Research Article

Multifaceted photoreceptor compositions in dual phototrophic systems – A genomic analysis

Janne A. Ihalainen [1,*], Batuhan Dogan [1], Moona Kurttila [1,†], Yonghui Zeng [2], Jan Dirk van Elsas [3] and Riitta Nissinen [1,4]

*1 - **University of Jyväskylä,** Nanoscience Center, Department of Biological and Environmental Science, 40014 Jyväskylä, Finland*
*2 - **University of Copenhagen,** Department of Plant and Environmental Sciences, 2100 Copenhagen, Denmark*
*3 - **University of Groningen,** Groningen Institute for Evolutionary Life Sciences, 9747 AG Groningen, the Netherlands*
*4 - **University of Turku,** Department of Biology, 20500 Turku, Finland*

***Correspondence to Janne A. Ihalainen:** janne.ihalainen@jyu.fi (J.A. Ihalainen)*
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Abstract

For microbes and their hosts, sensing of external cues is essential for their survival. For example, in the case of plant associated microbes, the light absorbing pigment composition of the plant as well as the ambient light conditions determine the well-being of the microbe. In addition to light sensing, some microbes can utilize xanthorhodopsin based proton pumps and bacterial photosynthetic complexes that work in parallel for energy production. They are called dual phototrophic systems. Light sensing requirements in these type of systems are obviously demanding. In nature, the photosensing machinery follows mainly the same composition in all organisms. However, the specific role of each photosensor in specific light conditions is elusive. In this study, we provide an overall picture of photosensors present in dual phototrophic systems. We compare the genomes of the photosensor proteins from dual phototrophs to those from similar microbes with "single" phototrophicity or microbes without phototrophicity. We find that the dual phototrophic bacteria obtain a larger variety of photosensors than their light inactive counterparts. Their rich domain composition and functional repertoire remains similar across all microbial photosensors. Our study calls further investigations of this particular group of bacteria. This includes protein specific biophysical characterization *in vitro*, microbiological studies, as well as clarification of the ecological meaning of their host microbial interactions.

Introduction

Photosynthesis performed by plants, algae, and cyanobacteria provide the organic carbon, and therefore the energy needed for all other life on earth. While in this case light energy is converted into chemical energy through carbon fixation, many microrganisms also have machinery to store energy through light-driven proton gradients across the bacterial membrane either via anoxygenic photosynthesis (chlorophototrophs) typically utilizing bacteriochlorophyll *a* (BChl *a*) molecules or retinal-rhodopsin-based proton pumping (retinalphototrophs). The light-driven redox reactions produce a proton-motive force for chemical energy production in the form of ATP.[1] Organisms that utilize light as an energy source but are still dependent on organic carbon from their host are called photoheterotrophs. Intriguingly, several bacterial strains have been reported with both of these phototrophic machinery. They are referred to as dual photoheterotrophs.[2]

As the name says, anoxygenic photosynthesis does not produce oxygen as a side product. Still, some anoxygenic phototrophs can live in aerobic environments. Typically, the biosynthesis of the photosynthetic apparatus is suppressed by oxygen. However, organisms that strictly need oxic conditions for photosynthetic apparatus production and for photosynthetic electron transport are called aerobic anoxygenic phototrophs (AAP).[1] These bacteria live in aerobic conditions and rely on diverse organic compounds as electron donors for the phototrophy.[3] AAP bacteria (AAPB) have been originally discovered in oligotrophic marine environments,[4] and are ubiquitous in both marine and freshwater ecosystems.[5,6] In aquatic ecosystems, they can constitute up to a third of the total bacterial populations. They are considered to play a significant role in carbon transformations in marine ecosystems.[3] AAPB have also been found in terrestrial systems, including soil biocrusts, Antarctic soils and plants.[7,8] AAPB have been detected in phyllosphere metagenomes, and are commonly present in epi- and endophytic microbial communities of diverse plant species in arctic and boreal regions.[8] Proteorhodopsin genes have been identified in all three domains of life, but they are mainly studied from marine bacteria.[9,10] Microbial rhodopsins are recognized to be present in about 50% of all “heterotrophic” bacteria living in the surface ocean.[10] Further, terrestrial and glacial proteorhodpsins have been reported,[11,12] but studies on terrestrial retinalphototrophs are rare.

Environmental factors impacting AAPB abundance, diversity and their photosynthesis are still relatively poorly understood. The AAP is driven by bacteriochlorophylls and as such they can utilize near infrared (NIR) light as an energy source. Rhodopsins consist essentially of one protein, opsin, and a maximum of two chromophores, a retinal and a carotenoid molecule, in the case of xanthorhodopsins.[13] The retinal has no cofactors and is produced in one metabolic step.[10] Under green light, the retinal pigment isomerizes and provokes a proton gradient across a membrane. Hence, in terms of excitation wavelengths, Chlorophyll *a* (Chl *a*) light absorption, taking place in plants, algae or cyanobacteria, do not disturb the functionality of AAPB or retinalphototrophs, and in principle, all photosystems can work in parallel in microbe-host systems. It is noteworthy that making rhodopsin-based proton pumps is rather ”cheap”, while making photosynthesis machinery for bacteria is ”expensive”, as the protein cluster for the latter is much larger than for the former one.[10,14] However, the latter can collect light much more efficiently and the turnover rate per absorbed photon is considerably higher than for a rhodopsin. The setting for host associated dual phototrophs - BChl *a* and retinal pigment containing bacterial systems in a Chl *a* based host - provides multifaceted requirements for bacterial photosensing. For example, they need to signal the fluency, intensity variability and spectral properties of light to their host. This in addition to sensing temperature and other external factors is necessary for optimal growth and functioning of microorganisms, and further benefits the well-being of the host. The wavelength sensitivity is conducted by using different photosensor proteins for each wavelength range.

Figure 1 lists the photosensors which detect light at each wavelength range. The general mechanism of all photosensors follows the same pattern. Photon absorption by the chromophore leads to physical changes of the chromophore and its nearby environment. The flavin-containing systems, Light-Oxygen-Voltage sensors (LOV), Blue-Light Using Flavin domain (BLUF), and Cryptocrome/Photolyase (CRY/PHR) complexes rely on the rupture of a covalent bond (LOV) or charge transfer processes (BLUF, CRY/PHR) after photon absorption, whereas the rhodopsins, xanthopsins and bacteriophytochromes (BphP), rely on the photoinduced isomerization process of the retinal, p-coumaric acid, and bilin chromophore, respectively.[15] The changes in the chromophore environment induce changes in the protein environment of the photosensory domain. The biochemical signaling is performed by the effector domain of the complex. The effector domain often binds (or separates) to (from) its cognate response regulator (RR) functioning as a gene expression regulator. Other mechanisms are for example binding to other induction or repression system (LOV), or cysteinyl-adduct formation (LOV), or direct DNA binding (Photolyases, LOV), to mention just a few mechanisms. In the case of histidine kinases, a phosphotransfer reaction between the HK unit and RR takes place.[16] To conclude, the photosensory domain architeture is rather conserved across the whole kingdom of life, whereas the effector domain variability is large. A plethora of functions are controlled by the effector domains and the interplay with their cognate RR or other suppressor/effector domains. Excellent studies on the variability of microbial photosensory systems and their functional mechanisms are available.[15,17–21]

In this study, we provide a genomic analysis of photosensor composition in dual phototrophic bacteria. We compare the presence and multiplicity of photosensors of bacteriophytochromes (BphP), Light-oxygen-Voltage (LOV), Blue light photosensor (BLUF), and Cryptochrome/Photolyase (CRY/PHR) systems, and photoactive yellow proteins (PYP) in dualphototrophic systems with those of similar species without dual phototrophic character (Fig. 1 and Tables 1 and S1). Further, we aim to pinpoint the differences and similarities of genome sequence levels of each photosensor among the dual phototrophic systems, some of which are newly discovered from plant associated bacteria.

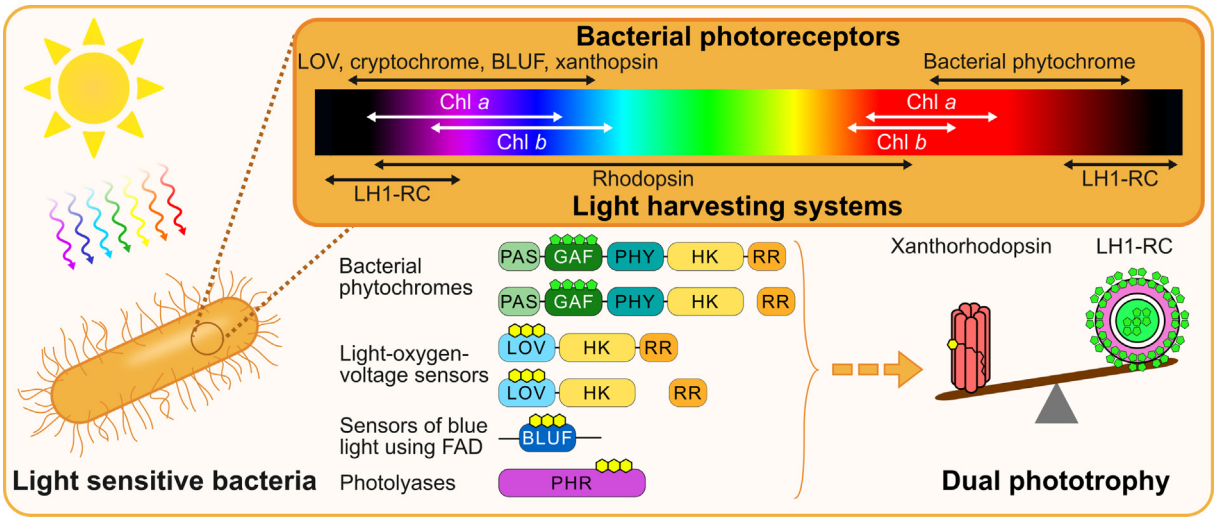

Fig. 1. Light, phototrophic systems and photoreceptors. In light sensing bacteria, photoreceptors and light harvesing systems work in parallel. The spectral regions utilized by bacterial photosynthesis systems, xanthorhodopsin and bacterial photosensors are marked as black arrows. The white arrows mark the spectral regions of "competing" absorbance of Chl *a* and *b* molecules The basic domain composition of the photosensors are shown as well. Rarely, a direct link between the phototrophic components with photosensors has been elucidated. Still, photoreceptors must play a decisive role which phototrophic system becomes active.

Results

Selection of dual phototrophic species - their origin and environmental conditions

We performed whole genome sequencing for selected *Sphingomonas* strains previously identified as AAPB, based on our NIR fluorescence analysis.[8] The AAP positive bacteria were confirmed by presence of the reaction center specific *pufM* gene. When a xanthorhodopsin gene was observed in addition to the *pufM* gene, the species was identified as dual phototrophic system, similar to AAP5 strain reported by Koblizek and colleagues.[2] Hence, dual phototrophs are strains which contained both AAP and xanthorhodopsin gene patterns.

We elaborated our analysis using the National Center for Biotechnology (NCBI) data base. We identified more dual phototrophic systems from the data base, but also strains with AAP genes only, strains with xanthorhodopsin genes only, and heterotrophic strains which did not contain any photoactive proton pumping system, but belonged to same genuses as those of photoheterotrophs. In our analysis, we have overall 62 strains, which contained more than 300 photosensors. All studied species with their access number, code used in this study, and source are presented in Table S1.

Table 1 lists strains under comparison in this study. All AAPs and dual phototrophs contain genes for light harvesting complexes (LH), either LH1 or LH1 and LH2 genes. We name as "Rho" strains those which contain only xanthorhodopsin gene. In addition to those, 23 similar strains but without phototrophicity are also listed. Peculiarly, strains TA304 and TA352 contain LH2 gene even though they lack photosynthetic reaction center genes,[12] and even more strikingly, OCH149 is a non-photoheterotroph but still contains LH2 gene.

Interestingly, the dual phototrophs found in this work mainly originate from cold and harsh conditions with perpetual growth in dynamically changing habitats where irradiance and temperatures vary significantly across seasons. The *Tardiphaga* strains are collected from soils of glaciers in North East Greenland, with extremely short growing seasons but with ample amount of light during the growth period.[12] The *Sphingomonas* strains defined as dual phototrophs are either plant associated bacteria originating from arctic plants, collected in the northern region of Finland (J1U1, M1U20),[22] or from Svalbard (L1CD2B). The *Sphingomonas* strains which are not plant associated bacteria, but are dual phototrophs, originate either from permafrost in arctic Canada region (CGMCC and SE61), or from high altitude alpine lake in Austria (AAP5).[2]

Photoreceptor gene collection

Dual phototrophy requires precise and elaborate photosensing capabilities, as the organisms need to control which of the photosystems is synthesised. In addition, several of the studied strains J1U1, M1U20 and L1CD2B, which all belong to the bacterial family Sphingomonas, live in changing light conditions as they have been

Table 1 The photoreceptor collection of the studied species, their origin and codes used in the phylogenetic trees. The NCBI accession numbers are listed in Supplementary Table S1. The dark grey color indicates the dual phototrophs, the grey the AAP systems and the light grey the systems with only xanthorhodopsins. The strains are ordered according to their total number of photosensor proteins.

Source	Genus	Strain	BLUF	LOV	BphP	CRY	PYP	Category	LH
High arctic permafrost Canada	Sphingomonas	SE61	10	2	2	2	1	Dual	LH1
Leaf of Oryza sativa	Methylobacterium	CBMB27	4	2	2	3	–	AAP	LH1
Stem surface of Oryza sativa	Methylobacterium	CBMB20	5	1	2	3	–	AAP	LH1
Antarctic sea ice	Octadecabacter	O307	5	–	–	5	–	Rho	–
High elevation lake Austria	Sphingomonas	AAP5	4	1	1	2	1	Dual	LH1
Antarctica soil	Sphingomonas	PAMC26645	3	2	3	2	–	None	–
Nodules of Lotononis bainesii	Methylobacterium	MS446	5	–	2	2	–	Dual	LH1
Greenland stream water	Gemmatimonas	TET16	4	–	1	4	–	AAP	LH1
High arctic permafrost Canada	Sphingomonas	CGMCC	3	1	1	2	1	Dual	LH1
Glacial ice, NE Greenland	Tardiphaga	TA278	1	–	5	2	–	Dual	LH1,LH2
Wastewater	Methylobacterium	C1	3	–	2	2	–	AAP	LH1
Arctic plant endosphere, Kilpisjärvi	Sphingomonas	S2H28	1	–	4	1	1	Rho	–
Glacial ice, NE Greenland	Tardiphaga	TA154	2	–	3	2	–	Dual	LH1,LH2
Contaminated soil	Methylorubrum	CM4	5	1	1	1	–	AAP	LH1
Arctic plant phyllosphere, Svalbard	Sphingomonas	L1CD2B	2	–	2	2	1	Dual	LH1
Malus prunifolia	Sphingomonas	AP4	2	1	2	2	–	None	–
Arctic sea ice	Octadecabacter	O238	2	–	–	4	–	Rho	–
Arctic plant endosphere, Kilpisjärvi	Sphingomonas	J1U1	1	–	3	2	–	Dual	LH1
Glacial ice, NE Greenland	Tardiphaga	TA304	1	–	3	2	–	Rho	LH2
Glacial ice, NE Greenland	Tardiphaga	TA352	1	–	3	2	–	Rho	LH2
Plant associated	Sphingomonas	AK	2	1	2	2	–	None	–
Tidal flat sediment	Roseobacter	YSTF	2	1	1	3	–	AAP	LH1,LH2
Arctic plant endosphere, Kilpisjärvi	Sphingomonas	M1U20	1	–	3	2	–	Dual	LH1
Cornfield topsoil Xinjiang	Sphingomonas	NX02	1	–	2	2	–	Dual	LH1
Freshwater water column	Limnohabitans	G32	3	–	–	3	–	AAP	LH1
Desert lake in north China	Gemmatimonas	AP64	2	–	–	3	1	AAP	LH1
Tidal flat area	Roseobacter	B14	1	1	–	3	–	AAP	LH1,LH2
Arctic plant endosphere, Kilpisjärvi	Sphingomonas	J5HS3a	1	–	1	2	1	Rho	–
Marine coastal samples	Dokdonia	MED134	1	–	1	2	–	Rho	–
Freshwater water column	Limnohabitans	B93	2	–	–	3	–	AAP	LH1
Arctic plant endosphere, Kilpisjärvi	Sphingomonas	S2U11	1	–	3	1	–	AAP	LH1
Freshwater water column	Limnohabitans	JIRII-31	2	–	–	2	–	AAP	LH1
Plant associated	Methylobacterium	AM1	2	1	1	1	–	AAP	LH1
Marine coastal samples	Congregibacter	KT71	2	–	–	3	–	AAP	LH1
Water column	Roseobacter	OCH149	1	1	–	3	–	None	LH2
Water column	Dinoroseobacter	DFL12	1	1	–	2	–	AAP	LH1,LH2
Root nodule	Tardiphaga	LMG	2	–	1	1	–	None	–
Unknown	Tardiphaga	OK246	2	–	1	1	–	None	–
Sea surface water, Japan	Roseobacter	AI77	–	–	1	3	–	AAP	LH1
Freshwater water column	Limnohabitans	15 K	1	–	–	3	–	AAP	LH1
Water column	Roseobacter	OCH114	1	1	–	2	–	AAP	LH1,LH2
Freshwater Reservoir	Limnohabitans	2D5	2	–	–	2	–	AAP	LH1
Root nodule	Tardiphaga	TA581	1	–	1	1	–	None	–
Root nodule	Tardiphaga	TA37S4	1	–	1	1	–	None	–
Unknown	Tardiphaga	OK245	1	–	1	1	–	None	–
Water column	Pelagibacter	SAR11	–	–	–	2	–	Rho	–
Soil of Hengshui Lake	Sphingomonas	WHSC8	1	–	1	1	–	AAP	LH1
Arctic plant endosphere, Kilpisjärvi	Sphingomonas	S3H21	–	–	1	1	1	Rho	–
Root	Tardiphaga	P911	1	–	–	1	–	None	–
Unknown	Sphingomonas	TY	–	–	1	1	–	None	–
Plant associated	Tardiphaga	VAF07	–	–	1	1	–	None	–
Sewage treatment plant	Gemmatimonas	T27	–	–	–	1	1	None	–
Antarctica soil	Sphingomonas	SO64	1	–	–	1	–	None	–
Plant associated	Sphingomonas	DCY99	–	–	1	1	–	None	–
Plant associated	Sphingomonas	CRA20	1	–	1	–	–	None	–
Freshwater lake	Sphingomonas	LM7	1	–	1	–	–	None	–
Alpine soil	Sphingomonas	DSM22537	1	–	–	1	–	None	–
Waste water bioreactor	Sphingomonas	CL51	1	–	–	–	–	None	–
Activated sludge	Sphingomonas	KC8	–	–	–	1	–	None	–

Table 1 (*continued*)

Source	Genus	Strain	BLUF	LOV	BphP	CRY	PYP	Category	LH
Oceanic crustal fluid	Gemmatimonas	AH-D16	–	–	–	–	–	None	-
Contaminated soil	Sphingomonas	MM1	–	–	–	–	–	None	–
Plant associated	Sphingomonas	DM2	–	–	–	–	–	None	–

Abbreviations: BLUF - blue light using flavin; LOV - light oxygen voltage; BphP - bacterial phytochrome; CRY - (CRY/PHR, cryptochrome/photolyase; PYP - photoactive yellow protein; Rho - xanthorhodopsin; LH - light harvesting complex

found either from plant leaf internal tissues (endosphere) or from plant leaf surfaces (phyllosphere) and therefore contain changing amounts of Chl molecules in their environment. The photosensing is performed by BLUF, LOV, BphP, Cryptochrome/Photolyase (CRY/PHR), and PYP photosensors. Table 1 shows the numbers of different photoreceptor proteins for each studied bacterial strain. Further, the Fig. 2 presents the average number of all photosensors as well as the average number of each photosensor in each photoheterotrophic category.

Table 1 and Fig. 2 demonstrate that the photoheterotrophs have a larger amount and variety of photosensors in comparison to heterotrophs, and dual photostrophs have the largest amount of photosensors. In our analysis, dual phototrophs have on average about eight photosensors whereas single phototrophs, i.e. AAP or xanthorhodopsin based phototrophs (”Rho” category), have about five to six photosensors. Heterotrophs (”none” category in Fig. 2A) have on average about three photosensor proteins. Fig. 2B shows that all phototrophs contain about two CRY/PHR photosensors on average, the dual phototrophs and AAPs have more BLUF sensors than xanthorhodopsin based photoheterotrophs. The dual phototrophs have also the largest average number of BphP systems. The average number of PYP and LOV domains is low on average; however, variation can be observed so that AAP and dual phototrophs have a slightly higher LOV domain value than heterotrophs. We could not identify any LOV domains for ”Rho” category.

Except for one case, all phototrophs contain multiple CRY/PHR, BLUF, or BphP genes. Several phototrophs contained two LOV domains, but many did not contain any LOV domains. The number CRY/PHR copies is generally two in each strain. Larger variation is observed with BLUF and BphP sensors. Often, with a relatively large number of BLUF genes a low number BphPs can be found, and vice versa. Some of the studied strains show genes for PYP. Often, PYPs are linked with BphPs.[23] However, in our search for gene locations, no strains' PYP genes located or linked to BphPs. Recently, a wider genomic analy-

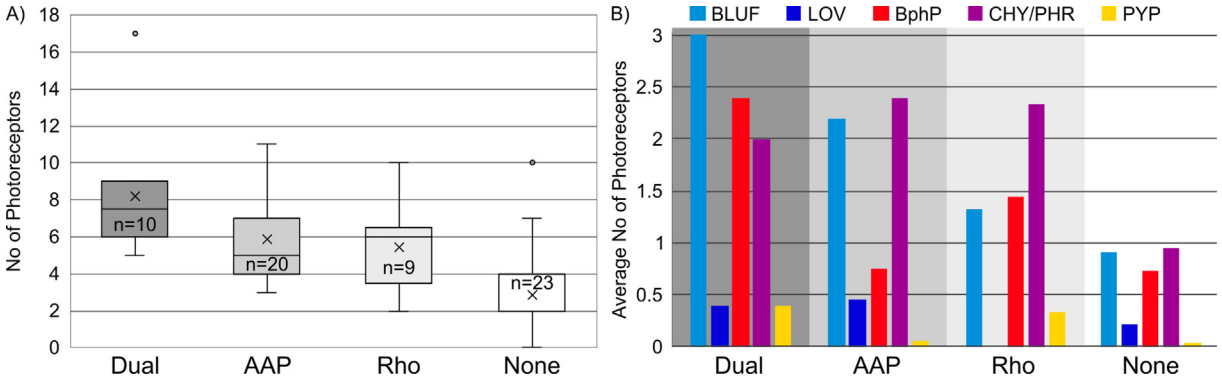

Fig. 2. Numerical distribution of photosensors according to different phototrophic groups. (A) The number of photosensor-encoding genes vary depending on the type of phototrophy. The median line show the skewed (asymmetric) distribution of the number of photosensors. In ”None” group, the median is 2. The first quartile marks one end of the box and the third quartile marks the other. The segment lines describe the highest and lowest number of the photosensors and the outliers which were observed in the case of ”Dual” and ”None” groups. On average (the cross), there are 8.2 photosensors in dual phototrophs while AAPB and xanthorhodopsin-based phototrophs (”Rho” category) have 5.9 and 5.4, respectively. In the heterotrophs (the ”None” group), which does not contain either xanthorhodopsin nor light harvesting system, the approximate average number is 2.9 photosensors. (B) The bar plot shows an average number of each type of photosensor in Dual, AAP, Rho and None groups. The studied Rho-based systems do not have any LOV domains.

sis on the spread of PYP was reported suggesting multiple functions for PYP protein, depending on the species.[24] It has also been suggested that PYP could act as a UV-sensor.[25] Rhodopsins as photosensors are not analyzed in this study. Yet, it is worth mentioning that in the case of J5HS3a (*S. faeni*), both xanthorhodopsin and sensory rhodopsin genes were detected, while coexistence of xanthorhodopsin and sensory rhodopsin genes could not be observed in other instances.

Comparison of the photosensory composition of the species in Table 1 identified a wider set of photosensory genes in the phototrophs, making it appealing to study the genomic sequences in more detail. In the Figs. 3–5 we provide the phylogenetic trees for CRY/PHR, LOV, BLUF, and BphP genes, respectively, of the strains studied, with the different categories highlighted. At first glance, one can see that the photosensor genes from each strain only partially cluster according to their taxonomy or to their photoheterotrophicity in the phylogenetic tree. When multiple photosensors are observed from the same strain, they often locate in different main branches. Still, across all the photosensors, the overall grouping remains according their genus. For example Sphingomonas always appear in the nearby branches (Figs. 3–5).

Cryptochromes and Photolyases

Cryptochromes and Photolyases marked in Table 1 were collected with a threshold of 35% sequence identity with *Dinoroseobacter shibae* (strain DFL 12) CRY/PHR sequence. The conservation of the FAD-binding domain's strong homology across all the strains we examined underscores the notion that cryptochromes and 6_4 PHRs, despite their diversity in bacterial hosts, may share a common functional heritage. This conservation hints at the importance of these photoreceptor proteins in the adaptation of bacteria to UV radiation-induced DNA damage[?]. The consistent presence of this homologous domain not only highlights the role played by cryptochromes and 6_4 PHRs in light-induced DNA repair but also suggests a shared evolutionary lineage among these proteins.[26,27] This shared ancestry, as evident from the robust FAD-binding domain homology, invites further exploration into the precise mechanisms governing the operation of these photoreceptors and their contribution to the adaptability of dual phototrophic bacteria. Additionally, the coexistence of FAD/NAD-based photosensors, such as those observed in AP64 6_4 PHR 1 and TET16 6_4 PHR, broadens the scope of photoreceptor diversity and their potential functional significance in bacterial UV radiation response. Furthermore, while all cryptochromes are categorized within the PhrB and DPRP (Deoxyribodipyrimidine photo-lyase-related protein) family, the inclusion of an N-terminal DNA photolyase domain within all 6_4 PHRs is another finding. This feature suggests a dual role for 6_4 PHRs, not only in light sensing but also in direct involvement in DNA repair processes.[28,29] The presence of such a domain provides a foundation for future research to unravel the specific mechanisms by which these photoreceptor proteins contribute to bacterial survival and adaptation in the face of UV radiation-induced DNA damage. In summary, the shared homology in the FAD-binding domain across diverse bacterial strains points to a unifying theme in the functionality of cryptochromes and 6_4 PHRs, encouraging a deeper exploration of their roles in the context of bacterial phototrophy and DNA repair mechanisms.

LOV domains - blue light sensing

The LOV domains, highlighted in Table 1, were meticulously curated using a stringent sequence identity threshold of 35% relative to the LOV domain of *Dinoroseobacter shibae* (strain DFL 12). These LOV domains exhibit a unique structural architecture comprising at least one of the modules from the PAS9-PAS3-GAF-HATPase-HWE_HK family. These functional modules include putative active sites, heme pockets, ATP binding sites, Mg binding sites, ATP-lid regions, and the signature G-X-G motif. This multifaceted architecture underscores the importance of these LOV domains as versatile light-sensing elements, potentially orchestrating a diverse range of biological responses in the organisms housing them.[30]

BLUF domains - blue light sensing

The BLUF domains marked in Table 1, were collected in accordance with the same 35% sequence identity threshold in comparison to the BLUF domain of *Dinoroseobacter shibae* (strain DFL 12), were consistently present in all the dual phototrophic strains examined. The conservation of this domain across the aligned strains suggests that these BLUF proteins belong to the group II BLUF proteins, denoting a shared ancestry and, potentially, a common functionality.[31] These BLUF domains also exhibit a distinctive set of conserved amino acids, specifically tyrosine, glutamine, and methionine, as depicted in Figure S8-10. Notably, a majority of the analyzed BLUF proteins are relatively short, comprising fewer than 200 amino acids. The absence of effector domains in these proteins implies that their signaling capabilities rely on changes in their oligomeric state, reflecting an elegant and efficient mechanism for translating light stimuli into biological responses.[31] However, it is worth highlighting that a few exceptions, namely NX02 BLUF 1, TET16 BLUF 1, C1 BLUF 1,

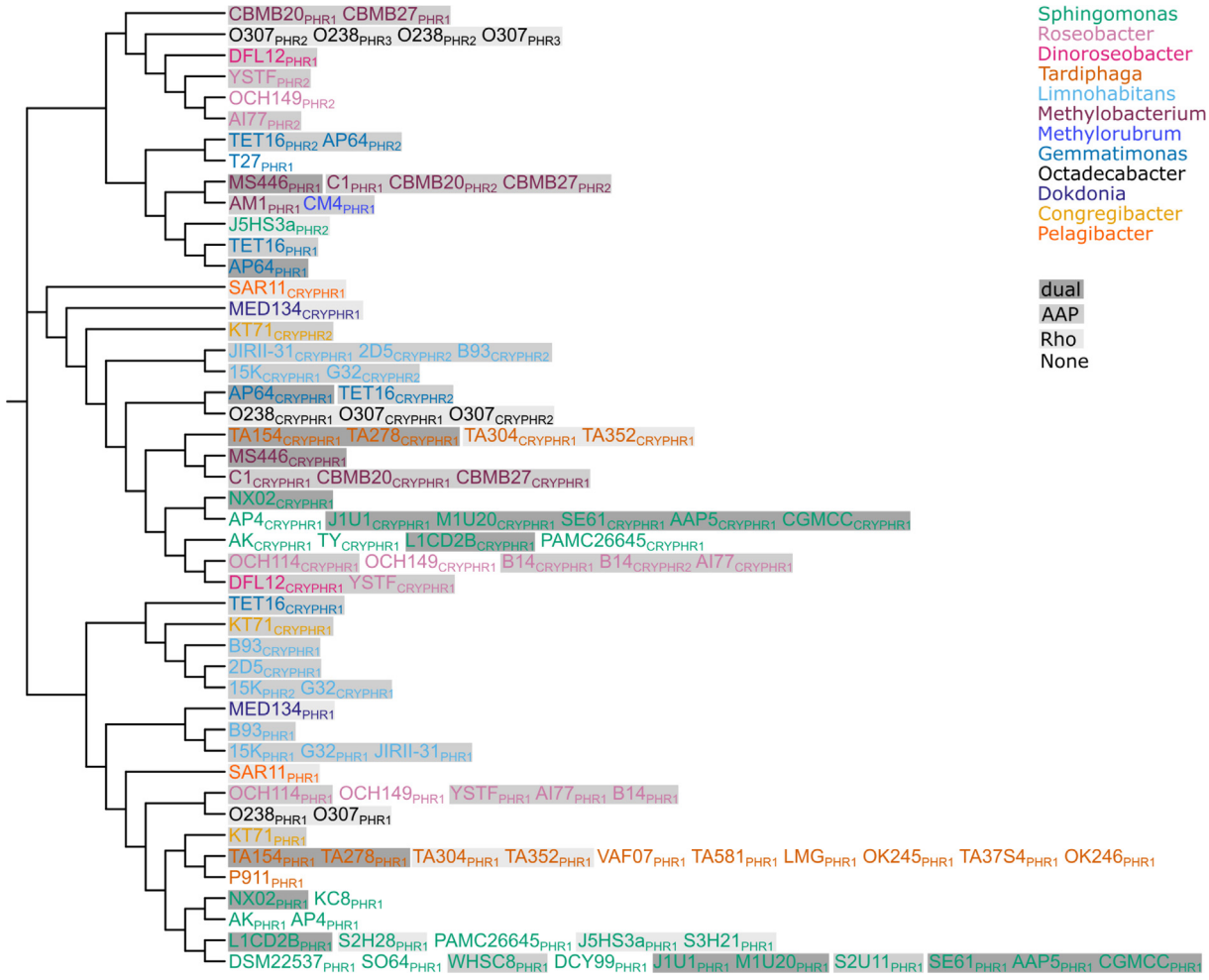

Fig. 3. Phylogenetic tree of CRY/PHR domains The phylogenetic tree is based on an alignment process, and the maximum likelihood trees show sequences with 85% or more sequence similarity in the same branch. The color coding reflects the genus of the strains (See Table 1) and the dual, AAP and Rho classes are marked with shades of grey. Branch length.s are not shown.

CBMB20 BLUF 1, and CBMB27 BLUF 1 domains, feature an additional C-terminal domain. According to protein family classification analysis provided by NCBI, these extra domains in NX02 BLUF 1 and TET16 BLUF 1 show homology to B12-binding domains. This suggests the possibility of an additional photosensor, such as CarH, which often plays a role in controlling carotenoid biosynthesis in various organisms.[32] Hence, it is conceivable that this dual phototrophic strain set may possess an extra layer of photoreceptor complexity, enabling them to fine-tune their responses to light in the context of carotenoid biosynthesis regulation. Furthermore, the NX02 strains exhibit notable variations in their N-terminal regions when compared to each other. This divergence in their N-terminal regions hints at potential adaptations or specialized functions within this particular group of organisms, and it warrants further investigation to unveil the intricacies of their light-sensing capabilities and how these variations may impact their overall responses to environmental stimuli.

Phytochromes - red light sensing

The phytochromes marked in Table 1 were collected with a threshold of 35% sequence identity with *Sphingomonas faeni* S3H21 phytochrome sequence. We restricted for an analysis where all bacteriophytochromes contained the conserved DIP-motive in the chromophore binding pocket.[33] Phytochromes are red – far-red light sensors. They have a bilin chromophore that undergoes cis–trans photoisomerization after absorbing light in the red or far-red region. Notably, the absorption spectra of the red absorbing state (Pr-state) and the far-red absorbing state (Pfr-state) vary exactly at the absorption wavelength of Chl *a* molecules with a very steep difference.[20] In canonical BphPs, the Pr-state is the resting state in darkness, and light activation drives the protein to the Pfr-state. Bathy type of BphPs, however, function conversely: in darkness the system is in Pfr state and far-red light activation drives the system to the Pr-state. A structure related genome

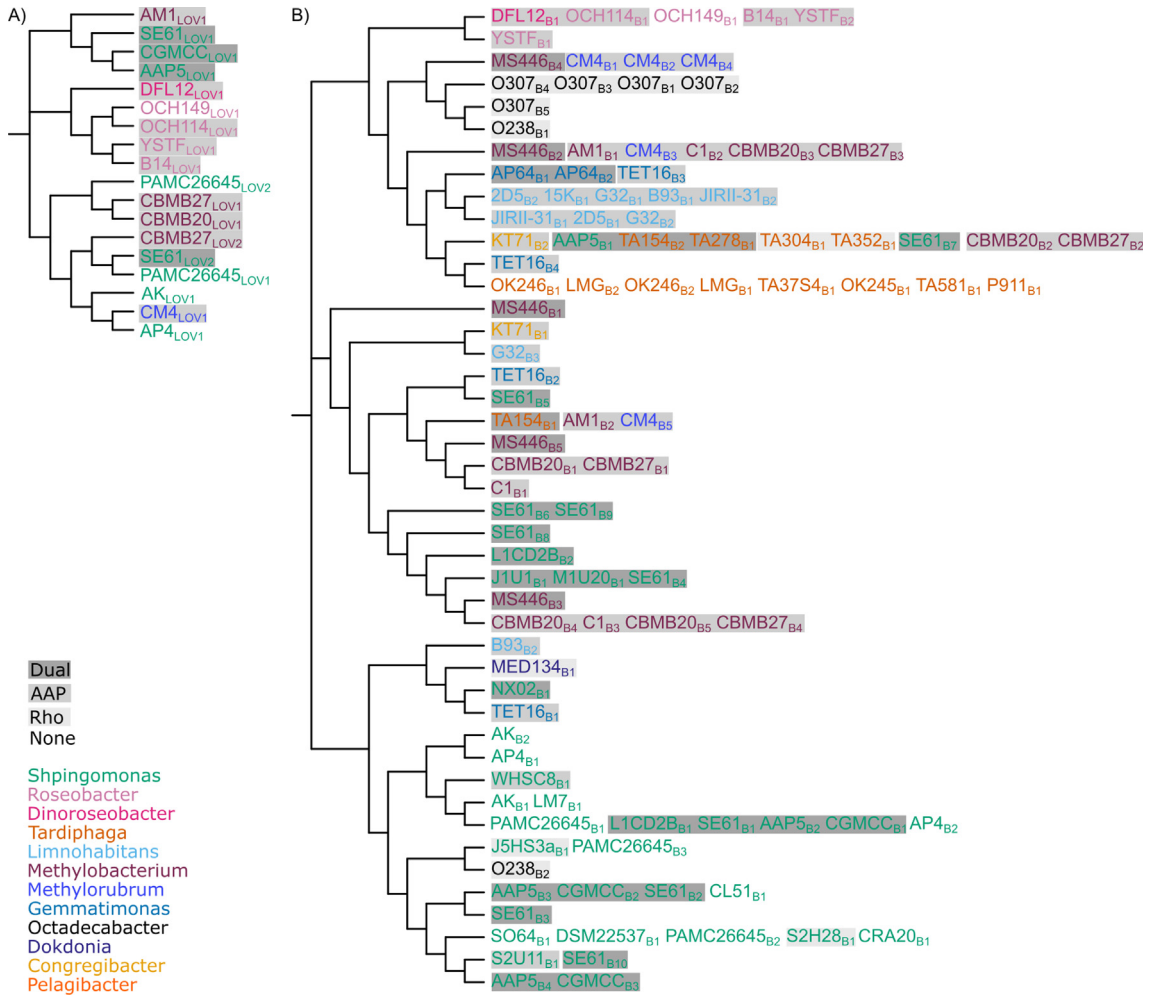

Fig. 4. Phylogenetic tree of LOV and BLUF domains The phylogenetic trees of LOV (A) and BLUF (B) are based on an alignment process, and the maximum likelihood trees show sequences with 85% or more sequence similarity in the same branch. The color coding reflects the genus of the strains (See Table 1) and the dual, AAP and Rho classes are marked with shades of grey. Branch lengths are not shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

comparison study by Velaquez-Escobar et al. suggested that RxxPRxSF motif in the so-called tongue region[34] and HExT motif indicates a histidine kinase activity of protein.[16] Following this guideline, the Figs. 5 and S11-15 and indicate that about two-thirds of dual phototrophic systems' BphPs may function as bathy BphPs. Observation of the phylogenetic tree indicates the lower half is completely bathy BphPs except for PAMC26645 BphP 2 and S2H28 BphP 2. Bathy BphPs can also be found on the second and sixth rows of the first branch. We note however, that the final decision of whether the particular strain is bathy or canonical needs to be characterized spectroscopically with isolated proteins, see for example in.[35] The HK proteins are typically divided into five subtypes (families), HisKA, HisKA_2, HWE_HK, HisKA_3, His_Kinase.[36] Typically BphPs are HisKA sensors.[36] The first branch (TA154 BphP 1 – CBMB27 BphP 2) are HisKA-family. The second branch (TA154 BphP 3 – PAMC26645 BphP 1) are HWE_HK family together with HATPase family. The third branch (TA278 BphP 2-SE61 BphP 1) are HWE_HK family. In the case of PAS-output domain containing strains the homologous superfamily comparison gives the PAS domains to be homologous with the PYP sensor domain, as observed with previous studies of BphP systems.[37,24] A tandem PYP-BphP construct has been linked to photosystem regulation,[38] and the PYP has been shown to accelerate the Pr-recovery once illuminated with blue light.[39] However, only MS446 BphP 1 includes the PYP-like domain inside of its PAS-domain. The function in these dual phototrops requires further investigation. Another detail about domain construction of the BphP sequences is that the AP4 BphP 2 and TA352 BphP 3 are actually PAS-less BphPs. All these observations brings attention to the rich variety of BphPs types in phototrophs.

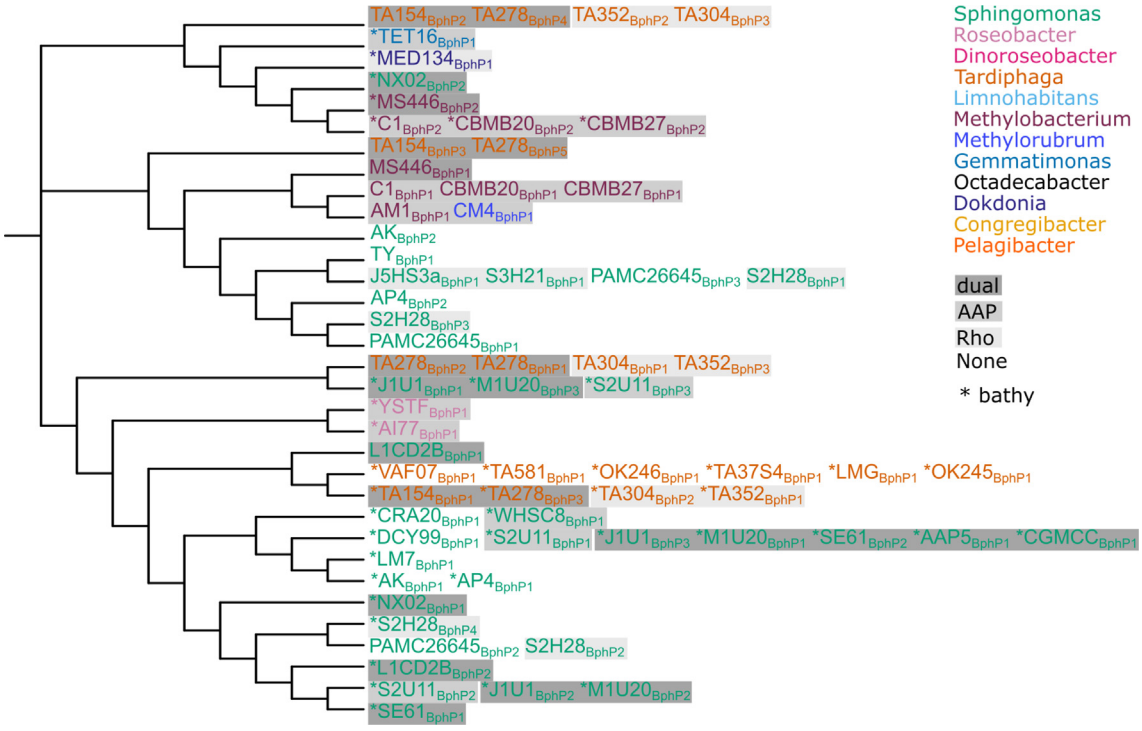

Fig. 5. Phylogenetic tree of BphP complexes. The phylogenetic tree is based on an alignment process, and the maximum likelihood trees show sequences with 85% or more sequence similarity in the same branch. The color coding reflects the genus of the strains (See Table 1) and the dual, AAP and Rho classes are marked with shades of grey. Based on the sequences, potential bathy BphPs are marked with an asterisk. Branch lengths are not shown.

Discussion

Dual phototrophs are a peculiar group of microbes which utilize a wide spectral range of photons for energy production either using proton pumping xanthorhodopsins or AAP (See Fig. 1). Intriguingly, these dual systems have also been detected in plant associated bacteria[8] some of which have very tight host association.[22] The putative role of dual phototrophy in arctic plant–microbe interactions warrants more research.

Our present analysis usually finds large subsets of photosensors in dual phototrophs covering a spectral range from UV to NIR region. It indicates more sophisticated and detailed light regulation in dual phototrophs compared to single phototrophs. Single phototrophs do show considerably larger light sensing capacity than with those phylogenetically similar bacteria without phototrophy capability.

For LOV, BLUF, CRY/PHR, and BphPs, multiple genes for the same photosensor type could be found from the studied strains. This agrees with the previous genomic studies.[19,17,40] Different effector domains or genomically nearby repressor proteins are often linked to the same photosensory protein. This became evident in our analysis where either HWE_HK, HisKA, or PAS output domains could exist in BphP from the same species (Fig. 5). In some cases a photosensor can act in a spectroscopically complementary manner, like in the case of Agp1 and Agp2 which function as canoncial and bathy BphP, respectively, in *Agrobacterium tumefaciens*.[41,42] However, in our study the majority of the dual phototrophic BphPs sequences seem to have bathy type of spectroscopic character. This, however, still needs to be proven experimentally.

It will be challenging to find the light conditions, and therefore photosensors, directly responsible for controlling either xanthorhodopsin or AAP biosynthesis pathways. Thus far, most of the AAP biosynthesis has been reported to take place in darkness.[4,43] However, Giraud et al. demonstrated that the wavelength dependence of BChl *a* production relates to Pfr spectum of BphPs, indicating that BphP is involved in the control of the photosystem production in anaerobic anoxygenic bacteria.[44] Further, Jaubert et al. reported a BTAi1P3 bacteriophytochrome was located in the photosynthesis cluster[45] and together its PpsR repression protein for LH4 production strongly suggests that the BTAi1P3 protein controls the photosynthesis cluster production. Thus, the location of the genome provides indications for the function of the photosensor. In our analysis, we found only two cases wherein BphPs were indeed discovered within the confines of the photosynthetic gene clusters (TA154 BphP 3 and TA278 BphP 5). Then, any

other photosensors, were not identified within the photosynthetic gene cluster or in close proximity to photosynthesis repression factors, specifically PpsR. This suggests a more divergent regulation mechanisms for the metabolism of the phototrophic systems. Indeed, recent studies have revealed general stress response transcription due to action of LOV HWE kinases in *Erythrobacter litoralis*,[46] or wide changes in gene regulatory patterns during light–dark-growth cycles were observed prior to rather minor and a delayed activation of photosynthesis gene expression for *D. shibae*.[43]

Dual phototrophs are found mainly in environments with harsh and cold conditions and strong seasonal changes in irradiance and temperature. So far, only Koblizek et al. have been able to report synergistic xanthorhodopsin and AAP production by tuning dark light-cycles and controlling temperature for Sphingomonas sp strain AAP5.[47] Lower temperatures but high light conditions favored xanthorhodopsin production, whereas low light conditions with a larger temperature range favored production of AAP. It therefore seems that in dual phototrophs, temperature sensing and light sensing should work in parallel. Studies on phytochromes acting as temperature sensors, either via their oligomerization capability or thermal reversion rates, are reported, mainly for plant phytochrome B system.[48,49] Temperature effects are also reported for prokaryotic systems.[50] It will be highly interesting to learn new microbial photosensors which can act as a thermal sensor in parallel with photosensing. This type of behavior may provide a link to bacteria - host interactions in harsh arctic environments. For this, collecting more dual phototrophic sequences from various types of environments and more detailed characterization of the photosensor is required.

Materials and methods

The strains J1U1, M1U20, and S2U11 were isolated from *Diapensia lapponica* endosphere, the strains S2H28, J5HS3a and S3H3 were isolated from *Oxyria digyna* endosphere, and the strain L1CD2B was isolated from *Bistorta vivipara* phyllosphere, according to the method described in Nissinen et al.[8,22] Briefly, phyllosphere bacteria were extracted by 3-min sonication of sterile-collected plant material in potassium phosphate buffer with surfactant. Endosphere bacteria were extracted subsequently by sterilizing the plant surface with 3 % sodium hypochlorite, triple water washing and maceration of the tissue in 20 mM KPi pH 6,5 buffer. The extracts were plated in a dilution series and grown on half-strength R2A medium with additional 0.7 % BD Bacto™ agar (Thermo Fisher Scientific, Waltham, MA, USA) adjusted to pH 6.5 with HCl, on 92 × 16 mm polystyrene Petri dishes (Sarstedt, Nümbrecht, Germany). Cells were grown for three days at room temperature after which they were placed in the fridge at +4 °C. For AAP and dual phototrophs, NIR-fluorescence signals of bacterial colonies could be detected after few weeks of incubation. Individual colonies were picked to produce pure cultures by transferring them to new plates. After incubation of about 10 the strains were transferred which were preserved in R2B, pH 6.5 with 30% glycerol at −80 °C.

Their genomic sequences were acquired using DNBseq at PR150 read length and 2 GB per sample at BGI (bgi.com). After sequencing, the reads were filtered by removing adaptor sequences, contamination and low-quality reads. Quality filtered and trimmed reads were assembled (18–200 contigs/genome) using Unicycler v0.5.0, and uploaded to the RAST Annotation Server[51] which was then utilized to search genes for reaction center (pufM), light harvesting, xanthorhodopsin, and individual photosensor. In parallel, NCBI Prokaryotic Genome Annotation Pipeline (PGAP)[52] was utilized for annotation. Additional genomes of previously documented strains were retrieved from the NCBI genome database and subjected to the aforementioned annotation processes. PGAP and RAST annotations for photosensors were supplemented with BLAST-P and a Biopython library function pairwise2.align.globalxx based searches with in–house python script on individual genomes.

BLUF, LOV and CRYPHR sequences from *Dinoroseobacter shibae*[43] strain DFL12 were compared with the sequences obtained from UniProt and used as a reference for other genomes in the study, with 35% identity threshold. Due to the absence of the BphP gene in DFL12 strain, the BphP sequence from *Sphingomonas faeni* S3H21 was employed as a point of reference, utilizing a 35% identity threshold for the purpose of BphP gene identification. Identified light sensor sequences were extracted and downloaded for alignments. In the aligment, the restricting conserved sequences for verification were GW-R-E, GGR, LLD, WFR, and EAL for CRY/PHR and NCRFLQ for LOV sequences, and TG and RH for BLUF sequences. For BphPs, DRV, DIP and CHH sequences were used. The protein classification was performed with NCBI's Conserved Domains and Protein Classification of protein families-database.[53] Maximum likelihood trees were constructed using MEGA v 11 and graphically adjusted using iTOL webserver.[54,55] Sequences with 85% or more sequence similarity were placed in the same branch by using an in–house Python script. The Python scripts used in the study are available per request.

DATA AVAILABILITY

Data will be made available on request.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jmb.2023.168412.

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† Current address: National Physical Laboratory, Hampton Road, Teddington, Middlesex TW11 0LW, UK.

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