Supporting Information

Mg-protoporphyrin IX monomethyl ester cyclase from *Rhodobacter capsulatus*: Radical SAM-dependent synthesis of the isocyclic ring of bacteriochlorophylls

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Table S1. Bacterial strains and plasmids used in this study

Strains and plasmids	Genotype or insert description	Reference
Strains		
Dinoroseobacter shibae DFL 12	wild-type	[1]
E. coli BL21 (λDE3)	BL21B F$^-$*dcm ompT hsdS*(r_B^- m_B^-) *gal* λ(DE3)	Stratagene; La Jolla, USA
E. coli DH10B	F$^-$ araD139 Δ(*ara, leu*)7697 Δ*lacX*74 *galU galK rpsL deoR* Φ80d*lacZΔ*M15 *endA1 nupG recA1 mcrA* Δ(*mrr hsdRMS mcrBC*)	Invitrogen, Carlsbad, USA
E. coli ST18	S17 λ*pir* Δ*hemA*	[2]
R. capsulatus SB1003	wild-type	[3]
R. capsulatus ZY5	*bchL*$^-$	[4]
R. capsulatus ZY6	*bchH*$^-$	[4]
R. capsulatus DB575	*bchE*$^-$	[5]
Plasmids		
pGEX-6P-1_*bchE*	*bchE* from *R. capsulatus* SB1003	this work
pRhoKs	*Rhodobacter* expression vector	[6]
pRhoKs_Gm	The gentamicin 3-acetyltransferase gene (GenBank: AFV59818.1) was implemented into pRhoKs.	this work
pRhoKs_Gm_*bchE*	The *bchE* gene from pGEX-6P-1_*bchE* was cloned downstream of the gentamycin resistance gene of pRhoKs_Gm. An *E. coli* specific ribosomal binding site was introduced.	this work
pRhoKs_Gm_*bchE*_C204A	Derivative of pRhoKs_Gm_*bchE.* The cysteine at position 204 was changed to alanine.	this work
pRhoKs_Gm_*bchE*_C208A	Derivative of pRhoKs Gm_*bchE.* The cysteine at position 208 was changed to alanine.	this work
pRhoKs_Gm_*bchE*_C211A	Derivative of pRhoKs_Gm_*bchE.* The cysteine at position 211 was changed to alanine.	this work
pRhoKs_Gm_*bchE*_T71A	Derivative of pRhoKs_Gm_*bchE.* The threonine at position 71 was changed to alanine.	this work
pRhoKs_Gm_*bchE*_T71K	Derivative of pRhoKs_Gm_*bchE.* The threonine at position 71 was changed to lysine.	this work
pRhoKs_Gm_*bchE*_E126K	Derivative of pRhoKs_Gm_*bchE.* The glutamic acid at position 126 was changed to lysine.	this work
pRhoKs_Gm_*bchE*_R202E	Derivative of pRhoKs_Gm_*bchE.* The arginine at position 202 was changed to glutamic acid.	this work
pRhoKs_Gm_*bchE*_L29R	Derivative of pRhoKs_Gm_*bchE.* The leucine at position 29 was changed to lysine.	this work
pRhoKs_Gm_*bchE*_V97R	Derivative of pRhoKs_Gm_*bchE.* The valine at position 97 was changed to arginine.	this work
pRhoKs_Gm_*bchE*_F210L	Derivative of pRhoKs_Gm_*bchE.* The phenylalanine at position 210 was changed to lysine	this work
pRhoKs_Gm_*bchE*_D248N	Derivative of pRhoKs_Gm_*bchE.* The aspartic acid at position 248 was changed to asparagine	this work
pRhoKs_Gm_*bchE*_E249Q	Derivative of pRhoKs_Gm_*bchE.* The glutamic acid at position 249 was changed to glutamine.	this work
pRhoKs_Gm_*bchE*_E250Q	Derivative of pRhoKs_Gm_*bchE.* The glutamic acid at position 250 was changed to glutamine.	this work
pRhoKs_Gm_*bchE*_E308Q	Derivative of pRhoKs_Gm_*bchE.* The glutamic acid at position 308 was changed to glutamine.	this work
pRhoKs_Gm_*bchE*_K320Q	Derivative of pRhoKs_Gm_*bchE.* The lysine at position 320 was changed to glutamine.	this work
pGEX-6P-1_lpor	*lpor* (gene Dshi_4160) from *D. shibae* DFL12	this work

Table S2: Primers used in this study.

Primers (1-7) were used to amplify the *lpor* gene from *D. shibae* DFL 12, *bchE* from *R. capsulatus* SB1003 and the gentamicin 3-acetyltransferase gene (GenBank: AFV59818.1). Primers (8-31) and their respective reverse complements were used to generate mutants of *bchE*.

Number	Primer name	Primer sequence
1	fw_BamHI_*lpor*	ggggcccctgggatccatgacccttgaccccatgccc
2	rv_XhoI_*lpor*	gatgcggccgctcgagtcaggcggcgaccggctc
3	fw_NdeI_Gm	gagatatacatatgttacgcagcagcaacg
4	rv_BamHI_Gm	gaattcggatccttaggtggcggtacttgggtc
5	fw_BamHI_*bchE* (pGEX-6P-1)	cccctgggatccatgcgcatccttttcgtccac
6	fw_SacI_RBS_*bchE*_SB1003 (pRhoKs_Gm)	gtagagctccgtcgacaaggagatataatgcgcatccttttcgtccac
7	rv_XhoI_*bchE*_SB1003	cggacgctcgagttattccgcggcgtcgacc
8	fw_*bchE*_C204A	cgagcaggtgaagggagcgccccgcgccatgttcgg
9	fw_*bchE*_C208A	ccactgcgagcagaacgaggcggtgaaggggcagc
10	fw_*bchE*_C211A	ccagaatttccactgcgaggcgaacgagcaggtgaagggg
11	fw_*bchE*_T71A	gacggcgtgatcgacgccacgccgatcaggtcgg
12	fw_*bchE*_L29R	cgtgggtcccctatcgtgcgggtcac
13	fw_*bchE*_V97R	gaatgcggtgcgccgtctgggcggcgtg
14	fw_*bchE*_F210L	gccccttcacctgctcgttatgctcgcagt
15	fw_*bchE*_D248N	tcttcatcctcgccaacgaggaaccgacg
16	fw_*bchE*_E249Q	catcctcgccgaccaggaaccgacgat
17	fw_*bchE*_E308Q	cgctcggcacccaggccgcggcgca
18	fw_*bchE*_K320	ctcgacctgttcaaccaggaaaccaccgtcg
19	fw_*bchE*_W214	cgcgccagaatttcgactgcgagcagaac
20	fw_*bchE*_W214K	aatcgcgccagaatttcttctgcgagcagaacgagc
21	fw_*bchE*_R202E	ggtgaaggggcagccctccgccatgttcgggatcg
22	fw_*bchE*_T71K	cgacggcgtgatcgacttcacgccgatcaggtcggg
23	fw_*bchE*_E126K	cagctcgaccatgatttccttgccctcgccgcggacg
24	fw_*bchE*_L29M	ccgtcgtgggtcccctatatggcgggtcacc
25	fw_*bchE*_V97M	gaatgcggtgcgcatgctgggcggcgtgc
26	fw_*bchE*_F210W	gccccttcacctgctcgtggtgctcgcagt
27	fw_*bchE*_D248A	ttcatcctcgccgccgaggaaccgacg
28	fw_*bchE*_E249K	tcatcctcgccgacaaggaaccgacgatc
29	fw_*bchE*_E250K	tcctcgccgacgagaaaccgacgatcaac
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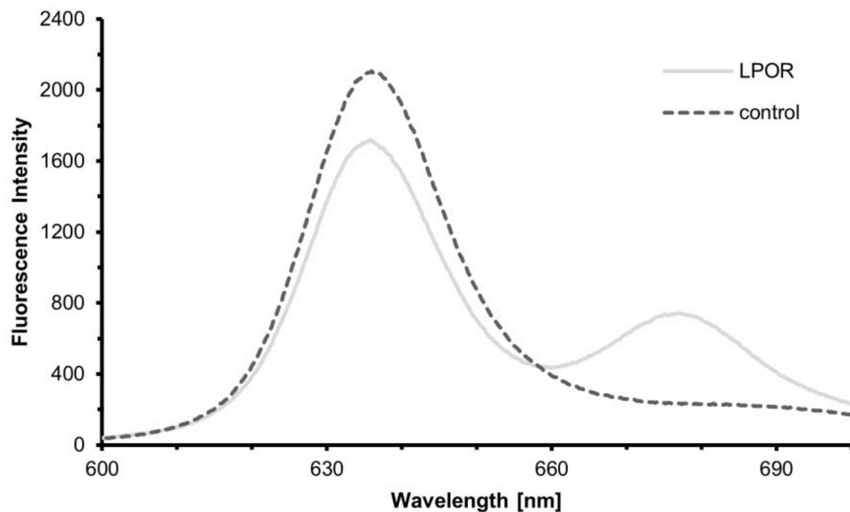

Figure S1: Enzymatic activity of *Dinoroseobacter shibae* LPOR.
An 250 µl activity assay containing 3.3 µM purified LPOR, 17 µM Pchlide and 2 mM NADPH was exposed to blue light for 2 min as also described under [7] (LPOR, *grey line*). A negative control in the absence of enzyme was processed in parallel (*dashed line*). The reaction was stopped by the addition of 250 µl acetone and subsequently centrifuged for 15 min at 12´000 g. Chlide formation was analyzed using a FP-8500 Spectrofluorimeter (Jasco, Tokyo, Japan).

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