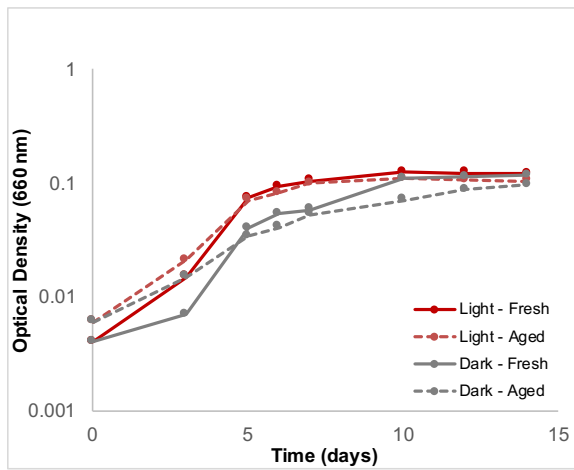


A.



B.

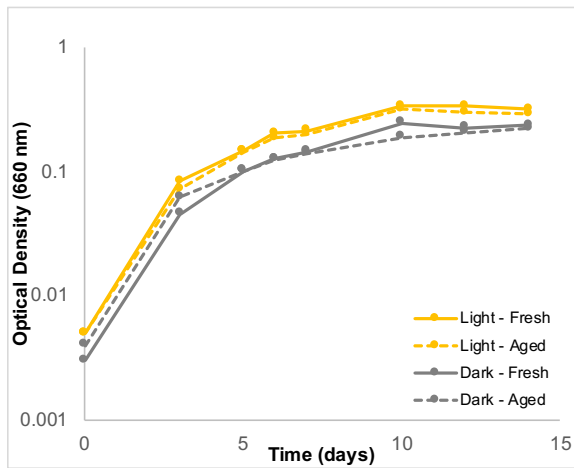


Figure S1. Growth in light-aged media. NSY medium was “aged” by exposure to white light for 4 days. Light-aged or fresh media were then inoculated with *Rhl. lacticola* (A) or *Aurantimicrobium* sp. strain Mo1 (B) and grown in white light or darkness as described in Materials and Methods. Although growth rates in fresh and aged media are not identical, the difference between growth in the light and growth in the dark is consistent.

Figure S2.

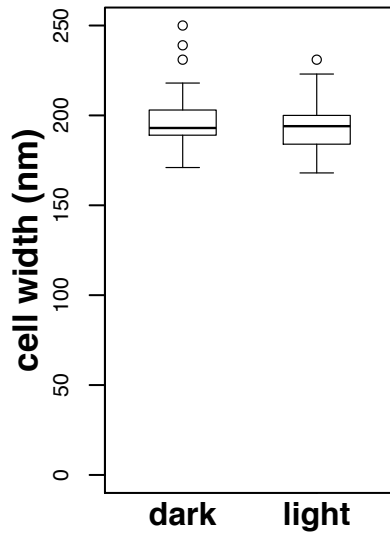


Figure S2. Cell widths are the same in light and dark. *Aurantimicrobium* sp. strain MWH-Mo1 was grown in white light or darkness, then imaged using SEM. NIH ImageJ was used to measure cell diameters in images; ~200 cells were measured. The average cell width (dark line) is the same in both conditions, and the distribution of measured values is also similar in both. Boxes include the 25th to 75th percentile of the data; whiskers represent $1.5 \times$ the interquartile range. Dots indicate cells whose width was outside that range. This plot was generated using the R function `boxplot()` and the default parameters for that function.

Figure S3.

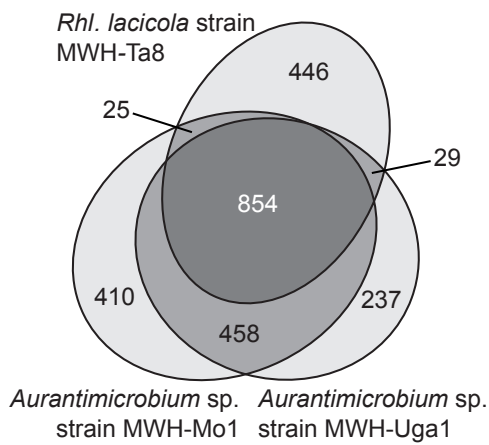
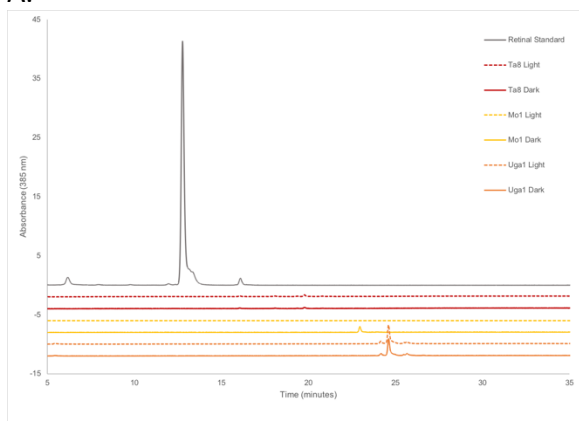


Figure S3. Whole genome comparisons. The predicted open reading frames in the genomes of *Rhl. lacticola*, *Aurantimicrobium* sp. strain MWH-Mo1, and *Aurantimicrobium* sp. strain MWH-Uga1 were compared using EFI-EST, and the overlaps were depicted using the program eulerAPE (<http://www.eulerdiagrams.org/eulerAPE/>). The two *Aurantimicrobium* spp. are very closely related (their 16S rRNA genes are 99.5% identical), and their genomes share a total of 1312 genes.

A.



B.

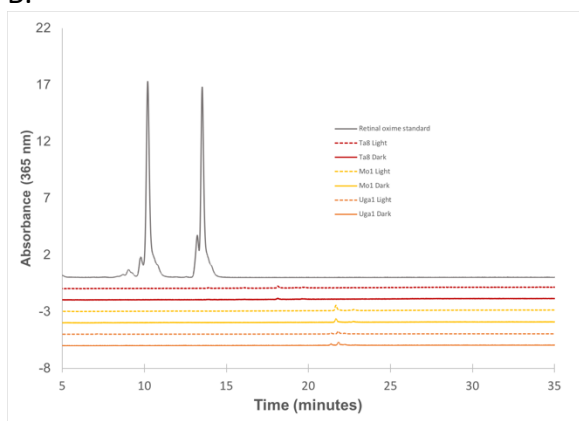


Figure S4. Retinal is not produced by any of the Actinobacteria investigated here. Cells (50 mL cultures) were grown in white light or darkness as described in Materials and Methods and pigments extracted for HPLC analysis. No peak corresponding to either free retinal or retinal oxime is observed in any sample. **A. Methanol extracts of cells.** The gray line is a commercial retinal standard with a single peak corresponding to free retinal (indicated by “R” in both A and B); no peak with the same retention time is observed in any of the pigment extracts from cells. **B. Retinal oxime derivatives.** When released from a rhodopsin, retinal is present not as free retinal, but as retinal oxime. The retinal oxime standard (indicated by “RO”) was produced by reacting the commercial retinal standard with hydroxylamine. To release any retinal from rhodopsins in cells, cells were lysed by sonication in methanol, then hydroxylamine was added to the lysate and incubated on ice for 1.5 hours. Cellular debris was then removed by centrifugation and filtration prior to loading on the HPLC. Fresh solvents were prepared between the samples shown in A and those in B, which changed the retention times by ~1.5 min.

Figure S5.

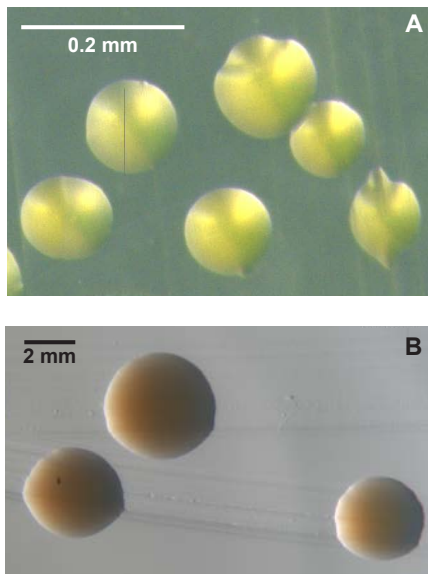


Figure S5. Colony pigmentation. (A) Colonies of *Aurantimicrobium* sp. strain MWH-Mo1 are bright yellow, and (B) those of *Aurantimicrobium* sp. strain MWH-Uga1 are orange, due to biosynthesis of carotenoids.

Figure S6.

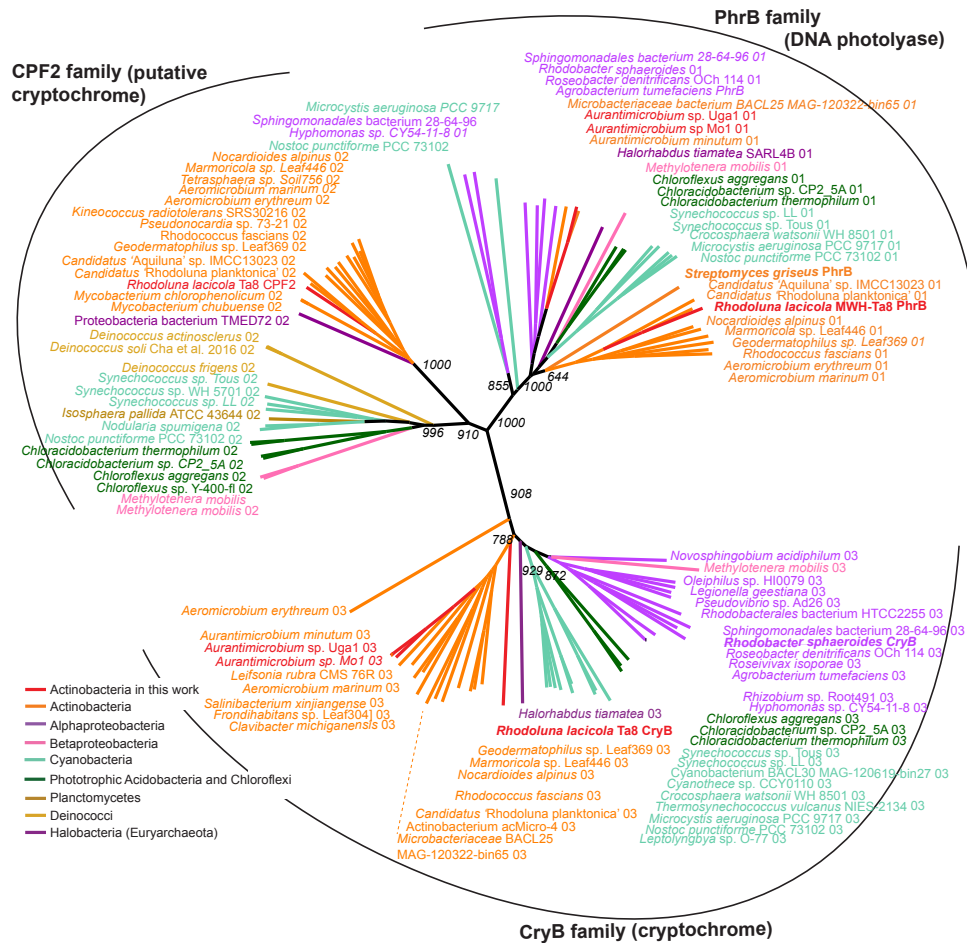


Figure S6. Phylogeny of cryptochrome-photolyase family (CPF) proteins found in Actinobacteria. Homologs of the three CPF proteins found in the *Rhl. ladicola* genome were aligned with ClustalW and a neighbor-joining tree was calculated with 1000 bootstraps, then visualized with Iroki. The three proteins clearly group separately, with the CryB-type cryptochromes separate from both DNA photolyases (PhrB) and the other putative cryptochrome (here labeled “CPF2”). Homologs of CryB are broadly distributed among Actinobacteria, phototrophic Acidobacteria, Archaea (primarily the halophilic Archaea), Cyanobacteria, and Proteobacteria (both phototrophic and not). CPF2 is separated into two clear groups, one of which includes Actinobacteria and a few Proteobacteria, and another that is found predominantly in Cyanobacteria and other phototrophs. The numbers after species names indicate whether the protein was found in the NCBI non-redundant database using the *Rhl. ladicola* photolyase (01), “CPF2” (02), or CryB homolog (03) amino acid sequence as the BLAST query. Accession numbers for the proteins used in this tree can be found in Table S4.

Figure S7.

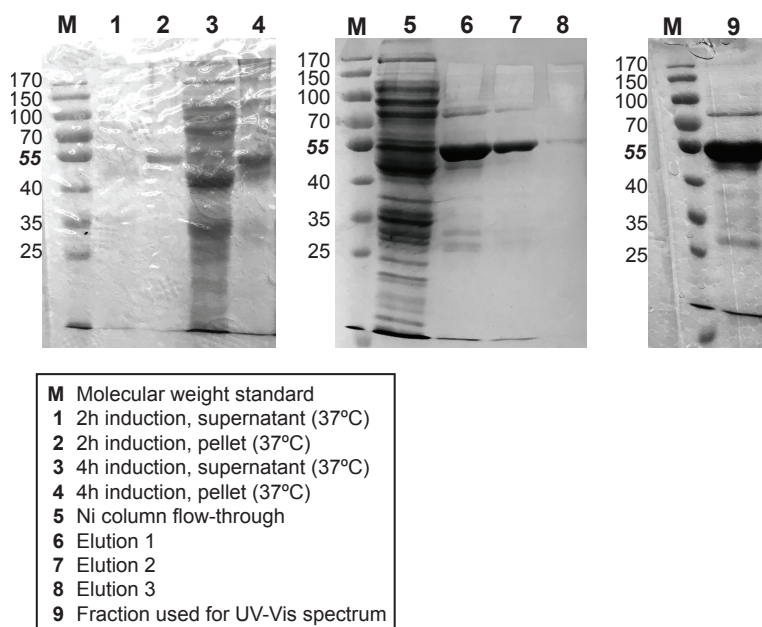


Figure S7. SDS-PAGE of overexpressed RS06470. Representative gels showing expression of RS06470. All lanes labeled “M” have the PageRuler Protein Ladder (10-180 KDa, ThermoScientific 26616). Lanes 1-4: Overexpression of RS06470 in *E. coli* at 37°C. Most protein produced at this temperature is insoluble, so growth temperature was reduced to ~23°C to decrease overexpression and increase soluble protein content. Lanes 5-8: After growth and induction at 23°C, the RS06470 protein was purified over a Ni column. RS06470 is predicted to have a molecular mass of 56 KDa. Most of the expressed protein elutes in the first two elution fractions. Lane 9: Fraction containing RS06470 used for UV-Vis spectroscopy.

Table S2. Sequences of known light-sensitive protein used as queries in genome searches. All three genomes (*Rhl. lacicola*, *Aurantimicrobium* sp. strain MWH-Mo1, and *Aurantimicrobium* sp. strain MWH-Uga1) were searched for predicted homologs of these proteins. Only the cryptochrome CryB from *Rhb. sphaeroides* and the DNA photolyase PhrB from *Streptomyces griseus* (in bold in the table above) had full-length alignments to predicted proteins in the genomes of *Rhl. lacicola* strain MWH-Ta8 and *Aurantimicrobium* sp. strains MWH-Mo1 and MWH-Uga1. Homologs of actinorhodopsin were identified in the genomes of *Rhl. lacicola* and *Aurantimicrobium* sp. strain MWH-Uga1, and homologs of heliorhodopsin were identified in both *Aurantimicrobium* species. None of the genomes encoded a homolog of the enzyme required for retinal synthesis from beta carotene.

Gene Name	Accession Number	Species	Function	Cofactor	Other domains	Locus tags of best hits in genomes
AerR	BAL96683.1	<i>Rubrivivax gelatinosus</i> IL144	blue light sensing	B12		
CarH	CAA79965.2	<i>Myxococcus xanthus</i>	blue light regulation of carotenoid synthesis	B12		
LitR	BAC68924.1	<i>Streptomyces avermitilis</i>	light sensing	B12		
MerR-B12 type	KRO35156	uncharacterized marine Actinobacterium	putative light sensitive regulator	B12	MerR domain	
MerR-B12 type	WP_067360985	<i>Micromonospora rosaria</i>	putative light sensitive regulator	B12	MerR domain	
PpaA	YP_353357.1	<i>Rhodobacter sphaeroides</i>	blue light sensing	B12		
CcaS	BAK51774.1	<i>Synechocystis</i> sp. PCC 6803	Green light sensing	bilin		
Cph1	WP_010873534.1	<i>Synechocystis</i> sp. PCC 6803	phytochrome, red light reponse	bilin	GAF domain	
Cph2	WP_351608.1	<i>Synechocystis</i> sp. PCC 6803	phytochrome, red light reponse	bilin		
Rcp1	Q55169.1	<i>Synechocystis</i> sp. PCC 6803	phytochrome, red light reponse	bilin		
BhpP (1)	YP_345384.1	<i>Rhodobacter sphaeroides</i>	phytochrome	bilin	EAL, GGDEF signal transduction domains	
BhpP (2)	YP_345327.1	<i>Rhodobacter sphaeroides</i>	phytochrome	bilin	PAS, GAF, phytochrome, EAL, GGDEF	

AppA	YP_351608.1	<i>Rhodobacter sphaeroides</i>	Antirepressor of PpsR, regulation of light-response	FAD		
BlrP1	AGT24399.1	<i>Klebsiella pneumoniae</i>	blue light sensing	FAD		
BlsA	ABO12652.2	<i>Acinetobacter baumannii</i>	blue light sensing	FAD		
Glr1749	BAC89690.1	<i>Gloeobacter violaceus</i>	DNA photorepair	FAD		
LovK	AAK22272.2	<i>Caulobacter crescentus</i>	blue light sensing	FAD		
PapB	CAE25966.1	<i>Rhodospseudomonas palustris</i>	light sensor; BLUF	FAD		
PhrB	WP_051804377.1	<i>Streptomyces griseus</i>	DNA photorepair	FAD		RHOLA_RS03920, AURMO_01673, AURUGA1_01549
YcgF	YP_002407889.1	<i>Escherichia coli</i>	blue light sensing	FAD		
CryB	WP_011339023.1	<i>Rhodobacter sphaeroides</i>	Blue light sensing	FAD, DLZ		RHOLA_RS06470, AURMO_00962, AURUGA1_00706
Cry1	SNC55619.1	<i>Vibrio cholerae</i>	cryptochrome-DASH family	FAD, folate	CRY-DASH	
LovK	AAK22272.2	<i>Caulobacter crescentus</i>	blue light sensing	FMN	LOV	
SL1	ABB56220.1	<i>Synechococcus elongatus</i> sp. PCC7942	blue light sensing	FMN	LOV	
SL2	ABB57385.1	<i>Synechococcus elongatus</i> sp. PCC7942	blue light sensing	FMN	LOV	
YtvA	AQR87275.1	<i>Bacillus subtilis</i>	Phototropin	FMN	LOV	
PpsR	AAF24278.1	<i>Rhodobacter sphaeroides</i>	blue light sensing	Heme		
PYP	ATZ73421.1	<i>Idiomarina</i>	Light sensing	p-coumaric acid		
Cry2	SNC55940.1	<i>Vibrio cholerae</i>	cryptochrome-like	photolyase domain		
LMIV_0353	EHY62896.1	<i>Listeria monocytogenes</i>	DNA photorepair	photolyase domain	Class I photolyase	
UM89_12670	KJJ41516.1	<i>Bacillus subtilis</i>	DNA photorepair	photolyase domain	Class III photolyase	
RcaE-like	U67397.1	<i>Synechocystis</i> sp. PCC 6803	blue light sensing	Phytochrome	PAS, GAF	

CarF	AAO22861.1	<i>Myxococcus xanthus</i>	blue light regulation of carotenoid synthesis	Protoporphyrin IX		
Mmc1_0735	ABK43256.1	<i>Magnetococcus</i> sp. strain Mmc1	putative blue light sensor		GAF/PAS	
PYP	ADV90265.1	<i>Thermochromatium</i>	PYP/phytochrome/cGMP		PAS, GAF, EAL, GGDEF	
ActR	FJ545221.1	<i>Rhodoluna lacicola</i>	Actinorhodopsin	Retinal		RHOLA_RS06005, AURUGA1_00474
HeR	MF737519.1	Uncultured Actinobacterium	Heliorhodopsin	Retinal		AURMO_01564, AURUGA1_00456
Blh/Brp	WP_070955078	<i>Rhodoluna planktonica</i> sp. MWH-Dar1	Putative beta carotene 15, 15' cleavage dioxygenase			

Supplementary Table S3. Protein domains searched for in actinobacterial genomes. Although proteins with homology to some of these domains were identified, none but the DNA photolyase or the CryB-related proteins appeared to be potential light sensors. Most had strong evidence for the annotations provided, such as RS04025, which has a B12 binding domain (PF02310) and is likely to be the methionine synthase in *Rhl. laticola*. However, some genes shared by all three genomes have intriguing combinations of PAS domains and signal transduction domains, and may be involved in signaling and/or gene regulation after light sensing.

Domain name	PFAM	Cofactor
BLUF	PF04940	FAD
CodY	PF06018	
EAL	PF00563	
GAF	PF01590	
GAF2	PF13185	
GAF3	PF13492	
GGDEF	PF00990	
HAMP	PF00672	
PAS	PF00989	
PAS 11	PF14598	
PAS 2	PF08446	
PAS 3	PF08447	
PAS 4	PF08448	
PAS 5	PF07310	
PAS 6	PF08348	
PAS 7	PF12860	
PAS 8	PF13188	
PAS 9	PF13426	
PHY	PF00360	bilin
B12 binding	PF02310	B12
B12 binding 2	PF02607	B12
MEKHLA domain	PF08670	p-coumaric acid
PYP	PF13188	p-coumaric acid
OCP	PF09150	carotenoid
Bac_rhodopsin	PF01036	retinal
photolyase	PF00875	flavin
photolyase	PF04244	flavin
FAD_binding_7	PF03441	FAD
cryptochrome	PF12546	flavin
DPRP	PF04244	flavin

Table S4. Accession numbers of amino acid sequences used in Figs. 5 and S6. Species names specified in Fig. S6 and the associated NCBI accession numbers. The numbers after the species names indicate the CPF clade to which that protein belongs: 01, PhrB-type DNA photolyase; 02, CryB-type cryptochrome; 03, “CPF2” family protein (uncharacterized).

Species Name	NCBI Accession Number
<i>Rhodoluna ladicola</i> MWH-Ta8 PhrB	2504662661
<i>Aurantimicrobium</i> sp strain MWH-Mo1 01	Mo1_00164
<i>Candidatus</i> 'Aquiluna' sp. IMCC13023 01	WP_007541646.1
<i>Rhodobacter sphaeroides</i> 01	WP_023003558.1
<i>Agrobacterium tumefaciens</i> PhrB	WP_080866077.1
<i>Aurantimicrobium minutum</i> 01	WP_096383072.1
<i>Aeromicrobium marinum</i> 01	WP_040320017.1
<i>Candidatus</i> 'Rhodoluna planktonica' 01	WP_070954614.1
<i>Nocardioides alpinus</i> 01	WP_091200075.1
<i>Microbacteriaceae</i> bacterium BAACL25 MAG-120322-bin65 01	KRO39476.1
<i>Crocospaera watsonii</i> WH 8501 01	WP_007304147.1
<i>Microcystis aeruginosa</i> PCC 9717 01	WP_002758099.1
<i>Synechococcus</i> sp. LL 01	WP_094591764.1
<i>Nostoc punctiforme</i> PCC 73102 01	WP_012408666.1
<i>Sphingomonadales</i> bacterium 28-64-96 01	OYZ13787.1
<i>Roseobacter denitrificans</i> OCh 114 01	WP_011568493.1
<i>Hyphomonas</i> sp. CY54-11-8 01	WP_034769403.1
<i>Geodermatophilus</i> sp. Leaf369 01	KQS60369.1
<i>Marmoricola</i> sp. Leaf446 01	WP_056540310.1
<i>Rhodococcus fascians</i> 01	WP_037185002.1
<i>Aeromicrobium erythreum</i> 01	WP_067859230.1
<i>Synechococcus</i> sp. Tous 01	OON12124.1
<i>Chloroflexus aggregans</i> 01	WP_012616155.1
<i>Chloracidobacterium thermophilum</i> 01	WP_058868137.1
<i>Chloracidobacterium</i> sp. CP2_5A 01	OYT73889.1
<i>Methylothera mobilis</i> 01	WP_015832830.1
<i>Halorhabdus tiamatea</i> SARL4B 01	WP_008525328.1
<i>Rhodoluna ladicola</i> MWH-Ta8 CPF2	2504662972
<i>Candidatus</i> 'Aquiluna' sp. IMCC13023 02	EIC91855.1
<i>Aeromicrobium marinum</i> 02	WP_007077203.1
<i>Rhodoluna planktonica</i> 02	WP_070955074.1
<i>Nocardioides alpinus</i> 02	WP_091199138.1
<i>Synechococcus</i> sp. LL 02	WP_094589955.1
<i>Nostoc punctiforme</i> PCC 73102 02	WP_012412678.1
<i>Geodermatophilus</i> sp. Leaf369 02	KQS60737.1
<i>Marmoricola</i> sp. Leaf446 02	WP_056539249.1
<i>Rhodococcus fascians</i> 02	WP_037152333.1
<i>Mycobacterium chubuense</i> 02	WP_048420367.1
<i>Pseudonocardia</i> sp. 73-21 02	OJY39528.1
<i>Mycobacterium chlorophenolicum</i> 02	WP_048472468.1
<i>Tetrasphaera</i> sp. Soil756 02	KRE61391.1

<i>Kineococcus radiotolerans</i> SRS30216, ATCC BAA-149 02	WP_012086202.1
<i>Aeromicrobium erythreum</i> 02	WP_067861656.1
<i>Proteobacteria</i> bacterium TMED72 02	OOU91956.1
<i>Synechococcus</i> sp. Tous 02	OON13070.1
<i>Synechococcus</i> sp. WH 5701 02	WP_006173128.1
<i>Chloroflexus aggregans</i> 02	WP_015940350.1
<i>Deinococcus frigens</i> 02	WP_051668791.1
<i>Chloroflexus</i> sp. Y-400-fl 02	WP_015909133.1
<i>Deinococcus soli</i> Cha et al. 2016 02	WP_081424584.1
<i>Nodularia spumigena</i> 02	WP_063873664.1
<i>Deinococcus actinosclerus</i> 02	WP_062157339.1
<i>Chloracidobacterium thermophilum</i> 02	WP_058867563.1
<i>Chloracidobacterium</i> sp. CP2_5A 02	OYT71351.1
<i>Isosphaera pallida</i> ATCC 43644 02	WP_013564012.1
<i>Methylothermobacter mobilis</i> 02	WP_019899883.1
<i>Rhodoluna laticola</i> MWH-Ta8 CryB	2504663175
<i>Aurantimicrobium</i> sp strain Mo1 03	Mo1_01237
<i>Rhodobacter sphaeroides</i> CryB	WP_011339023.1
<i>Agrobacterium tumefaciens</i> 03	WP_010974139.1
<i>Aurantimicrobium minutum</i> 03	BAU99214.1
<i>Leifsonia rubra</i> CMS 76R 03	EPR76568.1
<i>Frondehabitans</i> sp. Leaf304] 03	WP_055961530.1
<i>Salinibacterium xinjiangense</i> 03	WP_097060556.1
<i>Clavibacter michiganensis</i> 03	WP_086520529.1
<i>Aeromicrobium marinum</i> 03	WP_007077117.1
<i>Actinobacterium acMicro-4</i> 03	KGA05005.1
<i>Rhodoluna planktonica</i> 03	WP_070954309.1
<i>Nocardioides alpinus</i> 03	WP_091193811.1
<i>Microbacteriaceae</i> bacterium BACL25 MAG-120322-bin65 03	KRO39092.1
<i>Crocospaera watsonii</i> WH 8501 03	WP_007303106.1
<i>Leptolyngbya</i> sp. O-77 03	WP_068513283.1
<i>Microcystis aeruginosa</i> PCC 9717 03	WP_002757006.1
<i>Cyanothece</i> sp. CCY0110 03	WP_035798102.1
<i>Cyanobacterium</i> BACL30 MAG-120619-bin27 03	KRO92874.1
<i>Synechococcus</i> sp. LL 03	WP_094591397.1
<i>Thermosynechococcus vulcanus</i> NIES-2134 03	BAY52557.1
<i>Nostoc punctiforme</i> PCC 73102 03	ACC82051.1
<i>Novosphingobium acidiphilum</i> 03	WP_028639404.1
<i>Sphingomonadales</i> bacterium 28-64-96 03	OYZ14664.1
<i>Oleiphilus</i> sp. HI0079 03	WP_082873371.1
<i>Rhodobacterales</i> bacterium HTCC2255 03	WP_008033738.1
<i>Roseobacter denitrificans</i> OCh 114 03	WP_044032967.1
<i>Rhizobium</i> sp. Root491 03	KQY40850.1
<i>Pseudovibrio</i> sp. Ad26 03	WP_063312709.1
<i>Hyphomonas</i> sp. CY54-11-8 03	WP_034766300.1
<i>Legionella geestiana</i> 03	WP_028386762.1
<i>Roseivivax isopora</i> 03	WP_043767667.1

<i>Geodermatophilus</i> sp. Leaf369 03	KQS61106.1
<i>Marmoricola</i> sp. Leaf446 03	WP_056540174.1
<i>Rhodococcus fascians</i> 03	WP_037141299.1
<i>Aeromicrobium erythreum</i> 03	ALX06096.1
<i>Synechococcus</i> sp. Tous 03	OON11793.1
<i>Chloroflexus aggregans</i> 03	WP_015942013.1
<i>Chloracidobacterium thermophilum</i> 03	WP_014099386.1
<i>Chloracidobacterium</i> sp. CP2_5A 03	OYT72432.1
<i>Methylotenera mobilis</i> 03	WP_036317054.1
<i>Halorhabdus tiamatea</i> SARL4B 03	CCQ32593.1
<i>Aurantimicrobium minutum</i> 04	WP_096381351.1
<i>Microcystis aeruginosa</i> PCC 9717	WP_002756763.1
<i>Nostoc punctiforme</i> PCC 73102	WP_012413103.1
<i>Sphingomonadales</i> bacterium 28-64-96	OYZ13813.1
<i>Methylotenera mobilis</i>	WP_012777621.1