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Bacteriophytochrome controls carotenoid-independent response to photodynamic stress in a non-photosynthetic rhizobacterium, *Azospirillum brasilense* Sp7

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Ever since the discovery of the role of bacteriophytochrome (BphP) in inducing carotenoid synthesis in *Deinococcus radiodurans* in response to light the role of BphPs in other non-photosynthetic bacteria is not clear yet. *Azospirillum brasilense*, a non-photosynthetic rhizobacterium, harbours a pair of BphPs out of which AbBphP1 is a homolog of AtBphP1 of *Agrobacterium tumefaciens*. By overexpression, purification, biochemical and spectral characterization we have shown that AbBphP1 is a photochromic bacteriophytochrome. Phenotypic study of the ΔAbBphP1 mutant showed that it is required for the survival of *A. brasilense* on minimal medium under red light. The mutant also showed reduced chemotaxis towards dicarboxylates and increased sensitivity to the photooxidative stress. Unlike *D. radiodurans*, AbBphP1 was not involved in controlling carotenoid synthesis. Proteome analysis of the ΔAbBphP1 indicated that AbBphP1 is involved in inducing a cellular response that enables *A. brasilense* in regenerating proteins that might be damaged due to photodynamic stress.

Light has been the driving force of life on the planet earth, but it is also a threat to the survival of aerobic organisms due to its photodynamic effect[1]. Incidental formation of singlet oxygen ($^1O_2$), a highly reactive oxygen species (ROS), occurs in photosynthetic as well as non-photosynthetic cells by the simultaneous presence of light, oxygen and photosensitizers, which causes photooxidative stress to the cells[2]. The major light-dependent sources of singlet oxygen in a cell include energy transfer to molecular oxygen (O_2) from excited triplet state bacteriochlorophyll a (^{3}Bchl a*) in photosynthetic membranes, or excited endogenous- (e.g. porphyrins or other tetrapyrroles) or exogenous- (e.g. methylene blue, toluidine blue) photosensitizers[3]. Since light can severely harm living cells, it is important for the organisms to sense and appropriately respond to the light signals[4]. In both, photosynthetic and non-photosynthetic organisms, carotenoids protect cells from the photooxidative damage by scavenging the singlet molecular species of oxygen produced upon illumination[5].

Phytochromes are a widespread family of red/far-red responsive photoreceptors, which sense the quantity and quality of light in the photosynthetic and non-photosynthetic organisms, and transduce the physical signal into the biochemical message for responding to the ambient light conditions[6,7]. They play important role in adaptive processes in higher plants including seed germination, de-etiolation, neighbor preception and shade avoidance, and the transition from vegetative to reproductive growth (induction of flowering). Phytochromes exist in two spectral forms: red light absorbing form (Pr) and far-red absorbing form (Pfr). The Pr form phototransforms into the Pfr after absorbing red light. Pfr is again converted back to Pr state after absorbing far-red light[8]. The ratio of these two spectral forms determines the signaling state of the phytochrome.

A typical (R/FR) phytochrome consists of a conserved N-terminal PAS-GAF-PHY tridomain photosensory core, which is combined with a C-terminal catalytic domain with a histidine kinase (HK) or histidine kinase-related domain (HKRD)[9]. Phytochromes from different organisms have characteristic variations of this

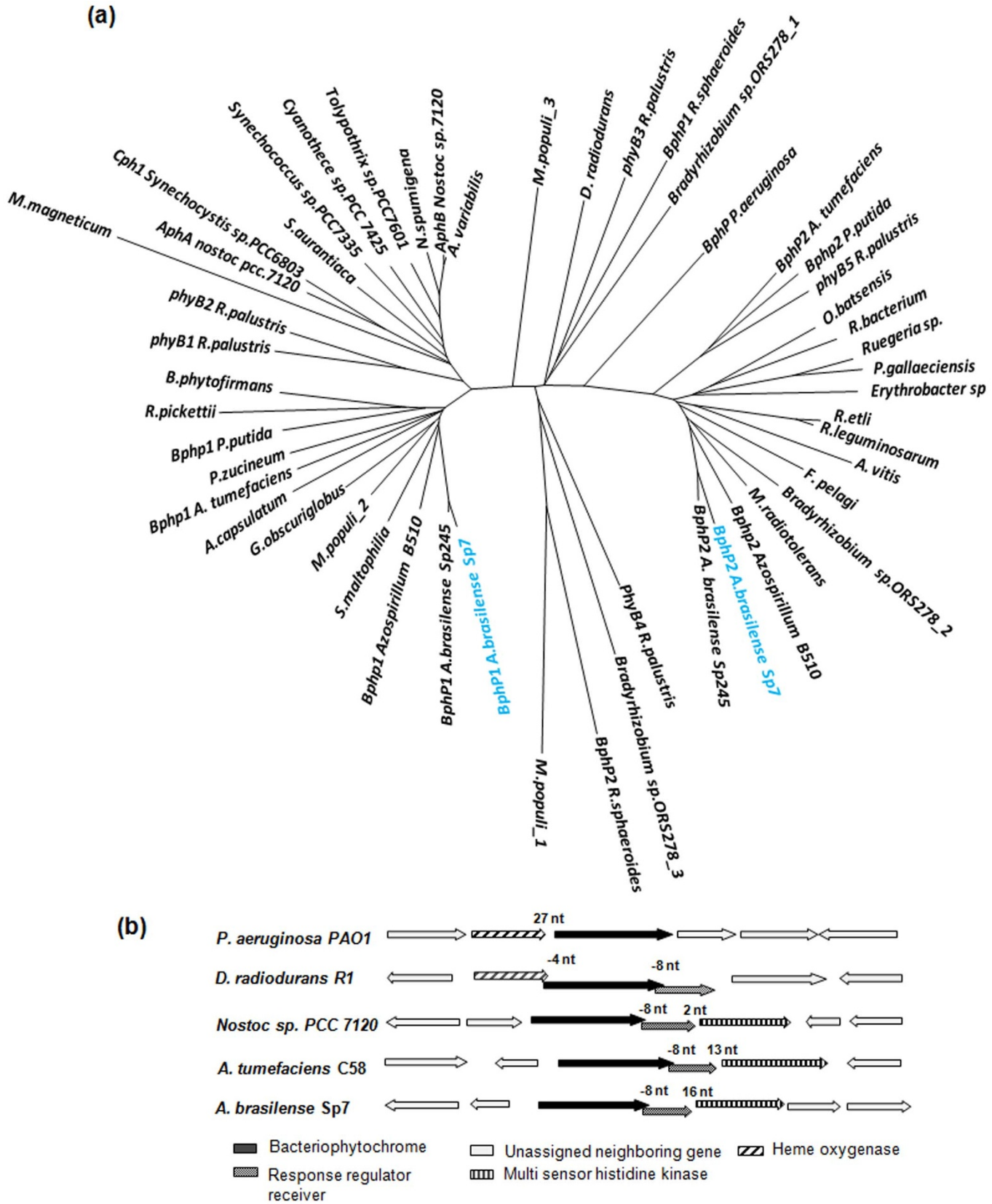

Figure 1 | (a) Phylogeny of bacteriophytochromes from different bacteria based on amino acid sequences retrieved from NCBI (accession numbers are provided in supplementary file). Neighbor-joining tree was built in MEGA version 5.04 with a 1000 bootstrap replications and Poisson model. The two bacteriophytochromes of *A. brasilense* Sp7 are marked in light blue. (b) Organization of genes around bacteriophytochrome (BphP) encoding gene in *A. brasilense* Sp7 and other bacteria. Direction of arrow indicates the orientation of genes. The nucleotide (nt) shows the distance between overlapping (minus sign) and closely associated gene.

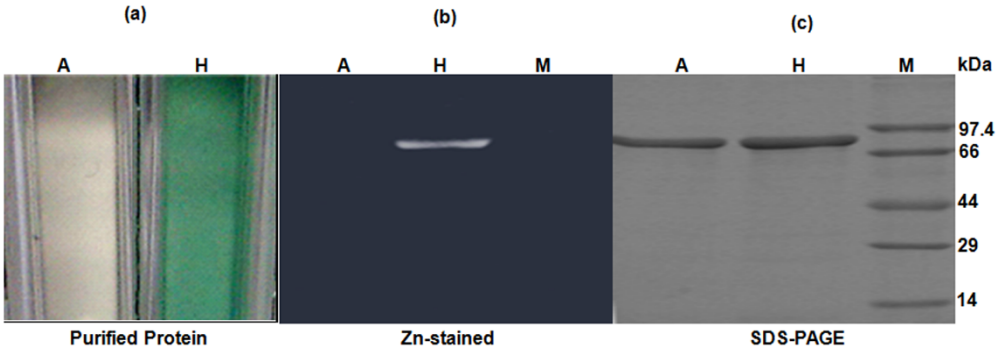

Figure 2 | Photochromic properties and zinc fluorescence of AbBphP1 apoprotein (A) and holoprotein (H). Visual appearance of purified apo- and holo-proteins (a), SDS-PAGE of apo- and holo-proteins (c) and fluorescence of apo- and holo-proteins in UV light after zinc staining (b).

architecture. Based on their distribution, photobiological properties and possible modes of action, phytochromes are divided into several families, which include plant phytochromes (Phys), cyanobacterial phytochromes (Cphs), bacteriophytochromes (BphPs), fungal phytochromes (Fphs) and unorthodox Phys, without apparent relationships[7,10]. The exact nature of the chromophore varies for different families of phytochromes: plant and cyanobacterial phytochromes covalently attach phytochromobilin (PΦB) and phycocyanobilin (PCB), respectively, to the Cys residues in the GAF domain, whereas BphPs and Fphs attach biliverdin IXα (BV) chromophores to a Cys residue in the PAS domain. The chromophore is autocatalytically assembled within the photosensory core, and the protein-chromphore interactions control the wavelength sensitivities of each phytochrome. Despite their spectral diversity, photoconversion between Pr and Pfr forms of each phytochrome is brought about by a Z-E isomerization about the C15=C16 double bond of the bilin chromophore.

Several bacteriophytochromes from photosynthetic and non-photosynthetic bacteria have been characterized biochemically, spectroscopically and structurally[11–17]. However, except for controlling the synthesis of photosynthetic machinery in photosynthetic bacteria[18,19], their role in the physiology of non-photosynthetic bacteria is not well known[20,21]. Elucidation of the role of bacteriophytochrome in carotenoid synthesis in *Deinococcus radiodurans* was the first example of the role of bacteriophytochrome in coupling the photodynamic stress with the induction of carotenoid synthesis in the photobiology of a non-photosynthetic bacterium[22]. Although bacteriophytochromes of *A. tumefaciens* are among the best characterized bacteriophytochromes, their biological roles are not known yet[6,21,23]. BphP of *P. aeruginosa* has also been characterized in detail, but its knock-out mutant did not reveal any phenotype[14]. However, transcriptomic and proteomic analyses indicated its possible involvement in the quorum sensing[14].

Azospirillum brasilense is a non-photosynthetic α-proteobacterium belonging to the family Rhodospirillaceae, which lives in the rhizosphere of a large number of non-legume crop plants and grasses, and promotes their growth by producing phytohormones[25]. The genome sequence of *A. brasilense* showed the presence of two genes encoding bacteriophytochromes. After the first report of the role of bacteriophytochromes in any non-photosynthetic bacteria in 1999[22], this is the second report describing a new role of bacteriophytochrome in another non-photosynthetic bacterium, *A. brasilense,* in coping with red light mediated photodynamic stress in dicarboxylate grown cultures by a mechanism that does not involve carotenoid biosynthesis.

Results

Organization of genes in bacteriophytochrome (BphP1) encoding operon in *A. brasilense* Sp7. Homology based search of *A. brasilense* genome database for phytochrome and a phylogenetic analysis of the prokaryotic phytochromes, including the representatives from cyanobacteria (Cphs) and photosynthetic as well as non-photosynthetic bacteria (BphPs), shows that *A. brasilense* genome harbours two putative genes encoding bacteriophytochromes (Fig. 1a), which were located in two different operons. Both of them were closely related to the two well characterized BphPs from the soil bacterium, *A. tumefaciens*; one showing homology to AtBphP1 (designated as AbBphP1), and the other to AtBphP2 (designated as AbBphP2). Organization of the gene encoding AbBphP1 showed synteny with *bphP* genes of *Nostoc* sp PCC7120 and *A. tumefaciens* in which *bphP* appears to be the first gene of a tricistronic operon followed by a gene encoding a putative response regulator (*bphR*), which overlapped with the *bphP* by 8 bp (Fig. 1b). The third gene encodes a putative hybrid multi-sensor histidine kinase (HK).

AbBphP1 is a photochromic bacteriophytochrome. To elucidate, if AbBphP1 is a photochromic bacteriophytochrome, the holoprotein was produced in *E. coli* by coexpressing a heme oxygenase along with AbBphP1 apoprotein. The apoprotein was also produced in *E. coli* cells lacking heme oxygenase expressing plasmid. Both, apo- and holo- proteins were expressed in soluble form, and purified using affinity- and gel filtration chromatography. The purified AbBphP1 apoprotein was colorless, whereas the holoprotein had a blue-green color indicative of bound BV and the consequent photochromicity (Fig. 2a). When protein extracts of *E. coli* expressing AbBphP1 holo- or apo-proteins were resolved in SDS-PAGE (Fig. 2c), stained with a Zn^{2+} solution, and exposed to UV light, Zinc-dependent fluorescence was observed (Fig. 2b) in the holoprotein, but not in the apoprotein, which indicated that BV was bound covalently in the AbBphP1 holoprotein, as observed in typical bacteriophytochromes.

In the dark (Pr state), holoprotein showed an absorbance maximum at 710 nm, which is a characteristic of Pr state of the bacteriophytochrome(s). Upon saturating illumination of 2 min with red light (λ_{max} = 695 nm), Pr state was phototransformed into Pfr state, which was evident by 50 % photobleaching, a red shift, and a new broad absorbance of holoprotein at 750 nm. Analysis of the difference (dark minus red light illuminated) spectra of AbBphP1 showed maximum difference at 710 and 750 nm (Fig. 3a). The changes induced by the red light were completely reversible upon incubation in the dark with recovery time of 40 min at room temperature (Fig. 3b). Photo-conversion of Pfr state to Pr state was accelerated with a recovery time of 25 min, when red-light irradiated sample was irradiated with far-red (750 nm) light (Fig. 3c).

AbBphP1 is a dimer. Propagation of signal from sensory module to the output module depends upon phytochrome dimerization[26]. Bacteriophytochrome from *D. radiodurans* dimerizes through PAS-GAF-PHY and HK domain[26]. Size exclusion chromatography (SEC) profile of the AbBphP1 also indicated dimerization of the holoprotein. Under native condition, the major fraction of the

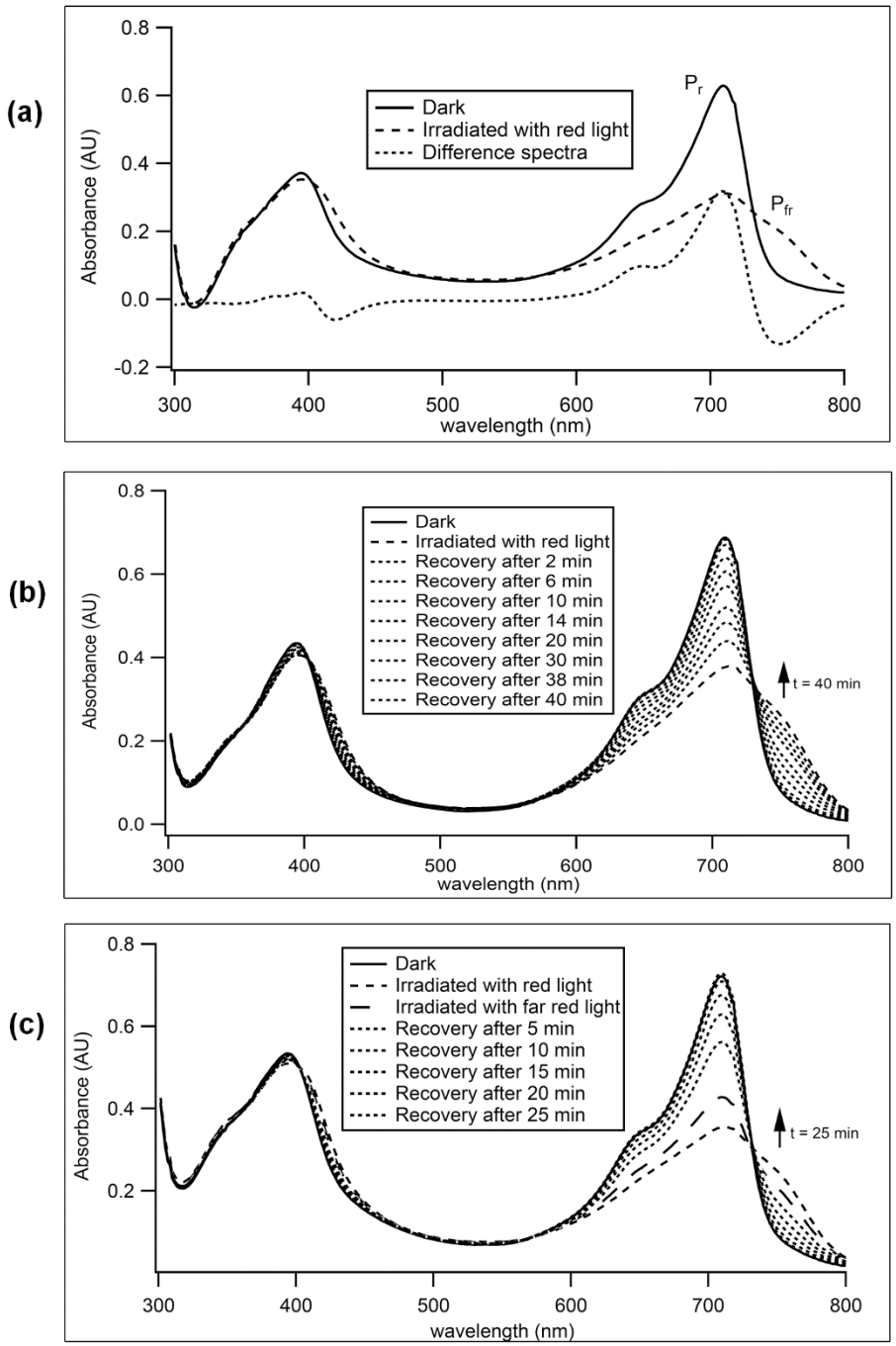

Figure 3 | Spectral characterization of recombinant AbBph1 protein. (a) UV-Visible spectra of holoprotein in the dark and after irradiation with red (695 nm) light. (b) Slow reversion to dark state of the recombinant holoprotein after irradiation with red light. (c) Fast reversion to dark state of the recombinant holoprotein after irradiation with red light followed by far-red light irradiation.

chromophore-bound AbBphP1 protein was eluted as dimer, whereas monomer and higher oligomer were eluted as minor fractions (Fig. 4a and b). Under native conditions, AbBphP1 had a size of 220 kDa, which was more than the dimer (170 kDa) of monomeric size (85 kDa). Larger than the predicted size of the phytochrome holoproteins have also been observed earlier due to the non-spherical shape of the holoprotein[27]. We observed a more prominent peak of the holoprotein monomers as compared to the apoprotein. This may be due to the limitation of SEC, as the interacting protein subunits, which are diluted during the separation, may dissociate during the analysis[28]. Quaternary structural characteristics of AbBphP1 were further confirmed by chemical cross-linking with glutaraldehyde, which also retained the protein as dimer. The presence of higher oligomeric species could be due to some degree of non-specific cross-linking (Fig. 4c). These experiments indicated that both apo- and holo- AbBphP1 monomers have a site each for dimerization of the photoreceptor in native condition. Modeling of AbBphP1 and protein-protein docking by Cluspro 2.0 also predicted it as a dimer, in which dimerization interface was made up of α helices and β sheets (Supplemental Fig. S1B). Some structural components of the chromophore binding pocket also seem to be involved in the dimerization site (Supplemental Fig.1C).

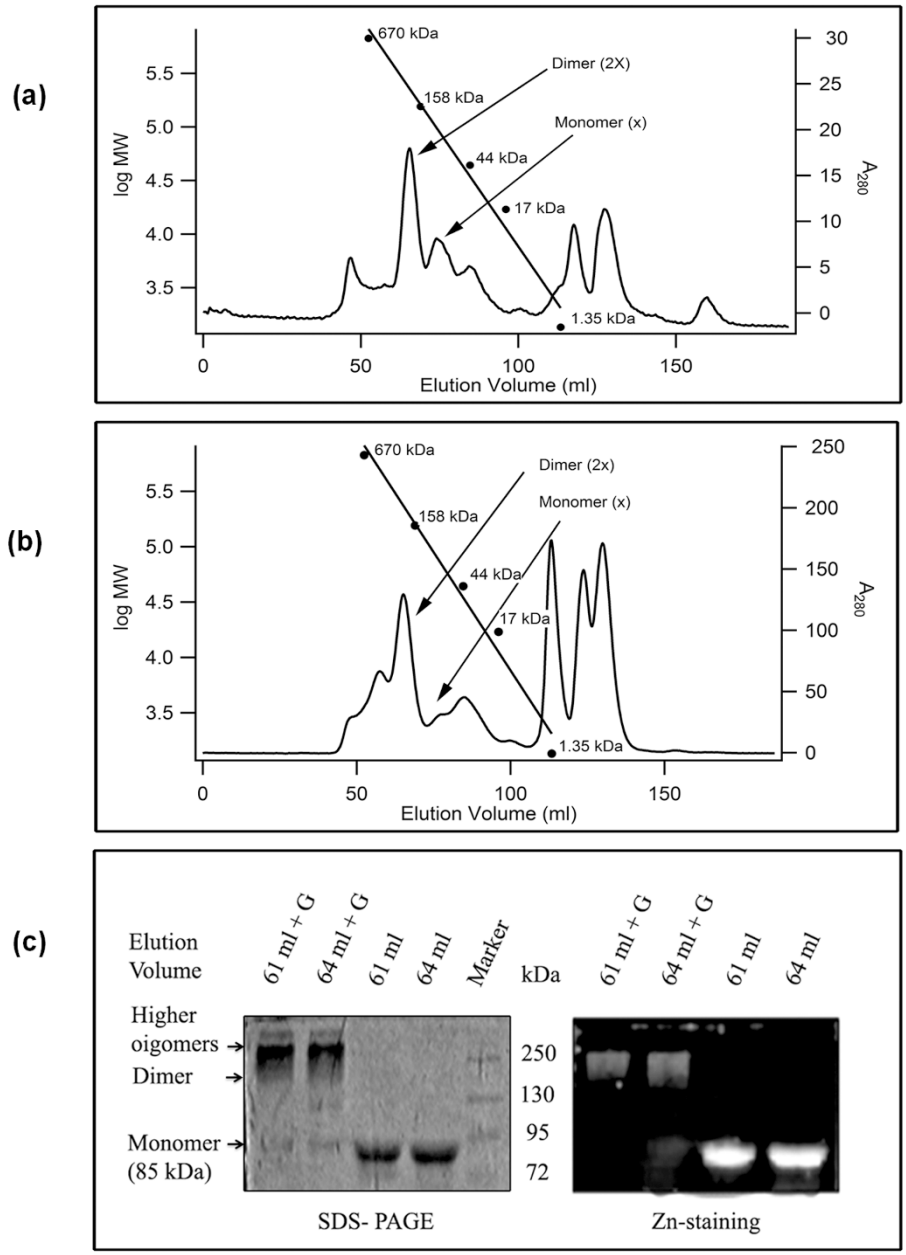

Figure 4 | Characterization of oligomeric states of AbBphP1 by size exclusion chromatography (SEC) of holoprotein (a) and apoprotein (b). FMN was used as a loading indicator, 120 ml -140 ml elution volume indicates FMN fraction. SDS-PAGE with Coomassie (left) and Zn (right) staining of glutaraldehyde (G) cross-linked SEC purified AbBphP1 holoprotein (c).

AbBphP1 is not involved in light-induced carotenoid synthesis. In order to understand the physiological role of AbBphP1 in *A. brasilense* Sp7, an in-frame deletion of the gene encoding AbBphP1 was constructed in *A. brasilense*. In view of the previous report that bacteriophytochrome is involved in red light-induced carotenoid synthesis in *D. radiodurans*, we first studied the role of AbBphP1 in carotenoid synthesis in *A. brasilense* Sp7. When *A. brasilense* Sp7 and ΔAbBphP1 mutant were grown in dark, red light or white light, the colonies grown in dark did not show pink color. In red light, both parent and ΔAbBphP1mutant showed very little pink color. However, in white light, there was maximum production of pink color both in parent and the mutant (Supplemental Fig S2). Absorption spectra of the methanolic extracts of the parent and ΔAbBphP1 mutant exposed to white light also showed that carotenoid content in the ΔAbBphP1 mutant was almost same as in the parent (Fig. 5).

AbBphP1 is required for optimal growth under red light in malate minimal medium. Both, *A. brasilense* Sp7 and ΔAbBphP1 mutant, grew equally well in the dark when their growth was compared in rich medium and in minimal medium (Fig. 6a). In white light also, both, wild type and the mutant, grew equally well, however, the mutant grew slower than the wild type in minimal malate medium. In red light, growth of the mutant was much slower than that of the wild type in minimal medium (Fig. 6a). But, in LB medium, both wild type and the mutant grew almost equally well in red light, indicating that ΔAbBphP1 mutant was sensitive to red light only in minimal malate medium. Although ΔAbBphP1 mutant displayed reduced chemotaxis towards malate in the minimal medium, this difference was observed both in dark as well as in red light. The ΔAbBphP1 mutant carrying a cloned copy of the AbBphP1 gene showed almost as good chemotaxis as the parent, indicating functional complementation (Fig. 6b).

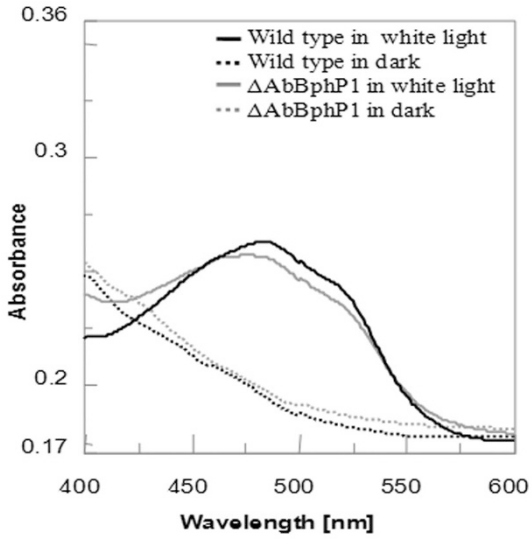

Figure 5 | Absorption spectra of methanolic extracts of carotenoids showing effect of darkness and white light on carotenoid content of *A. brasilense* Sp7 and ΔAbBphP1 mutant.

We also examined the effect of red light on the growth of *A. brasilense* Sp7 and its ΔAbBphP1 mutant, by epifluorescence microscopy, to understand if red light caused killing of ΔAbBphP1 mutant. When *A. brasilense* Sp7 and its ΔAbBphP1 mutant were grown in minimal medium in the dark, and the cells of the early stationary phase culture (at 15 h) stained with DAPI and propidium iodide, both showed blue color, indicating that all the cells were viable (Fig. 6c). When they were grown in white light, some (~ 5%) of the cells of ΔAbBphP1 showed yellowish fluorescence, indicating that the yellow fluorescing cells were dead, because propidium iodide can enter only in the dead cells. However, a large proportion of cells (~ 35 %) of the early stationary phase culture of ΔAbBphP1 grown in red light showed yellowish fluorescence (Fig. 6c). The wild type cells exposed to white light or red light did not show yellow fluorescing cells, indicating that they were not killed by red light in the minimal medium. The ΔAbBphP1(pSK5) strain, expressing cloned copy of AbBphP1 gene, was almost as resistant to killing by red light as wild type.

ΔAbBphP1 is sensitive to photodynamic stress by toluidine blue. Since red light is known to cause photodynamic stress, by evolving stressful levels of ROS[29], we tested the effect of different oxidative stress agents, viz paraquat (superoxide), hydrogen peroxide (peroxide) and toluidine blue plus light (singlet oxygen) on the growth of *A. brasilense* Sp7, ΔAbBphP1 mutant and ΔAbBphP1(pSK5). The zone of inhibition observed with toluidine blue plus light in the ΔAbBphP1 mutant was considerably larger than that observed in *A. brasilense* Sp7 or ΔAbBphP1(pSK5) (Fig. 7; Supplemental Fig. S3). But, the zones of inhibition observed with hydrogen peroxide and paraquat were similar in all the three strains (not shown), indicating that AbBphP1 plays major role in tolerating stress generated by singlet oxygen, but not the stresses caused by peroxide or superoxide.

Identification of differentially expressed proteins in *A. brasilense* Sp7 and ΔAbBphP1 mutant in response to red light. The comparison of the proteomes of *A. brasilense* and ΔAbBphP1 mutant grown in red light or dark, revealed at least 9 proteins, which were upregulated in *A. brasilense* in response to red light, but not in ΔAbBphP1 (Supplemental Fig. S4). The proteins, whose intensity varied more than 1.5 fold in the 2D gel, were identified by MALDI-TOF/TOF. A list of the proteins that appeared to be red light regulated in three out of three independent experiments is given in Table 1. The notable proteins upregulated in response to red light included DnaK, GroEL, AtpD, TufB, RpsA and OmaA.

Discussion

Although the genes encoding bacteriophytochromes are represented in the genomes of a large number of non-photosynthetic bacteria, the knowledge about their role is rather limited[14,17,22]. When we analyzed the genome of the non-photosynthetic rhizobacterium, *A. brasilense,* we found two ORFs encoding bacteriophytochromes, showing homology to a pair of BphPs found in *A. tumefaciens*. Bioinformatic analysis indicated, that like other phytochromes, AbBphP1 consists of an N-terminal chromophore binding domain (CBD), consisting of PAS, GAF and PHY sub-domains, and a C-terminal effector domain that includes HisKA and HATPase domains. The organization of the genes flanking *bphP* was also similar to that found in *A. tumefaciens* and *Nostoc* PCC7120, in which the gene encoding heme oxygense (BphO) is not in the vicinity of *bphP*[10]. In *A. brasilense* Sp7, *bphP1* is the first gene of a tricistronic operon, a response regulator and a multi sensor HK, being the second and the third genes, respectively. Thus, it is likely that all the three genes coordinate in mounting a response to light.

By cloning and overexpressing the AbBphP1 in *E. coli* BL21 DE3, expressing a heme oxygenase, we produced the typical blue colored recombinant holoprotein, which was purified and visualized by zinc induced fluorescence, and further confirmed by immunoblotting using 6XHis antibody (data not shown). Recombinant AbBphP1 kept in the dark, showed absorbance maximum at 710 nm, which is similar to that of *R. sphaeroides*[30]. Absorbance maximum of bacteriophytochrome at 710 nm has been attributed to the Pr state of these photoreceptors. Illumination of the dark-adapted AbBphP1 with red light showed spectral shift, suggesting photoconversion of Pr (710 nm) state to Pfr (750 nm) state. The spectral changes induced by the red light were completely reversible upon incubation in the dark. Illumination with far red light, however, resulted in very little photoconversion from Pfr state to Pr state. Size exclusion chromatography and protein cross-linking experiments suggest that like other bacteriophytochromes quarternary structure of AbBphP1 is also dimeric[26]. These characterizations indicated that AbBphP1 was a photochromic bacteriophytochrome in *A. brasilense* Sp7.

Although spectral properties and reversion kinetics of AbBphP1 were comparable to that of an unorthodox bacteriophytochrome BphG1 of *R. sphaeroides*[30], the BphG1 of *R. sphaeroides* has GGDEF and EAL as output domains, but AbBphP1, like most other phytochromes, possesses a histidine kinase as its output domain. Further, the gene encoding AbBphP1 is the first gene of a tricistronic operon, the gene encoding BphG1 seems to be bicistronic, as it shows an overlap of 79 nucleotides with BphO gene located upstream. Both, *R. sphaeroides* and *A. brasilense*, are closely related members of α-proteobacteria, the former is photosynthetic whereas the latter is non-photosynthetic.

In order to understand the biological role of AbBphP1 in *A. brasilense,* we constructed a mutant, in which the wild copy of the *bphP*1 was replaced by a mutant copy, having an in-frame deletion of 2229 bp in *bphP*1. Both, *A. brasilense* Sp7 and ΔAbBphP1 mutant produced almost equal amount of carotenoids in light, but failed to do so under dark. This indicated that although light induces carotenoid synthesis in *A. brasilense,* AbBphP1 is not involved in controlling carotenoid synthesis. This situation in *A. brasilense* is in contrast to *D. radiodurans*, where bacteriophytochrome is known to control carotenoid synthesis; it accumulated higher amounts of carotenoids under white/red light, whereas the synthesis of carotenoids was severely inhibited in the BphP1 mutant. The bacteriophytochrome found in *D. radiodurans* thus helps in protecting the bacterium from the adverse effects of intense visible light by controlling the synthesis of the deinoxanthin carotenoid[22,31]. Although AtBphP1 and AtBphP2 of *A. tumefaciens* are one of the most thoroughly

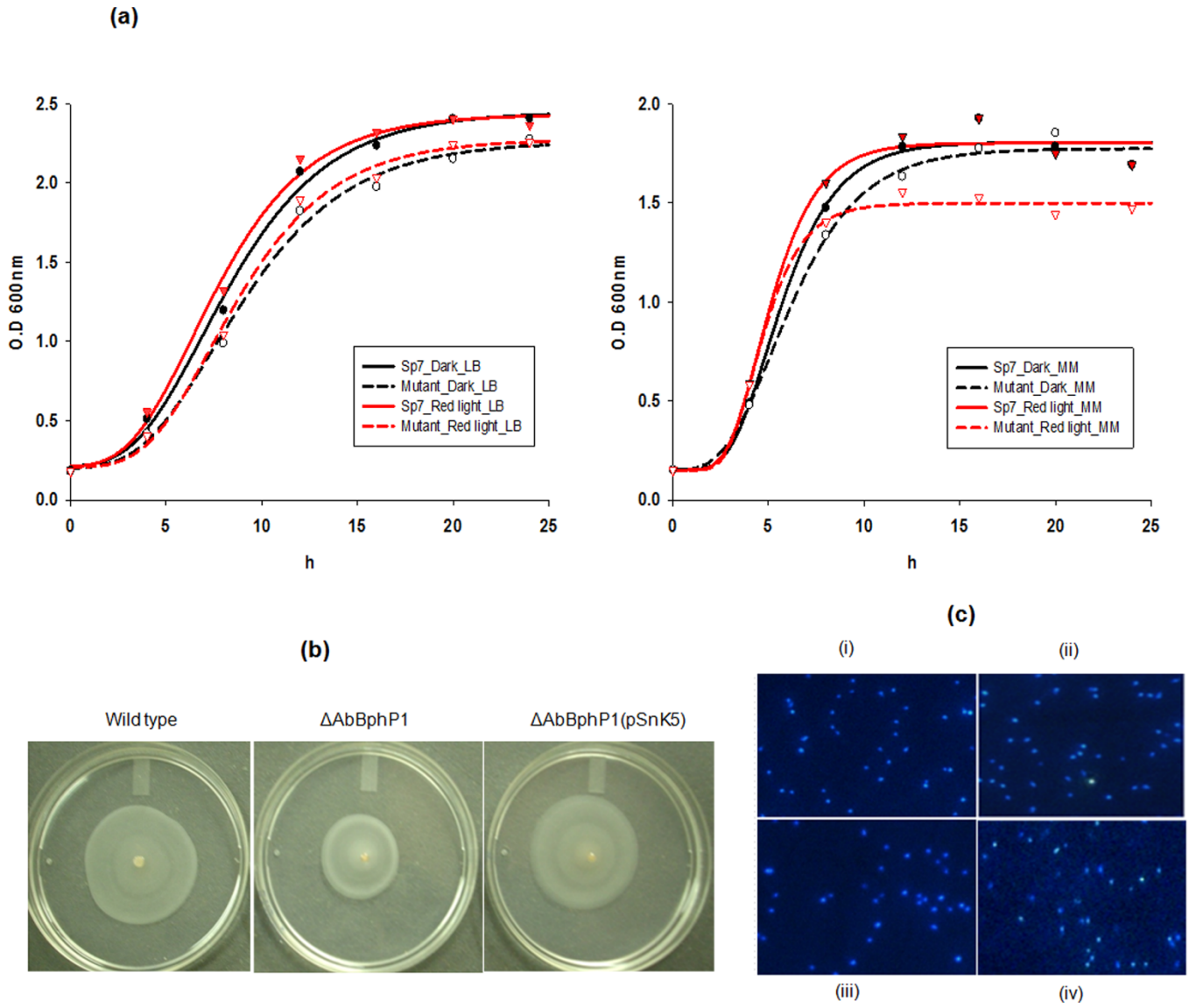

Figure 6 | (a) Growth curve of *A. brasilense* Sp7 and ΔAbBphP1 mutant in the dark and in red light in LB medium and minimal medium with 38 mM malate. (b) Swarm plates showing chemotaxis of *A. brasilense* Sp7, ΔAbBphP1 and ΔAbBphP1 (pSnK5) towards 10 mM malate in red light. (c) Epifluorescence microscopy images of *A. brasilense* Sp7 grown in dark (i) and red light (ii); ΔAbBphP1 mutant gown in dark (iii) and red light (iv) after staining with DAPI and propidium iodide as described in supplemental material.

investigated bacteriophytochromes[21,23,24], their biological role is not known yet.

Phenotypic characterization of the ΔAbBphP1 mutant showed that its growth was sensitive to red light, when it was grown on minimal medium containing malate as carbon source. The reduced growth of ΔAbBphP1 was due to the lethal effects of red light. This indicated an involvement of AbBphP1 in coping with the photodynamic stress, probably by sensing the light signal, and transducing it through response regulator to the downstream regulatory cascade to induce the proteins, which might be involved in responding to photodynamic stress, created by the red light. Why red light is more lethal to ΔAbBphP1 in minimal medium than in the rich LB medium, needs to be investigated further. We also observed that ΔAbBphP1 showed reduced swarming ability as compared to its parent. This difference in chemotaxis was observed in dark as well as under white or red light. This observation is in contrast to that found in *A. tumefaciens* where the swarming motility of ΔBphP1mutant on minimal medium did not differ from its parent under any light condition[21].

Our observation, that ΔAbBphP1 mutant was more sensitive to toluidene blue plus light than its parent, also indicated that AbBphP1 might be important in coping with the stress generated by ROS including $^1O_2$, which damages a large variety of biological molecules such as DNA, proteins and lipids[32]. Toluidene blue is a well known photosensitizer, which shows maximum phototoxicity to the Gram negative bacteria upon red light irradiation[33,34]. After the absorption of light, part of the energy is transferred to the triplet state, and then to the molecular oxygen to generate ROS. In this photodynamic action, singlet oxygen ($^1O_2$), the most reactive ROS, causes damage to the bacterial cell wall, membranes, proteins and nucleic acids[35,36]. The photooxidative stress in the cell induces pathways for the repair of DNA, and refolding of misfolded proteins[2,37]. The correct conformation of the misfolded proteins, which accumulate during heat shock in *E. coli*, is restored by the chaperones like GroEL, DnaK, DnaJ and GrpE[38,39].

Comparison of the effect of red light on the proteome of *A. brasilense* Sp7 revealed 3 fold, and 8 fold increase in the levels of DnaK (Hsp70) and GroEL (Hsp60) proteins, respectively, as compared to

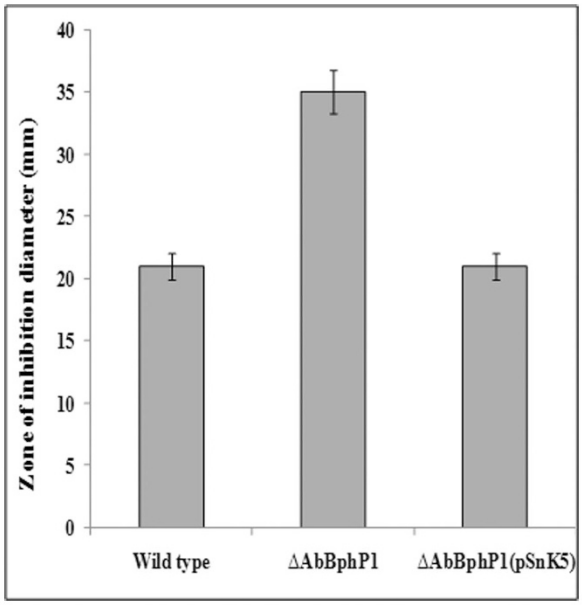

Figure 7 | Sensitivity of *A. brasilense* Sp7, ΔAbBphP1 and ΔAbBphP1 (pSnK5) to 5 mM toluidine blue plus light shown by zone of inhibition diameter on 1.5 % LB agar. Each bar represents the mean diameter of the zone of inhibition recorded in three independent assays performed in triplicate. Error bars indicate SD.

the dark. Such a response was not seen in the ΔAbBphP1 mutant. This indicated that these proteins might be involved in refolding of the misfolded, unfolded or aggregated proteins inside the cell, due to the photodynamic damage caused by the red light. Chaperonins, like DnaK and GroEL, which possess N-terminal ATPase domain and a C-terminal substrate binding domain, work in association with other co-chaperones in an ATP dependent manner[40]. The enhanced expression of the beta chain of F-type, H^+ transporting ATPase, observed in the wild type *A. brasilense* (but not in ΔAbBphP1 mutant) cells exposed to red light, indicates an increased demand for ATP to facilitate protein refolding by such HSPs[41]. Induction of GroEL and DnaK was also reported in *E. coli* in response to photodynamic therapy-mediated oxidative stress[37]. Accumulation of these HSPs along with other stress-associated proteins was also observed when *Synechocystis* and *Prochlorococcus*[42] were exposed to high light. In *R. sphaeroides* too, expression of the three alternative sigma factors *rpoE, rpoH$_{II}$* as well as *rpoH$_I$* is upregulated in response to photooxidative stress[43]. The observation, that several of the genes responding to $^1O_2$ are controlled by $RpoH_{II}$ as well as $RpoH_I$, indicated that the responses to $^1O_2$ and heat partially overlap in *R. sphaeroides*[44].

The discovery of photoreceptor proteins in non-photosynthetic bacteria, and their ability to sense and respond to light have opened many as yet unexplored issues in the photobiology of bacteria. Since blue and, to a lesser extent, red light can cause photooxidative stress in aerobic bacteria, it is useful for non-photosynthetic bacteria to harbour a light sensing system, which can enable them to activate an appropriate defense response to prepare them for damage by light[29]. Although the effect of red light on the physiology of photosynthetic prokaryotes is well known, there is very little information about the red light effects in non-photosynthetic bacteria. Unlike *D. radiodurans,* where bacteriophytochrome is involved in coping with photooxidative stress by inducing carotenoid synthesis, this is the first report showing the role of bacteriophytochrome in *A. brasilense* in tolerating the red light mediated photodynamic stress, without inducing carotenoids.

Methods

Bacterial strains, plasmids, chemicals and growth conditions. Bacterial strains and plasmids used in this study are listed in Supplemental Table S1. Plasmid pT7-HO1-1, expressing heme oxygenase gene *ho*1, was maintained in *E. coli* strains grown in Luria Bertani (LB) medium with kanamycin (40 μg/ml). Plasmid pET15b expressing AbBphP1 was maintained in *E. coli* DH5α and in *E. coli* BL21, and grown in LB medium having ampicillin (100 μg/ml). *A. brasilense* Sp7 was grown in minimal malate (MM) medium or LB broth, and maintained on MM Agar plates[45,46]. *E. coli* and *A. brasilense* Sp7 cultures were grown at 37°C and 30°C, respectively.

Bioinformatic tools/softwares are described in the Supplemental Material.

RT-PCR analysis of co-transcription of AbBphP1 gene with downstream genes. RNA was isolated from the cultures of *A. brasilense* Sp7 as described earlier[47]. The cDNA was prepared using RNaseH minus MMLV reverse transcriptase and random hexamers primer. PCR amplifications were performed using primers from the BphP intragenic region and 3′ end RR gene (RT1-RT2) in one set, and from BphP intragenic region and sequences at the 5′ end of the gene encoding multisensor histidine kinase (RT1-RT3) in the other set. PCR reactions were carried out as recommended by manufacturer (NEB). Primers used in this study are listed in Supplemental Table S2.

Cloning, over expression and purification of AbBphP1. The coding region for AbBphP1 was PCR-amplified from *A. brasilense* strain Sp7 by using primers designed to introduce *Nde*I and *Bam*HI sites before the start- and stop codons of AbBphP1, respectively. The *Nde*I-*Bam*HI digested PCR product was cloned into similarly digested pET15b (Novagen), resulting in the addition of a 6xHis-tag at the N-terminus. *E. coli* DH5α was used as host for cloning whereas *E. coli* BL21 (DE3) pLysS was used for protein expression. The pET15b clone having a gene encoding AbBphP1 was transformed into *E. coli* BL21 (DE3λ) harbouring the plasmid pT7-*ho*1-1, which expresses the heme oxygenase gene *ho1* of *Synechocystis* sp.[48]. It was expected that the

Table 1 | Identification of differentially expressed proteins in response to red light in *A. brasilense* and ΔAbBphP1 mutant by MALDI-TOF/TOF

Spot No	Protein	Matched Accession #	Function	Fold change	PI	M.W.	Mascot Score
Proteins upregulated by red light in *A. brasilense* Sp7							
S1	DnaK	gi|302381499	chaperon DnaK/Hsp70	3.0	4.77	67718	67
S2	RpsA	gi|288957134	small subunit ribosomal protein S1	2.0	5.13	62367	293
S3	GroEL	gi|288957187	chaperon GroEL /Hsp60	8.14	4.96	57702	212
S4	AcoB	gi|209963467	pyruvate dehydrogenase subunit beta	2.2	4.84	49523	166
S5	AtpD	gi|288959333	F-type H+ transporting ATPase beta chain	2.0	4.93	50036	527
S6	OmaA	gi|10798859	major outer membrane protein OmaA	2.5	4.78	40901	93
S7	TufB	gi|288957406	elongation factor EF-Tu	1.5	5.36	43169	139
S8	RfaG	gi|46241709	glycosyl transferase-like protein	3.0	6.11	153539	57
S9	LivK	gi|288962866	Branched chain amino acid transport system substrate-binding protein	1.75	6.47	40510	202
Proteins upregulated by red light in ΔAbBphP1							
S10	FusA	gi|288958721	Translation elongation factor EF-G	4.0	5.07	79305	123
S11	NusA	gi|17988250	Transcription elongation factor NusA	3.7	4.53	59658	45
S12	RplB	gi|23014085	Ribosomal protein L2	1.5	10.9	30319	106

heme oxygenase synthesized by pT7-*ho*1-1 would convert intracellular heme into biliverdin, which will covalently bind to the apoprotein to produce the AbBphP1 holoprotein. The transformants were selected on LB-agar plates containing appropriate antibiotics (50 μg/mL kanamycin or 100 μg/mL ampicillin).

A primary culture was inoculated in LB medium supplemented with appropriate antibiotics (50 μg/mL kanamycin or 100 μg/mL ampicillin). The secondary culture was prepared by inoculating an overnight grown primary culture into the terrific broth (TB), and growing at 37°C with shaking at 200 rpm until $O.D_{600}$ reached a value of about 0.6. At this point, the secondary culture was incubated on ice for 30 min, and expression was induced by adding 0.3 mM IPTG. The secondary culture was grown for another 48 h at 16°C. Cells were harvested, re-suspended in 1xPBS, and lysed by sonication. Lysed cells were centrifuged at 16000 g for 55 min to obtain soluble fractions containing protein of interest. Soluble fraction was filtered with 0.45 μm filter and purified with chelating sepharose fast flow beads with immobilized Co^{++} metal ion by immobilized metal affinity chromatography (IMAC), as per manufacturer's instructions (GE Healthcare). Further purification was achieved by size exclusion chromatography (SEC), using Akta Explorer (GE Healthcare) FPLC system with Hiload 16/60 superdex 200 preparative grade column (dead volume 0.8 ml) run at 0.8-1.0 ml/min with 1x PBS. The purified apoprotein and holoprotein were resolved in 12 % SDS-PAGE, and stained with 1 mg/ml zinc acetate solution. The zinc induced fluorescence of the biliverdin-bound BphP was observed in UV light.

Spectroscopic characterization of AbBphP1. Absorption spectra of the purified holoprotein and apoprotein were recorded at 25°C from 300 nm to 900 nm with a Cary Bio300 (Varian) UV-visible spectrophotometer using scan speed setting of 2500 nm/min. Spectra were recorded in the dark at different time intervals after saturating irradiation with red light (16 μW/cm²/nm) or far-red light (21 μW/cm²/nm) in a 10 mm light path quartz cuvette (outside the spectrophotometer). Red LED with λ_{max} = 695 nm, 80 nm bandwith (Conrad, Modul Alustar 10 GRAD, Germany) and far-red optical fibers with λ_{max} = 750 nm, 40 nm bandwith were used to generate red and far-red light, respectively.

Construction of ΔAbBphP1 mutant. We first constructed AbBphP1:Km knockout mutant by inserting a kanamycin resistance gene (Km) cassette in the gene encoding AbBphP1 and placing it into the *A. brasilense* Sp7 genome via homologous recombination. The Km disrupted AbBphP1 gene was later replaced by an in-frame deletion of the same gene once again via homologous recomobination, as described earlier[47]. Specific description of the procedure is described in Supplemental Materials.

Cloning of AbBphP1 gene in expression vector and mobilization in ΔAbBphP1 mutant. The gene encoding AbBphP1 was PCR amplified with DreamTaq (Fermentas), using primer pair BphP1-F' and BphP1-R' possessing *Bgl*II and *Hind*III restriction overhangs in their 5′ends, respectively. The PCR product was digested with *Bgl*II and *Hind*III, purified and ligated downstream to IPTG-inducible *lacUV5* promoter into a broad host range, low copy number (1 or 2 copy/cell) expression vector, pMMB206. The resulting plasmid, pSnK5, was conjugatively mobilized into ΔAbBphP1 mutant, and exconjugants selected on chloramphenicol plates.

Spectrophotometric estimation of carotenoids. *A. brasilense* Sp7 and ΔAbBphP1 were grown in the dark, red light (λ_{max} = 685 nm) and white light with shaking at 180 rpm, in an incubator shaker at 30°C, in minimal malate broth upto stationary phase. Cultures (50 ml) were then pelleted by centrifugation, washed twice, and resuspended in the normal saline solution (0.85 % NaCl in water) so as to obtain equal optical density at 600 nm. The cells were pelleted again, suspended in 5 ml 100 % methanol in Oakridge tubes, covered with aluminium foil, and incubated overnight at 25°C to extract the pigments[49]. Absorption spectra of the methanolic extracts were recorded between 300–600 nm in a UV-Vis spectrophotometer (V630, Jasco).

Growth and chemotaxis assay in dark and red light. To observe the effect of red light on colony morphology, *A. brasilense* Sp7 and ΔAbBphP1 mutant were streaked on LB agar plates, and incubated in dark or in red light at 30°C for 48 h. A red light emitting lamp of 250 V, 10 W (Max.), 45 lumens (Max.) with emission maxima at 685 nm was used as the source of red light, and the dark treatment was given by wrapping the plates with aluminum foil. Growth curves of the *A. brasilense* and its ΔAbBphP1 mutant were monitored in the dark as well as in red light. The pre-culture of the wild type and the mutant were grown overnight in MM medium and reinoculated to obtain an initial optical density of 0.1 at 600 nm in 50 ml LB medium or minimal medium containing 38 mM malate as carbon source in 250 ml capacity flasks. The culture flasks were incubated at 30°C with 180 rpm with shaking in an incubator shaker in red light and in dark. Red light treatment was given as above, and the dark treatment was given by wrapping the culture flasks with aluminum foil. Optical density of the culture was recorded at 4 h intervals up to 24 h, and the growth curves plotted in SigmaPlot version12.3 as per Gompertz-Equation[50], using data from three independent experiments, each set up in duplicate. Assay for the viability of cells treated with red light is described in supplemental material.

For chemotaxis assay, overnight cultures of *A. brasilense*, ΔAbBphP1 mutant and ΔAbBphP1(pSnK5), grown in minimal malate medium were centrifuged. The pellet was washed twice with minimal medium lacking any carbon source, and resuspended in the same medium and same volume. A drop of 5 μl suspension of wild type, ΔAbBphP1 mutant and ΔAbBphP1(pSnK5) was placed in the centre of semisolid minimal medium agar (0.2 %) plate, containing 10 mM malate as carbon source. The plates were incubated for 48 h in the dark or under the red light. The size of the chemotactic ring in the swarm plates was recorded by a digital camera.

Plate assay for sensitivity to reactive oxygen species. Precultures of *A. brasilense*, ΔAbBphP1 mutant and ΔAbBphP1(pSnK5) were allowed to grow up to the late exponential phase in LB medium with respective antibiotics. 100 μl preculture of each strain was mixed with 5 ml of LB agar, having only 0.5 % agar, poured on top of the LB agar (1.5 % agar) plates, and allowed to solidify. After 15 min, a 10 μl drop of 5 mM toluidine blue (TB), 2 mM H_2O_2 or 1 mM paraquat was placed in the centre of each agar plate, incubated for 3 days in white light at 30°C, and the zone of inhibition by each oxidative stress agent was measured.

Proteome analysis by 2-D gel electrophoresis. *A. brasilense* Sp7 and its ΔAbBphP1 mutant were grown in 50 ml MMAB medium in a 250 ml flask upto mid log phase ($O.D_{600nm}$ 1.5). These cells were harvested by centrifugation, and the pellet was used to prepare the protein sample. The detailed description of the procedure for proteome analysis is given in the Supplemental Material.

1. Elías-Arnanz, M., Padmanabhan, S. & Murillo, F. J. Light-dependent gene regulation in nonphototrophic bacteria. *Curr. Opin. Microbiol.* **14**, 128–135 (2011).
2. Ziegelhoffer, E. C. & Donohue, T. J. Bacterial responses to photo-oxidative stress. *Nat Rev Microbiol.* **7**, 856–863 (2009).
3. Sayed, Z., Harris, F. & Phoenix, D. A. A study on the bacterial photo-toxicity of phenothiazinium based photosensitisers. *FEMS Immunol. Med. Microbiol.* **43**, 367–372 (2005).
4. Hellingwerf, K. J. The molecular basis of sensing and responding to light in microorganisms. *Antonie Van Leeuwenhoek* **81**, 51–59 (2002).
5. Glaeser, J. & Klug, G. Photo-oxidative stress in *Rhodobacter sphaeroides*: protective role of carotenoids and expression of selected genes. *Microbiol.* **151**, 1927–1938 (2005).
6. Rockwell, C. N., Su, Y. S. & Lagarias, J. C. Phytochrome structure and signaling mechanisms. *Annu. Rev. Plant. Biol.* **57**, 837–858 (2006).
7. Rockwell, N. C. & Lagarias, J. C. A brief history of phytochromes. *Chemphyschem.* **11**, 1172–1180 (2010).
8. Fankhauser, C. The phytochromes, a family of red/far-red absorbing photoreceptors. *J. Biol. Chem.* **276**, 11453–11456 (2001).
9. Bellini, D. & Papiz, M. Z. Structure of a bacteriophytochrome and light-stimulated protomer swapping with a gene repressor. *Structure* **20**, 1436–1446 (2012).
10. Vierstra, R. D. & Karniol, B. Phytochromes in microorganisms. In: handbook of photosensory receptors. (W.R. Briggs and J. L. Spudich, eds). Wiley-VCH Press, Wehiheim Germany 171–195 (2005).
11. Yang X, Stojkovic E. A, Kuk, J. & Moffat, K. Crystal structure of the chromophore binding domain of an unusual bacteriophytochrome, RpBphP3, reveals residues that modulate photoconversion. *Proc. Natl. Acad. Sci. USA* **104**, 12571–12576 (2007).
12. Yang, X., Kuk, J. & Moffat, K. Crystal structure of *Pseudomonas aeruginosa* bacteriophytochrome: photoconversion and signal transduction. *Proc. Natl. Acad. Sci. USA* **105**, 14715–14720 (2008).
13. Cho, M. H., Yoo, Y., Bhoo, S. H. & Lee, S. W. Purification and characterization of a recombinant bacteriophytochrome of *Xanthomonas oryzae* pathovar *oryzae*. *Protein J.* **30**, 124–131 (2011).
14. Barkovits, K., Schubert, B., Heine, S., Scheer, M. & Frankenberg-Dinkel, N. Function of the bacteriophytochrome BphP in the RpoS/Las quorum-sensing network of *Pseudomonas aeruginosa*. *Microbiol.* **157**, 1651–1664 (2011).
15. Essen, L. O., Mailliet, J. & Hughes, J. The structure of a complete phytochrome sensory module in the Pr ground state. *Proc. Natl. Acad. Sci. USA* **105**, 14709–14714 (2008).
16. Ulijasz, A. T. *et al.* Cyanochromes are blue/green light photoreversible photoreceptors defined by a stable double cysteine linkage to a phycoviolobilin-type chromophore. *J. Biol. Chem.* **284**, 29757–29772 (2009).
17. van der Horst, M. A., Key, J. & Hellingwerf, K. J. Photosensing in chemotrophic, non-phototrophic bacteria: let there be light sensing too. *Trends Microbiol* **15**, 554–562 (2007).
18. Giraud, E. *et al.* A new type of bacteriophytochrome acts in tandem with a classical bacteriophytochrome to control the antennae synthesis in *Rhodopseudomonas palustris*. *J. Biol. Chem.* **280**, 32389–32397 (2005).
19. Wilde, A., Fiedler, B. & Borner, T. The cyanobacterial phytochrome Cph2 inhibits phototaxis towards blue light. *Mol. Microbiol.* **44**, 981–988 (2002).
20. Rottwinkel, G., Oberpichler, I. & Lamparter, T. Bathy phytochromes in rhizobial soil bacteria. *J. Bacteriol.* **192**, 5124–5133 (2010).
21. Oberpichler, I., Rosen, R., Rasouly, A., Vugman, M., Ron, E. Z. & Lamparter, T. Light affects motility and infectivity of *Agrobacterium tumefaciens*. *Env. Microbiol.* **10**, 2020–2029 (2008).
22. Davis, S. J., Vener, A. V. & Vierstra, R. D. Bacteriophytochromes: phytochrome-like photoreceptors from non photosynthetic eubacteria. *Science* **286**, 2517–2520 (1999).
23. Karniol, B. & Vierstra, R. D. The pair of bacteriophytochromes from *Agrobacterium tumefaciens* are histidine kinases with opposing photobiological properties. *Proc. Natl. Acad. Sci. USA* **100**, 2807–2812 (2003).

24. Scheerer, P. *et al*. Light induced conformational changes of the chromophore and the protein in phytochromes: bacterial phytochromes as model systems. *Chemphyschem.* **11**, 1090–1105 (2010).
25. Tien, T. M., Gaskins, M. H. & Hubbell, D. H. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of Pearl millet (*Pennisetum americanum* L.) *Appl. Env. Microbiol.* **37**, 1016–1024 (1979).
26. Li, H., Zhang, J., Vierstra, R. D. & Li, H. Quaternary organization of a phytochrome dimer as revealed by cryoelectron microscopy. *Proc. Natl. Acad. Sci. USA* **107**, 10872–10877 (2010).
27. Wagner, J. R., Zhang, J., Brunzelle, J. S., Vierstra, R. D. & Forest, K. T. High resolution structure of *Deinococcus* bacteriophytochrome yields new insights into phytochrome architecture and evolution. *J. Biol. Chem.* **282**, 12298–12309 (2007).
28. Noack, S., Michael, N., Rosen, R. & Lamparter, T. Protein conformational changes of *Agrobacterium* phytochrome Agp1 during chromophore assembly and photoconversion. *Biochem.* **46**, 4164–4176 (2007).
29. König, K., Teschke, M., Sigusch, B., Glockmann, E., Eick, S. & Pfister, W. Red light kills bacteria via photodynamic action. *Cell Mol. Biol.* **46**, 1297–1303 (2000).
30. Tarutina, M., Ryjenkov, D. A. & Gomelsky, M. An unorthodox bacteriophytochrome from Rhodobacter sphaeroides involved in turnover of the second messenger c-di-GMP. *J. Biol. Chem.* **281**, 34751–34758 (2006).
31. Lysenko, V. S. *et al*. Separation and mass spectrometry identification of carotenoid complex from radioresistant bacteria *Deinococcus radiodurans*. *J. Anal. Chem.* **66**, 1281–1284 (2011).
32. Briviba, K., Klotz, L. O. & Sies, H. Toxic and signaling effects of photochemically or chemically generated singlet oxygen in biological systems. *Biol. Chem.* **378**, 1259–1265 (1997).
33. Wilson, M. Lethal photosensitisation of oral bacteria and its potential application in the photodynamic therapy of oral infections. *Photochem. Photobiol. Sci.* **3**, 412–418 (2004).
34. Wang, X., Wang, H. & Huang, Z. Photosensitization induced by exposure to colour cathode ray tube monitor following topical 5-aminolaevulinic acid-based photodynamic therapy: a case report. *Acta. Derm. Venereol.* **90**, 546–548 (2005).
35. Maisch, T., Baier, J., Franz, B., Maier, M., Landthaler, M., Szeimies, R. M. & Bäumler, W. The role of singlet oxygen and oxygen concentration in photodynamic inactivation of bacteria. *Proc. Natl. Acad. Sci. USA* **104**, 7223–7228 (2007).
36. Mohammadi, M., Burbank, L. & Roper, M. C. Biological role of pigment production for the bacterial phytopathogen *Pantoea stewartii* subsp. *Stewartii*. *Appl. Environ. Microbiol.* **78**, 6859–6865 (2012).
37. St Denis, T. G., Huang, L., Dai, T. & Hamblin, M. R. Analysis of the bacterial heat shock response to photodynamic therapy-mediated oxidative stress. *Photochem. Photobiol.* **87**, 707–713 (2011).
38. Guisbert, E., Herman, C., Lu, C. Z. & Gross, C. A. A chaperone network controls the heat shock response in *E. coli*. *Genes Dev.* **18**, 2812–2821 (2004).
39. Gamer, J., Multhaup, G., Tomoyasu, T., McCarty, J. S., Rüdiger, S., Schönfeld, H. J., Schirra, C., Bujard, H. & Bukau, B. A cycle of binding and release of the DnaK, DnaJ and GrpE chaperones regulates activity of the *Escherichia coli* heat shock transcription factor sigma32. *EMBO J.* **15**, 607–17 (1996).
40. Bukau, B. & Horwich, A. L. The Hsp70 and Hsp60 chaperone machines. *Cell* **92**, 351–366 (1998).
41. Hartl, F. U. Molecular chaperones in cellular protein folding. *Nature* **381**, 571–579 (1996).
42. Mary, I., Tu, C. J., Grossman, A. & Vaulot, D. Effects of high light on transcripts of stress-associated genes for the cyanobacteria *Synechocystis sp.* PCC 6803 and *Prochlorococcus* MED4 and MIT9313. *Microbiol.* **150**, 1271–1281 (2004).
43. Nuss, A. M., Glaeser, J. & Klug, G. RpoHII activates oxidative stress defense systems and is controlled by RpoE in the singlet oxygen dependent response in *Rhodobacter sphaeroides*. *J. Bacteriol.* **191**, 220–230 (2009).
44. Nuss, A. M., Glaeser, J., Berghoff, B. A. & Klug, G. Overlapping alternative sigma factor regulons in the response to singlet oxygen in *Rhodobacter sphaeroides*. *J. Bacteriol.* **192**, 2613–2623 (2010).
45. Dobereiner, J. & Day, J. M. Associative symbiosis in tropical grasses: characterization of microorganism and dinitrogen fixing sites. *Int. Symp. on Nitrogen Fixation—Interdisciplinary Discussion*, In Newton W. E. and Nyman C. J. (ed.), Pullman, WA: Washington State University Press, 518–538 (1976).
46. Vanstockem, M., Michiels, K., Vanderleyden, J. & van Gool, A. Transfer and random integration of Tn5 in *Azospirillum*. *Azospirillum* III: *genetics, physiology, ecology*. In Klingmuller W. (ed.), Berlin Springer-Verlag 74–84 (1985).
47. Mishra, M. N., Kumar, S., Gupta, N., Kaur, S., Gupta, A. & Tripathi, A. K. An extracytoplasmic function sigma factor cotranscribed with its cognate anti-sigma factor confers tolerance to NaCl, ethanol and methylene blue in *Azospirillum brasilense* Sp7. *Microbiol.* **157**, 988–999 (2011).
48. Gambetta, G. A. & Lagarias, J. C. Genetic engineering of phytochrome biosynthesis in bacteria. *Proc. Natl. Acad. Sci. USA* **98**, 10566–10571 (2001).
49. Thirunavukkarasu, N., Mishra, M. N., Spaepen, S., Vanderleyden, J., Gross, C. A. & Tripathi, A. K. An extra-cytoplasmic function sigma factor and anti-sigma factor control carotenoid biosynthesis in *Azospirillum brasilense*. *Microbiol* **154**, 2096–2105 (2008).
50. Zwietering, M. H., Jongenburger, I., Rombouts, F. M. & van't Riet, K. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.* **56**, 1875–1881 (1990).

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Author contribution

[1]SK, VSS, MT, SA and HS performed the experiments. JPK and DVA provided some of the experimental facilities and inputs to the manuscript. [2]SK organized spectral characterization work and contributed in manuscript writing, AKT concieved the idea, coordinated the whole project and wrote the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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