanobacterial and cyanobacterium-like microscopic fossils, carbon isotopic data consistent with Rubisco-mediated CO_2-fixation being present and molecular data. It has proved important to have evidence from different sources, because of doubts raised as to whether the oldest structures were in fact biological. Chapter 2 also considers whether these organisms included O_2-producing photoautotrophic cyanobacteria much as known today, in spite of the fact that the Great Oxidation Event occurred a billion years later (~2,450 Ma ago). Whatever the sequence of changes may have been, they had a key role in the oxygenation of the atmosphere. Multicellularity in cyanobacteria is thought to have arisen between 2,450 and 2,220 Ma ago (Schirrmeister et al. 2011). Surprizingly, the extent to which cyanobacteria in later geological periods may have contributed to the petroleum deposits now being extracted seems much less clear (Chap. 16). It was concluded that in general the source, form, and distribution of oil-producing communities on the early Earth remains an enigma. However, there is considerable evidence for the involvement of free-living cyanobacteria and their symbiotic association with *Azolla* at particular sites with rich oil deposits.

Several authors have applied molecular data to assess the dates when modern cyanobacterial genera originated (e.g. Domínguez-Escobar et al. 2011). Although there is scope for discussion about the detailed conclusions, this approach should help geologists to consider their data more thoroughly, especially when morphologically complex cyanobacteria are present in geological samples. Such fossil materials are not only easier to equate with modern form-genera, but provide information on the environment where they were likely to have been growing, as is the case for *Palaeocalothrix* from the Precambrian described by Zhao-Liang (1984) (Chap. 22). In the case of hot and cold desert cyanobacteria, Bahl et al. (2011) concluded that the present-day distribution of taxa in the more extreme environments is determined by their ancient origins.

1.2.2 The Molecular Record

Until recently the majority of molecular studies on cyanobacteria focussed on taxonomic questions and phylogenetic relationships (Chaps. 7 and 22). Initially these were based on sequences of one particular gene or part of the genome, but studies began increasingly to combine information from several genes. When we introduced the previous "Ecology of Cyanobacteria" (Whitton and Potts 2000), there were complete sequence data for only a few cyanobacterial genomes. Now, such data are starting to be obtained more rapidly. Approximately 35 genomes were available with annotations for some 117,435 genes when Nakao et al. summarized the situation in 2010 and there were about 44 at the time of completing this chapter. Even where whole genome data are not available for a particular strain, genome sequences from other organisms can help to identify genes that interfere with phylogenetic reconstruction (Kauff and Büdel 2011). Extensive or complete genome sequences are driving significant advances in the understanding of not only phylogenetic relationships, but also the growth of populations *in situ* and in culture.

Phylogenetic analyses of 16S rDNA sequences led Schirrmeister et al. (2011) to suggest that all extant cyanobacteria may share a common ancestor, which was unicellular. Phylogenetic trees based on molecular data indicate that *Gloeobacter violaceus*, where the light-harvesting mechanism is restricted to the outer membrane of the cell rather than internal thylakoids, is the nearest living organism to that ancestor (see Kauff and Büdel 2011). However, Schirrmeister et al. also suggest that the majority of extant cyanobacteria descend from multicellular ancestors and that reversals to unicellularity have occurred at least five times. Among modern unicellular forms which have apparently originated from multicellular ones are the important marine *Synechococcus* and *Prochlorococcus* (Chaps. 5 and 20). Such concepts are a stimulus to research, but a great deal more evaluation of genomic information is needed before there is likely to be firm agreement about the detailed steps in cyanobacterial evolution. The fact that extant multicellular forms have similar, if not identical, morphologies to forms identified in early fossil records has sometimes surprized authors. However, if environmental factors influencing a past microbial community were closely similar to those of a modern one, there may have been little need for evolutionary change. The molecular evidence suggesting particular evolutionary steps needs to be compared with detailed environmental information for a particular geological time. This presents a huge challenge.

The consideration of multicellularity raises the question of the time of acquisition of heterocyst differentiation. Based on molecular data and theoretical modelling Rossetti et al. (2010) inferred, perhaps not unexpectedly, that terminally differentiated cyanobacteria evolved after undifferentiated species. The compartmentalization afforded by multicellularity is required to maintain the vegetative/heterocyst division. It is generally concluded that the heterocyst evolved only once because of the large number of steps involved (Henson et al. 2004), but the possibility of some dedifferentiation at various times should be borne in mind. There is a great deal of variation in heterocyst morphology and probably also functioning, which has as yet scarcely been investigated.

Based on a comparison of 58 contemporary cyanobacterial genomes, Larsson et al. (2011) concluded that the most recent common ancestor of cyanobacteria had a genome size of approx. 4.5 Mbp and 1,678–3,291 protein-coding genes, 4–6% of which are unique to cyanobacteria today. They concluded that there have been two routes of genome development during the history of cyanobacteria. One was an expansion strategy driven by gene-family enlargement which provides a broad adaptive potential. The other was a genome streamlining strategy which imposes adaptations to highly specific niches.

An important question absorbing the energy of many research groups is how a particular cluster or clusters of genes on a genome equates with particular phenotypes. The study by Larsson et al. (2011) led them to conclude that a few orthologues can be correlated with specific phenotypes, such as filament formation and symbiotic competence. Where organisms have diverged from each other relatively recently, they may be expected to have equivalent sets of genes in the same relative position on the genome. Recognition of this helps researchers to infer how portions of genomes are excised and transferred during the course of evolution. This approach (synteny) has provided useful insight for cyanobacteria. For instance, Stucken et al. (2010) compared the genomes with the smallest size of any filamentous species sequenced: *Cylindrospermopsis raciborskii* CS-505 i (3.9 Mbp) and *Raphidiopsis brookii* D9 (3.2 Mbp). Despite differences in their phenotypic features, these strains form a monophyletic group. The authors commented on the remarkable conservation in gene order between these genomes; differences in repetitive element content account for most of the difference in the genome. It was concluded that the lack of heterocysts in strain D9 is a secondary loss.

Local niche occupancy of any particular marine *Synechococcus* lineage appears to be driven by lateral gene transfer, in which specific genomic loci (islands) play a key role as a repository for transferred genes (Dufresne et al. 2008). This poses the question as to how important is the physical location of these islands. In a study with *Prochlorococcus* Kettler et al. (2007) asked whether flexible genes (as opposed to core genes) are located preferentially in island regions, and, if so, whether the most recently acquired genes are more likely to be island genes: are recently acquired genes directed to specific genomic loci? The authors identified genes that

appear to define high-light and low-light adapted phenotypes, but they also provided a detailed discussion and further questions relevant to cyanobacterial distribution and selection. "How many *Prochlorococcus* genotypes truly exist in the ocean, and what fraction of these has differential fitness at any point in time?" Information which Kettler et al. thought would be particularly enlightening is to understand the complete genome diversity of the 10^5 cells in a millilitre of ocean water, and, conversely, how widely separated in space two cells with identical genomes might be.

It would be hard to overstate the significance and complexity of these deliberations. For example, in the oligotrophic open ocean *Prochlorococcus* accounts for around half of all photosynthesis (Chaps. 5 and 20). Yet, remarkably, *Prochlorococcus* genomes lack catalase and other protective mechanisms that would appear essential for competition in the illuminated euphotic zone where reactive oxygen species are generated. It seems that genomic streamlining of *Prochlorococcus* through evolution was coincident with reliance on hydrogen peroxide-consuming members of the euphotic community (Morris et al. 2011). This emphasizes the importance of indirect biotic interactions in establishing niche boundaries and presumably driving genome evolution. Complex issues indeed, and it remains to be seen what further surprizes there are as studies continue on correlations between genomic form and function in cyanobacteria.

In addition to the examples already mentioned, molecular information has proved especially useful in a number of studies reported in this volume. This applies, for instance, to the study of the biosynthesis of microsporines, one of the types of molecule providing UV protection for cyanobacteria (Chap. 19). Balskus and Walsh (2010) used genome sequence data from different cyanobacteria in a genetic and molecular analysis of biosynthesis in *Anabaena variabilis* ATCC 29413. The mode of recruitment (reaction mechanism) of ATP-dependent peptide bond forming enzymes involved in this synthesis is apparently unprecedented in natural product biosynthesis. Of particular note is that the biosynthetic pathway is short, requiring only four enzymes. This is especially noteworthy in view of the ecological importance of tolerance to UV radiation.

1.3 Ecology: Current Challenges

1.3.1 Sensing the Environment and Other Organisms

An ability to detect and respond to variations in the environment is of key importance for the success of cyanobacteria on this planet and an understanding of which parts of the genome of a species are involved should prove a great stimulus for research. The following are aspects which seem of particular interest. Until recently most studies were concerned with light, N and P, but increasingly responses to the presence of other cells, whether of the same or a different species have gained attention. In the case of light, Castenholz (1983) concluded that most responses occur only after apparently random movements result in the long axis lying parallel to the light field. However, a range of photoreceptors are now known to exist in cyanobacteria for sensing light, such as the structure with carotenoid globules and rhodopsin-like pigment in the tip of the apical cell of a red-coloured *Leptolyngby*a (Albertano et al. 2000; Sect. 11.4.3). The first convincing evidence for chemotaxis came from Waterbury et al. (1985) for marine phycoerythrin-containing *Synechococcus* isolates showing swimming motility. The swimming behaviour, which was confined to open ocean isolates, showed a marked chemotactic response to various nitrogenous compounds (Willey and Waterbury 1989). The threshold levels for chemotactic responses were in the range 10^{-9}–10^{-10} M, which could be ecologically significant in the ocean. More recently the study of chemotaxis in cyanobacteria has focussed largely on the attraction of hormogonia to potential symbiotic partners. The first full account was for *Nostoc* and the liverwort *Blasia* (Knight and Adams 1996), but the phenomenon has now been shown for a range of associations (Nilsson et al. 2006; Chap. 23).

The study by Albertano et al. (2000) on the apical cell of a *Leptolyngbya* raises the question of the role of apical cells in this family, especially in species of *Phormidium*. At least a few trichomes of most Oscillatoriaceae populations have an apical cell which is in some way modified, such as being markedly pointed or with a decrease in colour. More distinct forms are a thickening at the end, a cap or an even more elaborate structure, the calyptra. It is unclear whether the thickening, cap and calyptra are distinct or there is a continuum between them, or whether they have different functions, but they are important characters used to distinguish species. Apart from this, there is a striking lack of detailed quantitative information on these structures, in spite of the fact that this could have been obtained any time in the past century. Although taxonomic accounts seldom make this clear, the specialized cell is present at only one end of a trichome. The frequency of trichomes with a modified end cell varies markedly between samples, so is presumably influenced by the environment. Should no trichome in a population possess a calyptra, it is impossible to comment on whether this is a genetic feature or merely a response to the environment. For the calyptra in particular, most field samples show only a few trichomes with this structure, so it is essential to check at least 20 trichomes to identify a sample.

The calyptra is probably the best known morphological structure in cyanobacteria about which nothing is known of its role. Perhaps the calyptra is involved in sensing light, as in the tip of the end cell of the cave *Leptolyngbya* studied by

Albertano et al. (2000), but it seems more likely that it is involved with another factor. We suggest that the most likely are phosphate gradients, the presence of other trichomes or possibly a combination of both. It is hard to understand how *Phormidium* mats with motile trichomes develop on submerged surfaces unless sensing between trichomes occurs. It is even more evident that sensing must occur between Rivulariaceae trichomes aggregating to form a colony (Chap. 22), though in this case the ends of the cells aggregating together are starting to differentiate a heterocyst. Is the heterocyst in this case involved in sensing other cells in addition to fixing N_2?

There have been many suggestions that quorum sensing is likely in be involved in cyanobacterial processes (e.g. Mann 2000) and some of the above must surely provide examples. Production of the toxin microcystin by *Microcystis* also seemed a likely example, so it was a surprize when several studies failed to find evidence for factors such as cell density changes influencing transcription of the microcystin gene cluster (Dittmann et al. 2001; Braun and Bachofen 2004; Pearson et al. 2004). ^{14}C studies with *M. aeruginosa* PCC 7806 showed that when an intracellular pool of microcystin was built up there was no significant export from the cells and the authors (Rohrlack and Hyenstrand 2007) interpreted this as a lack of evidence for quorum sensing. However, Chap. 7 shows how *Microcystis* responds in various ways to the presence of grazers, including changes in colony morphology and microcystin content. *Microcystis* strains exposed to zooplankton increased their cell specific toxin production (Jang et al. 2007; Sect. 7.5.1). Other aspects of signalling are discussed in Chap. 18, such as possible links between cyanobacterial-derived extracellular signalling molecules and phage physiology (Sect. 18.3) and autoinduction systems (Sect. 18.4). Evidence for the importance of quorum sensing in the regulation of surface phosphomonoesterase activity by epibiontic bacteria associated with *Trichodesmium* colonies was shown by Van Mooy et al. (2012), but it not yet clear how this influences P acquisition by *Trichodesmium* itself.

Microcystis is not the only cyanobacterium known to show morphological changes in response to the presence of grazers. Fialkowska and Pajdak-Stós (1997) found that when two *Phormidium* isolates from very shallow pools were subjected to grazing pressure by the ciliate *Pseudomicrothorax dubius*, both strains showed significant increases in the number of filaments terminating in an empty sheath. There was active withdrawal of a trichome inside a sheath when disturbed by grazers. *P. dubius* was unable to ingest trichomes enclosed in a sheath. *Phormidium* may be less efficient under these conditions, perhaps because of reduced nutrient uptake. However, possession of a sheath was also likely to have been important for these populations which occurred in an environment likely to become dried out intermittently. Another example of a response to a grazer is that of microcolony formation by a strain of *Cyanobium* sp. from single cells; this was induced by the presence of the photophagotroph, *Ochromonas* sp. DS (Jezberová and Komárková 2007). Colonies were characterized by hundreds of tubules (spinae), 100 nm to 1 μm long and 63 ± 6 nm wide on the surface of *Cyanobium* cells cultured together with *Ochromonas*. Such spinae have been reported a number of times on single-celled cyanobacteria, so perhaps this is a widespread response. In any case it seems probable that there are numerous other examples of cyanobacterial morphological responses to grazers waiting to be discovered. Perhaps because of their larger size, there is considerably more known about induced morphological and chemical responses of eukaryotic algae than cyanobacteria (see Van Donk et al. 2011).

1.3.2 Nitrogen and Phosphorus

While P has long been identified as the most common limiting nutrient in freshwater ecosystems (e.g. Schindler 1977), earlier studies focussed on uptake of P_i, because of the increased concentrations in lakes and rivers due to human activity and the resulting problems of cyanobacterial blooms. When considering how cyanobacteria and eukaryotic algae acquire P efficiently if the element is in short supply, some authors (e.g. Wagner and Falkner 2001) have considered only P_i, but others review all possibilities (Dignum et al. 2005). This matters, because, away from human activity, it seems likely that P acquisition from organic sources is more important than Pi for most cyanobacteria. It is difficult to be sure of the situation, because there are few really detailed studies on the P fractions present in freshwater and it is doubtful if any freshwater sample has ever been studied sufficiently to characterize all the P-containing molecules reaching concentrations of possible use for a cyanobacterium. There have been no studies on the possible presence and utilization of phosphonates in freshwater, in spite of their known importance for several marine cyanobacteria, such as *Trichodesmium* (Dyhrman et al. 2006). However, most filamentous cyanobacteria can obtain P from a wide range of organic molecules, though not necessarily all, and there are differences between species (Whitton et al. 1991, 2005). The overall situation with unicellular cyanobacteria is less clear, because of doubts about the relevance of data obtained with strains cultured with P_i for many generations. However, many strains can use phosphomonoesters, including the marine *Crocosphaera watsonii* (Dyhrman and Haley 2006). The evidence suggests that unicellular forms may be less successful at using phosphodiesters (Whitton et al. 1991). The next research step should be to relate the ability of particular strains to acquire different forms of P to the types and concentrations of the various molecules

in their natural environment. Inorganic precipitates and inorganic – organic complexes, such as the brown deposits among the mucilage of some planktonic colonial Chroococcales (e.g. *Cyanogranis ferruginea*), are likely to include P of potential use to the organism.

Although the problems associated with P concentrations in culture collection media different from the concentrations in the natural environment are a particular worry when considering unicellular strains, phenotypic and probably also genetic changes can also occur during prolonged subculture of filamentous species (Chap. 22). The difficulties originated from the fact that culture media mostly used a phosphate buffering system until the mid-1970s. For instance, the medium of Kratz and Myers (1955), which was often used for cyanobacteria over the next 20 years, has 158 mg L^{-1} PO_4-P. This needs to be borne in mind when reading research results obtained during this period, especially those concerning N_2 fixation. Although organic buffers started to be introduced in the 1970s, many media still had P concentrations well in excess of those likely in nature. BG-11 medium (Allen and Stanier 1968; Rippka et al. 1979) has been the most widely used medium for cyanobacteria, but sometimes without combined N ($BG11_0$). Both versions have 5 mg L^{-1} P, although more recently the concentration has sometimes been reduced to reduced 1 or 2 mg L^{-1} P. Even the well-known Chu No.10 medium (Chu 1942), which was designed for growing lake algae in the laboratory, has 2.87 mg L^{-1} P. All these and most other media listed by Andersen et al. (2005) still have P concentrations far higher than typical in nature. The N concentrations are usually also high, but below the value for the N:P ratio likely to lead to P limitation (16:1 molar, 7.2:1 by mass: Redfield et al. 1963), even if, in the case of a batch culture, a sufficiently high biomass is reached for this to occur.

1.4 Taxonomy and Nomenclature

It may seem a statement of the obvious to comment that different people have different reasons for wanting to name a cyanobacterium. However, cyanobacteria have sometimes had a reputation for being difficult to name and part of the reason for this comes from the fact that several different taxonomic approaches have been introduced at various times and none are ideal for all the needs of people requiring names. It is difficult to understand the present situation without knowing something of the past. The following account is mainly for those who know little about the subject; several reviews published in recent years provide much more detailed information.

Staff in water companies with a single reservoir, who will probably know the organism as a blue-green alga in an English-speaking country, merely want to give the same name to the same organism each time it is encountered. Larger environmental organizations require a more rigorous set of names which is consistent within their area, while ecologists conducting field surveys aim to use names which are consistent world-wide. Information about cyanobacterial populations in many of the world's ecosystems is still very limited and the need to provide detailed floristic lists for ecological studies is likely to increase greatly. Most of the generic and many of the species names used for this purpose originated in the second half of the nineteenth century and the first half of the twentieth century – what may be considered classical taxonomy. However, the names are increasingly being modified by results from molecular studies. Many of the problems for non-specialists are similar to those discussed with clarity and sympathy by Stace (2010) for field botanists wanting to name angiosperms, but who are not professional taxonomists.

Molecular data have provided a great stimulus not only for the broader questions of cyanobacterial evolution and phylogenetics discussed in Sect. 1.2.2, but also more straightforward matters of cyanobacterial taxonomy. They have helped to distinguish organisms which most phenotypic characters suggest are quite similar, a particular problem with many unicells; *Microcystis* provides an example (Chap. 7). Molecular data have also ensured the speedy acceptance of splits in well-known genera, where obvious morphological differences had been ignored in the past, as has occurred in the separation of *Cuspidothrix* (Rajeniemi et al. 2005) and *Sphaerospermum* (Zapomělová et al. 2009) from *Aphanizomenon.* However, molecular data have not always been used with sufficient care in phylogenetic and taxonomic studies. This is sometimes merely because of the rush to publish, but other times it comes from a failure to appreciate the significance of how taxa were originally described.

Many characters used in the original descriptions were ones which the organism had evolved to respond to what we might now consider as "stress" factors, although in most cases the original authors had no or little idea about such environmental factors. These include the various types of sheath pattern in terrestrial forms, many of which are responses to different cycles of water availability and the formation of sheath pigments to protect from UV damage. The influence of combined N on heterocyst formation by many cyanobacteria became increasingly clear during the 1970s (Wolk 1983), but recognition of the importance of P in inhibiting the formation of multicellular hairs in cyanobacteria has been less widely recognized (Chap. 22). Nevertheless, about 16% of the filamentous species listed by Geitler (1932) form such hairs.

Akinetes or other resting stages are needed to identify individual species in genera such as *Anabaena* and *Cylindrospermum*, so again these will only be seen if the correct environment is provided. It is essential to name an

organism when first isolated from nature if the name is to be used in phylogenetic analysis. Giving the correct name to a culture is even more of a challenge if there is a need to consider the whole population. This is essential, for instance, in Rivulariaceae colonies, which may contain more than one genotype (Berrendero et al. 2008), in *Microcystis* (Chap. 7) and probably also in all *Phormidium* (see above). Perhaps the hardest of all is to give the right name when the morphology of a cyanobacterium responds to the presence of a grazer.

The classical taxonomic information was first consolidated by Geitler (1932) and the approach to naming blue-green algae continued much the same for the next 40 or so years, although with a lot more information being incorporated, some of it based on experimental studies during the latter part of this period. The guidelines for naming organisms were provided by the International Code of Botanical Nomenclature (ICBN). In a series of monographs Francis Drouet set out to replace the pragmatic approach of the classical system with one based on reducing the number of genera to one relying solely on the most obvious characters (e.g. Drouet 1968), eventually reducing the number to nine (Drouet 1981). The monographs are an excellent source of nomenclatural information, but have little practical value for naming organisms. However, Drouet's views led to heated discussion at many symposia up to as late as 1991. There would be little need to mention them now but for the fact that an earlier version of Drouet's names was used for the colour pictures shown by Palmer (1962), which were subsequently reproduced in many editions of *Standard Methods for Water and Wastewater Treatment* published by the American Public Health Association. These pictures have probably been seen by more people than any others of cyanobacteria and are still on the walls of many water treatment laboratories around the world.

A third approach is that introduced by R.Y.Stanier, who became convinced during the mid-1970s that the classical approach was inadequate for critical research. A meeting of blue-green algal specialists – authors of floras and monographs – at Kastanienbaum by the Zürichsee in Switzerland gave him the chance to ask them to use light microscopy to name the genera of 20 cultures from the Pasteur Culture Collection. (B.A.W. was one of those involved.) Comparisons of the lists obtained showed that many organisms had been given more than one name and, in the case of several filamentous forms, *Oscillatoria*, *Lyngbya* and *Phormidium* for the same material. This added weight to the argument that organisms should be treated like bacteria, with isolation of individual cells or filaments to permit measurements of a wide range of characters. He therefore proposed that their taxonomy should follow the rules of International Code of Nomenclature of Bacteria, together with a change in name to cyanobacteria (Stanier et al. 1978); the practical methods were described by Rippka et al. (1979). Two editions of *Bergey's Manual of Determinative Bacteriology* have provided accounts of the genera which the authors of the cyanobacterial chapters thought could be characterized clearly at the time. The first (Castenholz and Waterbury 1989) relies largely on phenotypic characters, whereas the second (Castenholz 2001) makes considerable use of molecular information, especially sequence data.

The 1978 proposal by Stanier et al. led to nomenclatural problems which are still not fully resolved. The earlier steps towards doing this were reviewed by Oren (2004), who stressed the need for botanical and bacteriological taxonomists to use unified rules to describe new taxa. Oren (2011) assessed the contents of all the papers on cyanobacterial systematics and nomenclature published in the *International Journal of Systematic Bacteriology* and the *International Journal of Systematic and Evolutionary Microbiology* (and a predecessor bulletin). There have been only very few descriptions of new cyanobacterial taxa under the rules of the International Code of Nomenclature of Prokaryotes (ICNP) because of the difficulty of validly publishing new names of cyanobacteria under its rules. Most descriptions of new taxa are still published in the botanical literature. The situation had not changed much since Oren and Tindall (2005) considered how successful the system was in which Cyanophyta/Cyanobacteria can be named according to the provisions of either code. The problems include the fact that valid publication under the ICNP rules requires publication in a particular journal, whereas that of the ICBN has no such restriction. Another difference is that the ICNP requires the nomenclatural type of a species to be a viable type strain maintained in pure culture, while under the ICBN, non-living type specimens must be preserved permanently, although algal cultures preserved in a metabolically inactive state are acceptable as types. Neither system deals effectively with the problem of genetic shifts in cultures, which may even have occurred by the time the material is designated a type culture, nor the fact that they may only express characteristic features when present as a population.

At the same time as the bacteriological approach has been developing, there have been many reports of new taxa and nomenclatural revisions based on the ICBN rules, which have themselves been changing. This literature was brought together for the Chroococcales by Komárek and Anagnostidis (1999) and by the same authors for the Oscillatoriales in 2005. Often the previous revisions had also been made by them; some of these were made with more evidence to support them than others. The two volumes assemble a wealth of information and are essential for anyone making broad surveys. However, it would be a challenge to make such a survey without practical advice from others with experience of how their system is put into practice. There are, for instance, 109 species of *Phormidium* listed and the authors state that

200 species have been recognized and are identifiable. However, most morphological characters are influenced by the environment and often merge into those of other species.

Nomenclatural revisions in the past 10 years almost all incorporate molecular data and in many cases this is what led to the decision to make the change. Hoffmann et al. (2005) stressed that the taxonomic system needed to be continually revized and updated, but that system is essentially a continuation of the traditional system. Komárek (2010) reviewed the situation and emphasized the need for molecular data to have a central role, but also that phenotypic and ecological characters must be an integral part of the generic definition. He indicated that the molecular definition of a gene sequence corresponding to the genus should be based on a similarity index of ±95% using 16S rRNA sequencing. The species concept is "not uniform and must be modernized according to the diverse nature of genera". He regretted that "molecular cyanobacteriologists pay attention to the use of molecular methods for taxonomic articles, but unfortunately do not accept the results of modern investigations into cyanobacterial diversity in their studies and strain collections".

It seems probable that progress will continue much as indicated by Hoffmann et al. (2005), with a continual update of what originated from the classical system, but with an ever increasing contribution from molecular data. However, the situation with unicellular forms (in the broadest sense) is rather different from that of filamentous ones, where there are often quite a number of morphological characters. It might have been better if Stanier et al. (1978) had restricted their suggestion about change in nomenclatural code 1978 to the Chroococcales, with consideration of the filamentous forms, which are the main part of floristic surveys, being left until later. It will probably always be essential to rely on molecular data for reliable identification of many unicellular forms, but there is still a lot of potential for improving the traditional system enough to make it is possible to allocate a meaningful binomial name to the majority of filamentous forms found in nature based on their morphology.

Proposals to revize generic limits should be deferred until there are data for a sufficient number of strains. We believe that many nomenclatural changes have been introduced much too rapidly and this will inevitably lead to further revisions in a few years time. It is for this reason that not all recent nomenclatural changes have been included in the revized floristic account of cyanobacteria in the British Isles (Whitton 2011). However, in the long-term by far the most effective way to deal with the practical need for names in detailed field surveys is to use an interactive identification system based on morphology, although molecular comparisons should be sufficient for rapid checks on potential problem organisms. A early attempt at developing an interactive system was provided by Whitton et al. (2003) using the Lucid software prepared by University of Brisbane, Australia, and with the information on a CD. It should now be possible to provide a system which permits the storage of taxonomic information and records from as many countries as there are data, rapid conversion between different nomenclatural conventions and synonyms, a large number of images and rapid access to the internet. The information could also be linked to molecular records.

The increasing need to include sequence comparisons with the GenBank database in taxonomic comparisons and phylogenetic studies makes it is essential for the names to be correct. As pointed out by Komárek (2010), this is not always so, and some of the reasons for this have been explained above. It would be useful to have an index of reliability for every name in the database, although this would require retrospective assessment for the names already there. There is also a need for ecological relevance to be among the criteria used to decide which strains are used for complete genome sequencing. This should include detailed information about its original environment and morphology and the use of material which has had minimal chance for genetic change since first isolated.

Finally, we would point out that detailed descriptions of the morphology and cell contents visible with a light microscope of populations of filamentous forms at a field site can tell a lot about the environment at that site without even knowing the name of the organism. This comes from an understanding of the factors leading to the "stress" characters mentioned above and others details such as the relative abundance of cyanophycin (N storage) and polyphosphate (P storage) granules. The more morphologically complex the organism, the more information can be deduced.

1.5 The Future: How Cyanobacteria Can Contribute to Solving Real Problems

The variety of ways in which cyanobacteria are currently used for practical purposes is likely to surprize many readers of Chap. 26. Some of the places where cyanobacteria are harvested locally are well known, like the surrounds of Lake Chad, but others much less so, such as in Myanmar. It seems likely that there is a lot more to be reported about local use of natural material, especially from S-E. Asia. The commercial cultivation of *Arthrospira* ("Spirulina") continues to increase and what may have seemed at times rather wild suggestions for production have frequently turned into reality. Those of us who spent an afternoon at the 2005 Applied Phycology Symposium in Kunming listening to the plans of Chinese staff for managing large-scale cultivation on the Ordos Plateau in Inner Mongolia can now read in Chap. 25 about current production. Hopefully the comment by Lu et al. (2011) that a plan for a future annual production of 10^6 that has been sketched out will also become a reality.

Another success from the dry parts of China is the use of cyanobacterial inocula in the improvement of soils in semi-desert regions (Chap. 12). This has shown how important it is to study the ecology of natural soil biological crusts, select strains adapted to a particular area and then to find out how the material should be grown and applied to sites in that area. The sequencing of the genome of a strain of *Microcoleus vaginatus* (Starkenburg et al. 2011), one of the main species used in inocula, should assist in optimizing strains for a particular region.

There is a long history of cyanobacteria being applied to fields in other regions to increase soil fertility, especially the N status of rice fields. There has been considerable success in the use of *Azolla*, with its N_2-fixing symbiont, but most of the earlier studies on free-living cyanobacteria were too fragmentary to have much practical success. In particular there was often a failure to obtain an understanding of the ecology of local sites (Whitton 2000). However, some successes have been reported in recent years (Sect. 26.5.5). Although rice fields are much more complex ecosystems than soil biological crusts, it should be possible to manage the cyanobacterial populations of rice fields to enhance soil fertility effectively once the same critical and long-term approach is applied as has been done for semi-desert soils in China. The problems associated with cyanobacterial damage to outdoor monuments and archaeologically important surfaces in caves and other underground sites provide another example where detailed ecological research has helped to provide solutions (Chap. 11). Dealing with problems without such ecological understanding has sometimes done more harm than good and is still continuing to do so at many sites, especially large outdoor monuments in south, south-east and east Asia.

The use of barley (*Hordeum vulgare*) straw to control cyanobacterial blooms provides an example of an ecological problem where there have been many studies, but none adequate enough to ensure that the solution is always effective. The studies are summarized by Ó hUallacháin and Fenton (2010). Release of polyphenolics from rotting stems has been suggested to be the main factor involved (Pillinger et al. 1994; Everall and Lees 1997) and there is evidence for 1,000–3,000 molecular weight range polyphenolics being toxic to *Microcystis aeruginosa* (Waybright et al. 2009). However, evidence for other factors such as increases in zooplankton grazer density and microbial activity has been found for particular sites. In addition it is possible rotting barley straw might release other toxic molecules harmful to cyanobacterial blooms, since Wu et al. (2011) showed that periphyton biofilms could produce water-soluble allelochemicals such as indole and 3-oxo-a-ionone, which led to marked inhibition of cyanobacterial growth. Marked differences have been found in the responses of different planktonic cyanobacteria and eukaryotic algae (Brownlee et al. 2003).

Barley straw is now used widely in the British Isles to control cyanobacterial blooms, and, to a lesser extent, eukaryotic algae. It is also in increasing use elsewhere, though more often in ornamental ponds than reservoirs. Tests in North America have led to only mixed success (Boylan and Morris 2003; Geiger et al. 2005), perhaps due to different barley cultivars or higher rates of N fertilization in the barley fields reducing the lignin content of the straw. Nevertheless there is convincing evidence for success in shallow, well aerated waters in the British Isles, when a sufficient density of bales of straw is applied early enough for rotting to be well underway by the time a bloom population would normally start to increase – typically late spring. The value of straw used for this purpose is sufficient to influence borderline decisions by some farmers about the area to be planted for barley. There is great potential for making the barley straw methodology much more effective, but anyone planning a research project would be well advized to read the critical comments of Ó hUallacháin and Fenton (2010) on the weaknesses of previous studies.

In view of the widespread occurrence of cyanobacterial blooms in tropical and subtropical waters used for drinking water, the possibility that the straw of some rice cultivars might be used in a similar way should be tested. Evidence in support of this comes from an experimental study by Rice et al. (1980) showing that decaying rice straw had an inhibitory effect on cyanobacterial growth and N_2 fixation. In addition, anecdotal reports from deepwater rice farmers in Bangladesh to B.A.W. indicate that leaving rice straw to rot on soils after harvest at the end of the flood period decreases winter growths of cyanobacteria on the soil surface.

The intense interest in the potential of cyanobacteria for various products is leading to an exploration of the ways of optimizing the cell physiology of strains if it is to be used to produce the product, or the isolation and transfer of important cyanobacterial operons to non-phototrophic organisms if these are cheaper to grow on an industrial scale. Biofuel (Chaps. 16 and 26) is of course the most important product needed. Significantly, an alkane biosynthesis pathway from cyanobacteria as diverse as *Cyanothece* and *Nostoc* spp. is described recently by Schirmer et al. (2010). This pathway consists of an acyl–acyl carrier protein reductase and an aldehyde decarbonylase that together convert intermediates of fatty acid metabolism to alkanes and alkenes. Heterologous expression of the cyanobacterial alkane operon in *Escherichia coli* led to the production and secretion of long-chain alkanes and alkenes. Another approach is genetic modification of strains such as *Synechocystis* PCC 6803 wild type (SD100) to produce and secrete fatty acids (Liu et al. 2011). Since this involves changes to the cell walls, and cell density dependent changes may damage cell membranes, it remains to be seen how well such strains succeed, perhaps in competition with others, under rigorous environmental conditions.

Assessment of whole genome sequences would seem to be the most logical approach for long-term plans for genetic modification. The study carried out by Jones et al. (2011) on *Lyngbya majuscula* 3L provides an example. This strain belongs to a pantropical species and a worldwide genus that is the source of some 35% of all reported cyanobacterial natural products. In spite of the fact that some *L. majuscula* strains fix N_2 (Lundgren et al. 2003), no evidence for nitrogenase genes was found in *L. majuscula* 3L. However, this strain does produce curacin A, a tubulin polymerization inhibitor, and the molluscicide barbamide. Jones et al. suggested that *Lyngbya* metabolites are strain-specific and may be useful in delineating species. They showed that this species has a complex gene regulatory network with a large number of sigma factors and other regulatory proteins, it was concluded that this shows an enhanced ability for environmental adaptation or for forming microbial associations. More such detailed analyses of genome sequence are needed to provide a rational basis for assessing how strains interact with their environment and which ones are most likely to form products of potential biotechnological use.

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