Minireview

Anoxygenic phototrophic bacteria from extreme environments

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Abstract

A diverse group of anoxygenic phototrophic bacteria thrive in habitats characterized by extremes of temperature, pH, or salinity. These 'extremophilic' anoxygenic phototrophs are optimally adapted to the conditions of their habitats and are ideal model systems for defining the physiochemical limits of photosynthesis. Extremophilic phototrophs have provided new insight into the evolution of photosynthesis and play ecological roles as primary producers in their unusual habitats.

Abbreviations: BChl – bacteriochlorophyll; LH – light-harvesting; t_{opt} – optimal temperature for growth

Species of anoxygenic phototrophic bacteria that inhabit extreme environments have become increasingly popular as models for the study of basic problems in photosynthesis. These organisms are the 'extremophilic' phototrophs. In this minireview I will describe extremophilic anoxygenic phototrophs, including a brief history of their discovery and the extent of their diversity. I conclude with a discussion of how these organisms have benefited our understanding of photosynthesis and their relevance to issues of photosynthetic diversity, evolution, and ecology. Coverage of the major groups of extremophilic anoxygenic phototrophs will proceed along a historical path, with the exception of the acidophilic phototrophs, about which little is currently known. The acidophiles will be considered near the end of this review along with psychrophilic species, the most recent extremophilic anoxygenic phototrophs to have been isolated.

What is an extreme environment and what is an extremophile?

From a microbiological standpoint, extreme environments are habitats that we as humans consider extreme (Madigan and Marrs 1997; Madigan 2000). These include environments in which physical and chemical conditions differ significantly from those that humans consider 'normal': pH 7, moderate temperatures, and low salinity. I will consider an environment to be extreme if it displays, on a relatively constant basis; (1) a pH of less than 6 or greater than 8; (2) a temperature above 45 °C or below 20 °C; or (3) a salinity of greater than 4% w/v NaCl.

The issue of *constancy* is important here. Truly extreme environments are typically constant in their physiochemical properties, and thus the extreme is not simply a transient condition but rather an inherent part of the ecosystem. Indeed, it is this aspect of constancy that in large part forces an extremophilic lifestyle on the indigenous microflora. As a physiological response to this constancy, extremophilic prokaryotes typically show *optimal* growth under the extreme condition. That is, extremophiles do not just *tolerate* their extreme condition, but actually *require* it for optimal growth and metabolism (Madigan and Marrs 1997).

Extremophilic anoxygenic phototrophic bacteria

An early indication that phototrophic microorganisms inhabited extreme environments came from studies over 100 years ago by the Japanese botanist Miyoshi (1897) who showed that certain Japanese warm springs supported blooms of purple sulfur bacteria. Since that time a diverse array of thermophilic, halophilic, akaliphilic, haloalkaliphilic, acidophilic, and psychrophilic anoxygenic phototrophs have been isolated and characterized that grow optimally under the conditions defined previously for extreme environments.

The first extremophilic anoxygenic phototrophs to be discovered were in the 1960s and were either halophiles or acidophiles, including extremely halophilic species of the purple bacterium *Ectothiorhodospira*. At about the same time, *Chloroflexus aurantiacus* was discovered (the description papers on *Chloroflexus* were published in 1974 but the evidence that this organism was an anoxygenic phototroph was clear from a paper in 1971). Into the late 1970s, several new halophilic and haloalkaliphilic purple bacteria were discovered. In the 1980s and early 1990s, new thermophilic anoxygenic phototrophs were isolated, including *Thermochromatium tepidum* and *Chlorobium tepidum*. In the mid-1990s thermophilic heliobacteria were first described, and since then, a large diversity of alkaliphilic anoxygenic phototrophs have been isolated. Psychrophilic phototrophs have been described only very recently, with two representatives currently in culture.

I now elaborate on this overview with specific examples in each category of extremophiles (Table 1). I begin with the thermophiles and the filamentous phototroph *Chloroflexus*, an organism that has had a profound impact on the field of photosynthesis.

Thermophiles

Chloroflexus
Chloroflexus aurantiacus was the first thermophilic anoxygenic phototroph to be isolated. The discovery and most of the pioneering work on *Chloroflexus* was done by Beverly Pierson and Richard Castenholz (University of Oregon) in the late 1960s and early 1970s (Pierson and Castenholz 1971), leading to a detailed description of the species *C. aurantiacus* in 1974 (Pierson and Castenholz 1974a, b). The discovery of *Chloroflexus* introduced a new paradigm to the field of green bacteria. Suddenly, a green

bacterium was available that could respire in darkness, making possible studies of bioenergetic options and pigment/chlorosome biosynthesis. Indeed, compared to green sulfur bacteria such as *Chlorobium*, the metabolic and nutritional diversity of *Chloroflexus* (Madigan et al. 1974) seemed amazing. [Historical note: at the time of the discovery of *Chloroflexus* the science of molecular phylogeny was in its infancy; thus, this organism was considered, primarily on phenotypic grounds, such as its production of bacteriochlorophyll (BChl) *c*, to be a filamentous 'green bacterium'].

Although sharing pigment and ultrastructural features with green sulfur bacteria, we now know that *C. aurantiacus* is phylogenetically distinct from this group, forming its own deep lineage within the domain *Bacteria* (Oyaizu et al. 1987). Moreover, although *Chloroflexus* shares with green bacteria the ability to grow photoautotrophically using sulfide as electron donor (Madigan and Brock 1975), it does so employing a unique pathway for autotrophic CO_2 fixation, the hydroxypropionate pathway (Herter et al. 2002). This pathway is absent from all other anoxygenic phototrophic bacteria. Considering the apparent antiquity of *Chloroflexus* (Oyaizu et al. 1987), it is possible that the hydroxypropionate pathway was the first autotrophic pathway to have evolved in anoxygenic phototrophs.

Organisms that morphologically resemble *Chloroflexus* have been observed in stromatolites, ancient laminated microbial mats whose modern counterparts typically contain various *Chloroflexus* relatives (Nübel et al 2001; Ward et al. 1989). Thus, it is possible that *Chloroflexus*-like organisms were among the earliest, if not *the* earliest, phototrophic organisms on Earth. Since the discovery of *C. aurantiacus*, several other thermophilic or halophilic *Chloroflexus*-like organisms have been identified (Boomer et al. 2000, 2002; Gich et al. 2001; Nübel et al. 2001; Pierson and Castenholz 1995; Pierson et al. 1994) or isolated in pure culture (Hanada et al. 1995, 2002). At least one of these, *Roseiflexus castenholzii*, lacks BChl *c* and chlorosomes and is phylogenetically quite distinct from green (BChl *c*-containing) *Chloroflexus* species (Hanada et al. 2002). The filamentous phototroph *Heliothrix* (Pierson et al. 1984, 1985) shows similar properties to that of *Roseiflexus*, but this organism was not obtained in pure culture and thus little more is known about it.

From these studies it can be concluded that phenotypically diverse but phylogenetically related filamentous anoxygenic phototrophic bacteria exist in

Table 1. Extremophilic anoxygenic phototrophic bacteria

Class	Organism	Phylogenetic group	Bacterio-chlorophyll	Habitat	Special properties	Reference[h]
Thermophiles[a]	*Chloroflexus aurantiacus*	Green nonsulfur bacteria	c_s, a	Neutral to alkaline hot springs; marine microbial mats	Optimum 55 °C; growth up to 70 °C; aerobic and phototrophic growth; earliest phylogenetic lineage of phototrophs	Pierson and Castenholz 1974a, b
	Chloroflexus aggregans	Green nonsulfur bacteria	*c, a*	Japanese hot spring	Optimum 55 °C; growth up to at least 60 °C; differs in 16S rRNA sequence from *C. aurantiacus* by >5%	Hanada et al. 1995
	Roseiflexus castenholzii	Green nonsulfur bacteria	*a*	Alkaline Japanese hot spring	Optimum 50 °C; lacks Bchl *c* and chlorosomes; growth up to 60 °C; phylogenetically distinct from *C. aurantiacus*	Hanada et al. 2002
	Thermochroma-tium tepidum	Gamma proteo-bacteria	*a*	Neutral hot springs contain-ing sulfide, Yellowstone and other thermal areas	Optimum 49 °C; growth up to 56 °C; contains B920 LH I photocomplex	Madigan 1984, 1986; Imhoff et al. 1998b
	Chlorobium tepidum	Green sulfur *bacteria*	*c, a*	Acidic high sulfide hot springs, New Zealand and Yellowstone	Optimum 48 °C; growth up to 55 °C; very rapid growth; genome sequenced	Wahlund et al. 1991; Eisen et al. 2002
	Heliobacterium modesticaldum	Low GC gram-positive *Bacteria*	*g*[f]	Neutral to alkaline hot springs and volcanic soils	Optimum 52 °C; growth up to 57 °C; produces endospores	Kimble et al. 1995
Halophiles[b]	*Halochromatium salexigens*	Gamma proteobacteria	*a*	Saltern Salin de Giraud, France	Optimum 8–11% NaCl; growth up to 20%	Caumette et al. 1988

Table 1. Continued

Class	Organism	Phylogenetic group	Bacterio-chlorophyll	Habitat	Special properties	Reference[h]
	Halochromatium glycolicum	Gamma proteobacteria	*a*	Solar Lake, Sinai	Optimum 4–6% NaCl; growth up to 20%	Caumette et al. 1997; Imhoff et al. 1998b
	Marichromatium purpuratum	Gamma proteobacteria	*a*	Marine sponges; seawater	Optimum 5% NaCl; growth up to 7%	Imhoff and Trüper 1980
	Halorhodospira halophila	Gamma proteobacteria	*a*	Summer Lake, Oregon; Wadi Natroun soda lakes, Egypt	Optimum 15% NaCl; growth up to 30%	Raymond and Sistrom 1967, 1969; Imhoff and Suling 1996; Imhoff et al. 1978
	Thiohalocapsa halophila	Gamma proteobacteria	*a*	Saltern Salin-de-Giraud, France	Optimum 7% NaCl; growth up to 20%	Caumette et al. 1991; Imhoff et al. 1998b
	Rhodobium orientis	Alpha proteobacteria	*a*	Marine tidal pool	Optimum 4–5% NaCl; growth up to 8%	Hiraishi et al. 1995
	Rhodothalassium salexigens	Alpha proteobacteria	*a*	Evaporated seawater pool, Oregon coast, USA	Optimum 7% NaCl; growth up to 20%	Drews 1981; Imhoff et al. 1998a
	Rhodovibrio salinarum	Alpha proteobacteria	*a*	Portugese saltern	Optimum 4% NaCl; growth up to 20%	Nissen and Dundas 1984; Imhoff et al. 1998a
	Rhodovibrio sodomensis	Alpha proteobacteria	*a*	Dead Sea	Optimum 12% NaCl; growth up to 20%	Mack et al. 1993); Imhoff et al. 1998a
	Roseospira mediosalina	Alpha proteobacteria	*a*	Saline sulfur spring, Azebaijan	Optimum 4–7% NaCl; growth up to 15%	Kompantseva and Gorlenko 1984; Imhoff et al. 1998a
	Rhodovulum euryhalina	Alpha proteobacteria	*a*	Marine	Optimum 1–12% NaCl; growth up to 20%[g]	Kompantseva 1986; Hiraishi and Ueda (1994)
Alkaliphiles[c]	*Ectothiorhodo-spira haloalkali-phila*	Gamma proteobacteria	*a*	Soda lakes, Wadi Natroun, Egypt	Optimum pH 9; growth up to pH 10.5	Imhoff and Suling 1996; Imhoff et al. 1978
	Ectothiorhodo-spira vacuolata	Gamma proteobacteria	*a*	Soda lakes, Wadi Natroun, Egypt	Optimum pH 9; growth up to pH	Imhoff et al. 1981

Table 1. Continued

Class	Organism	Phylogenetic group	Bacterio-chlorophyll	Habitat	Special properties	Reference[h]
					10	
	Halorhodospira abdelmalekii	Gamma proteobacteria	*b*	Soda lakes, Wadi Natroun, Egypt	Optimum pH 8.5–9; growth up to pH 10.5	Imhoff and Trüper 1981
	Halorhodospira halochloris	Gamma proteobacteria	*b*	Soda lakes, Wadi Natroun, Egypt	Optimum pH 8.5–9; growth up to pH 10.5	Imhoff and Trüper 1977
	Heliorestis daurensis	Low GC Gram-positive *Bacteria*	*g*[f]	Lake Barun Torey, Siberia, Russia	Optimum pH 9; growth up to pH 10.5	Bryantseva et al. 1999a
	Heliorestis baculatum	Low GC Gram-positive *Bacteria*	*g*[f]	Lake Ostozhe, Siberia, Russia	Optimum pH 9; growth up to pH 10.5	Bryantseva et al. 2000b
	Thioalkalicoccus limnaeus	Gamma proteobacteria	*b*	Lake Verkhneye Beloe, Siberia, Russia	Optimum pH 9; growth up to pH 10.5	Bryantseva et al. 2000a
	Thiorhodospira sibirica	Gamma proteobacteria	*a*	Lake Malyi Kasytri, Siberia, Russia	Optimum pH 9; growth up to pH 10.5	Bryantseva et al. 1999b
	Rhodobaca bogoriensis	Alpha proteobacteria	*a*	Lake Bogoria, African Rift Valley, Kenya	Optimum pH 9; growth up to pH 10.5; lacks LH II	Milford et al. 2000
Acidophiles[d]	*Rhodoblastus acidophila*	Alpha proteobacteria	*a*	Acidic marsh-waters, soils, and lakes	Optimum pH 5.8; growth down to pH 4.8	Pfennig 1969; Imhoff 2001b
	Rhodophila globiformis	Alpha proteobacteria	*a*	Acidic warm sulfur springs, Yellowstone National Park	Optimum pH 5; growth down to pH 4.2	Pfennig 1974; Imhoff et al. 1984; Madigan et al. 2002
Psychrophiles[e]	*Rhodoferax antarcticus*	Beta proteobacteria	*a*	Microbial mats and lakewater, McMurdo Dry Valleys, Antarctica	Optimum growth temperature, 18 °C; growth down to 0 °C; no growth at 25 °C	Madigan et al. 2000

[a]Growth temperature optimum > 45 °C.
[b]Salinity optimum ≥ 4% NaCl.
[c]Growth pH optimum of 9 or greater. Some species listed are also halophiles. See also Figure 1, top left and right.
[d]Growth pH optimum < 6.
[e]Growth temperature optimum below 20 °C. See also Figure 1, bottom left and right.
[f]*Heliobacterium chlorum* has been shown to contain 8 (1) OH-chlorophyll *a* (Neerken and Ames 2001). Presumably this is true of other heliobacteria including the alkaliphilic species, but this has not been studied.
[g]In the original description of this organism (then named *Rhodobacter euryhalinus*), several strains were described. Some strains, such as the type strain, showed salinity optima below 3%, while others showed optima of from 5 to 12% (Kompantseva 1986). However, it is unclear whether all strains described in this study were of a single species. In retrospect, this seems unlikely.
[h]Only the primary reference(s) to the description of each organism is listed.

microbial mats that develop in hot springs, marine and alkaline environments. Based on electron micrographs of enrichment cultures, hypersaline environments in particular may contain many interesting new species (Pierson et al. 1994). In addition, although not extreme environments, freshwater lakes also contain *Chloroflexus*-like organisms (Gorlenko 1976; Pierson and Castenholz 1995; Gich et al. 2001).

There is little doubt that *Chloroflexus aurantiacus* has had a major impact on several areas of anoxygenic photosynthesis research. This organism revolutionized the study of many key aspects of green bacteria biology, in particular those of chlorosome biosynthesis (chemotrophically grown cells of *C. aurantiacus* lack chlorosomes and when switched to phototrophic conditions the steps in chlorosome synthesis could be tracked), autotrophic metabolism, and metabolic diversity. These topics are nicely reviewed in Pierson and Castenholz (1995). *C. aurantiacus* also has the distinction of being the most thermophilic of all known anoxygenic phototrophs, with growth occurring in some strains up to 70 °C (Pierson and Castenholz 1974a, b). It appears that this is the upper temperature limit for photosynthesis by anoxygenic phototrophs, and is near the limit for oxygenic photosynthesis (74 °C, Ward and Castenholz 2000).

Thermochromatium *and other thermophilic purple bacteria*

Following the descriptive work of Miyoshi (1897), mentioned previously, *Chromatium*-like organisms were identified from certain Yellowstone microbial mats (Castenholz 1969, 1977). But it was not until the early 1980s that *Thermochromatium* (originally *Chromatium*) *tepidum* was isolated in pure culture (Madigan 1984, 1986, 1993; Imhoff et al. 1998b). This phototroph is mildly thermophilic (t_{opt} ~50 °C) and contains a novel light-harvesting (LH) I photopigment complex that absorbs maximally near 920 nm (Garcia et al. 1986; Nozawa et al. 1986). Until recently (Glaser and Overmann 1999; Permentier et al. 2001), this was the longest wavelength-absorbing photocomplex containing BChl *a* known in anoxygenic phototrophs.

The *T. tepidum* LH I core antenna complex has been extensively studied in connection with the mechanism of energy transfer to the reaction center. A biophysical problem exists in this regard in that the absorption maximum of the LH I complex lies much more to the red than that of the reaction center. Despite this peculiarity, efficient energy transfer from the core antenna to the reaction center occurs (Kramer

and Amesz 1996). The photosynthetic reaction center of *T. tepidum* is similar in most respects to that of other purple bacteria, except for its increased thermal stability (Nozawa and Madigan 1991). To probe the mechanism behind this, the *T. tepidum* reaction center was crystallized, and key amino acid substitutions have been identified that may confer thermostability (Nogi et al. 2000). In addition, a thermophilic ribulose bisphosphate carboxylase (RubisCO, key enzyme of the Calvin cycle) was characterized from *T. tepidum* and shown to be thermostable up to at least 60 °C (Heda and Madigan 1988, 1989). However, the unusual light absorption properties of *T. tepidum* have been the focus of research on this organism in recent years and will likely continue to be so into the future. The genome of *T. tepidum* is currently being sequenced (M. Fonstein, Applied Genomics, personal communication), and when the genetic blueprint of this organism is available it should open additional avenues of research on this phototroph, especially in the area of thermostable enzymes.

Other mildly thermophilic purple bacteria (growth t_{opt} about 40 °C) have been cultured from hot spring microbial mats. These include the BChl *b*-containing species *Rhodopseudomonas* sp. strain GI, isolated from a New Mexico hot spring (Resnick and Madigan 1989), and *Rhodopseudomonas cryptolactis* (Statwald-Demchick et al. 1990) and *Rhodospirillum centenum* (*Rhodocysta centenaria*) (Favinger et al. 1989), both isolated from a Thermopolis (Wyoming, USA) hot spring. *R. centenum* in particular has been useful as a model organism for biochemical/genetic research on phototaxis and related issues of motility (see, for example, McClain et al. 2002).

Chlorobium tepidum

Chlorobium tepidum is a thermophilic (t_{opt} ~48 °C) green sulfur bacterium (family *Chlorobiaceae*). Strains of this organism have been isolated from relatively high sulfide (0.5–1 mM), acidic (pH 4–6) hot springs in Rotorua, New Zealand (Castenholz 1988; Castenholz et al. 1990; Wahlund et al. 1991), and Yellowstone National Park (Bedard et al. 2002).

For a number of reasons *C. tepidum* has found its way into the mainstream of research on green sulfur bacteria; indeed, it is now the organism of choice for biophysical, biochemical, and genetic studies of green bacteria. *C. tepidum* contains BChl *c*, grows rapidly (generation time about 2 h under optimal conditions) and uses thiosulfate as a photosynthetic electron donor (Wahlund et al. 1991). Dense cultures of $>5 \times 10^9$

cells/ml of *C. tepidum* are easily obtained overnight. In addition, the organism is a vigorous nitrogen-fixing bacterium (Wahlund and Madigan 1993). Since few examples of thermophilic nitrogen-fixing bacteria are known, the rapidly growing and high yielding *C. tepidum* presents an excellent opportunity for biochemical study of what should be a fairly heat stable nitrogenase (optimal nitrogenase activity occurs at 50 °C, Wahlund and Madigan 1993). Cells of *C. tepidum* also produce novel carotenoid glycosides (Takaichi et al. 1997). Since these carotenoids are absent from all non-thermophilic green sulfur bacteria, it is possible that they have functions relating to the thermophilic lifestyle of *C. tepidum*.

In addition to its ideal physiological properties for laboratory culture, the development of genetic transfer systems for *C. tepidum* based on conjugation (Wahlund and Madigan 1995) and transformation (Frigaard and Bryant 2001) offer powerful new tools for the study of green sulfur bacteria. Moreover, the genome sequence of *C. tepidum* is now complete (Eisen et al. 2002) (*C. tepidum* was the first anoxygenic phototroph to have its genome completely sequenced). These landmarks should stimulate rapid advances in the biochemical genetics of green bacteria, and the laboratory of Don Bryant, Penn State University, has already forged ahead in this area (Frigaard et al. 2002; Vassilieva et al. 2002). Several intriguing findings emerged from the genome sequence of *C. tepidum*, such as its nearly total lack of genes for regulatory functions, possession of RuBisco-like genes, and numerous archaeal homologs (Eisen et al. 2002). The *C. tepidum* genome sequence also revealed several unusual metabolic features involving sulfur and nitrogen metabolism, and genetically confirmed the reductive tricarboxylic acid cycle as the mechanism of CO_2 fixation in green bacteria (Eisen et al. 2002).

Heliobacteria

Heliobacteria were first discovered in the laboratory of Howard Gest in the early 1980s, and a fascinating historical account of this story can be found in Gest (1994). Heliobacteria are obligately anaerobic, endospore-forming anoxygenic phototrophs that produce BChl *g* and a form of green plant chlorophyll *a* (Amesz 1995; Madigan 2001a; Madigan and Ormerod 1995). Only one species of thermophilic heliobacteria is known, *Heliobacterium modesticaldum* (Kimble et al. 1995; Table 1). Strains of this organism have been isolated from both Icelandic and Yellowstone hot springs (Kimble et al. 1995; Stevenson et al. 1997). *H.*

modesticaldum resembles other heliobacteria in most aspects of its physiology, with the notable exception of its growth temperature optimum of 52 °C (Table 1). Like other heliobacteria, *H. modesticaldum* is an active N_2-fixing phototroph. And as previously mentioned regarding *Chlorobium tepidum*, examples of thermophilic nitrogen-fixing bacteria are rare. Thus the nitrogenase of *H. modesticaldum* might also be a good experimental subject for study of the biochemical basis of nitrogen fixation at high temperatures. In this connection, since *H. modesticaldum* can fix N_2 up to its growth temperature maximum of 56 °C (Kimble et al. 1995), its nitrogenase may actually be more thermostable than that of *C. tepidum*.

The photocomplexes of heliobacteria have been extensively studied because of their similarity to green plant Photosystem (PS) I (Amesz 1995; Neerken and Amesz 2001). Although *H. modesticaldum* has not been examined in this connection, one would predict that its PS I-like reaction center would be more thermostable than those of mesophilic heliobacteria. If true, this feature might be useful for purification of PS I-type reaction center complexes. Moreover, structural studies of purified *H. modesticaldum* reaction centers may yield insight into mechanisms of thermal stablility in PS I-type photocomplexes.

A summary of the basic properties of all of the thermophilic anoxygenic phototrophs discussed here can be found in Madigan (2001b) and Castenholz and Pierson (1995).

Halophiles, alkaliphiles, and haloalkaliphiles

More extremophilic anoxygenic phototrophs exist in these categories than in any of the others (Table 1). Some of these organisms have recently been reviewed (Imhoff 2001a), including both species that require high salt and those with only marine level salinity requirements.

In the 1970s and 1980s, Johannes F. Imhoff and Hans Georg Trüper (Bonn, Germany) isolated and characterized several halophilic and alkaliphilic purple bacteria (*Ectothiorhodospira* species) from soda lakes in the Wadi El Natroun in northwest Egypt. Following the advent of molecular phylogeny, many of these organisms were reclassified as *Halorhodospira* species (Imhoff and Suling 1996). These phototrophs contain either BChl *a* or *b*, are strongly alkaliphilic, and vary in their salinity requirements from marine to near saturating levels of NaCl, depending on the species (Table 1). From a phylogenetic standpoint these or-

ganisms cluster as a distinct clade within the gamma *Proteobacteria* and are related to purple sulfur bacteria that deposit sulfur inside the cell, such as *Chromatium* species (Imhoff and Suling 1996).

In addition to *Ectothiorhodospira* and *Halorhodospira* species, an assortment of halophilic but not alkaliphilic purple bacteria are also known from various hypersaline habitats throughout the world (Table 1). These include genera of purple sulfur bacteria such as *Halochromatium*, *Marichromatium*, and *Thiohalocapsa*, and purple nonsulfur bacteria such as *Rhodovibrio*, *Rhodothalassium*, *Rhodobium*, *Rhodovulum*, and *Roseospira* (Table 1). Many of these phototrophs have salt optima of about twice the salinity of seawater, although notable exceptions include the Dead Sea purple nonsulfur bacterium *Rhodovibrio sodomensis* (optimum growth at 12% NaCl), and the purple sulfur bacterium *Halochromatium salexigens* (optimum at 8–11% NaCl) (Table 1). *Halorhodospira halophila* is the most halophilic of the non-alkaliphiles, growing up to saturating levels of salt (Table 1).

In the mid to late 1990s, several new anoxygenic phototrophs have been isolated from soda lakes. These include not only purple bacteria, but also heliobacteria. Most of these purple bacteria differ dramatically from haloalkaliphilic *Halorhodospira* species in both phylogeny and physiology, requiring little if any NaCl for growth. These isolates, however, remain strongly alkaliphilic (Table 1). Many of these alkaliphilic phototrophs have been described as new genera, including *Rhodobaca* (Milford et al. 2000), *Thiorhodospira* (Bryantseva et al. 1999b), and *Thioalkalicoccus* (Bryantseva et al. 2000a) (all purple bacteria), and *Heliorestis* (Bryantseva et al. 1999a, 2000b) (heliobacteria) (Table 1). *Rhodobaca* is of particular interest because it lacks a peripheral (LH II) antenna photocomplex, a rarity among purple nonsulfur bacteria (Glaser and Overmann 1999), and produces several unusual carotenoids rendering phototrophic cultures of this organism yellow in color (Takaichi et al. 2001). *Rhodobaca* shows various metabolic peculiarities as well, including an inability to fix N_2 or to grow photoautotrophically, both of which are physiological hallmarks of purple nonsulfur bacteria (Milford et al. 2000).

Alkaliphilic heliobacteria (*Heliorestis* species) are currently represented with two species, *Heliorestis daurensis* and *Heliorestis baculata* (Table 1), and others in enrichment culture. A morphologically unusual coiled *Heliorestis* species was recently isolated from samples collected on an expedition to Lake Hamara, an Egyptian soda lake (Figure 1, top left and right) (Jung et al. 2002). This organism resembles cells present in the original enrichment cultures of *H. daurensis*, but differs dramatically in morphology from the pure culture of *H. daurensis* that was obtained (Bryantseva et al. 1999a). *H. daurensis* and the coiled heliobacterium are closely related phylogenetically, but differ in some physiological traits (Jung et al. 2002). Among other interesting properties, all alkaliphilic heliobacteria have been found to contain novel carotenoid glycosides absent from nonalkaliphilic heliobacteria (Takaichi et al. 2003). It is thus possible that these pigments are required for photosynthesis in heliobacteria under the extreme condition of high pH.

Haloalkaliphilic and alkaliphilic purple bacteria/heliobacteria typically show growth pH optima around 9, pH maxima about 10 (or slightly above), and are unable to grow at neutral pH (Table 1). Although some haloalkaliphilic *Archaea* such as *Natronobacterium* are more alkaliphilic than this (Tindall 2001), these anoxygenic phototrophs are among the most alkaliphilic, and in some cases the most halophilic, of all known *Bacteria* (Imhoff 1988, 1992). For these and other reasons, for example, their unique phylogeny (Imhoff and Süling 1996; Madigan 2001a), they should be of special interest to basic studies of photosynthesis.

Psychrophiles and acidophiles

The list of cold-loving and acid-loving anoxygenic phototrophs is thus far relatively short (Table 1). In terms of acidophiles, only two species, both purple nonsulfur bacteria, meet the previously mentioned criterion for acidophily (a pH optimum for growth of < pH 6). These species include *Rhodopseudomonas acidophila* (now renamed *Rhodoblastus acidophilus*, Imhoff 2001b) and *Rhodopila globiformis* (Table 1). Both of these organisms were isolated by the German microbiologist and pioneer in the isolation and characterization of anoxygenic phototrophic bacteria, Norbert Pfennig, about 30 years ago (Pfennig 1969, 1974). By contrast, research on psychrophilic phototrophs is very new and only a few representatives are known (Figure 1, bottom left and right).

Acidophiles
Rhodoblastus acidophilus is common in mildly acidic environments, such as bogs, marshes, and acidic lakes. In the original description of this organism, sev-
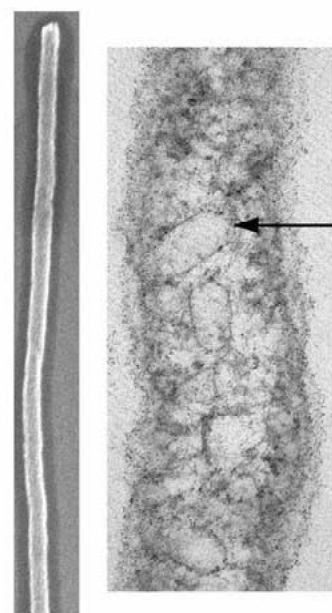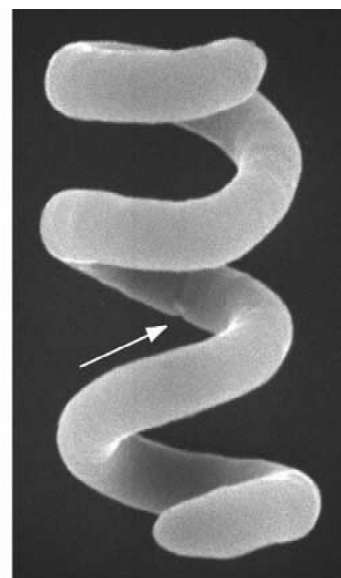
Figure 1. New anoxygenic phototrophic bacteria from extreme environments. *Top left:* Egypt. Photo of the author (second from right) along with a Bedouin family that kindly showed us a path to the shore of Lake Hamara (pH 10) Wadi Natroun, for sampling purposes. On far right is my guide and interpreter, Dr M.S.A. Shabeb, Aswan South Valley University. Photo courtesy of Dr Ahmed Shoreit, University of Assiut, who also accompanied us and kindly arranged for the trip to the Wadi. *Top right:* Scanning electron micrograph of a coiled *Heliorestis* species obtained by enrichment culture from Lake Hamara sediment. Arrow points to septum between two cells. The cell is about 1 μm in diameter. *Bottom left:* Antarctica. The author (far right) assists graduate student Matt Sattley in drilling through 5.8 m of ice on Lake Fryxell, McMurdo Dry Valleys, Antarctica, November, 2001. The Kukri Hills of the Taylor Valley are in the background. Lake Fryxell and other lakes in the Dry Valleys remain permanently frozen year round. After drilling a 4-inch diameter hole with the gas-powered drill, the hole was melted out to nearly one meter in diameter over the period of 2 days using a diesel-fired hot ethylene glycol circulating heater. Water samples could then be obtained at depth using a Niskin bottle. *Bottom right:* A new purple nonsulfur bacterium from Lake Fryxell. Left, scanning electron micrograph; right, thin section transmission electron micrograph. Note gas vesicles (arrow).

eral strains were isolated. Some strains contained a complement of orange/brown carotenoids while others contained purple/red carotenoids (Pfennig 1969). The strains seemed otherwise similar, and all showed a lower pH limit for growth near pH 4 (Pfennig 1969). *Rhodopila* (originally *Rhodopseudomonas*) *globiformis* was isolated from acidic warm sulfide springs (pH 3.5–4) along the Gibbon River in Yellowstone National Park (Pfennig 1974). The author has visited these springs on several occasions and has isolated additional strains of *R. globiformis* from them. *R. globiformis*-like organisms have also been obtained from Nymph Lake, a warm, highly acidic lake nearby the Gibbon River sulfide springs (Madigan et al. 2002). *R. globiformis* has a low pH optimum for growth, similar to that of *R. acidophilus* (Table 1), but is phylogenetically distinct from this organism and is thus far the sole described species in this genus.

So, is diversity in the acidophiles really limited to just two species? Based on what we know about diversity in other groups, this is unlikely. However, in the final analysis, the list of acidophiles will likely be much shorter than the list of alkaliphiles or thermophiles. A major reason for this may be the unsuitablility of acidic habitats for anoxygenic phototrophs. Highly acidic habitats commonly have high metal contents. The presence of excessive metal ions can be toxic, of course, but high concentrations of metals also tend to poise the E_0' of a habitat at a value sufficiently high to be unsuitable for anoxygenic photosynthesis. Interestingly, photosynthesis *per se*, is fully compatible with pH values significantly lower than those tolerated by *Rhodoblastus acidophila* and *Rhodopila globiformis*. For example, *Cyanidium*, a red alga (oxygenic phototroph), is present in Nymph Lake in Yellowstone and grows in culture at pH 2 (Rothschild and Mancinelli 2001). This leaves open the question of whether highly acidophilic anoxygenic phototrophs exist. Undoubtedly, if highly acidic anoxic habitats exposed to light are someday discovered, new acidophilic anoxygenic phototrophs will likely be found there.

Psychrophiles

Permanently cold environments are the most recent habitats to be explored for anoxygenic phototrophs (Madigan 1998). My laboratory has been studying lakes in the McMurdo Dry Valleys of Antarctica. These are closed basin lakes with a biology that is exclusively microbial, and remain permanently frozen under 4–6 m of ice (Priscu et al. 1998) (Figure 1, bottom left). The edges of these lakes thaw during the austral summer forming a moat, and when this occurs, microbial mats develop quickly in the open waters. These mats contain filamentous cyanobacteria, *Chloroflexus*-like organisms that contain BChl *c*, and purple bacteria (Madigan, unpublished results).

From mat samples and from the water column under the ice, we have isolated several phototrophic purple nonsulfur bacteria. The purple bacterium *Rhodoferax antarcticus* inhabits microbial mats and the water column of Lake Frxyell (77° S), the major lake we have been studying (Table 1) (Figure 1, bottom left). Lake Frxyell is stratified, with weakly saline waters overlain by freshwater. Sulfate reduction occurs in Lake Fryxell, resulting in a gradient of sulfide from micromolar levels at 9 m to nearly 1.5 mmol near the sediments (~19 m). Despite these nearly perfect conditions (except for temperature, of course, which remains constant at 0 °C) for the development of purple and green *sulfur* bacteria, no evidence for such organisms emerged from enrichment culture or nucleic acid probing studies (Achenbach et al. 2001). Instead, *R. antarcticus* and other purple *nonsulfur* bacteria appear to dominate anoxygenic photosynthesis in Lake Frxyell. Although *R. antarcticus* is not strongly psychrophilic (optimal growth occurs at 18 °C) it is the first anoxygenic phototroph to show distinct cold adaptation (Madigan et al. 2000). We have also enriched gas vesiculate purple nonsulfur bacteria from Lake Frxyell (Figure 1, bottom right) but these organisms, the first purple nonsulfur bacteria to contain gas vesicles, have yet to be thoroughly characterized.

In the Vestfold Hills areas of East Antarctica (68° S) a series of permanently frozen lakes exist near the coast. These lakes were formed from remnant seawater and are hypersaline, with large amounts of sulfide in the hypolimnion (Burke and Burton 1988a, b). We have isolated green sulfur bacteria resembling *Chlorobium* from water samples of the hypolimnion (Jung et al. 2001) and also have enriched purple sulfur bacteria that morphologically resemble *Thiocapsa* sp. (Madigan 1998). Both organisms grow best around 20 °C. In contrast to the Dry Valleys lakes, however, no evidence for purple *nonsulfur* bacteria has emerged from our enrichments of Vestfold Hills lake water. By contrast, Burke and Burton (1988a, b) reported that several nonsulfur purple bacteria were present in these lakes but no data on the properties of pure cultures or on cold adaptation were presented.

Permanently cold habitats clearly harbor anoxygenic phototrophs but thus far we lack evidence for

strongly psychrophilic species. However, our molecular probe studies using the *pufM* gene of purple bacteria indicate that several phylotypes of purple non-sulfur bacteria exist in Lake Frxyell (Achenbach et al. 2001; Karr et al. 2001), and it is hoped that some of these will show stronger psychrophilic tendencies. The challenge now is to get these organisms into axenic culture such that their cold adaptation and other physiological properties can be more carefully studied. Unfortunately, these are slow-growing phototrophs and thus patience is a necessary prerequisite for their study.

Ecology and evolution

Although it is understandable that the major interest in extremophilic anoxygenic phototrophs lies in basic research, we should not lose sight of the ecological importance of these fascinating organisms. I refer in particular here to the activities of extremophilic phototrophs as primary producers and nitrogen-fixers in their punishing habitats. For example, soda lakes support a relatively short, and primarily microbial, food chain (Jones et al. 1998), and often contain dense blooms of haloalkaliphilic purple bacteria (Imhoff et al. 1979). In these environments, anoxygenic photosynthesis may account for a major fraction of the total primary productivity. Another example of this exists with the thermophilic purple bacterium *Thermochromatium tepidum*. *T. tepidum* forms mats in certain Yellowstone hot springs, and carbon stable isotope studies have clearly shown the organism to be growing photoautotrophically *in situ* (Madigan et al. 1989). Since many chemotrophic prokaryotes also exist in these mats, *T. tepidum* is likely the foundation of the food chain for these simple microbial communities.

Evolution and exobiology are hot topics these days and extremophilic phototrophs are also relevant to these issues. There is little doubt that anoxygenic photosynthesis preceded, probably by about a billion years, the onset of oxygenic photosynthesis (Blankenship 2001). Because many modern extreme environments likely resemble habitats present on the early Earth (Madigan and Marrs 1997), anoxygenic phototrophs, especially extremophilic species, are particularly relevant models for the study of the evolution of photosynthetic systems. Indeed, molecular studies of anoxygenic phototrophs, both extremophilic and otherwise, have already yielded significant insight into the likely evolutionary development of photosynthesis

(Xiong et al. 2000; Blankenship 2001; Xiong and Bauer 2002). This trend will continue in even more significant ways as the era of genomics continues to unfold.

The search for life on other planets, in particular Mars or Jupiter's moon Europa, has relevant models here on Earth in the phototrophs that inhabit the permanently frozen lakes of the Antarctic Dry Valleys (Figure 1, bottom left and right). Because photosynthesis is such an ancient and adaptable process here on Earth, if microbial life once existed on Mars or Europa, photosynthesis probably played a role in supporting it. Perhaps new psychrophilic phototrophs obtained from Antarctic lakes will reveal some of the secrets of how photosynthesis can be sustained under constantly frigid conditions. Such data could be helpful when robots or humans eventually retrieve samples from the lakes that lie under the ice of Mars and Europa, and return samples to Earth for microbial exploration.

Summary/conclusions

In the past 40 years several new genera/species of extremophilic anoxygenic phototrophs have been discovered and characterized (Table 1). I believe that these organisms collectively have greatly expanded our knowledge of photosynthetic biodiversity, and continued studies will eventually define the environmental limits of photosynthesis. Moreover, extremophilic phototrophs represent new genetic resources for understanding the mechanisms of photosynthesis under extreme conditions. Genetic mining of these resources may assist in efforts to engineer crop plants to thrive in the climate changes that are already occurring and will continue to occur in the years ahead.

Extremophilic anoxygenic phototrophs have shown that anoxygenic photosynthesis is compatible with (1) temperatures up to at least $70\,^{\circ}\text{C}$ and down to $0\,^{\circ}\text{C}$ (and probably lower), (2) pH values as low as 3–4 or as high as 11, and (3) salinities from 0% NaCl to saturated ($\sim$32%) NaCl. Are these the absolute limits to anoxygenic photosynthesis? Unlikely. Exploration of extreme environments for anoxygenic phototrophs is still a young science and many chemically unique extreme environments likely await discovery. If any of these are anoxic and are exposed to light, however little, there is a chance that novel anoxygenic phototrophs will be found there that will extend the environmental boundaries for photosynthetic life.

It is hoped that a new generation of microbiologists with interests in anoxygenic phototrophs will pick up where the scientists cited in this review have left off, and continue to probe interesting extreme environments for signs of phototrophic life. If we are to truly understand photosynthesis, we must have a firm grasp of both the diversity of habitats that support this key biological process and the diversity of phototrophs that thrive there. Extremophilic anoxygenic phototrophs have already taught us much about photosynthesis and the future looks bright for additional exciting discoveries.

Acknowledgments

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