15

Functional Genomics of Anoxygenic Green Bacteria *Chloroflexi* Species and Evolution of Photosynthesis

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1. Introduction

In addition to the most recently reported aerobic anoxygenic phototrophic bacterium *Chloroacidobacterium thermophilium* [1], five phyla of phototrophic bacteria have been reported, including four phyla anoxygenic phototrophic bacteria (anaerobic and aerobic anoxygenic phototrophic Proteobacteria, filamentous anoxygenic phototrophs (FAPs), green sulfur bacteria and heliobacteria) and oxygenic phototrophic bacteria (cyanobacteria). According to 16S rRNA analysis, *Chloroflexi* species in FAPs are the earliest branching bacteria capable of photosynthesis [2,3] (**Fig. 1**), and the thermophilic bacterium *Chloroflexus* [Cfl.] *aurantiacus* among the *Chloroflexi* species has been long regarded as a key organism to resolve the obscurity of the origin and early evolution of photosynthesis. *Cfl. aurantiacus* can grow phototrophically under anaerobic conditions or chemotrophically under aerobic and dark conditions [4]. During phototrophic growth of *Cfl. aurantiacus*, the light energy is first absorbed by the peripheral light-harvesting complex chlorosomes, then transferred to the integral membrane B808-866 core antenna complex and finally to the reaction center (RC). *Cfl. aurantiacus* contains a chimeric photosystem that comprises some characters of green sulfur bacteria (chlorosomes) and anoxygenic phototrophic Proteobacteria (the B808-866 core antenna complex), and also has some unique electron transport proteins compared to other photosynthetic bacteria. The complete genomic sequence of *Cfl. aurantiacus* has been recently determined, analyzed and compared to the genomes of other photosynthetic bacteria [5].

Significant contributions of horizontal/lateral gene transfer among uni-cellular [6] and multi-cellular [7] organisms during the evolution, including the evolution of photosynthesis [8,9], have been recognized. Various perspectives on evolution of photosynthesis have been reported in literature [8-25], whereas our understanding of transition from anaerobic to aerobic world is still fragmentary. The recent genomic report on *Cfl. aurantiacus* [5], along with previous physiological, ecological and biochemical studies, indicate that the anoxygenic phototroph bacterium *Cfl. aurantiacus* has many interesting and certain unique features in its metabolic pathways. The *Cfl. aurantiacus* genome contains numerous aerobic/anaerobic gene pairs and oxygenic/anoxygenic metabolic pathways in the *Cfl. aurantiacus* genome [5], suggesting numerous gene adaptations/replacements in *Cfl. aurantiacus* to facilitate life under both anaerobic and aerobic growth conditions. These

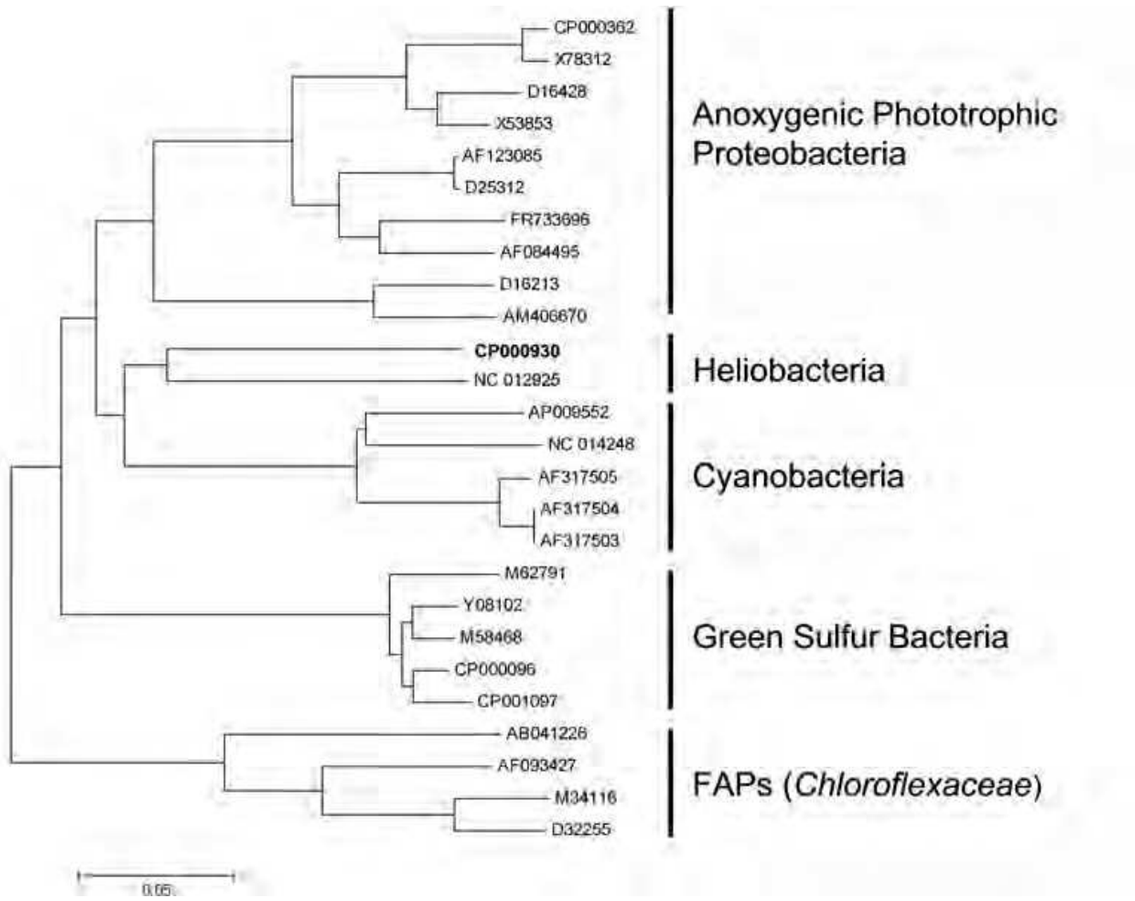


Fig. 1. Phylogenetic tree of photosynthetic bacteria.
The tree was constructed with un-rooted neighbor joining 16S rRNA dendrogram from five phyla of photosynthetic microbes, including cyanobacteria, heliobacteria, phototrophic anoxygenic Proteobacteria, green sulfur bacteria and filamentous anoxygenic phototrophs (FAPs). Bacterial names and accession numbers of 16S rRNA genes: (1) Phototrophic anoxygenic Proteobacteria: *Roseobacter denitrificans* OCh114 (CP000362), *Roseobacter litoralis* (X78312), *Rhodobacter capsulatus* (D16428), *Rhodobacter sphaeroides* 2.4.1 (X53853), *Rhodopseudomonas faecalis* strain gc (AF123085), *Rhodopseudomonas palustris* (D25312), *Rhodopseudomonas acidophila* (FR733696), *Rhodopseudomonas viridis* DSM 133 (AF084495), *Rubrivivax gelatinosus* (D16213); (2) heliobacteria: *Heliobacterium gestii* (AB100837), *Heliobacterium modesticaldum* (CP000930); (3) cyanobacteria: *Oscillatoria amphigranulata* str. 19-2 (AF317504), *Oscillatoria amphigranulata* str. 11-3 (AF317503), *Oscillatoria amphigranulata* str. 23-3 (AF317505), *Microcystis aeruginosa* NIES-843 (AP009552), *Nostoc azollae* 0708 (NC_014248); (4) green sulfur bacteria: *Chlorobaculum thiosulfatiphilum* DSM 249 (Y08102), *Pelodictyon luteolum* DSM 273 (CP000096), *Chlorobium limicola* DSM 245 (CP001097), *Chlorobaculum tepidum* TLS (M58468), *Chlorobium vibrioforme* DSM 260 (M62791); and (5) FAPs: *Chloroflexus aurantiacus* J-10-fl (M34116), *Chloroflexus aggregans* (D32255), *Oscillochloris trichoides* (AF093427), *Roseiflexus castenholzii* DSM 13941 (AB041226)

include duplicate genes and gene clusters for the alternative complex III (ACIII) [26,27], auracyanin (a type I blue copper protein) [28,29] and NADH:quinone oxidoreductase (complex I); and several aerobic/anaerobic enzyme pairs in central carbon metabolism (pyruvate metabolism and the tricarboxylic acid (TCA) cycle) and tetrapyrroles and nucleic acids biosynthesis [5]. Overall, genomic information is consistent with a high tolerance for oxygen that has been reported in the growth of *Cfl. aurantiacus*.

Phylogenetic analyses on the photosystems and comparisons to the genome and reports of other photosynthetic bacteria suggest lateral or horizontal gene transfers between *Cfl. aurantiacus* and other photosynthetic bacteria [3,30,31]. The *Cfl. aurantiacus* genome suggests possible evolutionary connections of photosynthesis. Here we probe some proposed lateral gene transfers using the phylogenetic analyses on important proteins/enzymes on chlorophyll biosynthesis, photosynthetic electron transport chain, and central carbon metabolism. Further, we also discuss the evolutionary perspectives on assembling photosynthetic machinery, autotrophic carbon assimilation and unique components on the electron transport chains of *Cfl. aurantiacus* and other phototrophic and non-phototrophic bacteria.

2. Results and discussion

a. Photosynthetic components

The photosystem of *Cfl. aurantiacus* is a chimeric system with contains a peripheral light harvesting complex chlorosomes and an integral membrane B808-866-type II RC (quinone-type) core complex. Chlorosomes are typically found in type I (Fe-S type) RC phototrophic organisms, such as green sulfur bacteria (GSBs) [32] and the recently discovered aerobic anoxygenic bacterium *Chloroacidobacterium thermophilium* [1], whereas the B808-866-RC core complex is arranged similarly to the LH-RC core complex in phototrophic Proteobacteria [33]. Thus, the *Cfl. aurantiacus* photosystem indicates little correlation between the RC type and light-harvesting antenna complexes in the assembly of the photosystem of anoxygenic phototrophic bacteria [8,34]. Two hypotheses, which are selective loss and fusion, for evolutionary of photosynthetic RCs have been proposed [8,35]. The phylogenic analyses and evolutionary perspectives of the integral membrane-RC core complex in *Cfl. aurantiacus* and other phyla of phototrophic bacteria are presented in several reports [8,36,37] for readers who are interested in further information. It is possible that during the evolution of photosynthesis chlorosomes were transferred between *Cfl. aurantiacus* and GSBs, which have larger chlorosomes and more genes encoding chlorosome proteins [38,39], and that the integral membrane core antenna complex and a type II RC in *Cfl. aurantiacus* were possibly transferred either to or from photosynthetic anoxygenic Proteobacteria.

b. Electron transfer complexes

Four copies of auracyanin genes have been identified in the *Cfl. aurantiacus* genome and two aurancyanin proteins have been characterized biochemically and structurally [28]. Auracyanin has also been biochemically characterized in *Roseiflexus castenholzii* [40], which only has one copy of aurancyanin gene in the genome [5]. The gene encoding a putative auracyanin has been identified in the genome of the non-photosynthetic aerobic thermophilic bacterium *Thermomicrobium roseum* DSM 5159, which is evolutionally related to *Cfl. aurantiacus* [41]. Genes encoding auracyanin may have been transferred either to or from

Thermomicrobium roseum. Further, higher plants, green algae and cyanobacteria operate the photosynthetic electron transport via a water-soluble mobile type I blue copper protein plastocyanin. Auracyanin may have evolved from or to plastocyanin in cyanobacteria.

Most of phototrophic bacteria use the cytochrome bc_1 or b_6/f complex for transferring electrons during phototrophic growth, whereas *Chloroflexi* species operate photosynthetic electron transport using a unique complex, namely alternative complex III (ACIII) [1,26,27]. Two sets of ACIII gene clusters, one containing seven genes and the other containing thirteen genes, have been identified in the *Cfl. aurantiacus* genome [5]. The seven subunit complex has been characterized biochemically [27]. In contrast, *Roseiflexus castenholzii*, which is a member of a familia *Chloroflexaceae* and phylogenetically closely related to *Cfl. aurantiacus* [42], contains only one copy of the ACIII operon with a six-gene cluster (Rcas_1462-1467) [5]. In addition to *Cfl. aurantiacus* and other members of *Chloroflexaceae*, genes encoding ACIII, which contains seven subunits [27], have also been identified in the *Chloroacidobacterium thermophilium* genome [1]. ACIII has also been identified in non-phototrophic bacterium *Rhodothermus marinu*s [43] and suggested to wide-spread in prokayrotes [44]. Genes encoding ACIII may have been transferred either from or to evolved from or to *Chloroacidobacterium thermophilium* (and/or *Rhodothermus marinu*s). Further, ACIII may have evolved from or to the cytochrome bc_1 or b_6/f complex.

NADH:quinone oxidoreductase (Complex I, EC 1.6.5.3) is known to be responsible for the electron transport in the respiratory chain. Two sets of the Complex I genes, one of which forms a gene cluster, have been identified in the *Cfl. aurantiacus* genome [5]. Two Complex I gene clusters have also been identified in some anaerobic anoxygenic phototrophic Proteobacteria (AnAPs), such as *Rhodobacter* [Rba.] *sphaeroides* and *Rhodopseudomonas* [Rps.] *palustris*, and gene expression profile in *Rba. sphaeroides* suggests that one of the gene clusters is responsible for photosynthetic electron transport during phototrophic and anaerobic growth and the other is required for the respiratory chain during aerobic and dark growth [45]. **Fig. 2** shows the phylogenetic trees constructed based on the amino acid sequences of the subunit F of Complex I (encoded by the *nuoF* gene) in phototrophic bacteria. The subunit F protein in **Fig. 2A** is encoded by the gene locus Caur_2901 in the gene cluster (Caur_2896 to Caur_2909), and the subunit F protein in **Fig. 2B** is encoded by the gene locus Caur_1185. No Complex I genes have been identified in the green sulfur bacteria, which cannot respire or grow in darkness. Note that one subunit F protein in *Cfl. aurantiacus* is more related to the protein in anoxygenic phototrophic Proteobacteria than to the protein in heliobacteria and cyanobacteria (**Fig. 2A**) and the other *Cfl. aurantiacus* subunit F protein is more related to the protein in heliobacteria and cyanobacteria than to the protein in anoxygenic Proteobacteria (**Fig. 2B**), suggesting different biological functions for two NADH:quinone oxidoreductase complexes found in the *Cfl. aurantiacus* genome.

c. (Bacterio)chlorophyll biosynthesis

AcsF (aerobic cyclase) and BchE (anaerobic cyclase) are suggested to be responsible for biosynthesis of the isocyclic ring of (bacterio)chlorophylls and conversion of Mg-protoporphyrin monomethyl ester (MgPMMe) to Mg-divinyl-protochlorophyllide *a* (PChlide) under aerobic and anaerobic growth conditions, respectively [46-51] (**Fig. 3A**). Both MgPMMe and PChlide are suggested to be photosensitizers of higher plants and green algae that produce reactive oxygen species in response to the excess light [52]. Both *acsF* (Caur_2590) and *bchE* (Caur_3676) are detected in the *Cfl. aurantiacus* genome [5]. AcsF has

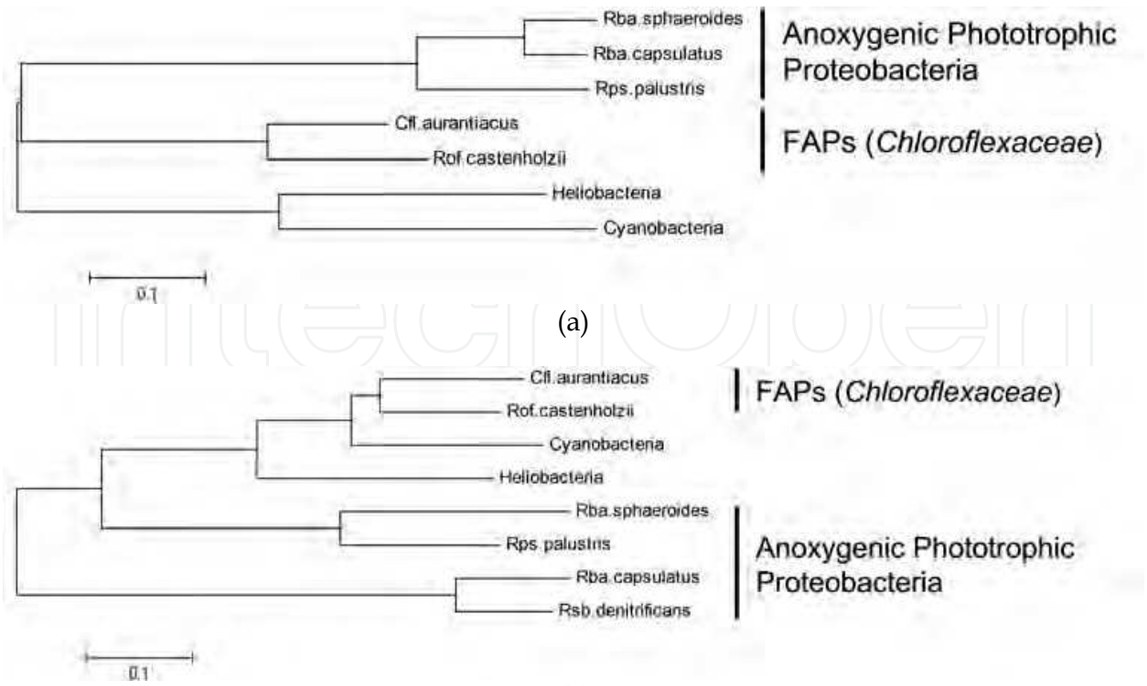
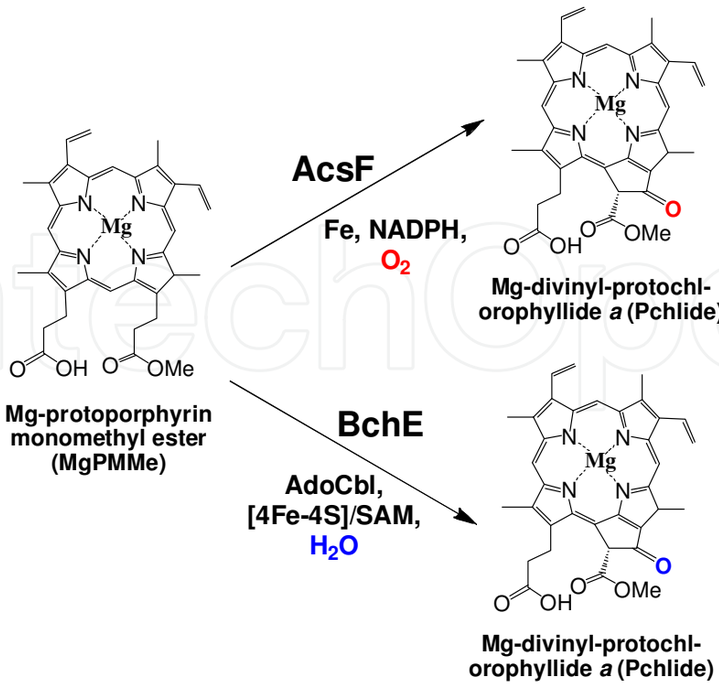
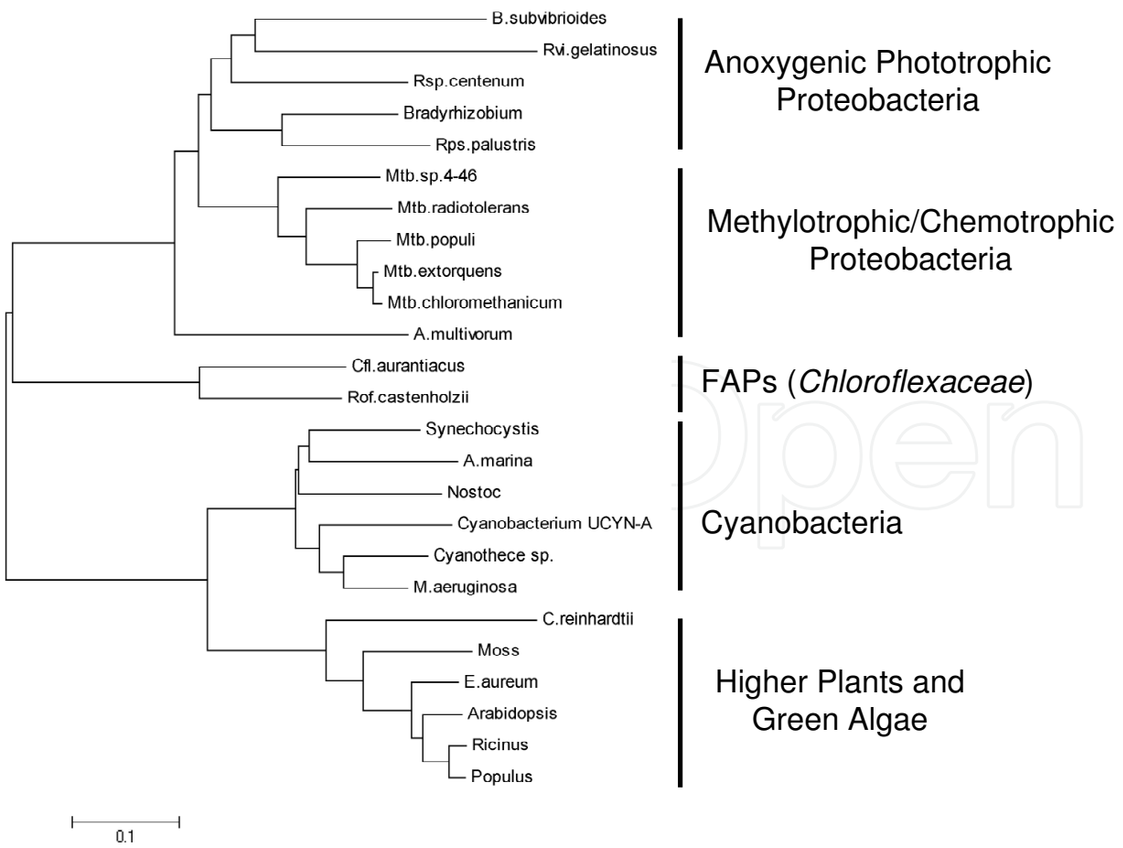


Fig. 2. Phylogenetic tree of the NADH:quinine oxidoreductase (Complex I) in phototrophic bacteria.
The subunit F proteins of *Cfl. aurantiacus*, *Roseiflexus* [Rof.] *castenholzii* (FAPs), *Rhodobacter* [Rba.] *sphaeroides* and *Rhodopseudomonas* [Rps.] *palustris* (anoxygenic Proteobacteria) in **Fig. 2A** and **2B** are encoded by different *nuoF* genes. Two Complex I are identified in *Cfl. aurantiacus*, *Rof. castenholzii*, *Rba. sphaeroides* and *Rps. palustris*, and one Complex I gene cluster is found in heliobacteria, cyanobacteria and some phototrophic anoxygenic Proteobacteria (e.g., *Rba. capsulatus* and *Roseobacter* [Rsb.] *denitrificans*). The trees are constructed based on amino acid sequences using the phylogenetic software MEGA5 [65] with un-rooted neighbor jointing method.

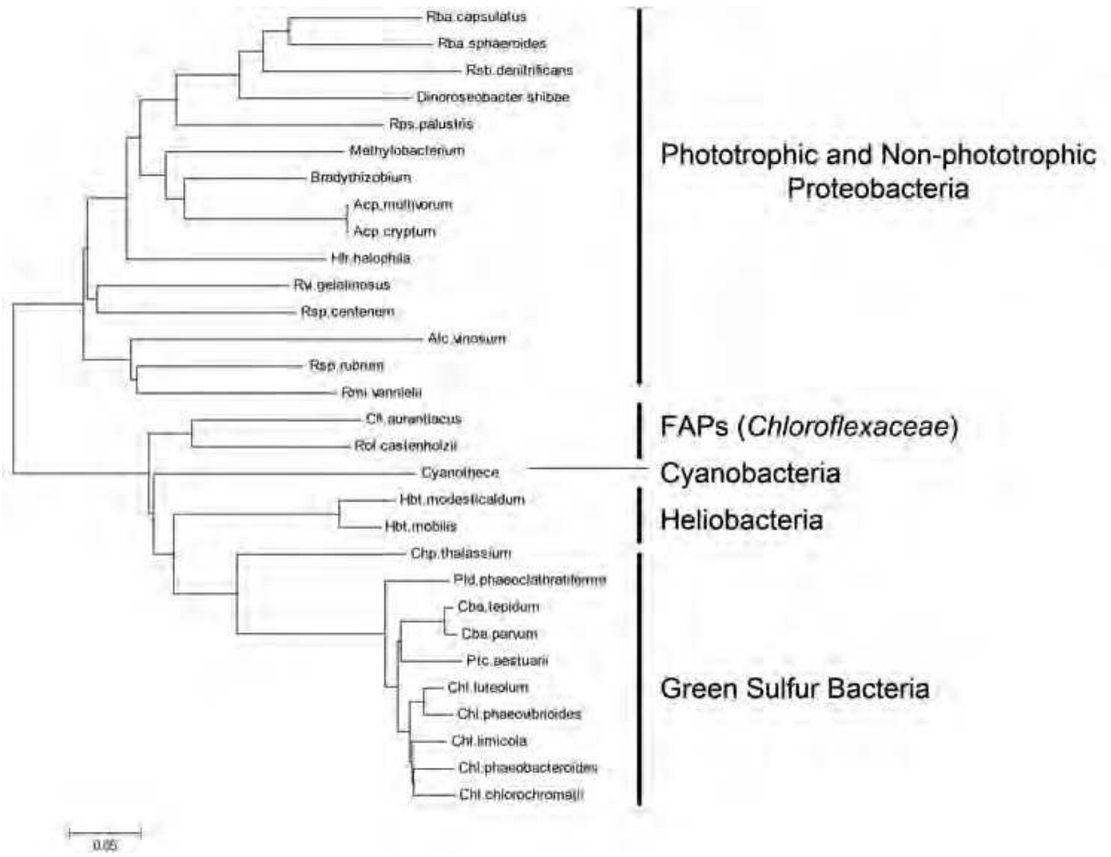
not been identified in any strictly anaerobic phototrophic bacteria (e.g., green sulfur bacteria and heliobacteria). In addition to Proteobacteria (including aerobic and anaerobic anoxygenic phototrophic Proteobacteria) and cyanobacteria, several non-phototrophic α-Proteobacteria also contain the *acsF* gene, including several facultative methotrophic bacteria (e.g., *Methylocella silvestris*, *Methylobacterium* [Mtb.] sp. 4-46, *Mtb. populi*, *Mtb. chloromethanicum*, *Mtb. radiotolerans* and *Mtb. extorquens*) and the environmental bacterium *Brevundimonas subvibrioides* (**Fig. 3B**). Roles of the gene encoding the putative AcsF in these non-phototrophic bacteria are unclear. AcsF has also been characterized for *Cfl. aurantiacus* grown under anaerobic conditions [50]. Together, the role of AcsF remains to be further understood. BchE is widely spread in all phyla of anoxygenic phototrophic bacteria (e.g., anoxygenic phototrophic Proteobacteria, green sulfur bacteria, heliobacteria and FAPs) and some facultative methyltrophic bacteria and cynaobacteria also contain the gene encoding the putative BchE (**Fig. 3C**). Experimental evidence indicates that the *bchE* genes in the cyanobacterium *Synechocystis* sp. PCC 6803 are important but do not contribute to the formation of the isocyclic ring of chlorophylls [47].


AcsF
Fe, NADPH,
O2
Mg-divinyl-protochl-orophyllide a (Pchlide)
Mg-protoporphyrin monomethyl ester (MgPMMe)
BchE
AdoCbl,
[4Fe-4S]/SAM,
H2O
Mg-divinyl-protochl-orophyllide a (Pchlide)

(a)


B.subvibrioides
Rvi.gelatinosus
Rsp.centenum
Bradyrhizobium
Rps.palustris
Mtb.sp.4-46
Mtb.radiotolerans
Mtb.populi
Mtb.extorquens
Mtb.chloromethanicum
A.multivorum
Cfl.aurantiacus
Rof.castenholzii
Synechocystis
A.marina
Nostoc
Cyanobacterium UCYN-A
Cyanothece sp.
M.aeruginosa
C.reinhardtii
Moss
E.aureum
Arabidopsis
Ricinus
Populus
0.1
Anoxygenic Phototrophic Proteobacteria
Methylotrophic/Chemotrophic Proteobacteria
FAPs (Chloroflexaceae)
Cyanobacteria
Higher Plants and Green Algae

(b)



(c)

Fig. 3. Reactions of aerobic cyclase (AcsF) and anaerobic cyclase (BchE) and the phylogenetic trees.
Conversion of MgPMMe into PChlide is suggested to be catalyzed by AcsF and BchE under aerobic and anaerobic conditions, respectively (A). The phylogenetic relationships of AcsF (B) and BchE (C) are shown. The trees are constructed based on amino acid sequences using the phylogenetic software MEGA5 [65] with un-rooted neighbor jointing method.

Phylogenetic analyses suggest that the *acsF* gene in *Cfl. aurantiacus* and other *Chloroflexaceae* species are more evolutionarily related to the genes in anoxygenic phototrophic Proteobacteria than to the genes in oxygenic phototrophs (cyanobacteria, green algae and higher plants) (**Fig. 3B**), and that the *bchE* gene in *Cfl. aurantiacus* is more evolutionarily related to the genes in strictly anaerobic phototrophs (green sulfur bacteria and heliobacteria) than to the genes in phototrophic and non-phototrophic Proteobacteria (**Fig. 3C**). It is possible that the *Cfl. aurantiacus acsF* gene was transferred either to or from Proteobacteria, and the *Cfl. aurantiacus bchE* gene was transferred either to or from heliobacteria and green sulfur bacteria. The phylogenetic analyses of AcsF and BchE in **Fig. 3** likely suggest horizontal gene transfers among phototrophic bacteria and also between phototrophic and non-phototrophic bacteria.

d. Central carbon metabolism

Here we analyze enzymes/gene products for pyruvate metabolism, which takes place in every living organism, and the TCA cycle. In contrast to other phyla of phototrophic

bacteria, *Cfl. aurantiacus* and other members of *Chloroflexaceae* are only bacteria containing both anaerobic and aerobic gene pairs for pyruvate and α-ketoglutarate metabolism: pyruvate/α-ketoglutarate dehydrogenase (aerobic enzymes) and pyruvate/α-ketoglutarate synthase (or pyruvate/α-keto-glutarate:ferredoxin oxidoreductase (PFOR/KFOR)) (anaerobic enzymes).

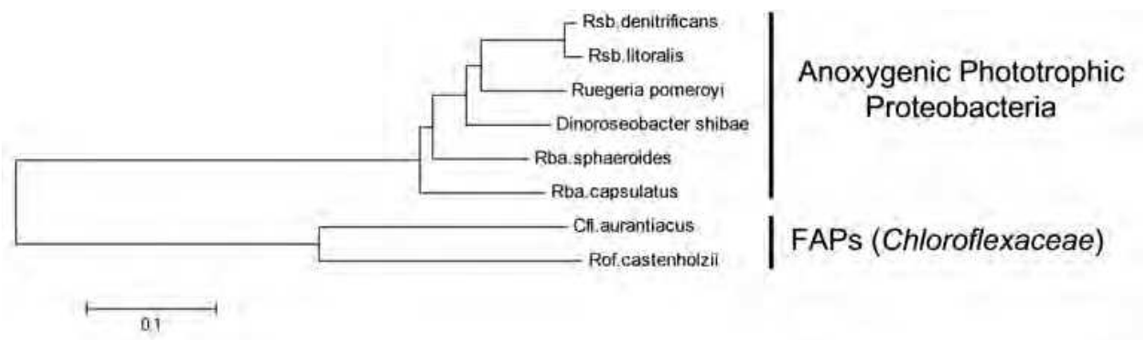
Fig. 4A shows the phylogenetic analyses of the E1 protein of α-ketoglutarate dehydrogenase **(**encoded by *sucA*) from FAPs and anoxygenic phototrophic Proteobacteria. Note that the *Cfl. aurantiacus* α-ketoglutarate dehydrogenase has higher sequence identities to many gram-(+) non-phototrophic *Bacillus* strains (~50%) than phototrophic anoxygenic Proteobacteria (~40%). Similar results also find in the sequence alignments of the E1 protein of pyruvate dehydrogenase, and the *Cfl. aurantiacus* enzyme has ~51-55% identities with *Thermobifida fusca*, *Streptomyces cattleya*, *Acidothermus cellulolyticus*, *Saccharopolyspora erythraea*, and *Sanguibacter keddieii* and ~38-44% or lower identities with the phosynthetic Proteobacteria and cyanobacteria (data not shown). These results support the horizontal gene transfer between microbial genomes. **Fig. 4B** shows the phylogenetic tree of the E1 protein of pyruvate dehydrogenase. The *Cfl. aurantiacus* enzyme is less related to cyanobacteria and anoxygenic phototrophic Proteobacteria.

Fig. 4C suggests that α-ketoglutarate synthase in *Cfl. aurantiacus* are more closely related to the enzyme in heliobacteria than in green sulfur bacteria. While the biochemical studies of the *Cfl. aurantiacus* α-ketoglutarate synthase have not been reported, the phylogenetic analyses of α-ketoglutarate synthase are consistent with the central carbon flow in these three phyla of photosynthetic bacteria: the green sulfur bacteria operate the reductive (reverse) TCA cycle, and *Cfl. aurantiacus* and heliobacteria have strong carbon flow via either a complete or a partial oxidative (forward) TCA cycle [34].

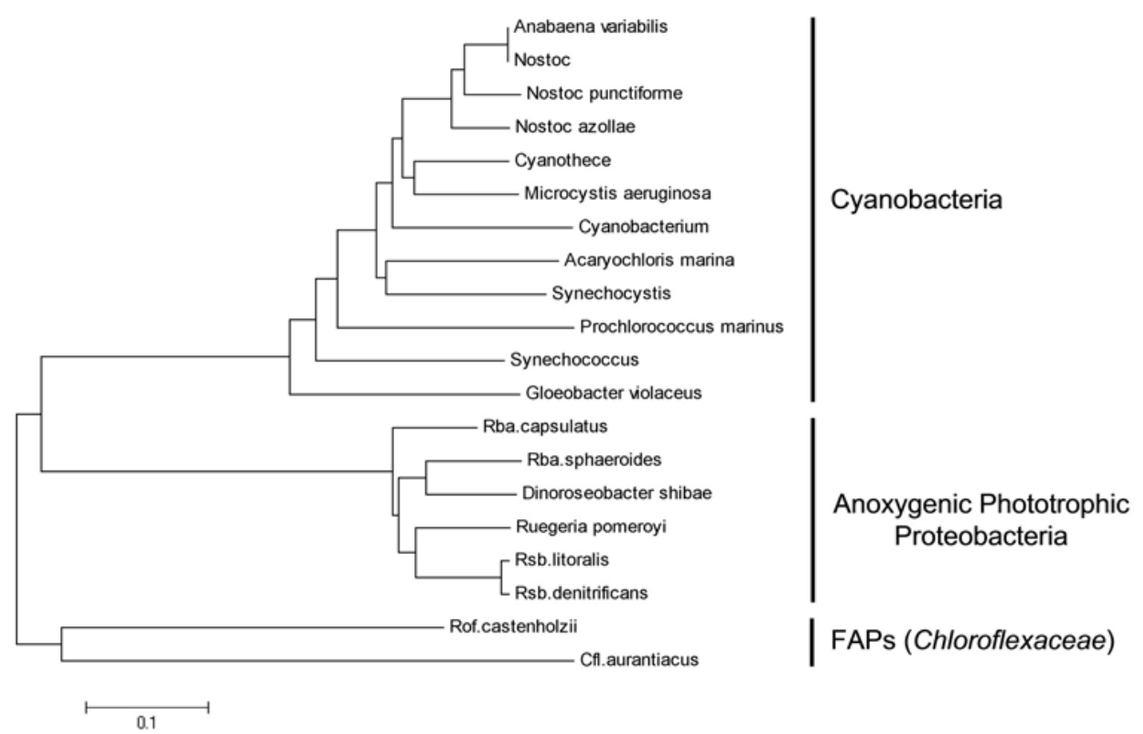
Fig. 4D suggests that pyruvate synthase in heliobcteria evolved prior to the enzymes in other phyla of photosynthetic bacteria, and that the enzyme in *Cfl. auranticus* is remotely related to the enzymes in GSBs and cyanobacteria, which are likely from the same origins, similar to the tree of the E1 protein of pyruvate dehydrogenase (**Fig. 4B**). Together, the phylogenetic analyses suggest pyruvate metabolism of anoxygenic phototrophic Proteobacteria is more related to cyanobacteria than to *Cfl. aurantiacus* (and perhaps FAPs). Compared to the experimental data, acetate can support the growth of *Cfl. aurantiacus* during anaerobic growth in the light and during aerobic growth in darkness [53], and acetate excretion has been reported during the pyruvate-grown heliobacteria [54,55] but not on other phyla of photosynthetic bacteria. *Cfl. aurantiacus* likely uses pyruvate synthase for assimilate acetyl-CoA. Since heliobacteria do not have pyruvate dehydrogenase, their pyruvate synthase is supposed to convert pyruvate to acetyl-CoA, which is then converted to acetate. Further, pyruvate synthase is essential for the growth of green sulfur bacteria because it is required to convert acetyl-CoA generated from the reductive TCA cycle to pyruvate, whereas the role of pyruvate synthase in oxygenic phototrophic bacteria (cyanobacteria) is not clear, as pyruvate synthase is sensitive to oxygen during biochemical characterization *in vitro*.

e. Autotrophic carbon assimilation

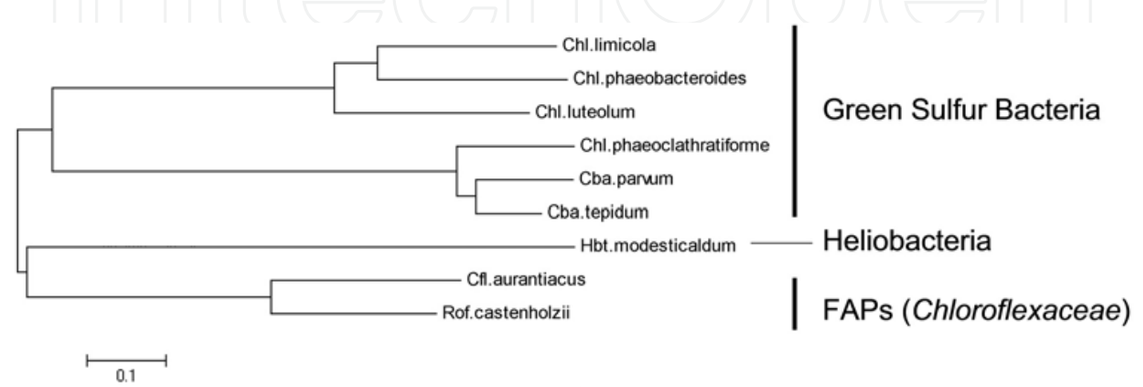
Cfl. aurantiacus can grow photoautotrophically and uses the 3-hydroxypropionate (3HOP) bi-cycle to assimilate inorganic carbon [5,56-58]. Both 3HOP bi-cycle and the widely distributed Calvin-Benson cycle can operate in both aerobic and anaerobic conditions.


Rsb.denitrificans
Rsb.litoralis
Ruegeria pomeroyi
Dinoroseobacter shibae
Rba.sphaeroides
Rba.capsulatus
Cfl.aurantiacus
Rof.castenholzii
0.1
Anoxygenic Phototrophic Proteobacteria
FAPs (*Chloroflexaceae*)

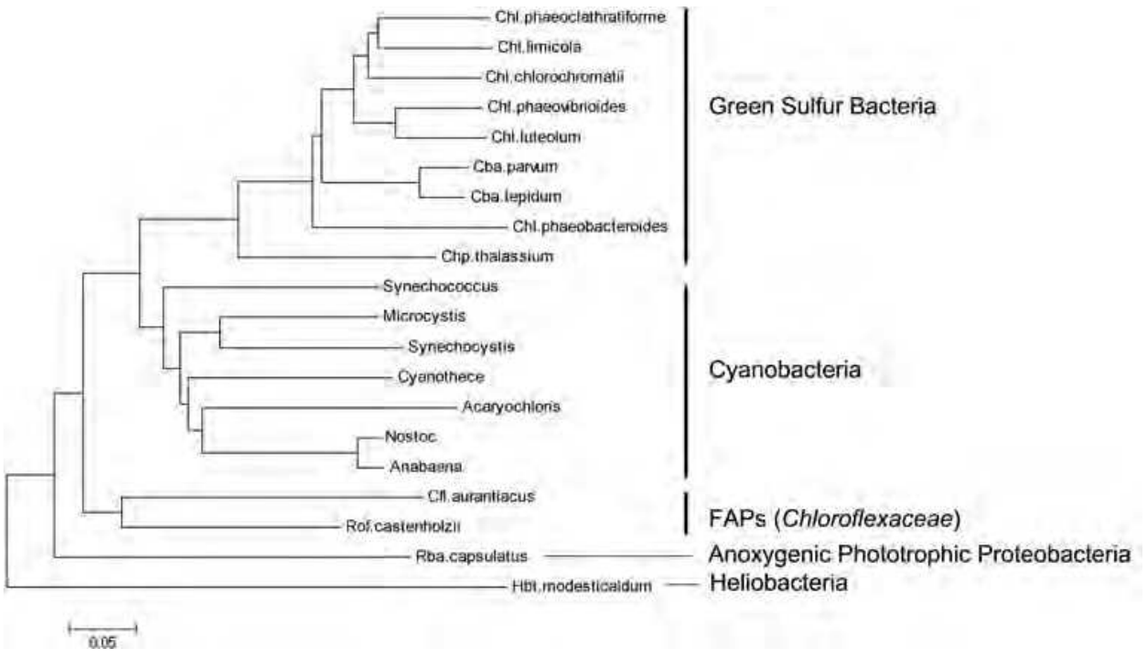
(a)


Anabaena variabilis
Nostoc
Nostoc punctiforme
Nostoc azollae
Cyanothece
Microcystis aeruginosa
Cyanobacterium
Acaryochloris marina
Synechocystis
Prochlorococcus marinus
Synechococcus
Gloeobacter violaceus
Rba.capsulatus
Rba.sphaeroides
Dinoroseobacter shibae
Ruegeria pomeroyi
Rsb.litoralis
Rsb.denitrificans
Rof.castenholzii
Cfl.aurantiacus
0.1
Cyanobacteria
Anoxygenic Phototrophic Proteobacteria
FAPs (*Chloroflexaceae*)

(b)


Chl.limicola
Chl.phaeobacteroides
Chl.luteolum
Chl.phaeoclathratiforme
Cba.parvum
Cba.tepidum
Hbt.modesticaldum
Cfl.aurantiacus
Rof.castenholzii
0.1
Green Sulfur Bacteria
Heliobacteria
FAPs (*Chloroflexaceae*)

(c)



(d)

Fig. 4. The phylogenetic trees of α-ketoglutarate dehydrogenase (A), pyruvate dehydrogenase (B), α-ketoglutarate synthase (C) and pyruvate synthase (D).
The trees are constructed based on amino acid sequences using the phylogenetic software MEGA5 [65] with un-rooted neighbor jointing method.

However, one significant problem leading to low photosynthesis efficiency of higher plants and oxygenic phototrophs is photorespiration and energy waste resulting from the interactions of oxygen with RuBisCO (ribulose 1,5-bisphosphate carboxylase/oxygenase) [12], the carboxylase in the Calvin-Benson cycle. Different from the Calvin-Benson cycle, the 3HOP bi-cycle assimilates bicarbonate instead of CO_2 (**Fig. 5A**). The 3HOP bi-cycle, which operates in *Cfl. aurantiacus* and most likely in other members of *Chloroflexaceae* [57], is similar to 3-hydroxypropionate/4-hydroxybutyrate (3HOP/4HOB) cycle reported in several archaea [59,60] (**Fig. 5B**). Several enzymes operate in both 3HOP bi-cycle and 3HOP/4HOB cycle, including enzymes for assimilating inorganic carbon: acetyl-CoA carboxylase and propionyl-CoA carboxylase. 16S rRNA analyses suggest that Archaea developed earlier than the bacteria capable of using light as the energy sources [3], so the 3HOP bi-cycle may have evolved from the 3HOP/4HOB cycle.

Other horizontal gene transfers can be also found in the autotrophic carbon assimilation on other members of *Chloroflexales*. For example, several strains in the family of *Oscillochloridaceae* assimilate inorganic carbon via the Calvin-Benson cycle and have an incomplete TCA cycle [61]. In addition to oxygenic phototrophs, anaerobic anoxygenic phototrophic Proteobacteria (AnAPs) also operate the Calvin-Benson cycle. In contrast to oxygenic phototrophs, poor substrate specificity of RuBisCO should not be a serious concern for anoxygenic phototrophs like AnAPs and *Oscillochloridaceae*. It is possible that the genes in the Calvin-Benson cycle in may transfer between *Oscillochloridaceae*, AnAPs and cyanobacteria. Furthermore, *Dehalococcoides ethanogenes* strain 195, a Gram-positive non-phototrophic bacteria in the subphylum 2 of *Chloroflexi* [62], uses (*Re*)-citrate synthase [63]

and has a branched TCA cycle [63,64]. Together, three members of the phylum *Chloroflexi*, *Cfl. aurantiacus*, *Oscillochloridaceae* and *Dehalococcoides ethanogenes* have distinct central carbon metabolic pathways.

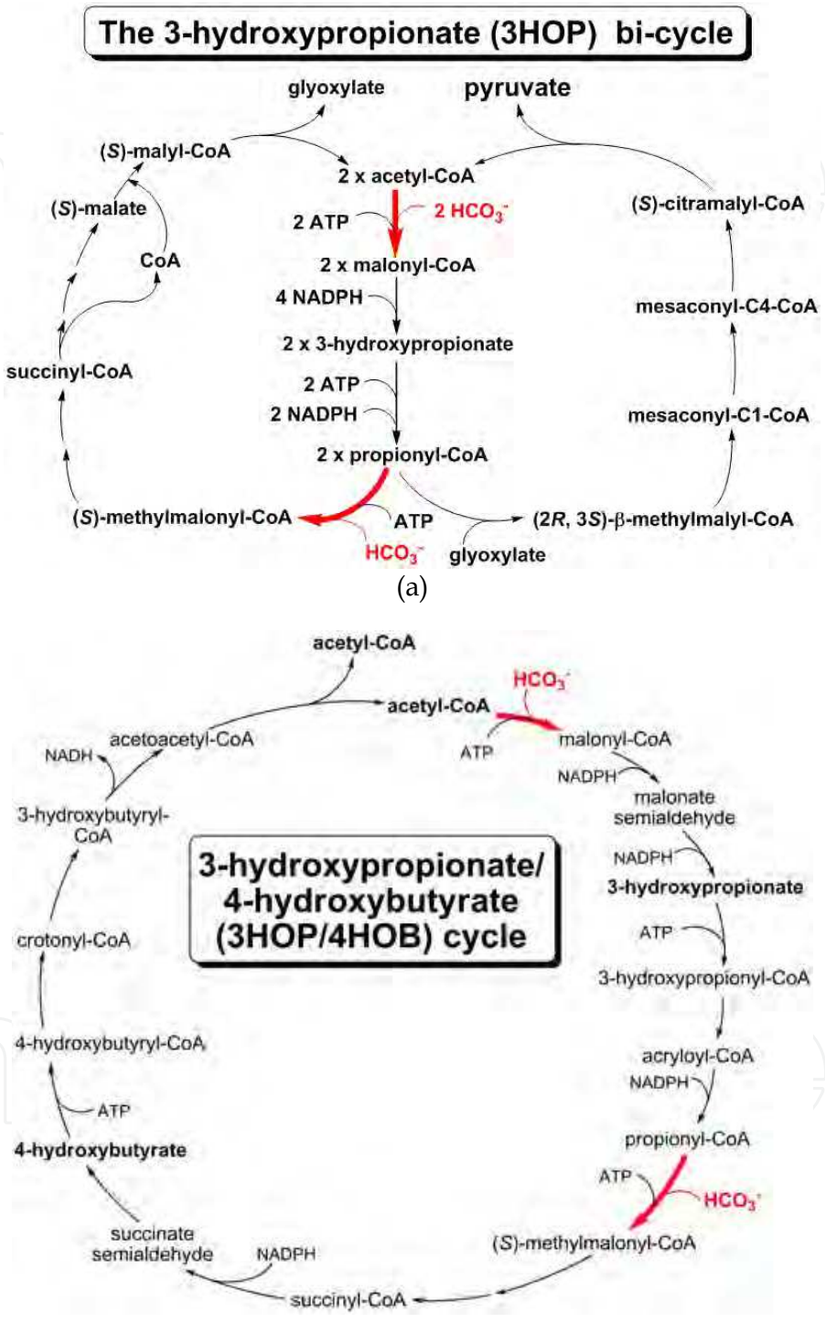


Fig. 5. Autotrophic carbon assimilations through 3-hydroxypropionate bi-cycle (A) and 3-hydroxypropionate/4-hydroxybutyrate cycle.
Several enzymes, including acetyl-CoA carboxylase and propionyl-CoA carboxylase, operate in both 3HOP bi-cycle and 3HOP/4HOB cycle.

3. Conclusions

Previous physiological, ecological and biochemical studies [4] as well as genomic analyses [5], indicate that the anoxygenic phototroph bacterium *Cfl. aurantiacus* has many interesting and certain unique features in its metabolic pathways. The evolutionary links of *Cfl. aurantiacus* and other phototrophic bacteria suggested from this report are summarized in **Table 1.** It has been recognized that the type II RCs were transferred between the *Chloroflexi* species (or FAPs) and the anoxygenic phototrophic Proteobacteria. Sequence alignments and phylogenetic analyses illustrated in this report suggest: (i) Some *Cfl. aurantiacus* enzymes in essential metabolic pathways are more related to the anoxygenic phototrophic Proteobacteria than other phototrophic bacteria, whereas other enzymes are more related to other phototrophic bacteria than anoxygenic phototrophic Proteobacteria; and (ii) some *Cfl. aurantiacus* enzymes in essential carbon metabolic pathways are more related to non-photosynthetic microbes than other phyla of phototrophic bacteria. Together, our studies support lateral/horizontal gene transfers among microbes, and suggest that photosynthesis is likely an adaption to the environments [9].

	Anoxygenic Phototrophic Proteobacteria	Green Sulfur Bacteria	Heliobacteria	Cyanobacteria
Photosynthetic Machinery				
Chlorosomes		√		
B808-866 core antenna complex	√			
Reaction center	√			
Electron Transport Chain				
Complex I -A[a]	more related		less related	less related
Complex I –B[b]	less related		related	more related
Auracyanin				√
Pigment Biosynthesis				
Aerobic cyclase (AcsF)	more related			less related
Anaerobic cyclase (BchE)	less related	more related	more related	?[c]
Central Carbon Metabolism				
Pyruvate dehydrogenase	√			√
Pyruvate synthase	related	more related	less related	more related
α-ketoglutarate dehydrogenase	√			
α-ketoglutarate synthase		less related	more related	

[a] The *Cfl. aurantiacus* complex I with clustered genes (**Fig. 2A**)
[b] The *Cfl. aurantiacus* complex I without clustered genes (**Fig. 2B**)
[c] The putative bchE genes in some cyanobacteria have been reported not to function as anaerobic cyclase.

Table 1. Evolutionary perspectives of selective proteins/enzymes/cellular complexes in *Cfl. aurantiacus* versus other phyla of phototrophic bacteria

4. Abbreviations

AnAPs:	anaerobic anoxygenic phototrophic Proteobacteria
AcsF:	aerobic cyclase
ACIII:	alternative complex III
BchE:	anaerobic cyclase
FAPs:	filamentous anoxygenic phototrophs (or green non-sulfur bacteria or green gliding bacteria)
GSBs:	green sulfur bacteria
3HOP bi-cycle:	3-hydroxypropionate bi-cycle
3HOP/4HOB cycle:	3-hydroxypropionate/4-hydroxybutyrate cycle
RC:	reaction center
RuBisCO:	ribulose 1,5-bisphosphate carboxylase/oxygenase
TCA cycle:	tricarboxylic acid cycle

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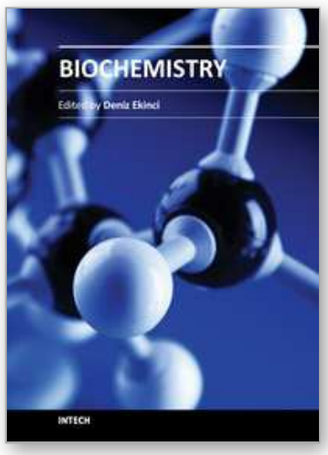

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Over the recent years, biochemistry has become responsible for explaining living processes such that many scientists in the life sciences from agronomy to medicine are engaged in biochemical research. This book contains an overview focusing on the research area of proteins, enzymes, cellular mechanisms and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in biochemistry. Particular emphasis is devoted to both theoretical and experimental aspect of modern biochemistry. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in biochemistry, molecular biology and associated areas. The book is written by international scientists with expertise in protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the book will enhance the knowledge of scientists in the complexities of some biochemical approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of biochemistry.

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