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Two Unrelated 8-Vinyl Reductases Ensure Production of Mature Chlorophylls in *Acaryochloris marina*

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De a _e fM ec, a B_ a d B_ ec ,U _e _ f S ef e d, S ef e d, U _ed K_ d _[a]; C ELSI I _, e, De a _e f C e _ca a d B_ ca E _ee _ ,U _e _ f S ef e d, S ef e d, U _ed K_ d _[b]

ABSTRACT

The major photopigment of the cyanobacterium *Acaryochloris marina* is chlorophyll *d*, while its direct biosynthetic precursor, chlorophyll *a*, is also present in the cell. These pigments, along with the majority of chlorophylls utilized by oxygenic phototrophs, carry an ethyl group at the C-8 position of the molecule, having undergone reduction of a vinyl group during biosynthesis. Two unrelated classes of 8-vinyl reductase involved in the biosynthesis of chlorophylls are known to exist, BciA and BciB. The genome of *Acaryochloris marina* contains open reading frames (ORFs) encoding proteins displaying high sequence similarity to BciA or BciB, although they are annotated as genes involved in transcriptional control (*nmrA*) and methanogenesis (*frhB*), respectively. These genes were introduced into an 8-vinyl chlorophyll *a*-producing Δ*bciB* strain of *Synechocystis* sp. strain PCC 6803, and both were shown to restore synthesis of the pigment with an ethyl group at C-8, demonstrating their activities as 8-vinyl reductases. We propose that *nmrA* and *frhB* be reassigned as *bciA* and *bciB*, respectively; transcript and proteomic analysis of *Acaryochloris marina* reveal that both *bciA* and *bciB* are expressed and their encoded proteins are present in the cell, possibly in order to ensure that all synthesized chlorophyll pigment carries an ethyl group at C-8. Potential reasons for the presence of two 8-vinyl reductases in this strain, which is unique for cyanobacteria, are discussed.

IMPORTANCE

The cyanobacterium *Acaryochloris marina* is the best-studied phototrophic organism that uses chlorophyll *d* for photosynthesis. Unique among cyanobacteria sequenced to date, its genome contains ORFs encoding two unrelated enzymes that catalyze the reduction of the C-8 vinyl group of a precursor molecule to an ethyl group. Carrying a reduced C-8 group may be of particular importance to organisms containing chlorophyll *d*. Plant genomes also contain orthologs of both of these genes; thus, the bacterial progenitor of the chloroplast may also have contained both *bciA* and *bciB*.

The proce of pho o n he i , in hich olar energ i con- er ed in o chemical po en ial energ , i relian pon ligh - ab orbing chloroph ll (Chl) pigmen ha are incorpora ed in o he an enna comple e of pho o rophic organi m . S r c ral modi ca ion o he e rap rrole macroc cle of he e Chl , hich in ence he pigmen -pigmen and pigmen -pro ein in erac ion i hin ligh -har e ing an enna comple e , are re pon ible for he peci c ab orp ion and energ ran fer fea re of he pho o - em (1 3).

Wi h he e cep ion of he marine c anobac erial *Prochlorococcus* pp. (4), he majori of Chl ed b o genic pho o roph carr an e h l gro p a he C-8 po i ion (8E), he prod c of an 8- in l red c a e (8VR) ac ing on a bio n he ic prec r or, 8- i- n l (8V) chloroph llide (Chlide) (5) (Fig. 1A). T o nrela ed cla e of 8VR are kno n o e i in o genic pho o roph , BciA and BciB.

BciA a r iden i ed hro gh creening m an of *Arabidopsis thaliana*; m a ion in he AT5G18660 loc led o he ac- c m la ion of 8V- ra her han 8E-Chl (6, 7), and recombinan pro ein prod ced in *Escherichia coli* a ho n o red ce 8V-Chlide o 8E-Chlide (6). S b eq en l , BciA ac i i ie ere demon ra ed for pro ein from rice (8), mai e and c c mber (9), he green lf r bac eri m *Chlorobaculum tepidum* (10), and he p r- ple pho o rophic bac eri m *Rhodobacter sphaeroides* (11). *In vitro* a a performed i h BciA- pe 8VR from ario pecie ho ed ha NADPH i a red c an for hi en me (8 10, 12).

Al ho gh al o ili ing 8E-Chl , he genome of he majori of c anobac eria do no con ain or holog of *bciA*, indica ing he e i ence of a econd, nrela ed 8VR. T o die on he model c anobac eri m *Synechocystis* p. rain PCC 6803 (*Synechocystis*) demon ra ed ha m an i h m a ion in open reading frame (ORF) lr1923 ere nable o gro nder high ligh in en i ie and acc m la ed 8V-Chl *a* (13, 14). S b eq en l , an or holog of lr1923 from he green lf r bac eri m *Chloroherpeton thalassium* a ho n o complemen he *Chlorobaculum tepidum bciA* m an , reco ering n he i of 8E-bac eriochloroph ll (BChl)

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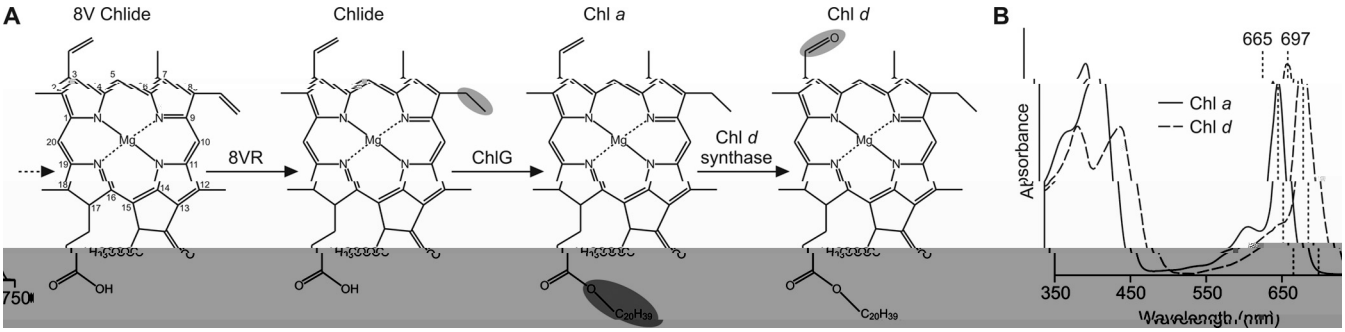

FIG 1 The erminal ep in he bio n he i of Chl *a* and *d*. (A) The prec r or 8V-Chlide (IUPAC n mbered) i red ced o Chlide b an 8VR prior o he addi ion of ph ol o he C-17 propiona e ide chain b Chl n ha e (ChlG). In *A. marina*, he c rren l niden i ed Chl *d* n ha e o idi e he C-3 in l gro p of Chl *a* o a charac eri ic form l gro p. (B) The Chl *d* n ha e-ca al ed o ida ion re l in a red hif in he Q ab orp ion ma im m of he pigmen from 665 nm o 697 nm (in me hanol).

and Chl in hi rain, con rming he ac i i of he econd, BciB, cla of 8VR (15). A d on he *in vitro* ac i i of he BciB- pe 8VR from *Chloroherpeton thalassium* ho ed ha he en me i an a in adenine din cleo ide (FAD)-con aining Fe-S pro ein, deri ing elec ron from red ced ferredo in (16).

Acaryochloris marina i he mo idel died organi m i- li ing Chl *d* for pho o n he i (17 19). Chl *d* differ from Chl *a* in ha i carrie a form l gro p a C-3 ra her han a in l gro p (17) (Fig. 1A), and o gen labeling e perimen con rmed ha Chl *a* i he direc bio n he ic prec r or of Chl *d* (20) (Fig. 1A). The pre - ence of he form l gro p red- hif he Q ab orp ion band of he nbo nd pigmen b appro ima el 30 nm compared o ha of Chl *a* (Fig. 1B), and Chl *d* a fo nd o acco n for 92% of he o al Chl con en of he cell (18). I ha al o been de ermined ha Chl *d* i ed no onl for ligh har e ing a an an enna pigmen b al o a pho ochemicall ac i e pecial-pair Chl in bo h pho- o em II (PSII) (21) and PSI (22, 23). The pigmen compo i ion of *A. marina* allo i o ef cien l har e far-red ligh o dri e pho o n he i , an adap a ion ha permi r i al in colonial a cidian (24) and microbial ma (25), here he pho o n he i- call ac i e radia ion i ab orbed b he Chl *a* (i h or i ho Chl *b*)-con aining pho o roph b far-red ligh i enriched (26).

While mo c anobac eria ili e BciB o pro ide red ced Chl for pho o n he i , a mall n mber in ead e BciA. Uniq el for c anobac eria eq enced o da e, bioinforma ic anal i re ealed ha he o eq enced genome of *Acaryochloris* pp. (*A. marina* MBIC11017 and *Acaryochloris* p. rain CCMEE 5410) con ain homolog of bo h *bciA* and *bciB*. Here e e pre ed he *A. marina* gene in a m an of *Synechocystis* nable o n he i e 8E-Chl *a* in an a emp o de ermine he her bo h ORF encoded f nc ional 8VR . He erologo e pre ion of bo h gene re ored he abili of he rain o gro nder high-ligh condi ion and o n he i e red ced Chl *a*. RNA and pro ein le el anal e of *A. marina* cell demon ra ed ha bo h BciA and BciB are pre en *in vivo*. We h po he i e ha o 8VR are emplo ed o en re ha onl Chl carr ing 8E gro p are n he i ed in he e rain ; po ible pen- al ie for he pre ence of 8V-Chl *d* are di c ed.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bac erial rain and pla - mid ed in hi d are li ed in Table 1. *E. coli* rain JM109 (27) ran formed i h pPD-FLAG (28) pla mid a gro n in a ro ar haker a 37 C in LB medi m pplemen ed i h 30 μg _ml^{-1} kanam cin. *Syn- echocystis* rain ere gro n pho oa o rophicall in a ro ar haker n- der modera e (50 μmol pho on _m^{-2} _ $^{-1}$)- or high (250 μmol pho on _ m^{-2} _ $^{-1}$)-ligh condi ion a 30 C in liq id BG-11 medi m (29) pple- men ed i h 10 mM TES [*N*- ri (h dro me h l)me h l-2-aminoe h- ane lfonic acid], pH 8.2. *A. marina* a gro n pho oa o rophicall in a ro ar haker nder modera e-ligh condi ion (50 μmol pho on _m^{-2} _ $^{-1}$) a 28 C in liq id MBG-11 medi m (25, 30) pplemen ed i h 10 mM TES, pH 8.2.

TABLE 1 S rain and pla mid ed in hi d

S rain or pla mid	Geno pe or charac eri ic	So rce or reference
E. coli JM109	Cloning rain for pPD con r c	Promega
A. marina MBIC11017	WT	R. Blanken hip[a]
Synechocystis rain		
PCC 6803	WT	R. Sobo ka[b]
Δ*bciB* m an	Em^r replacemen of cen ral por ion of lr1923 in WT	11
Δ*bciB*::*nmrA*(Am) m an	AM1_2394 and Km^r replacemen of *psbAII* in Δ*bciB* m an	Thi d
Δ*bciB*::*frhB*(Am) m an	AM1_2849 and Km^r replacemen of *psbAII* in Δ*bciB* m an	Thi d
Pla mid		
pPD-FLAG	Cloning i e and Km^r anked b *psbAII* p- and do n ream region ; Amp^r	28
pPD[*nmrA*]	AM1_2394 i h encoded Hi$_6$ ag cloned in o pPD-FLAG (NdeI/BglII)	Thi d
pPD[*frhB*]	AM1_2849 i h encoded Hi$_6$ ag cloned in o pPD-FLAG (NdeI/BglII)	Thi d

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Construction of *Synechocystis* mutants containing *A. marina* genes. The PCR primer ed in hi d are li ed in Table S1 in he pple-men al ma erial. The *frhB* gene a ampli ed from *A. marina* MBIC11017 genomic DNA ing primer frhBF and frhBR, i h he re er e primer encoding a C- erminal he ahi idine ag. The PCR prod c a dige ed and cloned in o he NdeI/BglII i e of pPD-FLAG ec or, and he re l-ing pla mid a named pPD[*frhB*]. The con r c ion of pPD[*nmrA*] a imilar o ha de cribed for pPD[*frhB*] e cep ha o erlap e en ion PCR a ed o genera e f ll-leng h *nmrA* con aining a ilen m a ion re-mo ing an in ernal NdeI i e fo nd in he na i e gene. The region p-and do n ream of hi re ric ion i e ere ampli ed ing he primer pair nmrA1F/nmrA1R and nmrA2F/nmrA2R, re pec i el . Primer nmrA1R and nmrA2F ere de igned o be in er el complemen ar o each o her and did no con ain he NdeI i e. The e amplicon ere ed a he empla e for o erlap e en ion PCR i h primer nmrA1F and nmrA2R, genera ing he f ll-leng h *nmrA*. The eq enced pla mid ere in rod ced in o he *Synechocystis* Δ*bciB* rain (11). Tran forman ere elec ed on olid BG-11 medi m con aining 10 μg_ml^{-1} kanam cin and f ll egrega ed b incremen all do bling he concen ra ion of an ibio ic o 80 μg_ml^{-1}. F ll egrega ed *Synechocystis* rain ere con rmed b colon PCR ing primer pPDCheckF and pPDCheckR.

Extraction and analysis of pigments. Chl ere e rac ed from *Synechocystis* cell pelle af er a hing in 20 mM HEPES (pH 7.2) b adding 9 pelle ol me of 0.2% (ol/ ol) ammonia in me hanol, or e mi ing for 30 , and inc ba ing on ice for 20 min. The e rac ere clari ed b cen rif ga ion (15,000 × *g* for 5 min a 4 C), and he perna an ere immedia el anal ed on an Agilen 1200 high-pre re liq id chroma-ograph (HPLC) em. Chl *a* pecie ere epara ed on a Phenomene Aq a C_{18} fè er e-pha e col mn (5-μm par icle i e, 125- pore i e, 250

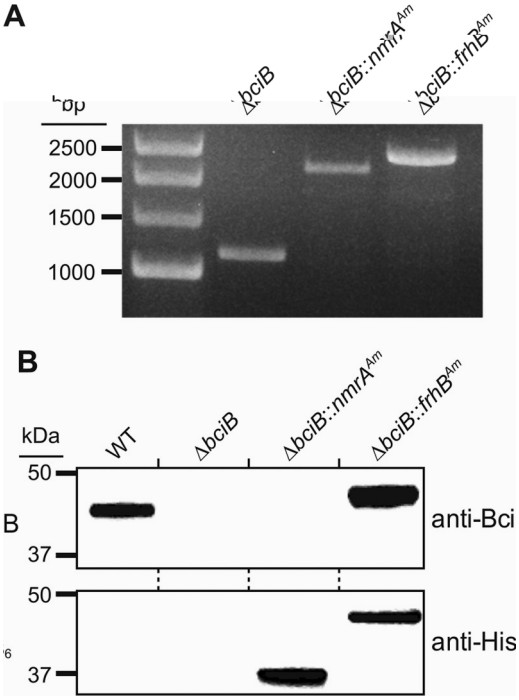

FIG 2 Con r c ion of *Synechocystis* rain de igned o e pre p a i e *A. marina* 8VR-encoding gene . (A) I ola ion of f ll egrega ed *Synechocystis* Δ*bciB* rain con aining gene from *A. marina*, con rmed b colon PCR amplif ing he *psbAII* loc . (B) E pre ion of recombinan pro ein a con- rmed b re ol ing membrane frac ion from he de cribed rain b SDS-PAGE, ran ferring o a membrane, and probing i h an i-BciB and an i-Hi $_6$ an ibodie .

ion ere re ol ed b SDS-PAGE and ran ferred o a pol i- n lidene di oride (PVDF) membrane, hich a probed i h an an ibod rai ed again *Synechocystis* BciB and, in he ab ence of an an ibod rai ed again BciA, a commercial an i-Hi $_6$ an i- bod (Be h l Labora orie , Inc.) (Fig. 2B). The blo indica e ha he recombinan pro ein are pre en , con rming he effec i e e pre ion of he *A. marina* gene hen nder he con rol of he *psbAII* promo er.

Functional testing of recombinant proteins. The rain e - pre ing *A. marina* gene , along i h he WT and Δ*bciB* rain , ere e ed for heir abili o gro nder high ligh . Pa che of cell ere inc ba ed nder con an ill mina ion on olid me- di m. A e pec ed, he Δ*bciB* rain a nable o gro nder high ligh , con i en i h pre io l p bli hed re l (11, 13, 14), hile complemen a ion i h bo h *nmrA* and *frhB* re ored he abili of he Δ*bciB* rain o gro nder he e condi ion , comparable o he gro h of he WT (Fig. 3A).

Chl from he e rain gro n in liq id medi m nder normal ligh ere e rac ed and anal ed b HPLC (Fig. 3B). The Chl e rac ed from he Δ*bciB* m an had a re en ion ime 0.4 min hor er han ha of Chl from he WT (Fig. 3B). Anal i of he ab orbance pro le of he e peak demon ra ed ha he Sore band ma im m from he Δ*bciB* pec r m i red hif ed b 11 nm rela i e o ha from he WT pec r m, indica ing ha i i he 8V form of he pigmen (11, 13, 14). The re en ion ime and ab or- bance pro le of he Chl peak from bo h he Δ*bciB*::*nmrA*(Am) and Δ*bciB*::*frhB*(Am) rain ere iden ical o ho e of he Chl peak from he WT (Fig. 3B). Therefore, e pre ion of ei her

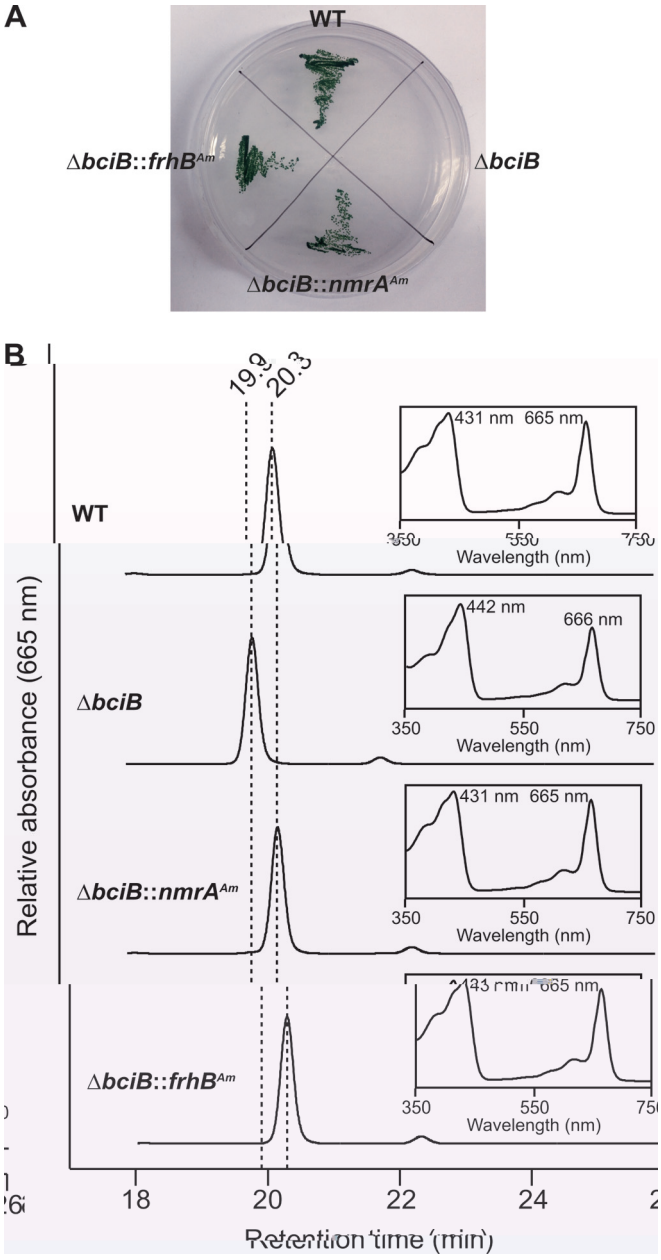

FIG 3 Gro h and pigmen anal i of de cribed rain of *Synechocystis*. (A) S rain e ed for gro h nder high ligh in en i on olid medi m. (B) HPLC el ion pro le and ab orp ion pec ra (in e) of Chl e rac ed from rain gro n nder modera e ligh in en i . Re en ion ime of 20.3 and 19.9 min and Sore ab orp ion ma ima a 431 and 442 nm are indica i e of 8E-Chl *a* and 8V-Chl *a*, re pec i el , in he HPLC ol en .

nmrA or *frhB* cce f ll reco er he WT a , i h re pec o 8E-Chl *a* n he i , and h e propo e ha he be rea igned a *bciA* and *bciB*, re pec i el .

Identi cation of 8VR utilized by *A. marina*. In order o de- ermine hich of he 8VR *A. marina* ili e for Chl bio n he i or he her bo h pro ein are emplo ed, ran crip ion of *bciA* and *bciB* a checked b RT-PCR, and he pre ence of he cogna e pro ein a de ermined b ma pec rome r . To al *A. marina* RNA a i ola ed from a c l re a mid-e ponen ial gro h

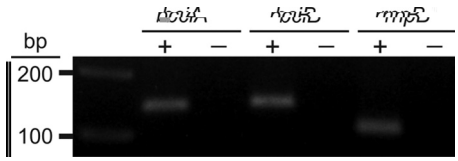

FIG 4 De ec ion of ran crip of *bciA* and *bciB* in *A. marina* b RT-PCR. Reac ion for *bciA* and *bciB*, along i h he *rnpB* ho ekeeping con rol, ere performed i h he incl ion (+) and omi ion (−) of re er e ran crip a e o en re ha ample ere no con amina ed b genomic DNA.

pha e, rea ed i h DNa e o remo e an genomic DNA, and ed a he empla e for one- ep RT-PCR in hich cDNA n he i and PCR ampli ca ion ere performed in a ingle reac ion. The ho ekeeping gene *rnpB*, encoding he RNA b ni of RNa e P, a incl ded a po i i e con rol. The amplicon genera ed b RT-PCR di pla ed a ingle band i h he e pec ed i e for all he hree gene hen anal ed b agaro e gel elec rophore i (Fig. 4): 140 bp for *bciA*, 142 bp for *bciB*, and 106 bp for *rnpB*. The ab ence of band in he no-RT con rol elimina ed he po ibili of genomic DNA con amina ion. Therefore, e can concl de ha bo h *nmrA* and *frhB* are ac i el ran cribed nder he condi ion e ed in *A. marina*. Ma pec rome r anal i a performed o erif he pre ence of NmrA and FrhB pro ein in *A. marina*. Pro-ein e rac ed from an *A. marina* hole-cell l a e ere rea ed i h a combina ion of endopro eina e L C and r p in o gener-a e pep ide fragmen hich ere hen anal ed b nano-liq id chroma ograph (LC)-MS/MS. Ma pec ra, con i ing of bo h pep ide ion ma e and heir prod c ion pro le , ere ed a inp for earching again he *A. marina* reference pro eome da aba e. In o al, 1,470 pro ein ere iden i ed, incl ding bo h NmrA and FrhB, a ho n in Table 2.

Phylogenetic analysis of BciA and BciB. Compari on of he ph logenie ob ained b ma im m-likelihood anal i of BciA and BciB amino acid alignmen i h ho e ob ained b anal i of 16S rRNA alignmen from he ame pecie are ho n in Fig. 5A and B, re pec i el . The ph logene ic po i ion of *A. marina* BciA and BciB are bo h broadl con i en i h ho e ho n for *A. marina* in he 16S rRNA ree , gge ing ha he *bciA* and *bciB* gene ha e no been acq ired b hori on al ran fer. Ho e er, he po i ion of *Synechococcus* pp. in he BciA ree and he clade con-aining he green lf r bac eria in he BciB ree are incon i en i h he 16S rRNA ph logen , indica ing ha here ma ha e been la eral ran fer e en d ring he e ol ion of bo h *bciA* and *bciB*.

DISCUSSION

Wi h he c rren ab ence of a gene ic em for arge ed m -agene i of *A. marina*, e ere nable o de ermine if he lo of a ingle 8VR-encoding gene, or lo of 8VR f nc ion ia di r p ion of bo h *nmrA* and *frhB*, o ld ha e a nega i e effec on iabili of he cell . Recen l , Wa abe and co orker ha e de cribed he r cce f l m agene i of *A. marina* cell ing a ran po on-ba ed em (38); he repor ed he i ola ion of a m an i h a ran po on in er ion m a ion in a gene in ol ed in mol bden m cofac or bio n he i . Thi m an co ld be f nc ionall comple-men ed ia in rod c ion of he WT cop of he di r p ed gene in *trans*. I i hoped ha f r her de elopmen of hi me hod ma ield a em for ro ine arge ed m agene i in *A. marina* and o her c anobac eria of in ere , allo ing he de ermina ion of fac-or in ol ed in far-red-ligh ili a ion, incl ding he bio n he-i of Chl *d*. F r her, iden if ing he gene in ol ed in ch a pro-ce i of igni can in ere i h he recen di co er ha ome rain of erre rial c anobac eria ili ing Chl *a* hen gro n in hi e ligh po e he abili o ini ia e n he i of Chl *d* and *f* hen c l red in far-red ligh , co pled i h he e en i e remod-eling of heir pho o n he ic comple e , a re pon e ermed far-red-ligh pho oacclima ion (FaRLiP) (39).

Lo of 8VR ac i i in *A. marina* o ld re l in he prod c-ion of 8V-Chl *a* and 8V-Chl *d*. 8V-Chl *a* domina e in *Prochloro-coccus* pp. (4) and in a recen l i ola ed rain of he marine e -kar o ic pro i *Alexandrium ostenfeldii* (40), and he nred ced form of he pigmen i olera ed in plan and c anobac erial m -an i h le ion in 8VR-encoding gene (6, 13, 14). 8V-Chl *d*, ho e er, ha e o be de ec ed in na re. Chl *d* a r repor ed a a minor pigmen in ario pecie of red microalgae (41), al ho gh i a la er de ermined ha *Acaryochloris* pp. a ached o he rface of he alga ere he r e o rce of he pigmen (42). Chl *a* can al o be readil o idi ed o Chl *d* d ring pigmen e rac-ion (43 45). F r her, a d b Lo ghlin e al. de ermined ha in l gro p of na rall occ rring Chl can pon aneo l o i-di e a C-3, ielding Chl *d*-like pigmen , and/or a C-8, ielding no el 8-form l er ion of he e Chl (46). The a hor mea red he Sore /Q ra io of he b ra e and prod c for each o ida-ion, comparing he ab orp ion in en i of he high-energ , bl e-mo -ab orbing band of he pigmen o ha of he lo er-energ , red-mo -ab orbing band. In ere ingl , Chl *a*, *d* and *f*, he la er carr ing a form l gro p a C-2, ha e Sore /Q ra io of <1.0, and he ra io of he 8V form of Chl *a* and *d* are 1.15 and 0.99, re pec i el . Ho e er, he Sore /Q ra io of bo h 8-form l Chl *a* and 8-form l Chl *d* are 2.34. If he o ida ion of he in l gro p a C-3 o ield Chl *d* occ r pon aneo l *in vivo* or if he en me ca al ing he o ida ion i no peci c for he C-3 in l gro p, he e 8-form l pigmen o ld be ili ed for ligh har e ing and pho ochemi r and ma re l in impaired red-ligh ab orp ion, h nega ing he ad an age *A. marina* hold in i ecological niche conferred b ing he far-red-ab orbing Chl *d*. Thi ma e plain h he o eq enced pecie in hi gen emplo o nrela ed 8VR : red ced Chl can be n he i ed d e o he pre ence of an al erna i e en me nder condi ion in hich one of he red c-an i limi ing; e.g., 8V red c ion b BciA ma domina e hen cell lar le el of ferredo in are deple ed nder iron-limi ing con-

TABLE 2 Iden i ca ion of BciA and BciB b pro eomic anal i

Pro ein	Ma (Da)	MOWSE core[a]	Seq ence co erage (%)	Pep ide [b]
BciA	36,780	216	27	R.ILVLGGTGTIGR.A, R.ATVAELVK.R, K.FLAEQVFK.N, R.QFYGVVSCLASR.T, R.ESGLIYSIVRPTAYFK.S, K.SVPPGFLNAIATVLGGIAK.I, R.LVDGSEEAERGDFAVF.-
BciB	45,492	58	7	R.TPEEVLAAR.V, R.SVQDSLGLEK.L, R.AGLQTFLETTSR.S

[a] The o 8VR ere iden i ed b da aba e earching i h a *P* al e of <0.05 indica ing igni cance, i h MOWSE core repre en ing he in er e of he probabili ha a ma ch i a random e en . The fal e-di co er ra e for hi earch a 0.75%.
[b] Tr p ic pep ide are ho n i h anking amino acid re id e epara ed b period .

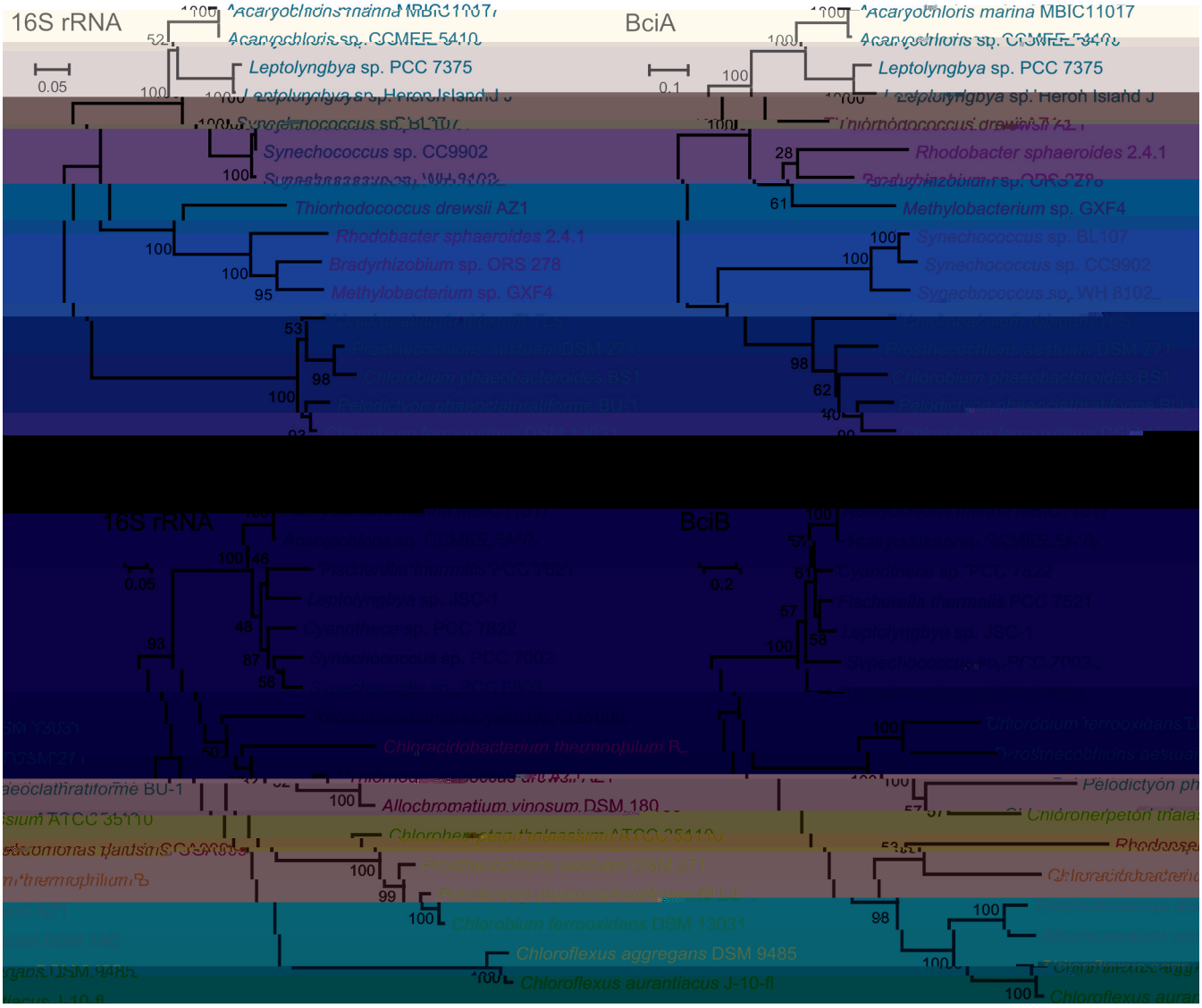

FIG 5 Phylogenetic relationship among 8VR protein sequences compared with parenthesized organism 16S rRNA phylogenies. Maximum-likelihood phylogenies of BciA (A) and BciB (B) homologs, compared with 16S rRNA phylogenies of the same organisms, are shown. The BciA and BciB trees were constructed from amino acid alignments using the PROTGAMMAAUTO model in RAxML version 8.2.4. The rRNA trees were constructed from nucleotide alignments using the GTRCAT model. The number on branches indicates the percentage bootstrap support from 100 replicates, and the calibration bar indicates the specified number of amino acid or nucleotide substitutions per site. Example organisms from cyanobacteria (cyan), purple nonsulfur bacteria (purple), green sulfur bacteria (green), green filamentous bacteria (amber), and *Acidobacteria* (red) are included.

diversity. In reconstructing the genomes of the cyanobacterial strains using the FaRLiP response sequenced hundreds of nonredundant multiple copies of 8VR-encoding genes. However, unlike in *Acaryochloris* spp., Chl *d* is not a dominant pigment, making up only 1 to 2% of the total Chl in the cell (47). We investigated the consequences of accumulation of 8V-Chl *d*, and possible 8-formyl Chl *d*, once the targeted genetic manipulation of *A. marina* is possible.

The distribution of unrelated enzymes to catalyze a single reaction would not be uncommon in photosynthetic organisms. The magnesium protoporphyrin monomethyl ester cyclase and Pchlide oxidoreductase enzymes in oxygenic photosynthetic organisms, each employing different reaction mechanism (48). As with *A. marina*, many strains of green sulfur bacteria appear to employ multiple 8VR forms for (B)Chl biosynthesis, containing either gene encoding enzymes of both classes or more than one copy of *bciB* (15). However, the activities of different conventional 8VR from the same organism had not been demonstrated until recently. In retrospect, the enzymatically required BChl biosynthesis in organisms containing BChl *a*, Chlide oxidoreductase (COR), is absolutely both 8V- and 8E-Chlide bearing, because in each case the product pigments carried an 8E group, demonstrating additional 8VR activities (49). All known BChl *a*-utilizing photosynthetic other than *Roseiflexus* spp. also contain a *bciA* gene (50). Removal of 8VR function in *Rhodobacter sphaeroides*, which naturally produces BChl *a*, re-

l ed in he i ch o he bio n he i of BChl *b*, he pigmen i h he lo e energ -ab orbing proper of an na rall occ rring pho opigmen (51), leading o he propo al ha m l iple 8VR ac i i ie en re again he forma ion of BChl *b* in he e organi m . The pre ence of m l iple 8VR in green lf r bac eria ma al o en re ha me h la ion of he C-8 gro p i po ible; dele ion of *bciA* in *Chlorobaculum tepidum* pre en ed hi me h la ion and re l ed in aberran a embl of he chloro ome, he peciali ed ligh -har e ing an enna in he e organi m (52). Similarl , e propo e here ha *Acaryochloris* pp. emplo o 8VR o pre en he n he i of pigmen de cien in red/far-red ab orp ion.

The genome of man plan pecie , incl ding *A. thaliana* and rice, hich rel on BciA for 8V gro p red c ion, con ain or holog of *bciB* hich appeared o ha e become red ndan in he e pecie . Ho e er, Meg ro e al. demon ra ed ha he *bciB* or holog in *A. thaliana* encode an en me in ol ed in he con er ion of Chl *b* back o Chl *a* (53), a proce impor an for greening, accli ma ion o ligh in en i , and ene cence in higher plan . Thi en me i propo ed o ha e e ol ed from a dia om BciB and no ca al e a ne ep in pigmen bio n he i (53). Of he e- q enced c anobac eria and prochloroph e , onl *Acaryochloris* pp. appear o con ain bo h *bciA* and *bciB*, and o r ph logene ic anal i indica e ha nei her of he gene a acq ired b la eral gene ran fer. The e ob er a ion ma pro ide in igh hen con- idering he c anobac erial progeni or of he chloropla .

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