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Diversity of Chlorophototrophic Bacteria Revealed in the Omics Era

Vera Thiel,[1] Marcus Tank,[1] and Donald A. Bryant[2,3]

[1]Department of Biological Sciences, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan; email: vthiel@tmu.ac.jp, mtank@tmu.ac.jp

[2]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, USA; email: dab14@psu.edu

[3]Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana 59717, USA

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Abstract

Because of recent advances in omics methodologies, knowledge of chlorophototrophy (i.e., chlorophyll-based phototrophy) in bacteria has rapidly increased. Chlorophototrophs currently are known to occur in seven bacterial phyla: *Cyanobacteria*, *Proteobacteria*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, *Acidobacteria*, and *Gemmatimonadetes*. Other organisms that can produce chlorophylls and photochemical reaction centers may still be undiscovered. Here we summarize the current status of the taxonomy and phylogeny of chlorophototrophic bacteria as revealed by genomic methods. In specific cases, we briefly describe important ecophysiological and metabolic insights that have been gained from the application of genomic methods to these bacteria. In the 20 years since the completion of the *Synechocystis* sp. PCC 6803 genome in 1996, approximately 1,100 genomes have been sequenced, which represents nearly the complete diversity of known chlorophototrophic bacteria. These data are leading to new insights into many important processes, including photosynthesis, nitrogen and carbon fixation, cellular differentiation and development, symbiosis, and ecosystem functionality.

Contents

Photosynthesis: biological process in which light energy is converted into chemical energy (ATP) and reducing power for reduction of CO_2 to produce biomass

Photolithoautotroph: an organism that uses light as its energy source, inorganic compounds as its source of electrons, and CO_2/HCO_3^- as its source of carbon

Cyanobacteria: bacteria that usually perform oxygen-evolving photosynthesis, using Photosystems I and II, and fix CO_2 by the Calvin-Benson-Bassham cycle

Phototroph(y): organism/process using light as an energy source

1. INTRODUCTION

Photosynthesis is the biological process in which light energy is used to produce chemical energy (ATP) and reductants, either reduced ferredoxin or NAD(P)H, which concomitantly are used to reduce CO_2 and produce biomass. The invention of photosynthesis was one of the seminal events in the evolutionary history of biology because it coupled biological energy production to an abundant and virtually inexhaustible energy source, the sun. In microbiology, organisms that perform photosynthesis are called photolithoautotrophs: These organisms obtain energy from light, electrons from the oxidation of inorganic compounds (e.g., H_2, H_2S, Fe^{2+}, nitrite, arsenite, H_2O), and carbon from inorganic sources (CO_2 and/or bicarbonate) (11, 66, 124). Arising ~3.5 Ga ago, photosynthesis resulted in an enormous increase in biomass production on Earth and coincidently produced oxidized compounds (e.g., sulfate and eventually oxygen) that could serve as electron acceptors for heterotrophic metabolism (111, 124). This resulted in extensive diversification of heterotrophs that could consume the biomass produced by these new primary producers. Photolithoautotrophs continue to play foundational roles in diverse ecosystems. Because they directly connect the energy of the sun to biomass production, and because cyanobacteria and their algal and plant descendants produce oxygen as a by-product, photolithoautotrophs are ultimately responsible for most life on Earth today (111).

Organisms that utilize light as an energy source are simply called phototrophs. There are two types of phototrophic bacteria and archaea: retinal-rhodopsin-based phototrophs (i.e., retinalophototrophs) and chlorophyll-based phototrophs (i.e., chlorophototrophs) (11). Note that all photosynthetic organisms are chlorophototrophs, but not all chlorophototrophs are photosynthetic.

1.1. Retinalophototrophs

Retinalophototrophs (e.g., halobacteria and diverse marine organisms including *Pelagibacter ubique*) use retinal-binding proteins, rhodopsins, to convert light energy into chemical energy

in the form of ion gradients (proton, sodium, or chloride) (85, 129). Rhodopsins, which include bacteriorhodopsin, proteorhodopsin, halorhodopsin, and xanthorhodopsin, are integral membrane proteins with seven transmembrane α-helices, one of which covalently binds retinal in a Schiff-base linkage to a conserved lysine residue (100). These proteins undergo a light-induced photocycle that is initiated by a *trans*-to-*cis* isomerization of the 13,14-double bond of the retinal moiety, and after directionally releasing an ion (e.g., proton or sodium release into the periplasm or chloride into the cytoplasm), they return to their original *trans* configuration in the dark (100). Knowledge of retinal-containing proteins and retinalophototrophs has increased dramatically in recent years (for recent reviews, see 85, 129). However, no known retinalophototrophs can couple light-driven ion gradient formation with the oxidation of an inorganic electron donor that can support CO_2 fixation, and these organisms are not further considered here.

1.2. Chlorophototrophs

Chlorophototrophs use chlorophylls (Chls) and/or bacteriochlorophylls (BChls) to capture light energy and to perform light-driven redox reactions (i.e., photochemistry) to produce protonmotive force for ATP production and reductants for CO_2 fixation in autotrophs. Fourteen major types of Chls and BChls (see **Table 1**) have been detected in chlorophototrophic bacteria, allowing these organisms to produce complex light-harvesting structures and to use electromagnetic radiation from ~350 to ~1,100 nm (10, 17, 40, 52). Radiation is converted into stable chemical energy by photochemical redox reactions catalyzed by pigment-protein complexes known as reaction centers (RCs). Two structurally related classes of RCs are known (72). Type-1 RCs employ [4Fe-4S] clusters as terminal electron acceptors, and they produce strong reductants and a weak oxidant, an oxidized "special pair" of Chls. Homodimeric type-1 RCs (50) are found in members of the phyla *Chlorobi*, *Acidobacteria*, and *Firmicutes* (**Figure 1**, **Table 1**, **Supplemental Figure 1**, and **Supplemental Table 1**). Type-2 RCs employ quinones as the terminal electron acceptors, and as a result, they produce a weak reductant (quinol) and a strong oxidant (oxidized Chl/BChl dimer) (84). Heterodimeric type-2 RCs are found in members of the phyla *Proteobacteria*, *Chloroflexi*, and *Gemmatimonadetes*. Nearly all cyanobacteria contain a heterodimeric type-1 RC, Photosystem I (PSI), and a heterodimeric type-2 RC, Photosystem II (PSII), which allow them to photooxidize water and produce oxygen as a by-product (72) (**Figure 1** and **Table 1**; also see **Supplemental Figure 1**).

Seven bacterial phyla, none of which is very closely related to the others phylogenetically (35, 74), contain members that are chlorophototrophs (**Table 1**, **Figure 1**, and **Supplemental Figure 1**). They thrive in nearly all naturally or artificially illuminated environments at temperatures below 75°C. Cyanobacteria were first described nearly 200 years ago (114), and purple bacteria were described approximately 180 years ago (80). Nadson (115) described the first green sulfur bacterium (GSB) (phylum *Chlorobi*) in 1906. Discoveries of the first chlorophototrophic members of the *Chloroflexi* (1974) (127), *Firmicutes* (1983) (48), *Acidobacteria* (2007) (12), and *Gemmatimonadetes* (2014) (172, 174) followed (**Figure 2**). In recent years, metagenomic and genomic sequencing methods have become increasingly important ways to discover and describe novel chlorophototrophic organisms. *Chloracidobacterium* (*Cab.*) *thermophilum* was the first chlorophototroph initially identified through metagenomics (12). Further studies of a similar hot spring mat community that produced *Cab. thermophilum* have identified 17 different chlorophototrophs (154; M. Tank, unpublished results) (see **Figure 3**); remarkably, only a few of these were known after ~50 years of cultivation studies. Metagenomic sequencing has the potential to identify new chlorophototrophic organisms, but to do so requires careful phylogenetic analyses of the genes for RCs and (B)Chl biosynthesis and the unambiguous association of these genes with established

Retinalophototroph(y): organism/process in which light-activated, retinal-containing, rhodopsin-like proteins produce an ion gradient

Chlorophototroph(y): organism/process using (bacterio)chlorophyll for energy production

Chl: chlorophyll

BChl: bacteriochlorophyll

Reaction center (RC): a (B)Chl-protein complex that performs photochemistry (light-induced charge separation)

Type-1 RC: produces a weak oxidant [oxidized (B)Chl] and a strong reductant (reduced [4Fe-4S] cluster)

Type-2 RC: produces a strong oxidant [oxidized (B)Chl] and a weak reductant (quinol)

Photosystem I (PSI): complex heterodimeric type-1 RCs that oxidize plastocyanin or cytochrome *c* and produce reduced ferredoxin and are found in cyanobacteria, algae, and plants

Photosystem II (PSII): complex heterodimeric type-2 RCs that oxidize water and reduce plastoquinone and are found in cyanobacteria, algae, and plants

Supplemental Material

Table 1 Overview of chlorophototrophic bacterial group

Group	Phylum	Oxygenic/ anoxygenic	Carbon metabolism	CO_2 fixation pathway	(B)Chls	Reaction center(s)	LH	Oxygen relationship	Genera #[a] (#)[b]	Species #[a] (#)[b]
Cyanobacteria	*Cyanobacteria*	Oxygenic	Autotroph	CBB	Chl *a*, divinyl-Chl *a*, and/or Chl *b*, divinyl-Chl *b*, Chl *d*, and/or Chl *f*	PS I and PS II	PBS	Aerobe	16[c] (>200[d])	21[c] (>800[d])
Purple sulfur bacteria	*Proteobacteria* (γ-class)	Anoxygenic	Autotroph/ heterotroph	CBB	BChl *a* or BChl *b*	Type 2	LH1, LH2 (LH3, LH4)	Anaerobe	29	74
Purple nonsulfur bacteria	*Proteobacteria* (α- and β-class)	Anoxygenic	Heterotroph/ autotroph	CBB	BChl *a* or BChl *b*	Type 2	LH1, LH2 (LH3, LH4)	Anaerobe	28	96
Aerobic anoxygenic phototrophic bacteria	*Proteobacteria* (α-, β-, and γ-class)	Anoxygenic	Heterotroph	None	BChl *a* [Zn-BChl *a* (few)]	Type 2	LH1 (LH2)	Aerobe	91	196
Chloracidobacterium thermophilum	*Acidobacteria*	Anoxygenic	Heterotroph	None	BChl *a*, BChl *c*; Zn-BChl *a*′; Chl *a*	Type 1 (Ho)	Csm	Aerobe	1	1
Gemmatimonas phototrophica	*Gemmatimonadetes*	Anoxygenic	Heterotroph	None	BChl *a*	Type 2	LH1, several	Aerobe	1	1
Heliobacteria	*Firmicutes*	Anoxygenic	Heterotroph	None	BChl *g*, 8^1-OH-Chl *a*	Type 1 (Ho)	None	Anaerobe	3 (4)	11 (12)
Chlorophototrophic *Chlorobi*	*Chlorobi*	Anoxygenic	Autotroph[e]	rTCA[e]	BChl *a*, Chl *a*, and BChl *c*, *d*, or *e* (*f*)[f]	Type 1 (Ho)	Csm	Anaerobe	4	20
Chlorophototrophic *Chloroflexi*	*Chloroflexi*	Anoxygenic	Heterotroph/ autotroph/ mixotroph	3-OH-PB (or CBB)[g]	BChl *a* and/or BChl *c* (*d*)	Type 2	Csm and/or LH1	Oxygen tolerant	4 (8)	6 (10)

Abbreviations: 3-OH-PB, 3-hydroxypropionate bi-cycle; BChl, bacteriochlorophyll; CBB, Calvin-Benson-Bassham cycle; Chl, chlorophyll; Csm, chlorosome; Ho, homodimer; LH, membrane-associated light-harvesting system; PBS, phycobilisome; PS, photosystem; rTCA, reverse tricarboxylic acid cycle.

[a] #, number of described genera/species (not including *Candidatus* genera/species).

[b] (#), number of described genera/species (including *Candidatus* genera/species).

[c] Recognized species in Living Tree Project.

[d] Based on NCBI Taxonomy (current August 2017).

[e] Except "*Candidatus* Thermochlorobacter aerophilum".

[f] Only in a mutant of *Chlorobaculum limnaeum* DSM1677^T.

[g] *Oscillochloris trichoides*.

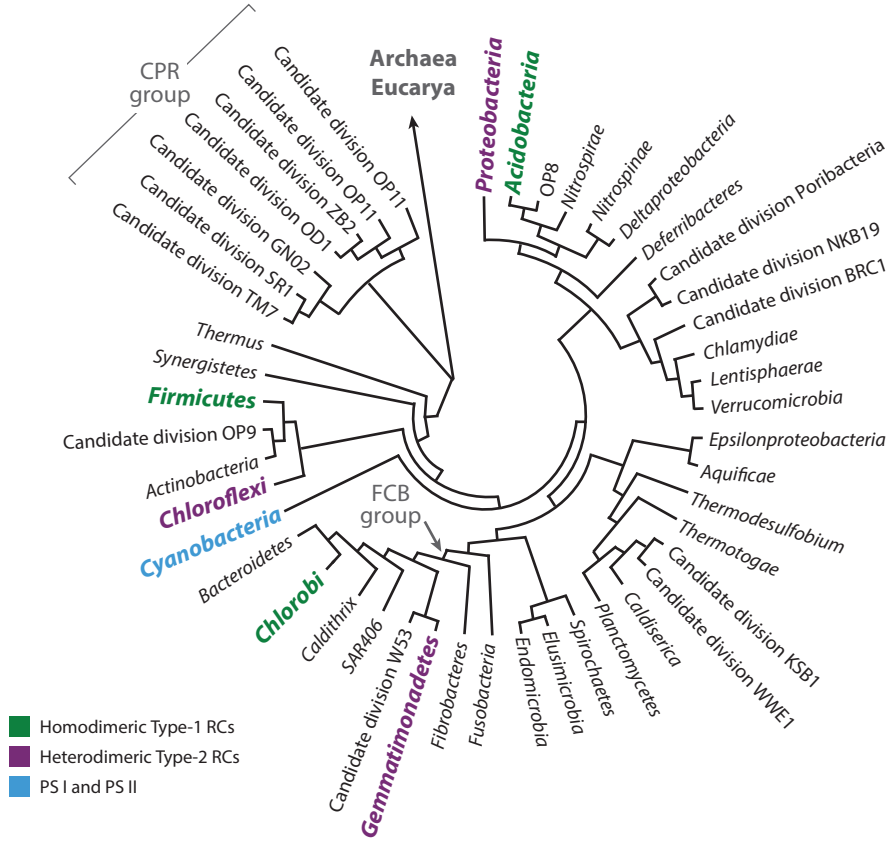

Figure 1

Overview tree of bacterial phyla containing chlorophototrophic members (*shown in color*). Phyla with homodimeric type-1 RCs are indicated in green; phyla with heterodimeric type-2 RCs are indicated in purple. Most cyanobacteria (*blue*) contain a type-1 RC (PS I) and a type-2 RC (PS II) allowing them to evolve oxygen. Adapted from a tree presented by Fischer et al. (35). Abbreviations: CPR, Candidate Phylum Radiation; FCB, *Fibrobacteres-Chlorobi-Bacteroidetes*; RC, reaction center; PS, photosystem.

phylogenetic markers. DNA sequencing can provide critical information about the likely physiology and metabolism of organisms, and that information, in combination with metatranscriptomic studies, can be used to develop appropriate conditions for enrichment cultures and isolation of axenic strains (151, 152). This review briefly summarizes progress in identifying and characterizing chlorophototrophic bacteria as well as the impact of omics methods on our knowledge of these organisms.

GSB: green sulfur bacteria

Metagenomics: sequence analysis of DNA obtained directly from an environmental sample or a mixed population of microorganisms

Metatranscriptomics: sequence analysis of messenger RNA obtained directly from an environmental sample

2. OXYGENIC CHLOROPHOTOTROPHS: *CYANOBACTERIA*

Cyanobacteria are aerobic, Gram-negative, oxygenic chlorophototrophic bacteria that emerged ~2.7 Ga ago and were first described in 1829 (114, 124). At that time, they were believed to resemble eukaryotic algae, resulting in the misleading taxonomic designation "blue-green algae," and phycologists generated their systematic descriptions using the Botanical Code of Nomenclature instead of the Bacterial Code. By 1979, researchers had described 150 genera and more than 1,000

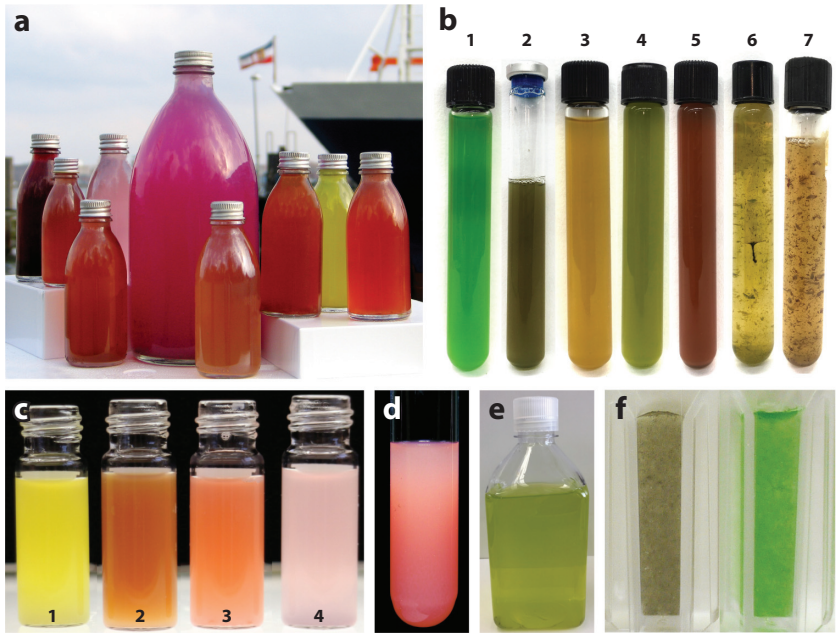

Figure 2

Varied colors of chlorophototrophic bacteria. (*a*) Selected PSB and PNSB (*Proteobacteria*). (*b*) Diverse chlorophototrophs: 1. *Synechococcus* sp. PCC 7002 (*Cyanobacteria*); 2. *Heliobacterium modesticaldum* ICE-1 (*Firmicutes*); 3. *Chloracidobacterium thermophilum* (*Acidobacteria*); 4. *Chlorobaculum tepidum* (BChl *c*; *Chlorobi*); 5. *Chlorobaculum limnaeum* (BChl *e*; *Chlorobi*); 6. "green" *Chloroflexus* sp. (*Chloroflexi*); 7. "brown" *Chloroflexus* sp. (*Chloroflexi*). (*c*) AAPB: 1. *Sphingomonas* sp. AAP2; 2. *Erytbrobacter longus*; 3. *Roseococcus thiosulfatophilus*; 4. *Roseobacter litoralis*. (*d*) *Gemmatimonas phototrophica*. (*e*) *Prochlorococcus* sp. (*Cyanobacteria*). (*f*) *Leptolyngbya* sp. JSC-1 (*Cyanobacteria*) grown under green light (*left*) and far-red light (*right*). Abbreviations: AAPB, aerobic anoxygenic phototrophic bacteria; BChl, bacteriochlorophyll; PNSB, purple nonsulfur bacteria; PSB, purple sulfur bacteria.

species, almost entirely on the basis of field observations (132). Over the past 40 years, bacterial taxonomy and phylogenetics have become closely intertwined, initially through the sequencing of 16S rRNA and more recently of entire genomes; remarkably, phylogeny still is not the basis for naming cyanobacteria. Thus, it is fair to say that cyanobacterial taxonomy is a mess, but it is slowly improving—thanks in large measure to omics studies and to the combined application of molecular methods and ecophysiological observations (33).

Presently, no consistent taxonomic scheme for cyanobacteria is available, and the number of species is uncertain. Estimates range from 2,700 to 5,000 for described and 6,380 to 8,000 for total species (56, 114). To place these numbers in perspective, the described cyanobacterial species represent 20–30% of all described prokaryotic species (114). Furthermore, none of these estimates considers the definition of species in the context of bacterial taxonomy. It is clear, however, that these estimates do not consider the number of ecologically distinct species (e.g., 3), which would unquestionably be many orders of magnitude greater than these estimates.

Despite many attempts to bring cyanobacteria under the rules of the Bacterial Code, the phylum *Cyanobacteria* has never been validly published under this code, and several taxonomic schemes for cyanobacteria exist. Comparative studies of 178 axenic strains in the Pasteur Culture Collection of Cyanobacteria led to their assignment to five sections, which were largely congruent with the cyanobacterial taxonomy that appeared in 2001 in Volume 1 of Bergey's Manual:

Oxygenic: oxygen-producing

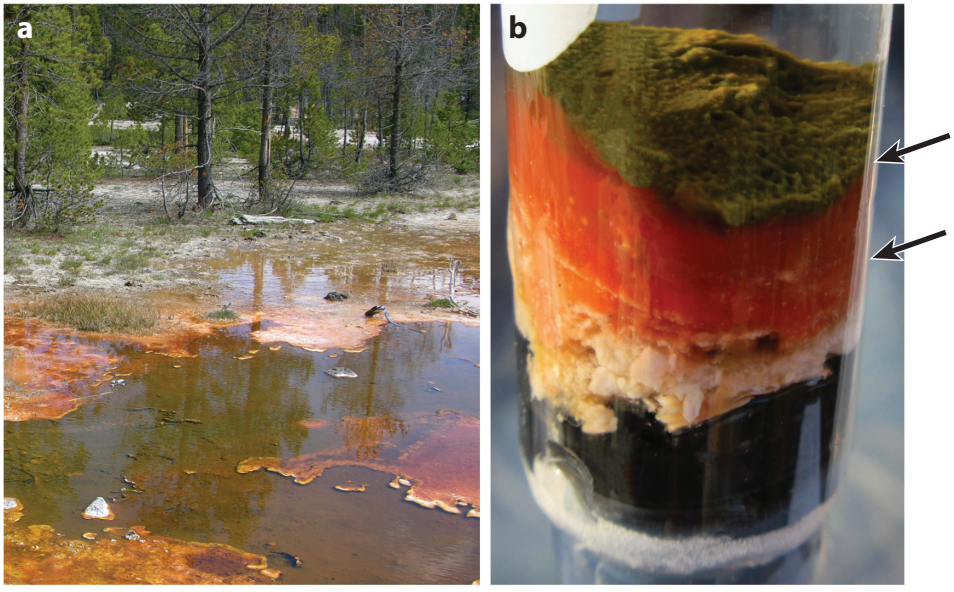

Figure 3

(*a*) Chlorophototrophic microbial mat associated with Octopus Spring, Yellowstone National Park, Wyoming, USA (temperature: ~45 to 60°C). (*b*) Section of the mat (~2 cm deep): Arrows indicate the upper green euphotic layer (1–2 mm deep) and the lower extent of the biologically active region of the orange-red undermat (extending ~5 mm below the upper green layer). Seventeen taxa, representing six of the seven known phyla containing chlorophototrophic bacteria (all except *Gemmatimonadetes*), live together in this mat community (154; M. Tank, unpublished results).

Subsection I (formerly *Chroococcales*), Subsection II (formerly *Pleurocapsales*), Subsection III (formerly *Oscillatoriales*), Subsection IV (formerly *Nostocales*), and Subsection V (formerly *Stigonematales*) (132). The NCBI Taxonomy database categorizes 17,948 entries into 8 orders, 49 families, 213 genera, and approximately 800 species (**Supplemental Table 1**) (**http://www.ncbi.nlm.nih.gov/taxonomy**). Online databases summarizing cyanobacterial taxa are also available, namely AlgaeBase (**http://www.algaebase.org/**) and CyanoDB (**http://www.cyanodb.cz/**). The CyanoDB database presently lists ~270 "validly described" cyanobacterial genera.

Supplemental Material

These taxonomic schemes do not reflect the current phylogeny based on 16S rRNA gene sequences (**Supplemental Figure 2**). The Pasteur Culture Collection (**https://www.pasteur.fr/en/public-health/crbip/distribution/pcc**) now contains more than 750 axenic strains of cyanobacteria, and the 16S rRNA genes of the majority of the strains have been sequenced. Polyphasic approaches that blend ecophysiological observations, observations with axenic cultures, and molecular sequencing methods are starting to produce a more coherent taxonomy—and phylogeny—for cyanobacteria (97). Modern methods even make it possible to amplify and sequence DNA from herbarium-type specimens to establish the relationships between herbarium-type specimens and axenic cultures. Using polyphasic approaches, Komárek (97) recently described nine clades of cyanobacteria: *Gloeobacterales*, *Synechococcales*, *Oscillatoriales*, *Chroococcales*, *Pleurocapsales*, *Spirulinales*, *Rubidibacter/Halothece*, *Chroococcidiopsidales*, and *Nostocales*. It is encouraging that by using polyphasic approaches the number of major groups is beginning to coalesce around approximately 9 to 12 major clades, including many that are similar to those that were historically recognized.

FaRLiP: far-red-light photoacclimation

Ecotype: an ecological species; a genetically distinct geographic population within a species that is adapted to specific environmental conditions

Genomics: study of the structure, function, evolution, and mapping of genomes (genetic material/chromosomes)

Supplemental Material

The 3.4-Mb genome of *Synechocystis* sp. PCC 6803, the first cyanobacterial genome and at the time only the third bacterial genome to be sequenced, was completed in 1996 (86). Presently, more than 550 cyanobacterial genomes, including representatives of all five taxonomic sections, have been sequenced (**Supplemental Tables 2** and **3**). The genomes of cyanobacteria range from approximately 1.6 to 13 Mbp with GC contents ranging from 30% to 69% (**Supplemental Table 2**). Although genome sequencing initially focused on model organisms, the diversity of organisms for which genomic information is available has expanded considerably. Genomic data indicate that cyanobacterial taxonomy/phylogeny is more complex than originally recognized, and on the basis of a combination of molecular and phenotypic criteria, the number of major groups has increased beyond the original five sections (33, 97, 139).

As genomic databases have grown, comparative methods could be applied to establish functional inferences concerning the distribution of inclusions, thylakoid structure, biochemical and metabolic traits, and other parameters that can aid in the identification and classification of organisms (e.g., 4, 53). For example, terrestrial cyanobacteria often grow in environments that are strongly enriched in light wavelengths greater than 700 nm (e.g., soil or under plant canopies where light is strongly filtered by Chl *a*), and many can acclimate to these conditions, a process known as far-red-light photoacclimation (FaRLiP) (42–44, 69–71, 120). Comparative genome analyses revealed a highly conserved cluster of 20 genes, which are paralogs of genes encoding core subunits of PSI, PSII, and phycobilisomes that are expressed in white light (42, 44). When cells capable of FaRLiP are grown in far-red light, a knot-less phytochrome (RfpA) activates a transcriptional activator (RfpB), which strongly activates the expression of the FaRLiP gene cluster (71). Additionally, the cells synthesize far-red-light-absorbing pigments, Chls *d* and *f*, as well as far-red-light-absorbing forms of allophycocyanin, which form specialized phycobilisome-like complexes (**Figure 2*f***) (42, 44, 69). Extensive remodeling of the photosynthetic apparatus to accommodate these changes confers the capacity to evolve oxygen and grow in far-red light (44). From the FaRLiP gene cluster, *chlF* encodes a highly divergent paralog of a PSII core subunit; ChlF is a photooxidoreductase that synthesizes Chl *f* from Chl *a* or Chlide *a* (70). Examples of cyanobacteria that can perform FaRLiP have been identified in all of the major taxonomic groupings (42, 44), but no extensive metagenomic survey of terrestrial cyanobacteria for FaRLiP has been performed.

Cyanobacteria of the genus *Prochlorococcus* were first described in a landmark paper in 1988 (**Figure 2*e***) (19). Together with marine *Synechococcus* spp., these bacteria are numerically dominant organisms in Earth's oceans (134), and they also dominate the genomic databases, accounting for approximately 30% of the cyanobacterial genomes sequenced to date (**Supplemental Tables 2** and **3**). These marine organisms may be responsible for ~5% of total global carbon fixation (and thereby of global-dioxygen production). Of the 12 major clades of *Prochlorococcus* sp., examples of 5 have been cultivated (6). Each is adapted to different light, temperature, and nutrient conditions. Quantitative PCR analyses have shown that temperature, light, nutrients, and competitor densities shape natural populations of these organisms (6, 82, 134). A similar distribution of ecotypes across physical and chemical gradients is observed for thermophilic *Synechococcus* sp. in microbial mat communities (3, 121). Comparative genomics of low-light- and high-light-adapted ecotypes of *Prochlorococcus* and *Synechococcus* sp. reveal differences in genome size as well as genomic islands possibly responsible for these niche adaptations (6, 121, 134). High-light-adapted ecotypes of *Prochlorococcus* spp. have smaller genomes, ~1.62–1.78 Mbp, whereas the genomes of low-light-adapted ecotypes are larger, ~1.7 to 2.6 Mbp (5, 6). *Synechococcus* spp. genomes are even larger, 2.2 to 2.86 Mb, but are still smaller than the average bacterial (3.69 ± 1.96 Mbp) or cyanobacterial genomes (5.33 ± 3.69 Mbp) (134). Further sequence analysis of worldwide samples has revealed that hundreds of distinctive individual genotypes can coexist in a milliliter of seawater (18, 88),

each with specific gene complements, which presumably confer selective advantages to each genotype. Remarkably, the global pangenome for *Prochlorococcus* spp. is now estimated to contain up to 80,000 genes (5, 6, 18, 22). In addition to affecting natural populations through lytic infection cycles, cyanophages may transfer genes among the various ecotypes, further enhancing the diversity of *Prochlorococcus* spp. that occur in the ocean (6). Finally, mathematical modeling studies suggest that *Prochlorococcus* spp. and the dominant, co-occurring heterotroph, *Pelagibacter ubique* (SAR11) (49), have a coevolved mutualism that maximizes their collective metabolic rate and carbon recycling through complementary carbon excretion and uptake pathways (9). These populations of organisms resemble the interactions that occur between mitochondria and chloroplasts.

Absence of chlorophototrophy among most bacterial (and archaeal) phyla is probably not the result of gene loss; instead, it probably results from lineages never having acquired the trait (**Figure 1** and **Supplemental Figure 1**) (111). Despite the enormous diversity of cyanobacteria, there is currently only one example of a secondarily nonphotosynthetic member of this group. Zehr and coworkers have identified and characterized the UCYN-A clade, "*Candidatus* Atelocyanobacterium thalassa," in detail (7, 160, 170). Two variant populations of this clade are photoheterotrophic cyanobacterial symbionts of prymnesiophyte algae (160) (**Supplemental Figure 2**). These symbionts have substantially reduced genomes, 1.44 and 1.48 Mbp, and have lost genes encoding PSII, ribulose 1,5-*bis*-phosphate carboxylase/oxygenase (RuBisCO), and enzymes of other metabolic pathways, but they have retained genes for nitrogenase and PSI, leading to an anoxygenic photoheterotrophic, nitrogen-fixing metabolism (7, 170). Though many genes have been lost, these genomes are still much larger than the 1.02-Mbp chromatophore genome of the intracellular symbiont of *Paulinella chromatophora* (118) and vastly larger than the 136-kb cyanelle genome of the glaucophyte alga *Cyanophora paradoxa* (144). "*Ca*. A. thalassa" contributes significantly to nitrogen fixation in the tropical North Atlantic Ocean, and its ecological impact is quite substantial, roughly equivalent in magnitude (~20% of oceanic N_2 fixation) to the colonial, N_2-fixing cyanobacteria of the genus *Trichodesmium* (112). UCYN-A is part of a clade that includes other ecologically important, unicellular N_2-fixing cyanobacteria, *Cyanothece* spp. and *Crocosphaera watsonii* (**Supplemental Figure 2**) (7, 170).

Although cyanobacteria have long been thought to be ancient organisms and are clearly quite diverse, no achlorophyllous members of this phylum were known. Recently, two deeply diverging, nonchlorophototrophic groups of bacteria that are related to the phylum *Cyanobacteria* were identified: *Melainabacteria* and *Sericytochromatia* (**Supplemental Figure 2**) (31, 138, 142). Melainabacteria were identified by metagenomic sequence analyses of human fecal and subsurface aquifer samples, but related organisms have also been detected in soil, groundwater, drinking water, freshwater lakes, wastewater treatment plants, calcium carbonate–containing microbialites, and animal guts (23, 31, 143). Through metagenomic analyses, researchers have identified a second group, *Sericytochromatia*, in a coal-bed methane well, an algae-associated biofilm in a laboratory bioreactor, and subsurface groundwater (142). Initial metabolic reconstruction analyses indicated that melainabacteria are anaerobic, nonphotosynthetic, and fermentative bacteria. The nitrogenase of a subsurface melainabacterium is unlike that found in chlorophototrophic cyanobacteria, which suggests the two lineages acquired nitrogen fixation independently (31). Although most melainabacteria appear to be strictly fermentative, the genomes of "*Candidatus* Obscuribacter phosphatis" and the *Chlorella*-predator *Vampirovibrio chlorellavorus* encode cytochromes and quinones, and these organisms should be capable of aerobic and/or anaerobic respiration in addition to fermentation (142, 143). Differences in the cytochrome complements and electron-transfer chains of respiring members of the *Melainabacteria* and *Sericytochromatia* suggest that these organisms gained respiration by horizontal gene transfer (HGT) after oxygenation of the atmosphere. Although one interpretation is that the common ancestor of the three cyanobacterial lineages was

Pangenome: all genes of a particular taxon

Anoxygenic: not oxygen-producing

Melainabacteria: early-diverging class of nonphotosynthetic bacteria within the phylum *Cyanobacteria*

HGT: horizontal gene transfer

Supplemental Material

nonphotosynthetic and fermentative, it is equally possible that members of the *Melainabacteria* and *Sericytochromatia* lost the capacity to synthesize Chls, components of the photosynthetic apparatus, and enzymes for CO_2 fixation after ancestral, chlorophototrophic (and possibly oxygen-evolving, i.e., *Oxyphotobacteria*) cyanobacteria arose (111).

PSB: purple sulfur bacteria

PNSB: purple nonsulfur bacteria

AAPB: aerobic anoxygenic phototrophic bacteria

ABC: aerobic BChl-containing

3. ANAEROBIC AND AEROBIC ANOXYGENIC CHLOROPHOTOTROPHS: *PROTEOBACTERIA*

The first descriptions of chlorophototrophic *Proteobacteria* date back to the mid-nineteenth century (80). After ~180 years of research, they now represent a highly diverse group of anoxygenic chlorophototrophs with more than 366 species in 16 orders and 30 families of *Alpha-*, *Beta-*, and *Gammaproteobacteria* (**Supplemental Table 1** and **Supplemental Figures 3** and **4**). The pioneering studies of Winogradsky and Molisch first described two physiologically distinct groups, the "*Thiorhodoceae*" and the "*Athiorhodoceae*," on the basis of their differing capacity to oxidize sulfur and to form microscopically visible polysulfide/sulfur globules (reviewed in 80). In addition to BChl *a* or *b*, both groups produce large amounts of carotenoids leading to reddish ("purple") coloration of the cells, varying from yellowish green to dark brown (**Figure 2**). The two groups were merged into the phenotypically and physiologically related group of purple bacteria with the two subgroups, purple sulfur bacteria (PSB) and purple nonsulfur bacteria (PNSB), which have been extensively reviewed (75, 80, and references therein). A third group of chlorophototrophic proteobacteria, the aerobic anoxygenic phototrophic bacteria (AAPB), was discovered in the coastal waters of Japan in 1979 (137). AAPB also produce BChl *a*, type-2 RCs, and carotenoids, but they differ from PSB and PNSB, which are anaerobic anoxygenic chlorophototrophs in which the biosynthesis of the photosynthetic apparatus is suppressed by oxygen. AAPB are mostly strict aerobic chemoheterotrophs and use light merely for auxiliary energy production under oligotrophic growth conditions (**Figure 2*c***) (169). A broader term for AAPB is aerobic (anoxygenic) BChl-containing (ABC) bacteria (95), which also include methylotrophs, *Rhizobia* (both *Alphaproteobacteria*), *Cab. thermophilum* (*Acidobacteria*) (151, 152), *Gemmatimonas phototrophica* (*Gemmatimonadetes*) (172), and "*Candidatus* Thermochlorobacter aerophilum" (*Chlorobi*) (104).

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Culture-dependent and culture-independent studies have shown that representatives of chlorophototrophic proteobacteria thrive in highly diverse habitats, including freshwater lakes, oceans, soils, hot springs, hypersaline springs, hydrothermal vents, symbionts, activated sludge, and wastewater treatment systems. They are physiologically diverse with species ranging from psychrophilic to thermophilic and preferences for acidic to alkaline conditions. Chlorophototrophic proteobacteria have contributed significantly to our knowledge of the biochemistry and molecular mechanisms of chlorophototrophy. Purple bacteria (e.g., *Allochromatium vinosum*, *Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, and *Rhodobacter sphaeroides*) have served as model organisms to understand the mechanisms of Chl-based light energy capture, conversion, and its molecular regulation (75). They have also been used to decipher the biochemistry of sulfur metabolism (30, 38, 167).

PSB are anoxygenic phototrophic members of the *Gammaproteobacteria* that utilize sulfide as preferred electron source for carbon fixation via the Calvin-Benson-Bassham cycle (150). The *Chromatiaceae* and *Ectothiorhodospiraceae* families within the order *Chromatiales* comprise 29 genera and 74 species (**Table 1**, **Supplemental Table 1**, and **Supplemental Figure 4*b***). During the oxidation of sulfide to sulfate, polysulfide is formed and stored as elemental sulfur in globules inside the cells in the case of the *Chromatiaceae* and extracellularly in the case of *Ectothiorhodospiraceae* (30, 38). PSB can be found in nearly all aquatic habitats in which sulfide and light are present. Members of the *Chromatiaceae* are mainly found in marine and freshwater habitats; members of the *Ectothiorhodospiraceae* are specifically adapted to saline and alkaline conditions and are associated

with soda lakes and other (hyper)saline environments. The main photosynthetic pigment in PSB is BChl *a*, although a few members, such as *Halorhodospira halochloris*, *Halorhodospira abdelmalekii*, *Thiococcus pfennigii*, and *Thioflavicoccus mobilis*, produce BChl *b*, which is specifically beneficial in sandy coastal sediments (**Supplemental Table 1**) (79). The distinctive purple-red coloration of PSB is due to carotenoids of the spirilloxanthin, rhodopinal, or okenone series (**Figure 2** and **Supplemental Table 1**) (148). Most described species have been discovered through isolation-based studies. Cultivation-independent approaches to the analysis of anoxygenic chlorophototrophic purple bacteria have mainly targeted the functional genes of the photosynthetic apparatus, such as the structural genes for the L and M subunits of the type-2 RCs (photosynthetic unit, fixed, *pufL*, and *pufM*). Cultivation-independent studies indicate that extreme environments such as hypersaline lakes in Chile contain a large diversity of undescribed bacteria with type-2 RCs (158). Phylogenetic analysis of *pufLM* gene sequences indicates a monophyletic origin for the PSB (153); however, HGT of the photosynthetic gene cluster has been suggested between the different proteobacterial classes (76, 126). Compared with other chlorophototrophs, PSB genomes were understudied until very recently. Isolated genomes have been used in support of proteomic and transcriptional analyses (e.g., 145, 167), but few comparative analyses have been conducted. The number of genomes for this group is now increasing rapidly, and representative genomes for more than half of the 29 PSB genera will soon be available (**Supplemental Table 1**). Comparative analyses may explain the evolution of chlorophototrophy and its loss within the *Chromatiales* (**Supplemental Figure 4*b***).

Photoferrotroph(y): organism/process performing light-driven oxidation of ferrous iron (Fe^{2+})

Supplemental Material

PNSB are facultatively anaerobic anoxygenic chlorophototrophic members of the *Alpha-* and *Betaproteobacteria*; 96 species in 28 genera have been described to date. Although the name implies otherwise, most PNSB are capable of oxidizing sulfide; however, they typically tolerate much lower concentrations (<0.5 mM sulfide) than PSB (up to 3–5 mM sulfide). Many PNSB are able to fix dinitrogen and can use H_2 to fix carbon, but they grow best under photoheterotrophic conditions. Similar to PSB, PNSB contain type-2 RCs, BChl *a* or *b*, and various carotenoids (**Supplemental Table 1**). They thrive in many mesophilic, circum-neutral, aquatic or terrestrial habitats, although some species are adapted to more extreme environments. *Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*, and *Rhodobacter capsulatus* are examples of the most commonly studied model organisms. The first PNSB genome to be sequenced was that of *Rba. sphaeroides* (20). Today, genomes are available for 35 of the 96 described PNSB species (**Supplemental Table 1**), and multiple genomes are available for some species. Genome and transcriptome studies have revealed essential genes and the genomic basis for metabolic versatility within this group; these studies have also revealed rapid evolution and specific adaptations among strains and ecotypes (21, 29, 51, 101, 119, 125, 140).

In addition to reduced sulfur compounds and H_2, some purple bacteria also use other electron donors, such as ferrous iron [Fe^{2+}], nitrite, and arsenite. Photoferrotrophy, the use of ferrous iron [Fe^{2+}] in photosynthesis, was discovered and characterized in several chlorophototrophic *Proteobacteria* isolates (15). Arsenotrophy, the coupling of arsenic reduction or oxidation to energy production, was first discovered in chemotrophs; the use of arsenite as an electron donor for anoxygenic photosynthesis, a process called photoarsenotrophy, has been analyzed in PSB of the genus *Ectothiorhodospira* (66). Some strains of *Thiocapsa* (PSB) and *Rhodopseudomonas* (PNSB) are capable of using nitrite as electron donor for photosynthesis (65, 124).

AAPB are a diverse group, and unlike most other anoxygenic chlorophototrophs, they produce BChl *a* under oxic conditions and require oxygen both for growth and for photosynthetic electron transport (**Figure 2*c***). *Roseobacter denitrificans* and *Erythrobacter longus*, the first two described species, were isolated from marine coastal areas approximately 40 years ago (135–137). Over the past few decades, more than 70 additional species have been described, and AAPB have

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been detected in diverse aquatic habitats such as oceans, freshwater lakes, and rivers around the world (**Supplemental Table 1**). AAPB also thrive in extreme conditions, including polar regions, soil crusts, hot springs, hypersaline spring systems, near hydrothermal vents, and in the presence of high concentrations of toxic metal(loid) oxides (26, 169). Originally, all known AAPB were members of the *Alpha-*, *Beta-*, and *Gammaproteobacteria*, with the majority belonging to the *Alphaproteobacteria* (**Supplemental Table 1**) (27, 28, 68, 95, 169). More recently, aerobic anoxygenic chlorophototrophs have also been described in other phyla, i.e., *Gemmatimonadetes* and *Acidobacteria*. Owing to their novelty, these species are discussed separately (see Section 6). In this review, we have included as putative AAPB all bacteria that produce BChl *a* and/or that have the genetic potential to produce PufLM-containing RCs, including *Methylobacterium*, *Agrobacterium*, *Rhizobium*, and *Bradyrhizobium* species (**Supplemental Tables 1–3**). An estimated ~500 proteobacterial genomes encoding PufLM-containing RCs have now been sequenced, ranging in size from ~2.5 to 8.5 Mbp with GC contents between 60% and 74% (**Supplemental Table 2**).

Similar to PNSB, proteobacterial AAPB contain BChl *a* in type-2 RCs and produce a variety of carotenoids, leading to quite varied coloration (see **Figure 2**). Unlike PNSB and PSB, AAPB are obligate heterotrophs, although some carbon fixation by anaplerotic reactions in the TCA cycle occurs (150, 168, 169). AAPB are aerobes, implying that their BChl biosynthesis is no longer repressed by oxygen and that oxygen-sensitive enzymes in the pathway have been replaced by oxygen-tolerant isozymes (40, 87). AAPB are numerous in all tested environments, and they are the third most abundant chlorophototrophs in the ocean (95, 169). Several comprehensive reviews of this fascinating group of phototrophs have recently appeared (e.g., 95, 96, 168, 169).

As many more strains are undergoing full genome sequencing, some species that were presumed to be aerobic nonphototrophic heterotrophs have been found to encode the genes necessary for fully functional pigment-protein complexes, RCs, and electron transfer (95). One of the latest examples is the obligately aerobic alphaproteobacterium *Elioraea tepidiphila*, which was originally described as a chemoheterotroph, although its genome includes the genes necessary for phototrophic growth (1, 154, 157, 159). Axenic cultures of the close relative "*Candidatus* Elioraea thermophila" produce BChl *a*, suggesting that the type strain of *E. tepidiphila* may not yet have been grown under appropriate conditions to allow expression of the genes necessary for production of the photosynthetic apparatus (154).

The increasing number of genome sequences for purple bacteria also aids the study of the evolution and polyphyletic distribution of chlorophototrophs. HGT may be responsible for the distribution of chlorophototrophy within the *Proteobacteria* as well as between distantly related phyla such as *Gemmatimonadetes* (116, 126, 146, 172, 173). In other cases, a regressive evolutionary pathway leading from photoautotrophic PNSB to chemoheterotrophic species via gene loss to produce anaerobic and aerobic photoheterotrophic intermediate species has been suggested (89). Recent comparative genome analyses indicate that gene loss has occurred in the course of evolution leading from chlorophototrophic AAPB or PNSB ancestors to chemotrophic descendants (96, 98, 175). An interesting possible example of HGT has recently been described in which *pufLM* genes similar to those of *Sphingomonas*-like organisms (AAPB) occur in *Hymenobacter* sp. isolate R-68361, a member of the phylum *Bacteroidetes* (147). Provided that further analyses confirm that R-68361 can actually synthesize BChl *a* and functional type-2 RCs, it is possible that *Bacteroidetes* will become the eighth phylum known to contain members capable of chlorophototrophy.

The *Roseobacter* clade (*Alphaproteobacteria*, *Rhodobacteraceae*) is a monophyletic clade and is one of the most dominant and successful bacterial lineages in pelagic environments, where it can represent up to 30% of bacterioplankton communities (141). The group is characterized by high genomic, physiological, and metabolic diversity, and despite the phototrophic lifestyle of the type species *Roseobacter litoralis*, members with chemoheterotrophic lifestyles dominate this clade,

possibly owing to regressive evolution of photosynthesis within this group (96). Nevertheless, a large number of the included genera contain ABC bacteria with genomes containing *pufLM* genes (see **Supplemental Table 1** and **Supplemental Figure 4**) (108, 131). Several available genomes for this group were analyzed in a phylogenomic study that questions the long-accepted monophyly of this group. This study revealed characteristic changes in genomic contents—gene losses and/or gene gains—correlated with adaptation to specific environments, such as marine or freshwater habitats (141).

Chlorosomes: sac-like antenna structures found in green bacteria containing up to 250,000 self-assembling BChl *c*, *d*, *e*, or *f* molecules

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4. ANAEROBIC AND AEROBIC ANOXYGENIC CHLOROPHOTOTROPHS: *CHLOROBI*

Chlorophototrophic GSB (*Chlorobiaceae*), one of the three major groups of anoxygenic green bacteria (13, 14), were discovered at the beginning of the twentieth century (115). The taxonomy, ecology, and ecophysiology of GSB have been extensively reviewed (14, 78, 110, 122, 163). GSB are characteristically low-light-adapted chlorophototrophs and are found in the deepest anoxic waters of lakes, inland seas, and other stratified aquatic habitats, where they can form massive blooms. They also occur in anoxic sediments, where sulfide is present but light levels limit the growth of other chlorophototrophs (78). Four genera of GSB are recognized: vibrioid or rod-shaped *Chlorobium* (*Chl.*); single-celled *Chlorobaculum* (*Cba.*); salt-requiring, prosthecate *Prosthecochloris*; and gliding and flexing rods, *Chloroherpeton* (78).

GSB are obligate photoautotrophs and are unable to grow in the dark. All green bacteria possess unique light-harvesting complexes called chlorosomes, which contain self-assembling, mostly nanotubular arrays of BChls *c*, *d*, *e*, or *f*; their photosynthetic apparatus also includes the BChl *a*–binding Fenna-Matthews-Olson protein and type-1 homodimeric RCs (10, 14). The characteristic green or brown color of GSB results from the dominant BChl present in chlorosomes. BChl *c*– and/or *d*–containing strains are green-colored and have chlorobactene and γ-carotene as major carotenoids, whereas BChl *e* and the bicyclic carotenoid, isorenieratene, lead to a distinctive brown coloration (**Figure 2*b***). Compared with green-colored GSB, brown-colored GSB are adapted to lower irradiances and are found at greater depths (163). However, pigmentation is not a phylogenetically informative trait. Most GSB oxidize reduced sulfur compounds (H_2S, polysulfide, elemental S^0, and sulfite), which most strains except *Chloroherpeton* sp. oxidize to sulfate. GSB couple the oxidation of reduced sulfur compounds with CO_2 fixation via the reverse tricarboxylic acid pathway for autotrophic growth. Elemental sulfur (polysulfide), produced as an intermediate in the oxidation of sulfide, is stored outside the cells as sulfur globules. GSB with the *sox* operon can also oxidize thiosulfate to sulfate (13, 14). For more detailed information on the photosynthetic apparatus as well as the biochemistry and physiology of GSB, the reader is referred to recent reviews (10, 13, 14, 38).

Some GSB are unable to use sulfide as electron donor for carbon fixation and instead use ferrous iron [Fe^{2+}], a process termed photoferrotrophy (also see Section 3 above; for a comprehensive review, see 15). Although molecular studies suggest that other examples exist (25), *Chlorobium ferrooxidans* and *Chlorobium phaeoferrooxidans* are currently the only described members of the *Chlorobi* with this capability (24, 64, 105). In contrast to *Chl. ferrooxidans*, which was isolated from a benthic habitat, *Chl. phaeoferrooxidans* was isolated from the water column of a ferruginous subbasin of Lake Kivu (East Africa), an analog of ferruginous oceans on Precambrian Earth (105). Photoferrotrophs may have been important primary producers on early Earth, and they may have been responsible for the deposition of banded iron formations from ferruginous but sulfide-poor oceans during the Precambrian period (15, 25).

Chlorobaculum tepidum (**Figure 2*b***) (formerly *Chlorobium tepidum*) has emerged as the laboratory model for the *Chlorobiaceae* because of its very rapid growth (doubling time, ~2 h) and its capacity

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for genetic modification by natural transformation and conjugation (36, 165). Most of the physiological and biochemical knowledge of GSB derives from studies on this moderately thermophilic, thiosulfate-oxidizing GSB (14, 110). *Cba. tepidum* was the first GSB to have its genome sequenced (34), although examples (~30 total) of all four recognized genera have followed (**Supplemental Table 1**) (13, 14, 24, 155). GSB genomes are small (1.97 to 3.13 Mb) and have moderate GC contents (44% to 57%) (**Supplemental Table 2**). Comparative genome analyses have generally confirmed the physiologically and metabolically similarities of GSB isolates (13, 14). Although chromosomal synteny of genes is not very highly maintained, GSB share a highly conserved core genome with all genes necessary for their anoxygenic phototrophic lifestyle by sulfur oxidation and CO_2 fixation (14). Some GSB genomes reflect a high proportion of HGT, e.g., up to 24% of all genes in *Cba. tepidum* (117). Phage-mediated transduction has been suggested as a possible mechanism of HGT (e.g., transfer the *sox* cluster for thiosulfate utilization) (14, 37). Although no phages infecting *Chlorobi* have been isolated, a recent metagenomic study provided strong evidence that a lytic DNA phage, whose sequence was obtained, may have been the vector for phage-mediated HGT in the natural GSB population in Lake Císo, a meromictic lake in Spain (106).

The first aerobic photoheterotrophic member of the *Chlorobi* was discovered through metagenomic analyses of a hot spring microbial mat community (104). The discovery of "*Ca*. Thermochlorobacter aerophilum" (**Figure 4**) broke the dogma concerning physiological conformity concerning GSB that had been created by cultivation-based studies. Although a chlorophototroph, this member of the *Chlorobi* is different from all known GSB. It cannot oxidize sulfur compounds and cannot fix carbon or nitrogen. Unlike all strictly anaerobic GSB, it relies on oxygen for important metabolic reactions and lives in an environment where oxygen tensions can reach 800% of saturation during the day (104).

In addition to free-living GSB, consortia comprising GSB cells surrounding a central chemotrophic and motile partner are commonly observed in many habitats where free-living GSB are also present (123). "*Chlorochromatium aggregatum*" and "*Pelochromatium roseum*" are representatives of multicellular consortial holobionts in which the chlorophototrophic partner can be either a green- or brown-colored GSB and the motile, heterotrophic partner is a betaproteobacterium from the candidate genus "*Candidatus* Symbiobacter" (103, 123, 164). Genome analyses identified a number of metabolic linkages between the two partners of "*Chlorochromatium aggregatum*," but perhaps the most striking inference was that the partners share a menaquinone/menaquinol pool and probably also their proton-motive force (103). In addition, syntrophic associations have been described between GSB and sulfate- or sulfur-reducing bacteria (78). Syntrophic interactions between a *Prosthecochloris* sp. and the chemoheterotrophic sulfur-reducing bacterium *Geobacter sulfurreducens* are based on direct intercellular electron transfer between species (58). In a process termed syntrophic anaerobic photosynthesis, the GSB is independent of sulfide as an electron source and receives its electrons indirectly from the oxidation of organic matter through its partner. Although both partners depend on each other under experimental coculture conditions, the still unknown mechanism(s) of electron transfer seems to be relatively nonspecific; the *Prosthecochloris* sp. strain is also able to receive electrons supplied by an electrode (58).

For about a century, GSB were the only known members of the phylum *Chlorobi*. Culture-independent studies using 16S rRNA gene analyses first indicated a greater diversity for the phylum than was previously known by revealing the existence of many uncultured members. Initially, a chlorophototrophic lifestyle was assumed for these distant relatives until the isolation of *Ignavibacterium album* (77, 102) and *Melioribacter roseus* (83, 130), early-diverging aerobic chemoorganoheterotrophic representatives of the phylum *Chlorobi*. GSB, which were once synonymous with the phylum *Chlorobi* in name and originally its only cultured representatives, belong to a broader phylum with much more diverse metabolic capabilities, which overlap

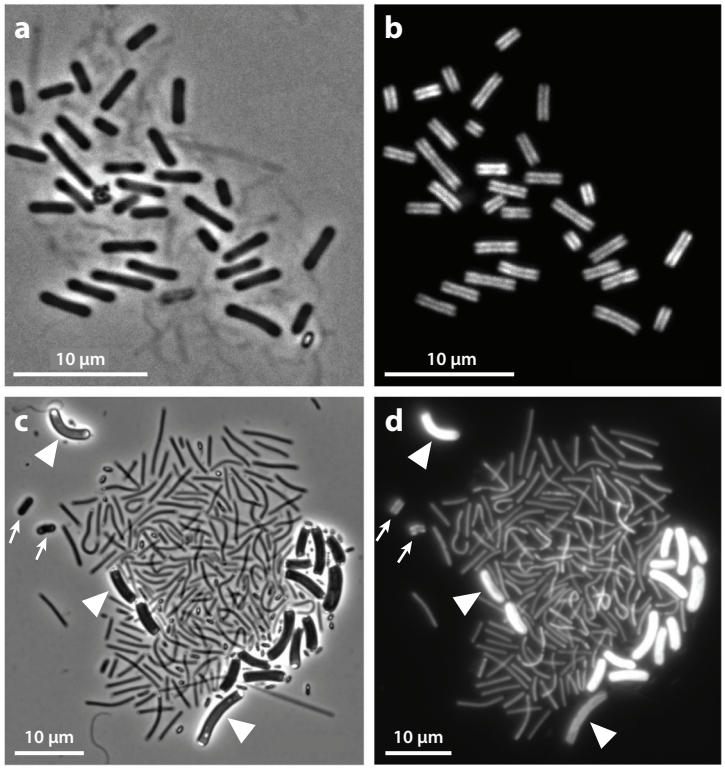

Figure 4

(*a*, *c*) Phase-contrast and (*b*, *d*) bacteriochlorophyll (BChl) *c* autofluorescence micrographs of (*a*, *b*) *Chloracidobacterium thermophilum* strain B^T from Octopus Spring (Yellowstone National Park, Wyoming, USA) and (*c*, *d*) "*Candidatus* Thermochlorobacter aerophilum." Additional phototrophs are marked with arrows (*Cab. thermophilum*) and triangles (*Synechococcus* spp.) in panels *c* and *d*.

somewhat with those of members of the phylum *Bacteroidetes* (67). With the proposal of the new class *Ignavibacteria* and the discovery of the first aerobic photoheterotrophic member of the *Chlorobi*, "*Ca*. T. aerophilum," the GSB now are restricted to the family *Chlorobiaceae* (class *Chlorobia*; order *Chlorobiales*) (102, 104) (**Supplemental Figure 5**).

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Genomes are available for *I. album*, *M. roseus*, and uncultured members of the OPB56 cluster referred to as "*Chlorobi* lineage 5" by Iino et al. (77). Genome sizes are 2.67 Mbp for OPB56 (67); 2.0 to 3.2 Mbp for GSB (14); and 3.3 and 3.7 Mbp for *M. roseus* (83, 130) and *I. album* (102), respectively. The facultative anaerobic lifestyle and metabolism of the achlorophyllous members contrast significantly with those of GSB (67, 102, 130). These three organisms lack genes required for oxidation of reduced sulfur compounds and cannot fix nitrogen. Amino acids are essential nutrients for the OPB56 member and *I. album* and probably serve as both nitrogen source and carbon/energy substrates (67, 83, 102, 130). The shared ability for anaerobic growth in chemotrophic and chlorophototrophic members of the *Chlorobi* is also based on different metabolic modes: fermentation and anaerobic respiration in chemotrophic members versus anaerobic chlorophototrophy in GSB.

Many of the genes involved in dissimilatory sulfur oxidation (*dsr*, *sat*, and *apr* genes) are also found in sulfate-reducing bacteria (30, 38). The polyphyletic distribution of dissimilatory sulfate

reduction (DSR) and *dsr* genes in *Bacteria* and *Archaea* is usually explained by HGT. Furthermore, on the basis of phylogenetic analysis of DsrMKJOP proteins of sulfur-oxidizing *Chlorobi*, which cluster with the proteins from sulfate-reducing prokaryotes, the presence of *dsr* genes in sulfur-oxidizing *Chlorobi* has also been attributed to HGT. A partial genome affiliated with a nonchlorophototrophic organism, presumably representing the first sulfate-reducing member of the phylum *Chlorobi*, was recently identified in a hot spring microbial mat metagenome (157, 159). A sulfate-reducing GSB could help to clarify the evolution of dissimilatory sulfur oxidation and supports the possible presence of *dsr* genes in an early-diverging ancestor within phylum *Chlorobi*.

FAP: filamentous anoxygenic phototroph (phylum *Chloroflexi*)

5. FILAMENTOUS ANOXYGENIC CHLOROPHOTOTROPHIC MEMBERS OF THE PHYLUM *CHLOROFLEXI*

Supplemental Material

The phylum *Chloroflexi* presently comprises eight classes: *Anaerolineae*, *Ardenticatenia*, *Caldilineae*, *Chloroflexia*, *Dehalococcoidia*, *Ktedonobacteria*, *Thermoflexia*, and *Thermomicrobia* (**Supplemental Figure 6**). To date, ~13 isolate genomes have been sequenced; these range in size from approximately 4.3 to 7.0 Mbp with GC contents between 54% and 67% (**Supplemental Table 2**). Based upon 10 additional genomes inferred from metagenomic analyses, evidence for a ninth class within the phylum *Chloroflexi* has recently been obtained (166). The newly proposed class would be a sister class to *Anaerolineae* and includes the chlorophototroph "*Candidatus* Roseilinea gracile" (see below), which was formerly thought to belong to *Anaerolineae* (94, 154, 157, 159).

The majority of chlorophototrophic members of the phylum *Chloroflexi* are found in the order *Chloroflexales*, one of two orders in the class *Chloroflexi* (**Supplemental Figure 6**). Both suborders *Chloroflexineae* and *Roseiflexineae* contain obligately or facultatively filamentous anoxygenic phototroph (FAP) bacteria, which can be differentiated by their color (*Chloroflexineae*: green; *Roseiflexineae*: reddish- or yellowish-brown) and the presence (*Chloroflexineae*) or absence (*Roseiflexineae*) of chlorosomes (**Figure 2*b***) (57). The members of the *Chloroflexales* are facultative aerobes and can grow as anaerobic photoheterotrophs in light and as aerobic chemoheterotrophs in dark. Some members have the ability to grow photoautotrophically and/or photomixotrophically under anoxic conditions in light (14, 156).

The *Chloroflexaceae* and *Oscillochloridaceae* (*Chloroflexineae*) contain straight or spiral-shaped green-colored FAP bacteria. All members contain chlorosomes and BChl *c* as their major photosynthetic pigment; BChl *a* (and sometimes BChl *d*) is additionally present. The family *Chloroflexaceae* contains a single described genus, *Chloroflexus*, with three validly described species to date: *Chloroflexus* (*Cfl.*) *aurantiacus* (127, 149), *Cfl. aggregans* (14, 61), and *Cfl. islandicus* (41). The type species, *Cfl. aurantiacus*, with the type strain J-10-fl^T, was the first isolated and described FAP (127), and it is the eponym for the phylum. Genomes are available for all *Chloroflexus* species as well as for isolates *Chloroflexus* sp. Y-396-1, *Chloroflexus* sp. Y-400-fl, and *Chloroflexus* sp. MS-G (14, 149, 156). *Chloroflexus* sp. MS-G was isolated from an alkaline, siliceous, nonsulfidic hot spring in Yellowstone National Park (Wyoming, USA), and on the basis of 16S rRNA gene sequence, it represents an undescribed species similar to strain Y-396-1. All described species are thermophilic, unbranched, multicellular filamentous bacteria that grow well at 55°C either anaerobically as photoheterotrophs or aerobically as chemoheterotrophs. All species also exhibit gliding motility and inhabit freshwater hot springs. Active cell aggregation has only been reported for *Cfl. aggregans*, which displays the fastest gliding motility (speeds of 1–3 $\mu m/s^{-1}$) (61). When grown under anoxic conditions, cell suspensions usually exhibit a greenish color because the cells contain chlorosomes containing BChl *c* with in vivo absorption maxima around 740 nm (**Figure 2*b***). Reduced sulfur compounds are not obligately required, which led to the former common name, green nonsulfur bacteria, for this lineage of chlorophototrophic *Chloroflexi*. All sequenced genomes of *Chloroflexus*

species contain genes encoding the enzymes of the 3-hydroxypropionate bi-cycle pathway for CO_2 fixation (14, 39, 92, 149, 150, 156). Autotrophic growth has been demonstrated in *Cfl. aurantiacus* strains OK-70-fl (DSM 636) (73) and *Chloroflexus* sp. MS-G (156).

Oscillochloris, *Chloronema*, and "*Candidatus* Chloroploca asiatica" (*Oscillochloridaceae*) are mesophilic, filamentous, freshwater bacteria that contain gas vesicles and an outer sheath. They form a distinctive, monophyletic clade separate from the thermophilic *Chloroflexaceae*. Cultures have a green or yellowish-green color, and cells contain chlorosomes and BChls *a* and *c* as photosynthetic pigments. The type genus and species is *Oscillochloris trichoides* (90); a 4.3-Mbp draft genome, as well as a detailed comparison of the genes for the photosynthetic apparatus and synthesis of BChls *a* and *c*, is available for this strain (55, 99). In contrast to other FAPs and in addition to the common photoheterotrophic growth of this group, members of this family grow photolithoautotrophically using the Calvin-Benson-Bassham cycle (reductive pentose phosphate pathway) (81, 90). The BChl *d*–containing *Chloronema giganteum*, isolated from a freshwater lake in Russia, was validly described but unfortunately the culture was lost (32). Recently, several phylogenetically similar, mesophilic FAP bacteria forming short, nonmotile, bundle-forming trichomes, "*Candidatus* Chloroploca asiatica", have been isolated in enrichment cultures from alkaline lakes and a sulfide spring in Russia (54).

Two other green FAPs have been identified. "*Candidatus* Chlorothrix halophila" was obtained as an enrichment culture from a hypersaline microbial mat in Guerrero Negro, Mexico (91) (**Supplemental Figure 6**); additionally, a novel green FAP, provisionally termed "*Candidatus* Chloranaerofilum corporosum," was recently found in a hot spring microbial mat in Yellowstone National Park (154, 157, 159). It was first detected in metagenomic analyses of the orange-colored undermat, and phylogenetic analysis of the 16S rRNA sequence as well as conserved signature indels determined it is a member of the suborder *Chloroflexineae* (57, 154). A partial genome sequence for "*Candidatus* C. corporosum" is publicly available in RAST (**http://rast.nmpdr.org/**; ID 6666666.236756) (157). This organism is only distantly related to known species of the phylum *Chloroflexi* (<91% nucleotide identity) and cannot reliably be affiliated with any member of the two families of this suborder. A partial genome for this organism was obtained through metagenomic analyses, and an enrichment culture has been obtained (154, 157; M. Tank, unpublished results). The novel FAP bacterium contains BChls *a* and *c* and grows at 52°C as long filaments of single cells that have a relatively large diameter of ~2 μm and are separated by obvious septa (154). Suggesting a capacity for photoautotrophic or photomixotrophic growth, the partial genome contains genes encoding for enzymes of the 3-hydroxypropionate bi-cycle. However, autotrophic growth has not yet been tested, and this strain is currently maintained under photoheterotrophic growth conditions.

Supplemental Material

In contrast to green FAPs, red FAPs currently do not form a monophyletic cluster (**Supplemental Figure 6**). The only available cultures of red FAP belong to the genus *Roseiflexus* (family *Roseiflexaceae*). However, additional red FAPs include *Heliothrix oregonensis* (128), whose type strain has been lost, and the uncultured species "*Candidatus* Roseilinea gracile" (see below). Red FAPs are (moderately) thermophilic and have been found in slightly sulfidic (<0.5 mM sulfide) hot springs, where they form orange-, reddish-, or pink-colored mats. These FAPs are characterized by the absence of BChl *c* and chlorosomes; their photosynthetic pigments are composed solely of BChl *a* and carotenoids (62, 128, 154, 157, 162). *Roseiflexus* (*Rof.*) *castenholzii*, the only validly described species of the genus *Roseiflexus*, was isolated from Nakabusa Hot Springs, Japan, where it forms a distinct, dense red or pink layer underneath mats of *Cyanobacteria* and *Chloroflexus* spp. at temperatures of 45.5–68.5°C at pH 7.8–8.2, with optimal growth at ~50°C and pH 7.5–8.0 in the laboratory (62). *Rof. castenholzii* grows photoheterotrophically under light anoxic conditions as well as chemotrophically under dark oxic conditions. Similar members of this genus have been observed or isolated from hot springs in

Yellowstone National Park (8, 154, 157, 159, 162). For example, *Roseiflexus* sp. RS-1 was isolated from the microbial mat of Octopus Spring (162). It was thoroughly characterized, and its genome was sequenced. However, it was never validly described as a new species, although its 16S rRNA gene sequence was only 96% identical to that of *Rof. castenholzii*. The original culture was lost, but closely related strains are available (M. Tank, V. Thiel & D.A. Bryant, unpublished data). Like *Rof. castenholzii*, the Yellowstone isolates contain BChl *a*, lack BChl *c* and chlorosomes, and grow photoheterotrophically or chemoheterotrophically under dark aerobic conditions. Photoautotrophic growth has not been observed in the laboratory, but the presence of genes encoding all necessary enzymes for the 3-hydroxypropionate bi-cycle as well as stable carbon isotopic compositions of FAP biomarkers suggest that photoautotrophic or photomixotrophic growth may occur in situ (92, 93, 162). Interestingly, *Roseiflexus* sp. RS-1 as well as "*Ca*. R. gracile" also contain rhodopsin genes with phylogenetic affiliation to light-driven, xanthorhodopsin-like H^+ pumps, which indicates the coexistence of retinal-based phototrophy and chlorophototrophy (94, 157). Another poorly described member of red FAP bacteria is the type isolate of Eikelboom morphotype 1851, *Kouleothrix aurantiaca*. On the basis of its 16S rRNA gene sequence, the presence of genes for chlorophototrophy, and its draft genome sequence, *K. aurantiaca* has been tentatively classified as a new member of the red FAPs (*Roseiflexineae*) (**Supplemental Figure 6** and **Supplemental Table 1**) (166).

Supplemental Material

Currently characterized members of the class *Anaerolineae* are exclusively achlorophyllous, chemoheterotrophic organisms. The possible existence of a chlorophototrophic member of the *Anaerolineae* was initially suggested by metagenomic analyses of hot spring microbial mats from Yellowstone National Park (94, 157). A metagenomic bin representing an *Anaerolinea*-like organism contains genes encoding a type-2 RC as well as enzymes for the synthesis of BChl *a*, but not BChl *c*, and two putative rhodopsin genes (157). The partial genome sequence for organism, provisionally named "*Candidatus* Roseilinea gracile," is publicly available in RAST (**http://rast.nmpdr.org/**; ID 6666666.201053 (157). In fresh samples from the hot spring microbial mats as well as in enrichments in the laboratory, very thin filaments (~0.2 μm in width) are observed with lengths of ~15–50 μm, and these filaments exhibit autofluorescence from BChl *a*, but not BChl *c*, as observed for *Roseiflexus* spp. strains (154). The taxonomic position of "*Candidatus* Roseilinea gracile" has recently been clarified by metagenomic studies of several closely related organisms from hot spring mat communities. These analyses indicate that "*Candidatus* R. gracile" is a member of a proposed ninth class, a sister class to *Anaerolineae*, in the phylum *Chloroflexi* (166).

6. ANOXYGENIC CHLOROPHOTOTROPHS: MEMBERS OF THE PHYLA *ACIDOBACTERIA*, *FIRMICUTES*, AND *GEMMATIMONADETES*

The three most recent phyla confirmed to contain chlorophototrophic bacteria are *Firmicutes*, *Acidobacteria*, and *Gemmatimonadetes* (**Table 1**, **Figure 1**, and **Supplemental Figure 7**). The phylum *Firmicutes* harbors the strictly anaerobic, anoxygenic photoheterotrophic heliobacteria that were discovered in 1983 (48). *Cab. thermophilum* (12) and *G. phototrophica* (172) were discovered less than 10 years ago and are presently the only described chlorophototrophic species in the *Acidobacteria* and *Gemmatimonadetes*, respectively (**Table 1** and **Supplemental Table 1**). A brief summary of major characteristics of these unusual chlorophototrophs follows.

6.1. Heliobacteria

Heliobacteria are chlorophototrophic members of the family *Heliobacteriaceae* within the phylum *Firmicutes* (class *Clostridia*, order *Clostridiales*) that contain BChl *g* in addition to small amounts

of 8^1-hydroxy-Chl *a* (63, 109, 110). They were discovered by serendipity when an unintended change in a standard enrichment medium (using deionized water instead of tap water) led to the enrichment and isolation from a soil sample of a brownish-green (**Figure 2*b***), nitrogen-fixing, spore-forming rod, *Heliobacterium chlorum* (48). Currently, in addition to the provisional taxon "*Candidatus* Heliomonas lunata," 11 validly described species of four heliobacterial genera are known (**Table 1** and **Supplemental Table 1**) (110). Like other members of the *Firmicutes*, heliobacteria have a Gram-positive cell wall with a thick layer of peptidoglycan and no outer membrane, and they form heat-resistant endospores that remain viable during stressful environmental conditions. Unlike all other *Firmicutes*, heliobacteria synthesize homodimeric, type-1 RCs, which constitute the simplest known photosynthetic apparatus, the structure of which was recently solved by X-ray crystallography (50). Heliobacterial cells lack both intracytoplasmic membranes and additional light-harvesting antenna complexes, and they are very sensitive to oxygen, which oxidizes BChl *g* (63). Neutrophilic heliobacteria can be found in various soils, including those associated with hot springs, and are classified into three genera: the type genus *Heliobacterium* with five described species as well as the two single-species genera *Heliobacillus* and *Heliophilum* (**Supplemental Table 1**). Alkaliphilic heliobacteria form a monophyletic cluster based on 16S rRNA gene sequences and are members of the genera *Heliorestis* and "*Candidatus* Heliomonas." The model organism for this group is *Heliobacterium modesticaldum*, which was isolated from a hot spring microbial mat in Iceland and is the only heliobacterial species whose genome has been sequenced (133). At 3.08 Mbp, it is moderate in size, and compared with most clostridia, it is notably high in GC content (57% versus ~21% to 54%). Consistent with its photoheterotrophic lifestyle, no genes encoding any carbon fixation pathway were found except for an incomplete reverse tricarboxylic acid cycle (110, 133, 150).

Supplemental Material

6.2. Chloracidobacteria

Cab. thermophilum is the first and currently the only chlorophototrophic member of the diverse but poorly characterized phylum *Acidobacteria*. *Cab. thermophilum* was first detected by metagenomic sequencing of microbial mats associated with slightly alkaline hot springs in Yellowstone National Park. Although early 16S rRNA gene surveys of these mats indicated the presence of an acidobacterium, identification of a bacterial artificial chromosome sequence containing both the rRNA operon and functional genes specific for chlorophototrophy, as well as the establishment of an enrichment culture, demonstrated that the acidobacterium was an obligate chlorophototroph (12, 154). With this enrichment culture, researchers were able to analyze the photosynthetic apparatus as well as the genome of the organism (45–47, 161). The total genome is 3.7 Mbp and comprises two chromosomes of 2.68 and 1.01 Mbp (45). Combined with classical microbiological techniques, knowledge gained from the genome and a diel metatranscriptomic analysis in situ (104) led to the isolation of an axenic culture and subsequent formal description of this new species (**Figure 2*b***) (151, 152).

Cab. thermophilum is unusual in several respects. It is the first oxygen-requiring ABC bacterium that contains a homodimeric type-1 RC, chlorosomes, and the Fenna-Matthews-Olson protein, which were previously known to occur only in strictly anaerobic GSB (45–47, 161). Interestingly, a second phototroph with a similar photosynthetic apparatus and the same oxygen relationship, "*Ca*. Thermochlorobacter aerophilum," was subsequently discovered in the same microbial mat (**Figure 4**) (104, 154) (see Section 4). In addition to the PscA and PscB polypeptides of the RC, *Cab. thermophilum* produces CbpA, an abundant 22-kDa carotenoid-binding protein that may be involved in photoprotection and/or light harvesting of the RC (161). BChl *c* and keto-carotenoids are the major light-harvesting pigments in the chlorosomes, whereas BChl *a*, Zn-BChl a′, and Chl *a* are found in the RC (**Figure 4*d***) (46, 47, 161). The presence of chlorosomes and BChl *c*

indicates that *Cab. thermophilum* is adapted to low light; it thrives at irradiance values between 20 and 50 μmol photons m^{-2} s^{-1}. *Cab. thermophilum* does not grow under anoxic conditions and requires oxygen for essential enzymatic reactions (e.g., for BChl and carotenoid biosynthesis and the synthesis of tyrosine from phenylalanine). Although this photoheterotroph lacks key enzymes of all known carbon fixation pathways, bicarbonate is essential for growth and is presumably used in anaplerotic reactions including the synthesis of 2-oxoglutarate from succinyl-CoA (151, 152). As inferred from genomic analysis, *Cab. thermophilum* strictly depends on all three branched-chain amino acids, lysine, and vitamin B_{12} as well as reduced sulfur sources (e.g., thioglycolate, cysteine/methionine, sulfur, or thiosulfate) for growth. Surveys show that *Cab. thermophilum* is found in slightly alkaline hot spring microbial mats around the world at temperatures between 40°C and 68°C (16, 60, 107, 151, 152; M. Tank, unpublished results). Analyses of the 16S rRNA genes of *Chloracidobacterium* spp. strains obtained from different locations suggest that several new species may occur in the genus *Chloracidobacterium* (**Supplemental Figure 7**). Cultivation experiments with different axenic strains of *Cab. thermophilum* from Mushroom Spring (Yellowstone National Park) as well as 16S rRNA surveys indicate the occurrence of ecotypes with different adaptations to temperature and perhaps other environmental factors (113, 152, 154).

Supplemental Material

6.3. *Gemmatimonas phototrophica*

The bacterial phylum *Gemmatimonadetes* has recently been shown to contain a novel ABC member. Only the *G. phototrophica* AP64 strain (**Figure 2*d***) has been described to date (**Supplemental Figure 7**). It was identified by high-throughput fluorescence screening and isolated from a freshwater lake in the Gobi Desert in North China in 2011 (172–174). The discovery of this novel, aerobic, anoxygenic photoheterotrophic bacterium extended the number of bacterial phyla containing chlorophototrophic members from six to seven. *G. phototrophica* is a mesophilic oligotroph; the slow-growing, red-colored, rod-shaped bacterium is most closely related to *Gemmatimonas aurantiaca*, an aerobic chemoheterotroph. *G. phototrophica* contains BChl *a* and a fully functional type-2 RC. In this organism, chlorophototrophy acts only as an auxiliary energy source that amends a chemoheterotrophic lifestyle, making this bacterium a mesophilic, aerobic, facultative photoheterotroph. The *G. phototrophica* genome is 4.7 Mbp (**Supplemental Table 2**), and it encodes all genes for chlorophototrophy in a 42.3-kb gene cluster, which appears to have resulted from HGT from a chlorophototrophic proteobacterium (172). According to metagenomic database analyses, chlorophototrophic *Gemmatimonadetes* occur in diverse freshwater and terrestrial environments, where they account for up to 12% of chlorophototrophic microbial communities; however, related organisms have not yet been detected in marine habitats (171–174). Analyses of *G. phototrophica* have provided the first clear evidence for exchange of chlorophototrophy by HGT between distantly related taxonomic bacterial groups, which provides new insights into the evolution of bacterial photosynthesis. Evidence of HGT by plasmid transfer among more closely related strains of marine *Roseobacter* spp. (*Alphaproteobacteria*) has also been reported (126). Finally, a possible example of HGT of genes related to chlorophototrophy between a *Sphingomonas*-like organism (AAPB; *Alphaproteobacteria*) and a member of the phylum *Bacteriodetes*, *Hymenobacter* sp. isolate R-68361, has recently been described (147). If this isolate is able to synthesize BChl *a* and produce functional RCs, *Bacteriodetes* will become the eighth bacterial phylum with members that can perform chlorophototrophy.

7. OUTLOOK

Genomic methods, including metagenomics, metatranscriptomics, and single-cell genome sequencing, are leading to a very rapid increase in our knowledge of the ecophysiology, metabolism,

and evolution of chlorophototrophic bacteria. In combination with traditional microbiological methods, the information gained from molecular studies can provide key insights for the cultivation of novel chlorophototrophs (and other bacteria as well). Further surveys of microbial communities that occur in strong sunlight, especially those with complex microbial mat communities, are likely to reveal additional chlorophototrophs as well as new symbioses with other bacteria and eukaryotes. Finally, the rapid expansion of molecular data is creating new opportunities to build rational and lasting taxonomies for cyanobacteria, which have long been problematic in this regard.

SUMMARY POINTS

1. Chlorophyll-dependent phototrophs, i.e., chlorophototrophs, occur in seven bacterial phyla: *Cyanobacteria*, *Proteobacteria*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, *Acidobacteria*, and *Gemmatimonadetes*.
2. Thanks to genomic sequencing of isolates as well as metagenomic methods, more than 1,100 chlorophototroph genomes have been sequenced during the past 20 years.
3. Genomic data are leading to unprecedented insights into many important processes, including photosynthesis, nitrogen and carbon fixation, cellular differentiation and development, symbiosis, and ecosystem functionality.
4. The combination of genomic information with traditional microbiological methods provides powerful insights for the cultivation and isolation of novel (phototrophic) microorganisms.
5. Metagenomic analyses are revealing that chlorophototrophic microbial communities are remarkably complex and can comprise dozens of species and hundreds (or thousands) of ecotypes, which contribute to ecosystem function and robustness.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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