Invited Review

Aerobic anoxygenic photosynthetic bacteria with zinc-bacteriochlorophyll

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(Received May 17, 2001; Accepted August 4, 2001)

Naturally occurring chlorophyllous pigments, which function as the cofactor in the early photochemical reaction of photosynthesis, have been proven beyond question to be magnesium-complexed porphyrin derivatives. Phototrophic organisms that use (bacterio)chlorophylls ([B]Chls) containing metals other than Mg were unknown for a long time. This common knowledge of natural photosynthesis has recently been modified by the striking finding that a novel purple pigment, zinc-chelated-BChl (Zn-BChl) *a*, is present as the major and functional pigment in species of the genus *Acidiphilium*. *Acidiphilium* species are obligately acidophilic chemoorganotrophic bacteria that grow and produce photopigments only under aerobic conditions. Although the mechanism of photosynthesis with Zn-BChl *a* in *Acidiphilium* species is similar to that seen in common purple bacteria, some characteristic photosynthetic features of the acidophilic bacteria are also found. The discovery of natural photosynthesis with Zn-BChl has not only provided a new insight into our understanding of bacterial photosynthesis but also raised some interesting questions to be clarified. The major questions are why the acidophilic bacteria have selected Zn-BChl for their photosynthesis and how they synthesize Zn-BChl and express photosynthetic activity with it in their natural habitats. In this article we review the current knowledge of the biology of *Acidiphilium* as aerobic photosynthetic bacteria with Zn-BChl *a* and discuss the interesting topics noted above.

Key Words—*Acidiphilium*; aerobic photosynthetic bacteria; zinc-bacteriochlorophyll

Introduction

Chlorophylls (Chls) and bacteriochlorophylls (BChls) are porphyrin derivatives chelated with magnesium, and represent a class of lipophilic pigments involved in natural photosynthesis. There has been no rational explanation for why extant phototrophic organisms have used Mg exclusively as the central metal with which

the chlorophyllous pigments are complexed. Apart from this question, phototrophic organisms that use Chls or BChls containing metals other than Mg were unknown for a long time. Recently, this common understanding of natural photosynthesis was unexpectedly modified by the new finding that a novel purple pigment, zinc-chelated-BChl (Zn-BChl) (for abbreviations, see Takaichi et al. (1999)), was discovered in a specific group of obligately aerobic bacteria (Hiraishi et al., 1998; Wakao et al., 1996, 1999).

It has been satisfactorily demonstrated that BChls are present not only in facultatively and obligately anaerobic anoxygenic phototrophic bacteria but also in large numbers of species of obligately aerobic bacteria

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that do not grow anaerobically in the light. These aerobic BChl-producing bacteria are called "aerobic photosynthetic bacteria" (Shiba, 1989; Shiba and Harashima, 1986) or "aerobic phototrophic bacteria" (Shimada, 1995; Yurkov and Beatty, 1998). In a strict sense, the term phototrophy means the utilization by organisms of light energy for growth and survival. In this context, it is unclear whether all aerobic BChl-containing bacteria are actually phototrophic or photosynthetic. For this reason, they are often called merely "aerobic BChl-containing (ABC) bacteria" (Imhoff and Hiraishi, 2001). All established species of obligately aerobic bacteria classified into this category are positioned phylogenetically in the purple bacteria now classified as the phylum *Proteobacteria*.

The natural occurrence of Zn-BChl *a* has so far been known in a limited group of aerobic acidophilic proteobacteria including species of the genus *Acidiphilium*. The major photopigment in *Acidiphilium* was first identified tentatively as Mg-BChl *a* on the basis of preliminary spectral analyses (Kishimoto et al., 1995a; Wakao et al., 1993). However, more intensive studies revealed that all previously known species of *Acidiphilium* contained Zn-BChl *a* as the major photopigment (Hiraishi et al., 1998; Wakao et al., 1996). In a representative species, *Acidiphilium* (*Acp.*) *rubrum*, Zn-BChl *a* was thought to be functional in a fully active photosynthetic reaction center and the core light-harvesting (LH 1) complex which is likely analogous to those of the phototrophic purple bacteria (Wakao et al., 1996). Light-stimulated CO₂ fixation was also found in *Acp. rubrum* (Kishimoto et al., 1995b).

In view of these collective observations, it is reasonable to recognize *Acidiphilium* species as being actually photosynthetic. In this paper, therefore, we use the term "aerobic photosynthetic bacteria" to describe these bacteria. The discovery of Zn-BChl *a* in *Acidiphilium* species has provided a new insight into natural photosynthesis and the evolution and ecophysiology of the ABC bacteria. Here we review the current knowledge of the biology of *Acidiphilium* as aerobic photosynthetic bacteria with Zn-BChl *a*.

1. Overview of Aerobic Bacteriochlorophyll-Containing (ABC) Bacteria

One of the most characteristic features of the ABC bacteria is their inability to grow phototrophically under anoxic conditions, unlike the "true" anoxygenic pho-

totrophic bacteria (for reviews, Shiba, 1989; Shiba and Harashima, 1986; Shimada, 1995; Yurkov and Beatty, 1998). Thus, all species of the ABC bacteria synthesize BChl *a* and other photopigments only under aerobic growth conditions, even though some species are capable of growing anaerobically with an auxiliary terminal oxidant (Arata et al., 1988). In addition to these traits, the ABC bacteria share a number of common characteristics, such as aerobic chemoorganotrophy as the preferred mode of growth, low levels of BChls, and strong inhibition by light of BChl synthesis under normal growth conditions. Moreover, fully active reaction center and LH 1 complexes with BChl are present in all the species studied so far (Nishimura et al., 1996; Takamiya et al., 1987; Yurkov et al., 1998). Peripheral antenna (LH 2) complexes are absent in most species. In addition to lipophilic carotenoids as the photopigments, polar carotenoids are present in most species of the ABC bacteria. However, *Acidiphilium* species lack any type of polar carotenoids.

The ABC bacteria are a new physiological group relatively recently discovered. The presence of BChls in strictly aerobic bacteria was first reported in a methylotrophic bacterium (Sato, 1978; Sato and Shimizu, 1979) now classified as *Methylobacterium rhodensianum* (Green et al., 1988) and in a newly isolated aerobic marine bacterium (Harashima et al., 1978; Shiba et al., 1979) for which the new genus *Erythrobacter* was proposed (Shiba and Shimizu, 1982). Since then, large numbers of strains of ABC bacteria have been isolated from marine, freshwater, and terrestrial environments, and most of these bacteria have been described as new genera and species. The currently known species of ABC bacteria are listed in Table 1, and a phylogenetic tree of these bacteria based on 16S rDNA sequences is shown in Fig. 1. All species of ABC bacteria, except β -proteobacterium *Roseateles depolymerans*, belong to α -*Proteobacteria* (class "Alphaproteobacteria"), in which they are further divided into four major subgroups, the so-called α -1, α -2, α -3, and α -4 groups. *Acidiphilium* species form a major phylogenetic cluster together with members of the genera *Acidisphaera*, *Craurococcus*, *Paracraurococcus*, and *Roseococcus* within the α -1 group (order "Rhodospirillales"). Among the genera of the ABC bacteria so far described, only two genera, *Acidiphilium* and *Acidisphaera*, include obligately acidophilic bacteria. *Acidiphilium* species always produce Zn-BChl *a* as the main photopigment, whereas *Acidisphaera* strains

Table 1. Genera and species of aerobic BChl-containing (ABC) bacteria and occurrence of Zn-BChl.

Phylogenetic group (class and order) and genus ^a	Species ^b	Type or reference strain	Reference	Presence of Zn-BChl
"Alphaproteobacteria" "Rhodospirillales" (α -1) <i>Acidiphilium</i>	<i>A. acidophilum</i>	Strain ATCC 27897 = DSM 700	Harrison (1981); Hiraishi et al. (1998)	+ ^c
	<i>A. angustum</i> (= <i>A. rubrum</i>)	Strain KLB = ATCC 35903	Wichlacz et al. (1986)	
	<i>A. cryptum</i> *	Strain Lhet2 = ATCC 33463 = DSM 2389	Harrison (1983)	+
	<i>A. multivorum</i>	Strain AIU 301 = JCM 8867 = DSM 11245	Wakao et al. (1994)	+
	<i>A. organovorum</i>	Strain ATCC 43141	Lobos et al. (1986)	+
<i>Acidisphaera</i> <i>Craurococcus</i> <i>Paracraurococcus</i> <i>Roseococcus</i> "Rhizobiales" (α -2)	<i>A. rubrifaciens</i> *	Strain OP = ATCC 35905	Wichlacz et al. (1986)	+
	<i>C. roseus</i> *	Strain HS-AP3 = JCM 10600	Hiraishi et al. (2000b)	(+)
	<i>P. ruber</i> *	Strain NS130 = JCM 9933	Saitoh et al. (1998)	–
	<i>R. thiosulfatophilus</i> *	Strain NS89 = JCM 9931	Saitoh et al. (1998)	–
		Strain RB-3 = DSM 8511	Yurkov et al. (1994)	nd
<i>Bradyrhizobium</i> <i>Methylobacterium</i>	<i>Bradyrhizobium</i> sp. ^d	Strain ORS278 = LMG12187	Chaintreuil et al. (2000)	nd
	<i>M. extorquens</i>	Strain TK 0001 = NCIB 9399 = DSM 1337	Urakami and Komagata (1984); Bousfield and Green (1985)	–
	<i>M. radiotolerans</i>	Strain ATCC 27329 = NCIB 10815 = IAM 12098	Green and Bousfield (1983)	–
	<i>M. rhodesianum</i>	Strain NCIB 12249 = DSM 5687	Green et al. (1988)	–
	<i>R. dehamense</i> *	Strain Och 254 = JCM 10543	Suzuki et al. (2000)	nd
<i>Rhodobacterales</i> (α -3) <i>Roseibium</i> <i>Roseinatronobacter</i> <i>Roseivivax</i> <i>Roseobacter</i>	<i>R. hamelinense</i>	Strain Och 368 = JCM 10544	Suzuki et al. (2000)	nd
	<i>R. thiooxidans</i> *	Strain ALG1 = DSM 13087	Sorokin et al. (2000)	nd
	<i>R. halodurans</i> *	Strain Och 239 = JCM 10272	Suzuki et al. (1999a)	–
	<i>R. halotolerans</i>	Strain Och 210 = JCM 10271	Suzuki et al. (1999a)	–
	<i>R. denitrificans</i>	Strain Och 114 = ATCC 33942 = DSM 7001	Shiba (1991)	–
<i>Roseovarius</i> <i>Rubrimonas</i> <i>Stappia</i> ^e "Sphingomonadales" (α -4) <i>Blastomonas</i>	<i>R. litolaris</i> *	Strain Och 149 = ATCC 49566 = DSM 6996	Shiba (1991)	–
	<i>R. tolerans</i> *	Strain EL-172 = DSM 11457	Labrenz et al. (1999)	–
	<i>R. cliftonensis</i> *	Strain Och 317 = JCM 10189	Suzuki et al. (1999b)	–
	<i>S. stellulata</i> *	Strain IAM 12621 = ATCC 15215	Uchino et al. (1998)	–
	<i>B. natatoria</i> *	Strain ACM 2507 = DSM 3183 = ATCC 35951	Sly and Cahill (1997); Hiraishi et al. (2000a)	–
<i>"Citromicrobium"</i>	<i>B. ursincola</i> (= <i>Erythromonas ursincola</i>)	Strain KR-99 = DSM 9006 = JCM 10397	Yurkov et al. (1997); Hiraishi et al. (2000a)	–
	<i>"C. bathoceanense"</i>	Strain JF-1	Yurkov et al. (1999)	nd

Table 1. (Continued)

Phylogenetic group (class and order) and genus	Species ^b	Type or reference strain	Reference	Presence of Zn-BChl
<i>Erythrobacter</i>	<i>E. litoralis</i> <i>E. longus</i> *	Strain T4=DSM 8509 Strain OCh 101=IFO 14126=ATCC 33941 =DSM 6997	Yurkov et al. (1994) Shiba and Shimidu (1982)	— —
<i>Erythromicrobium</i> <i>Porphyrobacter</i>	<i>E. ramosum</i> * <i>P. neustonensis</i> * <i>P. tepidarius</i> <i>S. sibiricus</i> *	Strain E5=DSM 8510 ACM 2844=DSM 9434 Strain OT3=DSM 10594 Strain RB16-17	Yurkov et al. (1994) Fuerst et al. (1993) Hanada et al. (1997) Yurkov et al. (1997)	— — — nd
<i>Sandaracinobacter</i> "Betaproteobacteria" "Burkholderiales" <i>Roseateles</i>	<i>R. depolymerans</i> *	Strain 61A=DSM 11813	Suyama et al. (1999)	—

^a The class and order names are based on information from Garrity and Holt (2001).

^b Type species are asterisked.

^c Symbols: +, present; (+), trace amount present in cells grown with 0.1 mM Zn²⁺; —, absent; nd, not determined.

^d The BChl-containing bradyrhizobia have been suggested to represent a separate genus, together with *Agromonas oligotrophica* and *Blastobacter denitrificans*, from the genus *Bradyrhizobium* (Willems et al., 2001).

^e *Stappia* was not described as a genus of ABC bacteria (Uchino et al., 1998), but has been found to contain BChl *a* (Hiraishi, A., unpubl.).

Fig. 1. Phylogenetic tree of aerobic anoxygenic BChl-containing (ABC) bacteria together with some facultative phototrophic bacteria (asterisked) based on 16S rDNA sequences.

The tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) with *Roseateles depolymerans* as an outgroup to root the tree. Accession numbers for the 16S rDNA sequences used are given in parentheses behind species and strain names. Bootstrap values with 1,000 resamplings are given at branching points. Scale=10% nucleotide substitution rate.

produce Zn-BChl only when grown with 0.1 mM Zn²⁺ added (Hiraishi et al., 2000b).

The ABC bacteria contain much lower amounts of BChls than do anaerobic phototrophic bacteria, and in some cases, produce only trace amounts too small to detect by standard spectrophotometric measurement (Hiraishi et al., 1998, 2000a). It has been shown that some strains belonging to the same genera as pro-

posed for the ABC bacteria, as for example, strains of *Roseovarius varians* (Labrenz et al., 1999), fail to produce BChls in detectable amounts under the tested conditions. This is the case in some *Acidiphilium* strains (see Table 3). It is unclear at this time whether all these strains are actually non-BChl producers or whether they express genes for BChl synthesis, if any, under specific environmental conditions. Apart from

this question, we are puzzled over the problem of what biological significance or ecophysiological merit the ABC bacteria with a low level of BChls have.

Recent reports have indicated that ABC bacteria comprise significant proportions of the total microbial community in marine environments and play an important role in the cycling of organic and inorganic carbon in the ocean (Kolber et al., 2000, 2001). Probably, these bacteria are capable of photosynthetic light utilization for their survival in oligotrophic environments.

2. Phylogeny and General Characteristics of *Acidiphilium*

2.1. Historical view of systematics

The genus *Acidiphilium* was created by Harrison (1983) with *Acp. cryptum* as the type and only species to accommodate strictly aerobic chemoorganotrophic acidophilic strains isolated from acidic environments such as mine drainage. In the 1980's, several new species of *Acidiphilium*, including *Acp. angustum*, *Acp. facilis*, *Acp. organovorum*, and *Acp. rubrum* (Lobos et al., 1986; Wichlacz et al., 1986), were described, but none of these descriptions included any information about BChl production or other photosynthetic properties. The phylogenetic affiliations and photosynthetic features of *Acidiphilium* have received intensive scrutiny during the past decade. The report of Wakao et al. (1993) was the first to demonstrate the occurrence of photopigments in *Acidiphilium* species. Similar observations were reported by Kishimoto et al. (1995a). Phylogenetic analyses based on 16S rDNA sequences revealed that all these species of *Acidiphilium* formed a major phylogenetic cluster in the α -*Proteobacteria* (Lane et al., 1992; Sievers et al., 1994) and that the genus *Acidiphilium* consisted of BChl-producing species and non-BChl-producing ones genetically distinguishable from each other (Kishimoto et al., 1995b). This finding led to the proposal to remove the non-BChl producing species, i.e., *Acp. facilis* (Wichlacz et al., 1986) and *Acp. aminolytica* (Kishimoto et al., 1993), from the genus *Acidiphilium* and to transfer them in the new genus *Acidocella* (Kishimoto et al., 1995b). As a result, the genus *Acidiphilium* has been emended to include BChl-producing species only. Following this, the major photopigment of all *Acidiphilium* species was demonstrated to be Zn-BChl *a* by Wakao et al. (1996) and Hiraishi et al. (1998).

At this time the genus *Acidiphilium* consists of the

following six species: *Acp. acidophilum* (formerly *Thiobacillus acidophilus*), *Acp. angustum*, *Acp. cryptum*, *Acp. multivorum*, *Acp. organovorum*, and *Acp. rubrum* (Table 1). However, 16S rDNA sequence comparisons and DNA-DNA hybridization studies have shown that *Acp. angustum* and *Acp. rubrum* form a genetically coherent group at the species level (Hiraishi et al., 1998; Kishimoto et al., 1995b; Wakao et al., 1994). These two species names should be subjective synonym. Since *Acp. multivorum*, *Acp. cryptum*, and *Acp. organovorum* are very closely related to one another, DNA-DNA hybridization assays as well as phenotypic tests are essential to identify them.

2.2. Cell morphology and ultrastructure

Cells of *Acidiphilium* species are straight or slightly curved rods with rounded ends (Fig. 2). They vary from 0.4 to 0.8 μm in width and from 0.9 to 3.0 μm in length under optimal growth conditions, but cell size and shape are influenced by the carbon sources and by physico-chemical growth conditions. Cells are generally motile, but some strains are nonmotile. Electron microscopy of negatively stained cells shows that motile cells have single polar or subpolar flagella (Fig. 2b). Two lateral flagella are occasionally found in *Acp. cryptum* (Harrison, 1989) and *Acp. multivorum* (Wakao et al., 1994).

While intracellular chromatophore-like structures are found in some species of the ABC bacteria including *Methylobacterium rhodesianum* (Sato and Shimizu, 1979), *Roseobacter denitrificans* (Harashima et al., 1982), and "*Photorhizobium*" strain BTAi1 (Evans et al., 1990), all *Acidiphilium* species may lack such internal membrane systems. Thin-section electron microscopy of *Acp. rubrum*, being the most remarkable in BChl production among the established species of *Acidiphilium*, showed that any type of intracytoplasmic membrane was absent in the cells, most of which contained poly- β -hydroxybutyrate and polyphosphate granules (Matsuzawa et al., 2000) (Fig. 3). Accumulation of poly- β -hydroxybutyrate as a storage compound in *Acp. rubrum* was reported by Akiyama et al. (1999).

Matsuzawa et al. (2000) reported that *Acp. rubrum* produced electron dense granules that were associated with the inner part of the cytoplasmic membrane when grown with 0.1% FeSO_4 . An energy-dispersive X-ray analysis showed that these membrane-bound electron-dense granules contained iron. The relationship between this structure and the intracellular mag-

Fig. 2. General cell morphology of *Acidiphilium* species.

(a) Scanning electron micrograph showing cells of *Acp. rubrum* ATCC 35905^T (photo by A. Hiraishi); (b) transmission electron micrograph showing cell with a single polar flagellum of *Acp. cryptum* ATCC 33463^T (photo by N. Wakao). Scale=1 μm.

Fig. 3. Transmission electron micrograph of thin-section of *Acp. rubrum* ATCC 35905^T cells (photo by Y. Matsuzawa).

Arrows indicate polyphosphate (left) and poly-β-hydroxybutyrate (right) granules. Scale=1 μm.

netite produced in *Acp. rubrum* (Itoh et al., 1998) is an interesting subject awaiting further studies.

2.3. Growth and physiology

Members of the genus *Acidiphilium* are strictly aerobic chemoorganotrophic bacteria that require high acidity for growth. They grow in the pH range of 2.5 to 5.9 (optimum 3.5), some as low as 1.5–2.0, but not at pH 6.1 and above. The temperature range for growth is 20 to 40°C with an optimal temperature of 30–35°C in most strains. The type species *Acp. cryptum* and its closest relatives, *Acp. multivorum* and *Acp. organovorum*, exhibit a doubling time of 3 to 9 h, whereas *Acp. acidophilum* and *Acp. rubrum* grow much more slowly with a doubling time of more than 10 h under optimal growth conditions. Liquid cultures and colonies on agar media are cream to pale pink at early phases of

growth but turn red to brown in older stages. *Acidiphilium* species, as well as all other ABC bacteria, are unable to grow anaerobically in the light. In addition, light-stimulated aerobic growth is not found or not significant. However, aerobic ¹⁴CO₂ uptake by *Acidiphilium* species is enhanced clearly by light (Kishimoto et al., 1995b), and the uptake rate in the light may be elevated with increasing BChl content of the cells (Wakao, N., unpubl.). Strains of another genus of the acidophilic ABC bacteria, *Acidisphaera*, grow faster under continuous incandescent illumination than in darkness (Hiraishi et al., 2000b). Viability of *Acidiphilium* and *Acidisphaera* cells under starved conditions is maintained for a long time by illumination (Hiraishi, A., unpubl.).

Acidiphilium species use simple organic compounds as carbon and energy sources for growth. Sugars and sugar alcohols, such as D-xylose, D-glucose, D-fructose, and mannitol, are good substrates supporting growth of all species. Growth in glucose-mineral media is inhibited by low concentrations of acetate or lactate. In addition, succinate and fumarate inhibit growth of some species. No growth occurs on ordinary complex media containing peptone, even if the pH of the media is adjusted to around 3.5. Yeast extract at a low concentration of less than 0.1% (w/v) enhances growth of *Acidiphilium* species, but at 0.3% and above it has inhibitory effects on the growth (Harrison, 1981; Kishimoto et al., 1990). This is due to the effects of organic acids contained in the yeast extract (Kishimoto et al., 1990). No species of *Acidiphilium*, except *Acp. acidophilum*, exhibit chemolithotrophic growth with reduced sulfur compounds as energy sources, but may have the ability to oxidize elemental sulfur to sulfate

(Harrison, 1981). A number of studies have demonstrated sulfur-dependent chemolithotrophy of and sulfur oxidation by *Acp. acidophilum* (Guay and Silver, 1975; Harrison, 1981; Hiraishi et al., 1998; Meulenberg et al., 1992; Pronk et al., 1990).

High resistance to heavy metals, such as copper, nickel, and zinc, is one of the most striking characteristics of *Acidiphilium* species (Mahapatra and Banerjee, 1996). They are capable of growing in the presence of 100 mM Fe^{2+} or 100 mM Al^{3+} . Although the addition of these metals stimulates heterotrophic growth significantly, it is unlikely that Fe^{2+} and other metal ions serve as electron donors for chemolithotrophic growth. Some strains of *Acidiphilium* species including *Acp. cryptum* are capable of growing with the reduction of Fe(III) coupled to the oxidation of a variety of substrates under aerobic or anaerobic conditions (Johnson, 1998; Johnson and McGinness, 1991; Küsel et al., 1999; Pronk and Johnson, 1992). Küsel et al. (1999) reported that *Acp. cryptum* JF-5 produced unusual blebs on the cell surface during the reduction of Fe(III) as the terminal electron acceptor. The available information provides circumstantial evidence that they may corespire oxygen and Fe(III) under aerobic conditions. However, further studies in this interesting area are clearly necessary.

Acp. multivorum has the ability to oxidize arsenite to arsenate, whereas all other species lack this property (Wakao et al., 1994). *Acp. multivorum* has a number of plasmids of different sizes, one of which is 56 kbp and encodes its arsenic resistance. The arsenic resistance (*ars*) operon from the *Acp. multivorum* plasmid was cloned and sequenced (Suzuki et al., 1998).

2.4. Habitats and ecology

Strains of *Acidiphilium* species have been isolated from acidic mineral environments, such as pyritic mine drainage, pyritic coal refuse, copper and uranium mine tailings, and acidic soil (Banerjee et al., 1996; Harrison, 1984, 1989; Kishimoto and Tano, 1987; Manning, 1975; Tuttle et al., 1968; Wakao et al., 1994; Wichlacz and Unz, 1981; Wichlacz et al., 1986; Hiraishi, A., unpubl.). Culture-independent rRNA approaches including environmental 16S rDNA cloning and in situ hybridization have demonstrated the occurrence of *Acidiphilium* species as the major populations in mine drainage (Peccia et al., 2000) and acidic geothermal springs (Burton and Norris, 2000). As several strains of *Acidiphilium* species were isolated or found as con-

taminants from cultures of the iron-oxidizing chemolithotroph *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*, see Kelly and Wood (2000)) (Guay and Silver, 1975; Harrison, 1981, 1989; Harrison et al., 1980; Johnson and Kelso, 1983; Lobos et al., 1986), they may aid leaching through the consumption of organic matter inhibitory to the growth and activity of *Acidithiobacillus ferrooxidans*. Although most *Acidiphilium* species are obligately heterotrophic, their natural habitats seem to be oligotrophic environments with poor organic matter. The ability of *Acidiphilium* to perform photosynthesis with Zn-BChl *a* may be an advantage to grow and survive in such oligotrophic acidic environments.

In addition, the ability of *Acidiphilium* species to reduce Fe(III) with the expenditure of a variety of organic substrates indicates that they may be of ecological significance in the biogeochemical cycling of iron at oxic-anoxic interfaces in acidic environments. In the biological mine drainage treatment process, the overwhelming majority of the microbial population present is represented by *Acidithiobacillus ferrooxidans*, which plays the primary role in oxidation of Fe(II) to Fe(III). However, chemoorganotrophic bacteria including *Acidiphilium* species always coexist as minor populations in this process (Hiraishi, A., unpubl.). The major role of these chemoorganotrophs is most likely to scavenge trace amounts of organic compounds, but another role in Fe(III) reduction may be taken into consideration.

3. Photopigments and Quinones

3.1. Spectral properties of *Acidiphilium* photopigments

One of the most outstanding characteristics of *Acidiphilium* species is the production of photopigments with Zn-BChl *a* as the major component. The presence of Zn-BChl *a* can be roughly estimated by measurement of in vivo and in vitro absorption spectra. For example, membrane preparations of *Acp. rubrum* have absorption maxima at 377, 486, 515, 549, 590, 792, and 864 nm (Fig. 4a). This spectral pattern is very similar to that of the membrane preparation from *Rhodospirillum rubrum*, a typical species of Mg-BChl *a*-containing phototrophic bacteria, but the former spectrum differs from the latter in blue shifts of absorption peaks in the near infrared region. The dominant occurrence of Zn-BChl *a* in *Acidiphilium* is also indicated by

Fig. 4. Absorption spectra of photopigments of *Acp. rubrum* ATCC 35905^T (solid lines) and *Rhodospirillum rubrum* ATCC 17100^T (broken lines).

(a) Membrane preparations; (b) purified BChls (Zn-BChl *a* and Mg-BChl *a*).

Table 2. Absorption maxima (nm) and relative intensities of the B, Q_x, and Q_y bands of Zn-BChl *a* and Mg-BChl *a* in different organic solvents.

Solvent	Zn-BChl <i>a</i>			Mg-BChl <i>a</i>		
	B	Q _x	Q _y	B	Q _x	Q _y
Acetone	354.0 (99.6)	562.0 (29.4)	762.0 (100)	358.5 (94.7)	579.0 (27.4)	769.0 (100)
Benzene	355.0 (68.0)	556.5 (20.2)	775.5 (100)	361.0 (79.0)	580.5 (25.2)	781.0 (100)
Diethyl ether	353.0 (80.1)	559.0 (23.6)	762.5 (100)	357.0 (76.7)	573.5 (22.3)	770.5 (100)
2-Propanol	357.0 (91.4)	571.5 (29.3)	768.0 (100)	362.5 (84.8)	586.5 (21.4)	775.5 (100)

Information from Kobayashi et al. (1998b).

an absorption maximum at 762–763 nm of the acetone-methanol or diethyl ether extract, which is blue-shifted by 7–8 nm compared to the absorption maximum of Mg-BChl *a* in the corresponding near infrared region (see Fig. 4b and Table 2). For this property, Zn-BChl *a* was tentatively called P763 before the exact determination of its chemical structure (Wakao et al., 1996).

3.2. Structure and characteristics of Zn-BChl *a*

The structure and characteristics of naturally occur-

Fig. 5. Molecular structure of Zn-BChl *a*.

ring Zn-BChl *a* have been studied mainly using that purified from *Acp. rubrum* (Akiyama et al., 1998b; Kobayashi et al., 1998a, 1999; Wakao et al., 1996). Based on the results of HPLC, ICP measurement, mass spectrometry, CD spectroscopy, and NMR, the chemical structure of Zn-BChl *a* was demonstrated to be the same as that of Mg-BChl *a* esterified with phytol, except that Zn is present in place of Mg as the central metal (Fig. 5). Because of the high structural similarity of Zn-BChl *a* to Mg-BChl *a*, there are similarities in absorption maxima in organic solvents between the two (Table 2), although Zn-BChl *a* is characterized by its absorption maxima blue-shifted as noted above. Artificially Zn-transmetalated BChl *a* has the identical spectral pattern (Scheer and Hartwich, 1995).

The available information about the physico-chemical characteristics of Zn-BChl *a* provides a basis for profiling natural photosynthesis with this pigment. One of the most interesting properties of Zn-BChl *a* is high resistance to acid compared to other chlorophyllous pigments. Zn-BChl *a* is pheophytinized 10⁶-fold slower than Mg-BChl *a* (Kobayashi et al., 1998b). In fact, it is difficult to fully convert Zn-BChl *a* to bacteriopheophytin (BPhe) by treatment with 1 N HCl, which is commonly used for pheophytinizing Mg-BChl and Mg-Chl. The acid tolerance of Zn-BChl *a* is one of the possible reasons why the acidophilic bacteria selected this pigment to perform photosynthesis under strongly acidic conditions.

The photochemical and electrochemical traits of Zn-BChl *a* can be evaluated on the basis of the available information on those of different transmetalated Chl *a* molecules including Zn-Chl *a* (Watanabe and Kobayashi, 1991; Watanabe et al., 1985). It has been shown that there is no marked difference in light-harvesting efficiency between Zn-Chl *a* and Mg-Chl *a*. In addition, Zn-Chl *a* has a fluorescence quantum yield

Table 3. Photopigment contents of different species of *Acidiphilium* and *Acidisphaera* grown under optimum growth conditions.

Species	Strain	Pigment content (nmol · g ⁻¹ dry wt) ^a					Presence of <i>puf</i> genes ^b
		Zn-BChl <i>a</i>	Mg-BChl <i>a</i>	BPhe	Spir	Rhod	
Genus <i>Acidiphilium</i>							
<i>A. acidophilum</i>	ATCC 27897 ^T	2.3	0	0.8	3.3	0	+
<i>A. angustum</i> (= <i>A. rubrum</i>)	ATCC 35903 ^T	180	26	14	120	0	+
<i>A. cryptum</i>	ATCC 33463 ^T	57	5.1	5.1	37	1.5	+
<i>A. multivorum</i>	AIU 301 ^T	67	8.8	5.9	54	0	+
<i>A. organovorum</i>	ATCC 43141 ^T	6.7	0.4	0.9	6.0	0.4	+
<i>A. rubrum</i>	ATCC 35905 ^T	810	120	61	510	0	+
<i>A. rubrum</i>	St1-5	170	25	13	100	0	nd
<i>A. rubrum</i>	St1-7	130	20	11	82	0	nd
<i>Acidiphilium</i> sp.	MS-09	0	0	0	+	nd	nd
<i>Acidiphilium</i> sp.	MS-16	0	0	0	+	nd	nd
Genus <i>Acidisphaera</i>							
<i>A. rubrifaciens</i>	HS-AP3 ^T	0	90	6.3	+	nd	+
<i>A. rubrifaciens</i>	HS-AP16	0	35	2.4	+	nd	+
<i>A. rubrifaciens</i>	YO-704	0	84	5.4	+	nd	+

^a Information from Hiraishi et al. (1998, 2000b) and Hiraishi, A. (unpubl.). Spir, spirilloxanthin; Rhod, rhodovibrin.

^b Information from Nagashima et al. (1997), Hiraishi et al. (1998), and Hiraishi, A. (unpubl.).

nd, not determined.

lower than, but comparable to that of, Mg-Chl *a*. Moreover, Zn-Chl *a* exhibits a similar feature to Mg-Chl *a* in the relationship of the redox potential with the electronegativity of the central metal. These data indicate that Zn-BChl *a* should work well in place of Mg-BChl *a* as the cofactor in natural photosynthesis (Kobayashi et al., 1999).

3.3. Other pigments and quinones

In addition to Zn-BChl *a* as the major pigment, Mg-BChl *a*, BPhe, and carotenoids are present in *Acidiphilium* species. The molar ratio of Zn-BChl *a*:Mg-BChl *a*:BPhe in the cells is relatively constant among species; for instance, *Acp. rubrum* ATCC 35905 produced them at a molar ratio of 13 : 2 : 1 (Hiraishi et al., 1998; Wakao et al., 1996) (see also Table 3). Spirilloxanthin is the sole carotenoid component of the antenna complex. Rhodovibrin occurs in trace amounts in some strains of *Acidiphilium*, but is probably an intermediate in the biosynthesis of spirilloxanthin. Unlike other genera of the ABC bacteria, such as *Erythrobacter* and *Porphyrobacter*, *Acidiphilium* contains no other carotenoids than those bound to the antenna and the reaction center complexes.

All species of *Acidiphilium* contain ubiquinones with Q-10 as the major respiratory quinone (Hiraishi et al., 1998; Katayama-Fujimura et al., 1982; Wakao et al., 1994). Ubiquinone-10 accounts for 93–99% of the total quinone content of whole cells. Any other quinone structural classes, such as menaquinones and rhodoquinones, may be absent.

4. Biosynthesis of Zn-BChl *a*

4.1. Factors affecting Zn-BChl *a* production

The cellular content of Zn-BChl *a* as well as of other photopigments differs greatly from species to species. The total BChl content of *Acidiphilium* species grown under optimal growth conditions ranges from 0.002 to 0.9 μmol · g⁻¹ dry wt of cells (Table 3). Such a great variation in the BChl content is found in other ABC bacteria. Therefore, an HPLC analysis in addition to standard spectrophotometric measurement is essential to detect BChls from the ABC bacteria. Since the type strain of *Acp. rubrum* is the most remarkable for Zn-BChl *a* production among the *Acidiphilium* strains so far tested, this organism has received most intensive study with respect to BChl biosynthesis.

Fig. 6. BChl production by *Acp. rubrum* ATCC 35905^T as a function of external pH (Hiraishi, A., unpubl.).

Histograms indicate the sum of Zn-BChl *a* and Mg-BChl *a* produced. Open circles indicate the molar ratio of Mg-BChl *a* to Zn-BChl *a*.

The major environmental factors affecting BChl production by *Acidiphilium* are oxygen, light, pH, and nutrient availability. *Acidiphilium* species produce Zn-BChl *a* as well as Mg-BChl *a* only under aerobic-dark conditions, indicating the strong requirement of energy produced via aerobic respiratory electron transport for the pigment production. Light inhibits photopigment production completely under normal growth conditions, as is the case in other genera of the ABC bacteria. The BChl content and the Zn-BChl/Mg-BChl ratio in *Acidiphilium* strains increases with decreasing external pH (Masuda et al., 1998; Hiraishi, A., unpubl.) (Fig. 6). This is suggestive of the suitability of Zn-BChl *a* for photosynthesis in more acidic environments. Carbon limitation is effective in increasing the BChl content of *Acidiphilium* species as well as of *Acidisphaera rubrifaciens* (Fig. 7).

Culture media routinely used for growth of *Acidiphilium* contain a trace amount of Zn ion, as this component is derived only from yeast extract supplemented (usually 0.5 g·L⁻¹) as the growth factor. It is therefore of special interest to note whether the Zn-BChl content of the acidophilic ABC bacteria depends upon the concentration of external Zn ion added. The addition of ZnSO₄ up to 0.1 mM exerted no effects on the Zn-BChl content of *Acp. rubrum*. On the other hand, *Acidisphaera rubrifaciens* produced a small amount of Zn-BChl *a* in addition to Mg-BChl *a* in the presence of 0.1 mM Zn²⁺ (Hiraishi et al., 2000b).

4.2. Biochemical and molecular aspects

In the biosynthetic pathway of BChls in typical purple bacteria, an Mg atom is introduced into protoporphyrin IX by magnesium chelatase composed of three

Fig. 7. BChl production by *Acp. rubrum* ATCC 35905^T (a) and *Acidisphaera rubrifaciens* HS-AP3^T (b) as a function of carbon source concentrations in growth medium (Hiraishi, A., unpubl.).

Acp. rubrum ATCC 35905^T and *Acidisphaera rubrifaciens* HS-AP3^T were grown with 0.01–0.3% glucose and sodium gluconate, respectively, as the sole carbon source.

subunits coded in *bchD*, *bchI*, and *bchH* genes. For the biosynthesis of Zn-BChl *a*, three possible mechanisms can be assumed. First, a Zn chelatase is actually present in *Acidiphilium*. Second, the Mg chelatase can also catalyze Zn insertion into protoporphyrin IX when a Zn ion is available. Third, after the insertion of Mg into protoporphyrin IX, the Mg atom is exchanged for a Zn atom during subsequent BChl biosynthesis steps.

Masuda et al. (1999) have searched for *bchD*, *bchI*, and *bchH* genes which are known to code Mg-chelatase subunits in purple bacteria and found respective homologues in the *Acp. rubrum* genome. After cloning, they showed that the products of these genes have Mg-chelatase activity from functional complementation of *Rhodobacter capsulatus* BChl biosynthesis mutants. They also detected Mg-protoporphyrin IX monomethylester in cell extract of *Acp. rubrum*, while neither Zn-protoporphyrin IX monomethylester nor Zn-protoporphyrin IX were detected. Thus, it is likely that Zn-BChl *a* is formed by a substitution of a Zn ion for the Mg ion at some step(s) in the BChl synthesis pathway after formation of Mg-protoporphyrin IX monomethyl ester (Fig. 8).

Fig. 8. Proposed biosynthetic pathway of Zn-Bchl *a*.

As noted above, culture media commonly used for *Acidiphilium* contain only trace amounts of zinc. Therefore, if a non-enzymatic reaction is involved in zinc insertion into protoporphyrin IX to form Zn-Bchl *a* in *Acidiphilium* species there may be a Zn-incorporating and -concentrating system in them that enables the occurrence of high local concentrations of Zn ion in the cell.

5. Photosynthetic Apparatus

5.1. Reaction center

As described in Section 3.1., the membrane preparation of *Acp. rubrum* shows a similar but slightly blue-shifted near-infrared absorption spectrum to those of purple phototrophic bacteria lacking in the peripheral (LH 2) type of antenna complex. This suggests that *Acp. rubrum* has the reaction center and the core-antenna complex (LH 1) of the purple bacterial type and the major pigment in these pigment-protein complexes is Zn-Bchl *a*. However, it was possible that the reaction center, where photochemical reaction is conducted, contained Mg-Bchl *a* instead of Zn-Bchl *a*, since this bacterium has small amount of Mg-Bchl *a* (Hiraishi et al., 1998).

Treatment of the membranes with a detergent, lauryldimethylamine-N-oxide, followed by chromatographic separation, yielded a fraction showing a similar absorption spectrum to that of the purple bacterial reaction center (Shimada et al., 1998). However, the broad absorption band around 850 nm is significantly blue-shifted from that of the special pair of Bchl *a* in the purple bacterial reaction center (Fig. 9a). The absorption band corresponding to the accessory BChls is observed at 793 nm, also blue-shifted from those in the typical purple bacteria. Chemical oxidation of the presumed reaction center preparation by potassium

Fig. 9. Absorption spectra of the pigment-proteins of *Acp. rubrum*.

(a) Reaction center preparation (solid line). Corresponding spectrum of *Rhodobacter sphaeroides* R-26 reaction center is also shown (broken line). (b) LH 1 preparation (solid line). Subunit form obtained by 1% SDS treatment (broken line).

ferricyanide induces absorption decrease in the 850 nm band and blue-shift of the 793 nm band which can be reversed by reduction with sodium ascorbate. These absorption changes are consistent with those observed in the membranes upon flash excitation (Wakao et al., 1996). Thus, this fraction is identified as the reaction center complex of *Acp. rubrum*. Pigment analysis of the reaction center preparation clearly shows the absence of Mg-Bchl *a* in this fraction. Four

Zn-BChl *a* and two BPhe *a* molecules per one carotenoid, spirilloxanthin, have been found, suggesting that the four Mg-BChls *a* in the purple bacterial reaction center are replaced by Zn-BChls *a* in the *Acidiphilium* reaction center and Zn-BChls *a* are functioning as the special pair and the accessory BChls in this reaction center (Akiyama et al., 1998a; Shimada et al., 1998).

An interesting feature of the *Acidiphilium* reaction center is the incomplete bleaching of the 850 nm band upon oxidation. In the reaction centers of typical purple bacteria, the absorption band of the special pair around 865 nm is bleached almost completely by photo or chemical oxidation. Although the magnetic circular dichroism (MCD) spectrum indicates the dimeric nature of the primary donor Zn-BChls *a* in the *Acidiphilium* reaction center, the magnitude of the oxidation-induced bleach around 596 nm (Q_x band) suggests that the cation formed by charge separation is localized on a single Zn-BChl *a* of the special pair. Analysis for spectral components of absorption and circular dichroism spectra also indicates a weaker interaction between the special pair BChls than that in purple bacterial reaction centers (Mimuro et al., 2000). Thus, it is likely that the spatial arrangement of the special pair BChls in the *Acidiphilium* reaction center is different from those in the reaction center of typical purple bacteria. The substitution of L168His residue close to one of the special pair BChl in the purple bacterial reaction center to Glu residue in the *Acidiphilium* reaction center (see below) seems to intimately correlate with the altered arrangement of the special pair.

5.2. Antenna complex

The magnitude of photobleach of the reaction center in membranes of *Acp. rubrum* is dependent on the intensity of the excitation light. The dependency is similar to that observed in a typical purple bacterium, *Rhodospirillum rubrum*, indicating that *Acp. rubrum* has a light-harvesting system which is as efficient as those of purple bacteria (Wakao et al., 1996). As expected from the absorption spectrum of the membranes (Fig. 4a), no indication of the presence of LH 2-type (peripheral) antenna complex in the *Acp. rubrum* membrane has been obtained. By chromatographic or electrophoretic fractionation of the membrane lysate, only one type of antenna complex showing a single near-infrared absorption band at 863 nm has been isolated. Since genes homologous to those of LH 1 (core antenna)

apoproteins have been found with those for reaction center proteins as described below, the isolated antenna has been identified as the LH 1 complex of *Acp. rubrum*. The reversible dissociation into subunit form by detergent treatment also confirms the nature of this complex to be of the LH 1 type (Fig. 9b).

The dominance of Zn-BChl *a* in this LH 1 complex has been suggested by the blue-shifted absorption maximum of both multimeric and subunit forms compared with those of the common purple bacteria. Actually, no Mg-BChl *a* can be detected in the isolated LH 1 complex. Thus, the small amount of Mg-BChl *a* present in the *Acp. rubrum* cells has been lost during the isolation procedure. It is likely that the LH 1 apoprotein as well as reaction center apoprotein had been specified for Zn-BChl binding, which is also suggested by the substitution of a conserved amino acid residue shown below.

5.3. Photosynthetic electron transfer

On flash excitation of *Acp. rubrum* cells, an absorption decrease is observed around 550 nm showing that *c*-type cytochrome(s) are functioning as the immediate electron donor to the light-excited reaction center. Analysis of the reaction center gene has shown that the *Acidiphilium* reaction center has a tetraheme cytochrome subunit as do many purple bacteria. Although this subunit is not present in the isolated reaction center preparation, the fast photo-oxidation of *c*-type cytochrome in membranes shows that this subunit is functioning in vivo. In intact cells, the re-reduction of the photo-bleached *c*-type cytochrome can be observed indicating the operation of a photosynthetic electron transfer system in *Acp. rubrum*. In the soluble fraction obtained after cell disruption, the presence of soluble *c*-type cytochromes can be detected (Narita, A., et al., unpubl.). Since photoinhibition of respiration can be observed, it is likely that *Acidiphilium* has a photosynthetic electron transfer system similar to those of purple bacteria. It should be investigated, however, whether *Acidiphilium* has *b/c* type complex which is active in proton transfer against the extremely high external H^+ concentration.

6. Genes for Photosynthetic Apparatus

Genes for reaction center proteins of *Acidiphilium* species were sought and sequenced using probes designed on the basis of the conserved nucleotide se-

Fig. 10. Structure of *puf* genes and deduced amino acid sequences of *Acp. rubrum*.

(a) Boxes B, A, L, M, C indicate the positions of genes coding for the b and a subunits of LH 1 complex and the L, M and cytochrome subunits of the reaction center, respectively. A possible ORF and a BChl biosynthesis gene (*bchZ*) are also indicated. (b) Alignment of amino acid sequences around Glu L168 of the reaction center L subunit. His L173 which is the ligand for the Mg atom in one BChl of the special pair is marked by an asterisk. The 168th Glu residues of *Acidiphilium* and corresponding His residues of other purple bacteria are underlined. (c) Alignment of amino acid sequences of α subunit of LH 1. BChl-binding His residue is marked by an asterisk. The 39th Asp residues of *Acidiphilium* and corresponding Asn residues of other purple bacteria are underlined.

quences of the reaction center genes (*puf* genes) of purple bacteria as the PCR primers (Nagashima et al., 1997). The deduced amino acid sequences showed that *Acidiphilium* species have genes for L and M subunits of the reaction center which is highly homologous to those of common purple bacteria. Flanking regions of the L and M genes have also been sequenced in *Acp. rubrum* ATCC 35905 and *Acp. angustum* ATCC 35903. Comparison of the sequences with those of *puf* genes of purple bacteria shows that the reaction center LM genes are flanked by a gene for BChl synthesis (*bchZ*), genes for LH 1 apoproteins and a gene for tetraheme cytochrome subunit of the reaction center.

Thus, *Acp. rubrum* has a *puf* operon similar to those of other purple bacteria (Fig. 10a). Since *Acp. rubrum* also has a similar gene cluster for photopigment biosynthesis including *bchB*, *bchD*, *bchF*, *bchH*, *bchI*, *bchL*, *bchN*, *bchP*, and a carotenoid biosynthesis gene (*crtI*) (Masuda et al., 1999), it is likely that *Acidiphilium* has a large photosynthetic gene cluster as revealed in some purple bacteria (Igarashi et al., 2001).

In the alignment of the deduced amino acid sequence of the reaction center subunits, all except one amino acid residue functioning in the cofactor binding in the reaction center are conserved in *Acidiphilium*. The only significant difference is the substitution of

L168His residue that is hydrogen-bonded to the acetyl-carbonyl group of one BChl of the special pair in the purple bacterial reaction center (Mattioli et al., 1994) (Fig. 10b). This residue is replaced by Glu residue in the *Acidiphilium* reaction center which may significantly affect the spatial arrangement of the special pair. Since the radius of the Zn atom is larger than that of the Mg atom, the Zn atom in the Zn-BChl *a* may protrude from the surface of the macrocycle of the bacteriochlorin ring. The substitution of the L168His residue with Glu may correspond to this difference in the shape of BChls.

Noteworthy substitution of an amino acid residue is also seen in the LH 1 apoprotein. A conserved Asn residue adjacent to the highly conserved Trp residue at the C-terminal side from the BChl-binding His residue of the α subunit is replaced by Asp residue (Fig. 10c). The Asn residue is possibly interacting with BChl molecules in purple bacterial antenna proteins as well as the adjacent Trp residue which is shown to interact with B850 BChl in the LH 2 complex of purple bacteria (McDermott et al., 1995).

7. Concluding Remarks and Future Perspectives

The finding that Zn-BChl *a* is present as the main and functional photopigment in *Acidiphilium* was one of the most remarkable achievements in recent photosynthetic research. The existence of Zn-BChl-containing photosynthetic organisms had not been expected before this finding, although it is not surprising because Zn-(B)Chl *a* can work well in place of Mg-(B)Chl *a* in photosynthesis in view of its photochemical and electrochemical nature. At any rate, the discovery of naturally occurring Zn-BChl raised interesting questions, some of which are still to be clarified in future studies.

Why *Acidiphilium* species have selected Zn-BChl *a* rather than Mg-BChl *a* for their photosynthesis is of major concern but not yet fully understood. One of the possible explanations for this is the physico-chemical stability of Zn-BChl *a* in relation to the natural habitats of *Acidiphilium*. Since Zn-porphyrin derivatives are more stable than the Mg-containing ones, they have been used in studies of artificial photosynthesis (Osuka et al., 1993; Wasielewski and Niemczyk, 1984). As noted above, the rate of pheophytinization upon treatment with H₂SO₄ or HCl is much smaller for Zn-BChl *a* than for Chl *a* and Mg-BChl *a* (Kobayashi et

al., 1998b). Because of its stability, Zn-BChl *a* may have an advantage in photosynthesis especially under strongly acidic environments. Since the special pair BChls of the reaction center and the antenna BChls in LH 1 complex in purple bacteria are located near the periplasmic side of the cytoplasmic membrane, they may be affected to some extent by external pH. This may be also true for *Acidiphilium* species, since they have homologous pigment-protein complexes to those of common purple bacteria.

Another possible explanation for the occurrence of Zn-BChl in the acidophilic bacteria is the availability of zinc ion under strongly acidic conditions. Since zinc becomes insoluble as its hydroxide complex at neutral pH, neutrophilic phototrophic bacteria encounter difficulty in obtaining zinc even if they prefer to use Zn in place of Mg for their BChls. Conversely, the solubility of zinc is markedly increased under acidic conditions (<pH 4), and the magnesium-porphyrins become easily pheophytinized. In fact, the Zn-BChl/Mg-BChl ratio in *Acp. rubrum* is increased with decreasing pH of the culture medium as noted above. However, some chemotrophic bacteria are known to produce zinc complexes of porphyrins in growth media at neutral pH (Kajiwara et al., 1995; Toriya et al., 1993) and the intracellular pH of acidophilic bacteria including *Acidiphilium* was reported to be neutral in general (Hsung and Haug, 1975; Matin, 1999; Zychlinsky and Matin, 1983a, b). Although it is likely that acidic habitats may provide more favorable conditions for Zn-BChl production by photosynthetic organisms living therein, the relationship between specific Zn-BChl requirement for photosynthesis and acidic conditions as natural habitats awaits further study.

Where and how the zinc substitution takes place during the biosynthesis of Zn-BChl *a* in *Acidiphilium* is another interesting question. Masuda et al. (1998) have provided evidence that Zn-BChl *a* is formed by a substitution of a Zn ion for the Mg ion at some step(s) in the BChl synthesis pathway after formation of Mg-protoporphyrin IX monomethyl ester. At present, nothing is known about the presence of a distinct zinc chelatase or "metal-exchange enzyme" involved in this reaction. Zinc is known to be easily inserted into porphyrin without any catalyst, but the zinc insertion becomes more difficult as the macrocyclic ring is reduced from chlorin to bacteriochlorin (Tamiaki et al., 1998). Zinc insertion by direct metal exchange into magnesium-complexed bacteriochlorin may require substan-

tial heat energy for completion. If zinc substitution proceeds without involvement of any enzyme, one can assume that it is by spontaneous insertion into a hypothetical pheophytinized intermediate after formation of Mg-protoporphyrin IX monomethyl ester.

Apart from a number of questions to be clarified, it may be logical to conclude that the capacity for photosynthesis with Zn-BChl *a* of *Acidiphilium* is a result of its adaptation to acidic mineral environments in the evolutionary process. The gene structure for proteins of photosynthetic apparatus of *Acidiphilium* shows not only some characteristic features, as seen in the substitution of the L168His residue by Glu, but also highly conserved amino acid sequences among the *Acidiphilium* species in contrast to some intragenic 16S rRNA heterogeneity (see Fig. 1). The *puf* gene sequences of neutrophilic and mesophilic purple bacteria within a single genus, for example those of *Rhodobacter*, seem to be much more variable. It is of special interest to note whether the highly conservative gene structure of *Acidiphilium* has been formed under such an environmental selection pressure as high acidity. Finally, interesting questions are how *Acidiphilium* species express photosynthetic genes in their natural habitats and how the photosynthesis with low BChl levels contribute to the life of them. These questions are at issue in all other species of the ABC bacteria.

Acknowledgments

We thank K. Inoue, S. Itoh, M. Mimuro, K. V. P. Nagashima, S. Takaichi, K. Takamiya, H. Tamiaki, and N. Wakao for providing us with unpublished data and stimulating discussions. This work was supported in part by a grant-in-aid from the Ministry of Education, Science, Sports and Culture, Japan (No. 09309008).

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