Bioscience, Biotechnology, and Biochemistry

ISSN: 0916-8451 (Print) 1347-6947 (Online) Journal homepage: http://www.tandfonline.com/loi/tbbb20

The regulatory mechanism underlying light-inducible production of carotenoids in nonphototrophic bacteria

Hideaki Takano

To cite this article: Hideaki Takano (2016): The regulatory mechanism underlying light-inducible production of carotenoids in nonphototrophic bacteria, Bioscience, Biotechnology, and Biochemistry, DOI: 10.1080/09168451.2016.1156478

To link to this article: http://dx.doi.org/10.1080/09168451.2016.1156478

Published online: 11 Mar 2016.

Bioscience, Biotechnology, and Biochemistry, 2016

Award Review

The regulatory mechanism underlying light-inducible production of carotenoids in nonphototrophic bacteria

Hideaki Takano*

Applied Biological Science and Life Science Research Center, College of Bioresource Sciences, Nihon University, Fujisawa, Japan

Received November 14, 2015; accepted January 31, 2016
http://dx.doi.org/10.1080/09168451.2016.1156478

Light is a ubiquitous environmental factor serving as an energy source and external stimulus. Here, I review the conserved molecular mechanism of light-inducible production of carotenoids in three nonphototrophic bacteria: *Streptomyces coelicolor* A3(2), *Thermus thermophilus* HB27, and *Bacillus megaterium* QM B1551. A MerR family transcriptional regulator, LitR, commonly plays a central role in their light-inducible carotenoid production. Genetic and biochemical studies on LitR proteins revealed a conserved function: LitR in complex with adenosyl B_{12} ($AdoB_{12}$) has a light-sensitive DNA-binding activity and thus suppresses the expression of the *Crt* biosynthesis gene cluster. The *in vitro* DNA-binding and transcription assays showed that the LitR–$AdoB_{12}$ complex serves as a repressor allowing transcription initiation by RNA polymerase in response to illumination. The existence of novel light-inducible genes and the unique role of the megaplasmid were revealed by the transcriptomic analysis of *T. thermophilus*. The findings suggest that LitR is a general regulator responsible for the light-inducible carotenoid production in the phylogenetically divergent nonphototrophic bacteria, and that LitR performs diverse physiological functions in bacteria.

Key words: nonphototrophic bacteria; carotenoid; MerR family regulator; vitamin B_{12}

Light is a general environmental factor that affects biological functions including photosynthesis, circadian rhythms, and secondary metabolite production, which are observed in various organisms including eukaryotes and phototrophic bacteria.[1,2] The molecular mechanism behind transmission of a light signal to gene expression machinery in the above organisms has been reported previously.[3–5] In contrast, little is known about the photoresponse phenomena and their regulatory mechanism in nonphototrophic bacteria because of the unavailability of light stimuli[2]; however, the increased amount of genomic information on diverged bacterial genera revealed wide distribution of a homologous protein similar to the plant-type blue-light receptor LOV, which uses flavin as a light-sensing molecule.[1] The common presence of the LOV family suggests that the light-sensing and light response ability also generally occur in nonphototrophic bacteria.

Carotenoids are terpene compounds produced by a wide variety of organisms including plants,[6] fungi,[7] algae,[8] and bacteria.[9] Carotenoids have an antioxidant activity and thereby protect the cells from photo-oxidative damage by scavenging harmful agents such as singlet and triplet molecular species produced after illumination.[10] The light-inducible carotenoid production is a representative phenotype of the light response present in various organisms including not only plants, fungi, and phototrophic bacteria but also nonphototrophic bacteria.[11] Therefore, many organisms use carotenoids to protect the cells from sunlight-derived reactive oxygen species. Thus, induction of carotenoid production by light saves energy in the dark.

In nonphototrophic bacteria, carotenoid production is mainly classified into three types: (i) light-inducible,[12] (ii) constitutive,[13] and (iii) cryptic.[11,14,15] The light-inducible carotenoid production (the focus of this study) is observed in several bacterial species including the gliding Gram-negative bacterium *Myxococcus xanthus* and Gram-positive bacteria, *Mycobacterium* spp.[16,17] The regulatory mechanism in *M. xanthus* has been well-studied; two regulators play a central role in the light-inducible carotenoid production: the MerR family transcriptional regulator CarA[18–20] and extracytoplasmic function (ECF)-type sigma factor CarQ.[21–23] Nevertheless, the light-sensing mechanism of this bacterium remained unclear when my colleagues and I started this research. With respect to the light-inducible carotenoid production in *Mycobacterium marinum*, the detailed molecular mechanism has not been elucidated yet although the involvement of a MarR family-type regulator was suggested.[17]

Our work is focused on the regulatory mechanism behind the light-inducible carotenoid production in

*Email: takano.hideaki@nihon-u.ac.jp
This review was written in response to the author's receipt of the JSBBA Award for Young Scientists in 2015.

nonphototrophic bacteria for the following reasons: we are interested in (i) how a light signal is transmitted to the level of gene expression machinery, and (ii) the molecular mechanism underlying the light-inducible carotenoid production, which is poorly understood. We believe that elucidation of the gene expression mechanism will provide new knowledge about bacterial responses to the environment and how bacteria are awoken by illumination or sunlight. This review highlights the current knowledge about the molecular mechanism of the light-inducible carotenoid production in nonphototrophic bacteria.

I. Light-inducible carotenoid production in *Streptomyces coelicolor* A3(2)

Streptomyces not only has the ability to produce a variety of secondary metabolites including bioactive compounds, such as antibiotics and antitumor drugs, but also has a complex life cycle similar to that of fungi.[24,25] Carotenoids are some of the secondary metabolites produced by many *Streptomyces* spp. and related bacteria.[26,27] We originally found that the carotenoid production in *S. coelicolor* A3(2), a model organism for genetic studies,[28] takes place in a light-responsive manner when we studied a strain that is deficient in two pigment antibiotics: actinorhodin and prodigiosin.[29] This strain had a yellow color when it was left on top of a desk for several days; this finding prompted us to assume that the pigmentation of *S. coelicolor* is caused by the illumination. We again incubated the strain on top of the desk and in the drawer of the desk. The strain that was incubated on top of the desk developed a yellow color, whereas the strain in the desk drawer formed white colonies. We assumed that this phenomenon occurs in response to illumination and recognized that carotenoid production had been hidden by the pigment antibiotics.

The putative *crt* biosynthesis gene cluster of *S. coelicolor* is composed of two convergent operons: *crtEIBV* and *crtYTU* (Fig. 1). Our preliminary chemical analysis showed that the yellow pigment is isorenieratene, a carotenoid previously found in other actinomycetes such as *S. griseus* and *Mycobacterium smegmatis*. According to the results of transcriptional analysis, the light-induced phenomenon is regulated at the transcriptional level; the transcription of *crtE* and *crtY*, the first gene of the operon, was induced by illumination, whereas their transcription was extremely weak in the dark. These findings suggested that this bacterium retained a regulatory system for light signal transduction.

Two transcriptional regulatory proteins are encoded in the flanking region of the *crt* biosynthesis gene cluster of *S. coelicolor:* (i) a MerR family transcriptional regulator (designated as LitR; light-inducible transcriptional regulator) and (ii) ECF-type sigma factor (designated as LitS). LitR, consisting of 330 amino acid residues, contains a DNA-binding domain at the N terminus and vitamin B_{12} (synonym cobalamin, Cbl)-binding domain at the C terminus. Generally, MerR family proteins serve as positive or negative regulators whose activity is regulated by the association with a ligand that is directly related to the genes that are regulated by the MerR regulator.[30,31] Therefore, it appears that Cbl serves as a ligand for LitR. In contrast, LitS is involved in the promoter recognition by the RNA polymerase holoenzyme.

Fig. 1 depicts a hypothetical regulatory model of the light-inducible carotenoid production in *S. coelicolor* according to our transcriptional analyses. Such an analysis with S1 nuclease mapping showed that the transcription of *crtE*, *crtY*, and *litS* is induced by illumination, while the *litR* transcription is slightly increased by illumination. The knockout of *litR* caused constitutive expression of *litR* itself but abrogated the transcription of *crtE*, *crtY*, and *litS*. This result indicates that LitR represses the *litR* gene but enhances the expression of *litS*. Our experiments were hampered by the difficulties with preparation of an active recombinant LitR protein: biochemical analysis of the LitR protein is necessary for elucidation of its properties.

The *litS* knockout abrogated the light induction of the *crtE* and *crtY* genes but had no effect on the expression of *litR* or *litS* itself. This finding suggested that σ^{LitS} serves as a sigma factor directing the promoter recognition by *crtE* and *crtY*. We successfully reproduced the σ^{LitS} function by an *in vitro* runoff transcriptional assay: the RNA polymerase holoenzyme containing σ^{LitS} specifically directs the transcription initiated from the promoters preceding *crtE* and *crtY*. The alignment of these two promoters provided the consensus sequence recognized by σ^{LitS}, a possible consensus sequence, GCAT(G/C)-(16 or 17 bp)-C(G/C)(G/C)AA (G/C) in the region -35 to -10.

The central role of LitR and σ^{LitS} in the regulatory system was also supported by the genetic analysis involving a heterologous host, *S. griseus*, a streptomycin producer.[32] This bacterium is a suitable host because of its inability to produce carotenoids despite retaining the *crt* biosynthesis gene cluster on the chromosome.[14] The light-inducible transcription was monitored with a reporter plasmid, which carries *litR*, *litS*, and melanin biosynthesis genes *melC1-melC2* fused with the light-inducible promoter. An *S. griseus* transformant carrying the reporter plasmid showed melanin (brown pigment) production during illumination, whereas melanin was not produced during culturing in the dark. The light-dependent melanin production was abrogated by the deletion of *litR* or *litS* from the reporter plasmid. This evidence points to two things: (i) the light-inducible transcription is activated only by two proteins including σ^{LitS}-RNA polymerase holoenzyme and LitR, and (ii) LitR is a transcriptional regulator with a photosensory function because illumination has no effect on the activity of the σ^{LitS}-RNA polymerase holoenzyme in the *in vitro* transcription assay.

The existence of a putative B_{12}-binding domain at the C terminus of LitR suggests that Cbl serves an antenna molecule that receives the light signal. Cbl is a cobalt (Co)-containing tetrapyrrole that absorbs blue and green light (Fig. 2). Our gene disruption experiment revealed the involvement of Cbl in the regulatory mechanism: an *S. coelicolor* mutant deficient in *cobDQN*, which encodes enzymes involved in a later step of Cbl biosynthesis, showed constitutive carotenoid production at a low level, and the transcription of *crt* biosynthesis genes was weak, in line with the

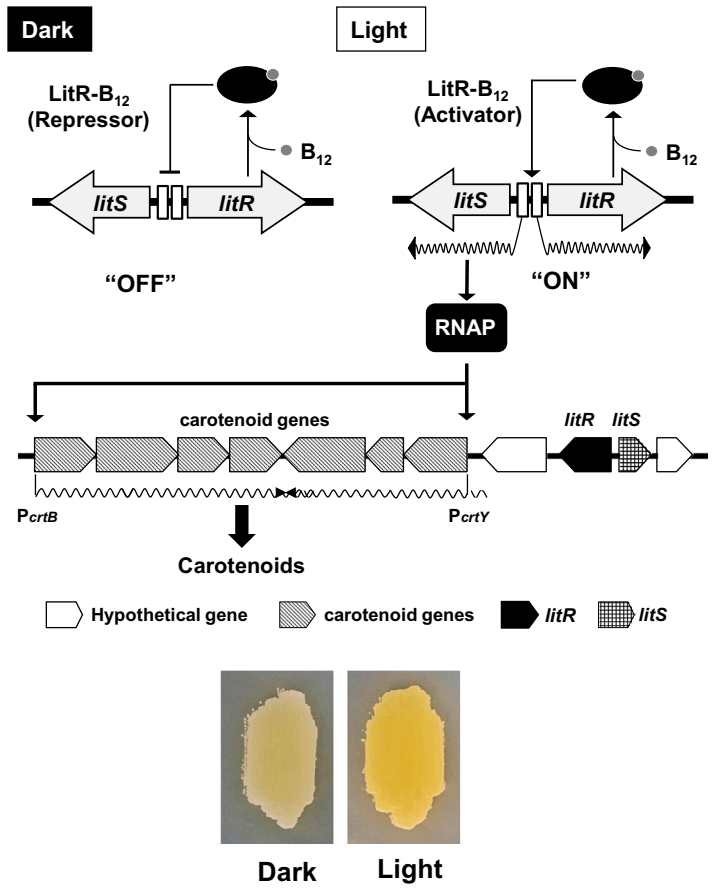

Fig. 1. The proposed regulatory mechanism of light-inducible carotenoid production in *S. coelicolor* A3(2).
Notes: LitR, a MerR family regulator, represses the transcription initiation for *litR* itself and for an extracytoplasmic function (ECF)-type sigma factor gene *litS* in the dark. Illumination transforms the LitR protein into the activator form, which then enables the expression of *litS*. Subsequently, the RNA polymerase holoenzyme containing σ^{LitS} directs mRNA synthesis in *crt* operons.

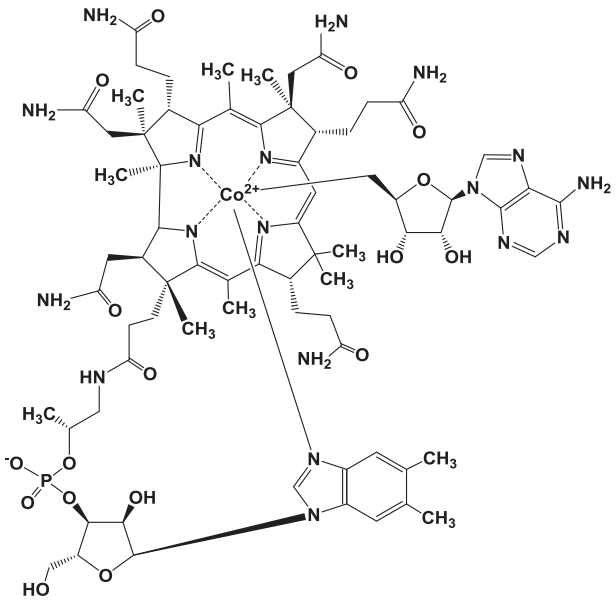

Fig. 2. Chemical structure of Adenosyl B_{12} ($AdoB_{12}$).
Notes: Among B_{12} derivatives, $AdoB_{12}$ served as a light-sensing ligand of the LitR protein. It is known that the Co-C bond of $AdoB_{12}$ is sensitive to light.

phenotype.[33] Addition of 0.1 μM MeCbl to the medium and genetic complementation both restored the ability to perform light-induced carotenogenesis in the mutant. This result shows that Cbl serves as a light-sensing cofactor of LitR by binding to the C-terminal domain. In addition, the mutation in *cbl* genes causes a defect in the morphological differentiation and in production of two pigment antibiotics in *S. coelicolor*, although the mutation mostly did not affect the viability in a rich culture medium.[34] The pleiotropic role of Cbl in the development of *Streptomyces* indicates that Cbl participates in primary metabolism and serves as a signaling compound involved in the signal transduction supporting the complex life cycle.

A protein homologous to LitR is found in almost every *Streptomyces* spp. whose genome has been completely sequenced; however, ~60% of *Streptomyces* species do not produce carotenoids in a light-dependent manner (our unpublished observation). The same is true for *Bacillus* as described below. The inconsistency between retention of *litR* and the phenotype suggests that the role of LitR is not confined to the control of carotenoid production and indicates that LitR has diverse functions.

II. Wide distribution of *litR* homologs in nonphototrophic bacteria

The genomic information on phylogenetically diverged bacteria is publicly available; hence, a comparative genomics approach is a powerful tool for the

exploration of bacterial functions *in silico*.[35] Our genomic search revealed wide distribution of *litR* homologs in phylogenetically diverged bacterial genera including not only Gram-positive but also Gram-negative bacteria (Fig. 3).[32] Approximately 30% of the bacterial genera retain the gene encoding a *litR* homolog in their genomes, including the bacteria studied in the field of applied microbiology, for example, *Thermus*, *Bacillus*, and *Pseudomonas*, and in clinical research, for example, *P. aeruginosa* and *Burkholderia* known as pathogens. The common presence of a LitR homolog indicates that nonphototrophic bacteria generally are equipped with the light-responsive system.

Photoresponse-related genes are located in close proximity to the *litR* homolog. These genes are *crt* biosynthesis gene cluster, *phr*, and bacteriorhodopsin in *Thermus* sp. CCB_US3_UF1[36] and *LOV* in *Pseudomonas* spp.[37] Phr is a DNA photolyase that repairs pyrimidine dimers (generated by UV) using light as an energy source. LOV is a homolog of a plant blue-light sensor, phototropin. Proteorhodopsin, a homolog of bacteriorhodopsin, associates with a retinal molecule and serves as a light-driven proton pump.[38,39] In contrast to the diversity of photoresponse-related proteins, the *litR* homolog is conserved. This situation indicates that LitR may serve as a light sensor to regulate their transcription after illumination.

The other interesting finding is multiplicity of LitR homologs in a single bacterial genome: three homologs in *Pseudomonas* spp., two homologs in *Actinosynnema mirum*, a nocardicin producer[39,40]; three homologs in *Kitasatospora setae*,[41] a setamycin producer; and five homologs in *Amycolatopsis mediterranei*,[42] a rifamycin producer. LitR lies outside the *crt* biosynthesis operon or cluster; this situation is suggestive of the diversity of LitR functions. The bacteria retaining multiple *litR* homologs are mainly soil habitants; this finding suggests that *litR* genes provide an advantage for survival in the soil niche.

Of these *litR*-retaining bacteria, we selected *Thermus thermophilus* HB27[43,44] and *Bacillus megaterium* QM B1551[45,46] as representatives of Gram-negative and

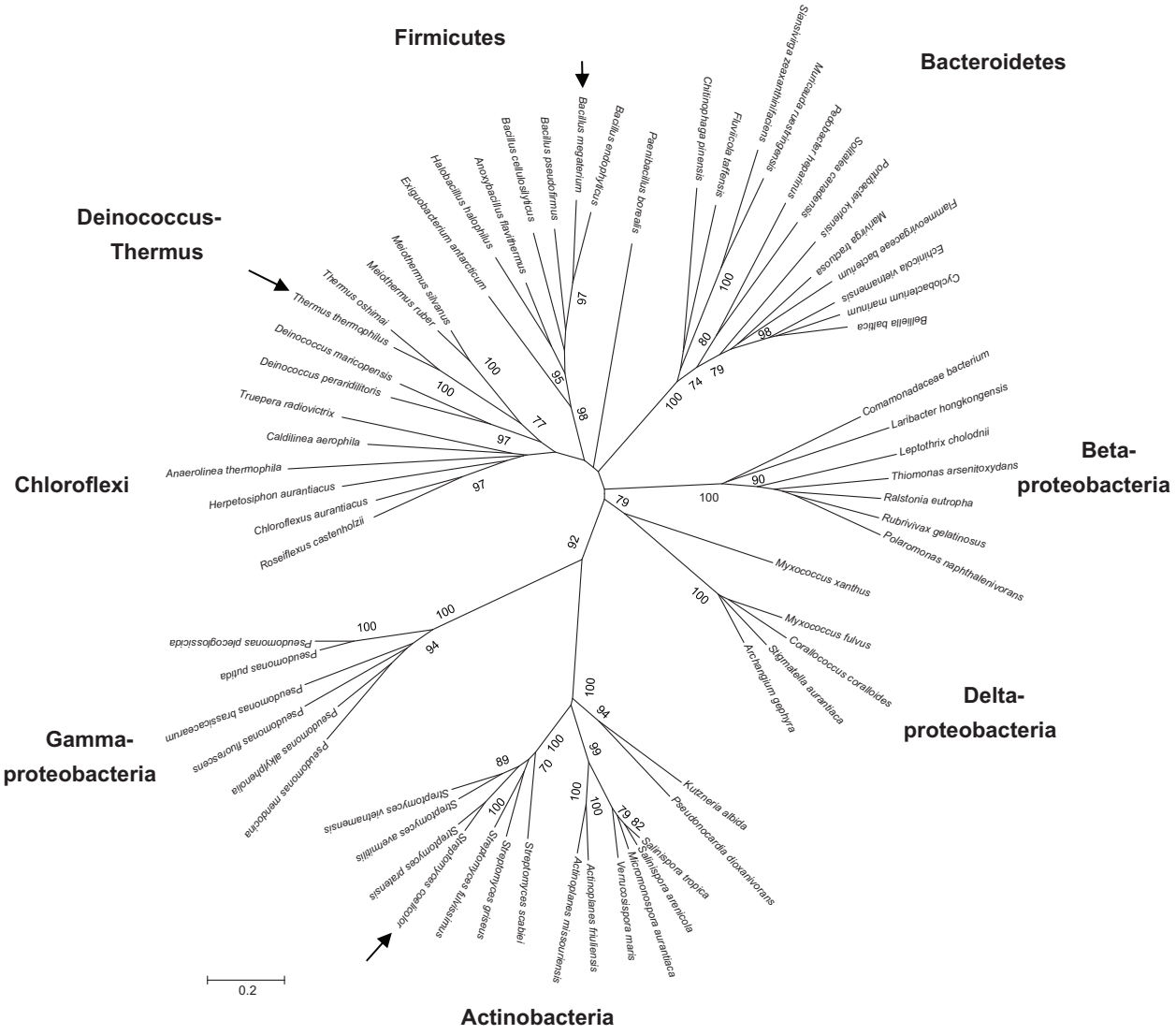

Fig. 3. The neighbor-joining phylogenetic tree based on amino acid sequence of the B_{12}-binding domain of LitR homologs from Gram-positive and Gram-negative bacteria. Arrowheads indicate LitR proteins characterized in our study.

Notes: Numbers at nodes are bootstrap values (expressed as percentages of 100 resampled datasets; only values > 70% are shown). The scale bar shows 0.2 amino acid substitutions per amino acid site. These amino-acid sequences were aligned using the ClustalW2.1 software. Evolutionary analyses were conducted in the MEGA 5.05 software.[60]

Gram-positive bacteria, respectively, because both strains play an important role in the field of applied microbiology. Our intensive studies on the regulatory mechanism are described below.

III. LitR from the Gram-negative bacterium *T. thermophilus* HB27

T. thermophilus HB27 is a high-GC Gram-negative thermophilic bacterium isolated from hot springs whose proteins have been used for the studies of enzyme properties and protein structure because of their high stability. This bacterium harbors a 1.85-Mb chromosome and a large (0.26-Mb) circular plasmid pTT27. The large plasmid pTT27 carries the genes of a LitR homolog and *crt* biosynthesis enzymes in an adjacent region.[47] The *litR* gene is a part of an operon structure with a gene encoding the cyclic AMP (cAMP) receptor protein (CRP)/fumarate and nitrate reduction regulator (FNR) family transcriptional regulator (designated as LdrP; light-dependent regulatory protein), whose function as a positive regulator is activated by the binding of cAMP.[48] The gene organization suggests that LdrP serves as a positive regulator like LitS in *S. coelicolor*.

T. thermophilus produces a yellow pigment called thermozeaxanthin, a zeaxanthin derivative (glycosylated).[49] The biosynthesis is known to be constitutive; however, our research based on the presence of a *litR* homolog in the genome of *T. thermophilus* HB27 revealed that this bacterium produces thermozeaxanthin in a light-responsive manner. It is likely that the light-dependent phenomena had been overlooked owing to the leaky regulation of expression of the corresponding genes. Our gene disruption experiment showed that *crt* genes, which are located far from *litR*, are responsible for the synthesis of thermozeaxanthin. The light-inducible carotenoid production was also observed in phylogenetically close bacteria belonging to the *Thermus* genus; these findings indicate that the capacity for light sensing is widespread within this genus.

Fig. 4 shows a proposed regulatory model of the light-inducible carotenoid production in *T. thermophilus* HB27. Our transcriptional analysis revealed that the light-inducible carotenoid production is regulated at the transcriptional level. Hence, the transcription of *crtB* encoding a phytoene synthase is remarkably induced by illumination, whereas the light induction does not occur in the dark. Genetic analysis of knockout mutants revealed that two tandem transcriptional regulator genes, *litR* and *ldrP*, play a central role in the light-responsive regulation. Disruption of *ldrP* causes a loss of *crtB* transcription but does not affect the transcription initiated from the *litR* promoter. This result leads to two conclusions: (i) LdrP is not involved in the expression of *litR* and *ldrP* itself and (ii) LdrP is an activator specific to *crtB* transcription. On the other hand, the *litR* knockout leads to constitutive transcription of *crtB* and *litR* itself indicating that LitR serves as a transcriptional repressor for both genes.

The function of the LdrP protein was successfully demonstrated with an *in vitro* runoff transcriptional assay: *Thermus* RNA polymerase holoenzyme specifically directs the *crtB* transcription in the presence of a recombinant LdrP protein but does not in the absence of LdrP. The CRP/FNR family proteins generally require cAMP to assume the active form; however, the transcriptional activation by LdrP occurs in the absence of cAMP. This result is supported by three-dimensional structure of LdrP reported by Agari *et al.*[50] this structure does not afford sufficient space for the incorporation of cAMP, and the structure of LdrP is similar to that of the *Escherichia coli* CRP–cAMP protein complex.[51] These findings suggest that LdrP is a cAMP-independent CRP/FNR family protein and is in the active form constitutively.

As described above, we identified the mechanism behind the transcriptional activation of *crtB*, but it is still unclear how the light induction of *crtB* transcripts takes place. The knockout of *litR* caused the constitutive transcription of *crtB* and *litR*, indicating that the fundamental function of LitR is a repressor that inhibits *crtB* transcription in the dark. Hence, we assumed that it was important to elucidate the mechanism underlying LitR inactivation by illumination. We used a recombinant LitR protein prepared in an *E. coli* expression system. In agreement with the fact that LitR retains a B_{12}-binding domain at the C terminus, LitR has an ability to associate with B_{12} derivatives including hydroxocobalamin (OHB_{12}), methyl Cbl, and 5′-deoxyadenosylcobalamin ($AdoB_{12}$). Among these B_{12} derivatives, LitR that is associated with $AdoB_{12}$ showed a strong and specific DNA-binding activity toward the intergenic promoter region of *litR* and *crtE* in the dark. On the other hand, the DNA-binding activity of LitR–$AdoB_{12}$ during illumination drastically decreases.

In parallel with our study, Ortiz-Guerrero *et al.*[52] reported that a chimeric protein—CTt2 consisting of the N-terminal DNA-binding domain of CarH and the C-terminal Cbl-binding domain of LitR of *T. thermophilus* HB8—is a light-sensitive DNA-binding protein, according to the function of the ligand: $AdoB_{12}$. Light causes photolysis of $AdoB_{12}$ bound to CTt2, leading to the conversion of $AdoB_{12}$ to OHB_{12} due to the chemical cleavage of the Co–C bond of $AdoB_{12}$, which abrogates its DNA-binding activity. The photolysis of $AdoB_{12}$ also induces a dramatic subunit dissociation of CTt2; the tetramer of CTt2–$AdoB_{12}$ assumes monomeric structure in response to illumination. Furthermore, Díez *et al.*[53] reported that the full-length native protein TtCarH (a LitR homolog: a single-residue substitution) from *T. thermophilus* HB8 has a light-sensitive DNA-binding activity. These results clearly showed that LitR/CarH family proteins are novel coenzyme B_{12}-based photosensors.

Our analyses provided detailed information on the main switch for the light-inducible transcription, which is located in the intergenic region between *litR* and *crtB*. As shown in Fig. 5, the LitR–$AdoB_{12}$ complex directly binds to the promoter region -55 to -94 relative to the transcriptional start site of *crtB*. The putative sequences of the region -35 to -10 of *litR* promoter and *crtB* promoter are similar to the σ^A-dependent promoter of *T. thermophilus*. In contrast, LdrP activation requires the region -51 to + 1 relative to the transcriptional start site of *crtB*, which contains a potential binding sequence (AGTGT[N7]GCAAAA) that has partial

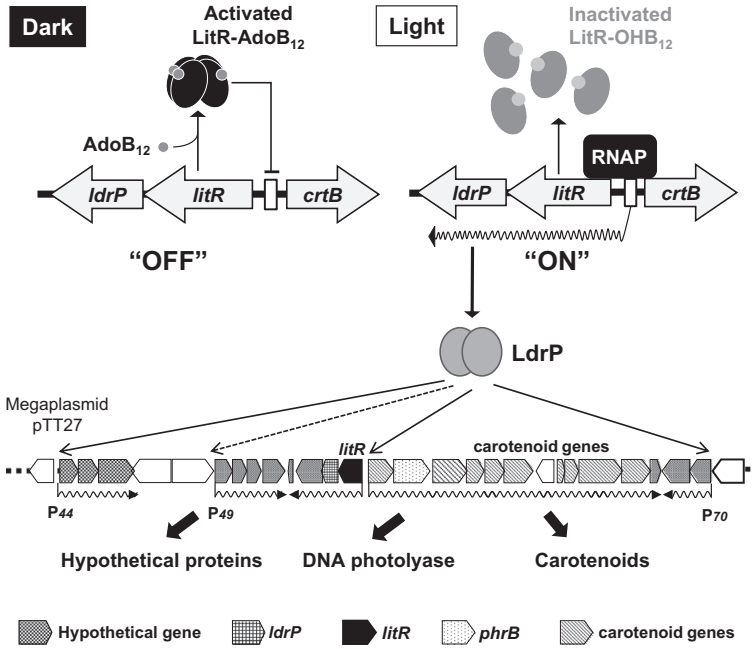

Fig. 4. The working model of the light-inducible carotenoid production in *T. thermophilus* HB27.

Notes: LitR-AdoB$_{12}$ binds to the intergenic region of *litR* and *crtB*, repressing the transcription of both genes in the dark. During illumination, the absorption of light by the tetrameric AdoB$_{12}$-LitR complex causes its dissociation into monomers probably because of photolysis of AdoB$_{12}$. This event allows for the expression of LitR and LdrP. Subsequently, LdrP recruits the RNA polymerase holoenzyme (RNAP) to initiate transcription of the *crt* operon, *phrB* (DNA photolyase), and some hypothetical genes.

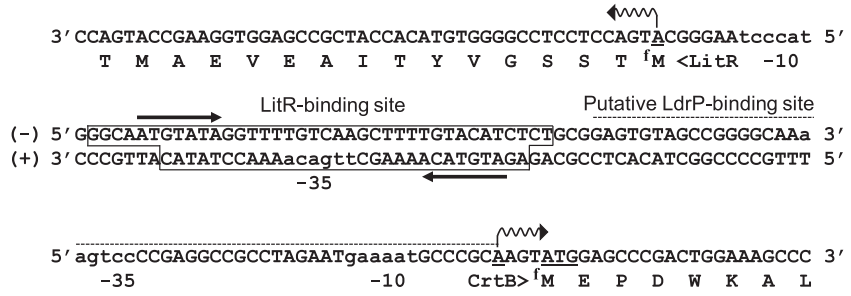

Fig. 5. The main switch for the light-inducible gene expression in *Thermus thermophilus* HB27.

Notes: The promoter is the intergenic region between *litR* and *crtB*. The LitR-binding site is boxed, and the putative LdrP-binding site is indicated by dashed lines. Transcriptional start sites are indicated by wavy arrowheads. The putative region -10 to -35 is shown by small letters. LitR regulates the bidirectional gene expression by binding to a single site.

similarity with the consensus [WWGTGA(N5–7) ACACWW] for the cAMP-independent CRP/FNR regulator of *T. thermophilus*. This finding suggests that the LitR–AdoB$_{12}$ complex binds to a single site, regulating the bidirectional transcription of *litR* itself and *crtB*.

IV. LitR of the Gram-positive bacterium *Bacillus megaterium* QM B1551

B. megaterium is a low-GC, endospore-forming Gram-negative soil bacterium.[45,46] The genetic research on the *Bacillus* genus has been extensive owing to the ability of these bacteria to form endospores and produce useful enzymes and secondary metabolites.[54] The carotenoid production in this genus has also been observed, and several chemical structures were elucidated[55–57]; however, there are no reports on the light responsiveness. During our screening of light-responsive soil-dwelling bacteria, we originally found that a number of strains of *B. megaterium*, *Bacillus sphaericus*, and *Bacillus pumilus* produce a carotenoid-like yellow pigment in response to illumination.[58] This phenomenon suggests that a light-responsive regulatory mechanism also exists in this genus.

The genome of *B. megaterium* QM B1551 retains a *litR* homolog (*BMQ_4356*) and a putative *crt* biosynthesis gene cluster consisting of two operons, *crtI1-crtI2-crtB* (*BMQ_0654–0656*) and *BMQ_2952–2949*. Unlike in *S. coelicolor* and *T. thermophilus*, the *litR* gene in *Bacillus* is located in a locus far from *crt* genes. LitR of *B. megaterium* QM B1551 consists of 303 amino acid residues, showing 28% amino acid sequence identity to LitR of *T. thermophilus*. Our preliminary genetic analysis using the knockout mutant revealed that (i) the LitR homolog negatively regulates light-inducible carotenoid production, (ii) a *cbl* knockout mutant causes constitutive and weak carotenoid production, and (iii) *crtI1-crtI2-crtB* represents the carotenoid biosynthesis genes. Our evidence suggests that

the LitR homolog also plays a crucial role in the light-dependent carotenoid production in this bacterium, and this function depends on $AdoB_{12}$. With respect to the chemical structure of the carotenoid produced by *B. megaterium* QM B1551, we were unable to identify this compound owing to its high light sensitivity, although the *litR* mutation results in 10-fold greater amounts of the carotenoid compared with the wild type.

Our biochemical study revealed that the *Bacillus* LitR recombinant protein has many properties similar to those of *T. thermophilus:* (i) LitR associates with $AdoB_{12}$, (ii) $AdoB_{12}$–LitR is a photosensitive DNA-binding protein, (iii) LitR–$AdoB_{12}$ directly binds to the promoter region of *crt* biosynthesis genes and *litR* itself, (iv) LitR–$AdoB_{12}$ serves as a transcriptional repressor, (v) LitR–$AdoB_{12}$ forms a homo-oligomer, and (iv) LitR–$AdoB_{12}$ undergoes subunit dissociation in response to illumination. The characteristic of this LitR that is different is the subunit structure: the tetramer of *Bacillus* LitR–$AdoB_{12}$ dissociates into dimers, whereas the tetramer of *Thermus* LitR–$AdoB_{12}$ dissociates into monomers. This result was confirmed by two independent analytical methods: gel filtration chromatography and analytical ultracentrifugation.

Fig. 6 represents a working model illustrating the molecular mechanism underlying light-inducible carotenoid production in *B. megaterium* QM B1551 according to our genetic and biochemical analysis. $AdoB_{12}$-LitR (recombinant protein) specifically binds to the promoter region of *crt* and *litR*, repressing their transcription in the dark. Exposure to light transforms the LitR–$AdoB_{12}$ complex to the inactive form, and this event leads to dissociation from the target promoter. Subsequently, the RNA polymerase holoenzyme directs transcription of carotenoid biosynthesis genes, and finally, the expressed *crt* enzymes catalyze carotenoid biosynthesis. Our analysis also revealed detailed promoter structures preceding the *crtI1* and *litR* genes. The DNA site bound by LitR was determined by DNase I footprint analysis: for the *litR* promoter, the region -36 to -67 of the sense strand and region -27 to -69 of the antisense strand relative to the transcriptional start site; for *crtI1* promoter: the region -13 to + 22 of the sense strand and region -15 to + 18 of the antisense strand. Alignment of two LitR-binding sequences yields a definitive consensus sequence, 5′-TG(T/A)A(C/T)A-N_{16}-T(G/A)TA(C/T)A-3′. Determination of the transcriptional start point showed that the regions -10 and -35 of *crt* and *litR* promoters are similar to that of the promoter of σ^A, an essential sigma factor.

The proposed regulatory model of light-inducible carotenoid production in *B. megaterium* seems to be simpler than that of other bacteria such as *S. coelicolor* and *T. thermophilus*. This is because a gene encoding a transcriptional regulator or sigma factor is not located in the region adjacent to *litR*. The simplicity of the light-inducible regulatory mechanism in *B. megaterium* was demonstrated by two experiments: (i) *in vivo* genetic analysis of *Bacillus subtilis* 168 and (ii) an *in vitro* runoff transcriptional assay involving minimal components. In the *in vivo* analysis of *B. subtilis* 168, a heterologous host that lacks a *litR* homolog and *crt* genes, the *B. subtilis* transformant retaining the *litR* gene showed light-inducible transcription. This result indicates that LitR can be responsible for the light dependence of the *crt* transcription without other regulatory proteins. Subsequently, an *in vitro* runoff transcriptional assay reproduced the light-dependent transcription observed *in vivo*. The presence of a non-illuminated LitR–$AdoB_{12}$ complex strongly and specifically inhibits the mRNA synthesis that starts from the *crt* promoter and is directed by the σ^A-containing RNA polymerase holoenzyme. Meanwhile, the illuminated LitR–$AdoB_{12}$ allows the σ^A-RNA polymerase to synthesize mRNA. This is the first *in vitro* evidence showing that a member of the LitR/CarH family, LitR-$AdoB_{12}$, serves as a light-sensitive repressor.

The *in vitro* runoff assay also provided information on the light wavelength and type of Cbl required for the LitR repressor activity. Among Cbl derivatives, $AdoB_{12}$ was the only Cbl that conferred the repressor activity on LitR. In contrast, light wavelengths of 365, 450, and 530 nm inhibit the LitR-repressor activity, whereas illumination at 633 nm does not affect the activity. The light wavelengths that inactivate $AdoB_{12}$–LitR activity

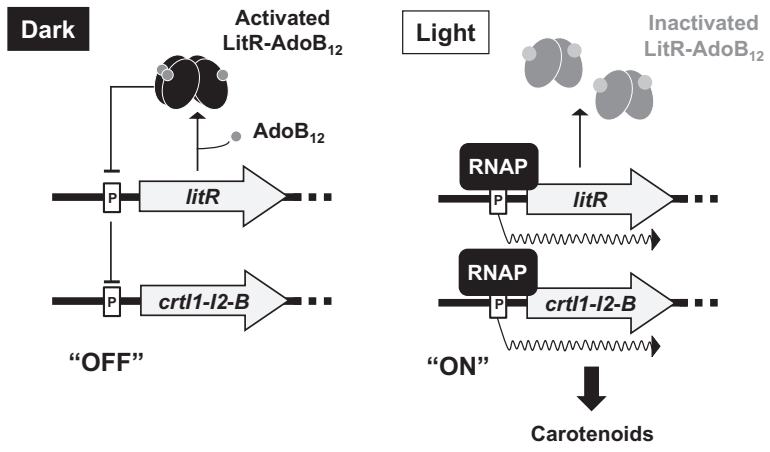

Fig. 6. The working model of light-inducible carotenoid production in *B. megaterium* QM B1551.

Notes: In the dark, LitR-$AdoB_{12}$ associates with the promoter region of *litR* itself and *crtI1*, preventing the transcription initiation. In contrast, illuminated LitR-$AdoB_{12}$ loses the DNA-binding activity because of the photolysis of $AdoB_{12}$, and this event allows the RNA polymerase holoenzyme containing σ^A to direct transcription of the *crt* operon.

are consistent with the absorption spectrum of $AdoB_{12}$. Collectively, these results show that the $AdoB_{12}$–LitR complex serves as a blue and green light-sensitive repressor.

V. Light-inducible gene cluster of *T. thermophilus* HB27

To elucidate the diverse functions of LitR, we performed transcriptomic analysis in *T. thermophilus* and identified 19 LitR-dependent genes that showed 2.0- to 27.5-fold greater transcriptional activity in the *litR* mutant than in the wild-type strain in the dark.[59] As shown in Fig. 4, LitR-dependent genes that are clustered around *litR* itself form four putative operons. The genes encoding a protein with known functions are the five following genes: a methyltransferase (TT_P0045), the α/β-hydrolase fold protein (TT_P0046), short-chain dehydrogenases/reductases (TT_P0049), flavin-containing oxidoreductase (TT_P0050), and YceI-like protein from *E. coli* (TT_P0051). The other products of the light-inducible gene are hypothetical proteins; therefore, the relations of these gene products with light are not known. Therefore, these genes may be novel light-inducible genes. The 30% genes retained by the megaplasmid encode photoresponse-related proteins; 45 of 251 genes are *litR*, *ldrP*, *crt* genes, a B12 biosynthesis gene cluster, and the aforementioned novel light-inducible genes. This finding suggests that the major role of the megaplasmid is to protect the cell from light stress.

Our transcription experiments *in vivo* and *in vitro* demonstrated that a recombinant LdrP protein directly activates the *crtB* promoter and *TT_P0044* promoter (P*44* in Fig. 4). We also identified the minimal region (in the promoter) that is required for the transcriptional activation by LdrP in this same assay. The nucleotide sequence alignment and the mutation analysis of conserved nucleotides yielded a predicted consensus sequence: 5′-(A/G)(T/C)(T/G)TNGCCN(T/G)NG(G/C)NNA-3′, for the LdrP recognition site. The present evidence indicates that the light-dependently expressed LdrP, which is regulated by LitR, serves as a master switch for the set of light-inducible genes.

VI. Concluding remarks and future perspectives

Our research revealed that LitR serves as a general photosensory transcriptional regulator in the light-inducible carotenoid production of nonphototrophic bacteria including not only Gram-negative but also Gram-positive bacteria. The wide distribution of *litR* among bacterial species also indicates that the light stress response during illumination is essential for survival in the harsh soil environment. A major lesson from our study is that light as a general environmental factor can activate a dormant gene responsible for a useful bacterial function. Our research identified novel light-inducible genes in the above bacteria; this means that the LitR family has diverse functions. The progress of our research may uncover a novel light-sensing mechanism and its diversity among bacterial species. This research is also expected to elucidate the interaction of bacteria and sunlight based on the wide distribution and divergent functions of LitR and its homologs.

Acknowledgments

I am most grateful to Prof. Emeritus Teruhiko Beppu and Prof. Kenji Ueda for their continuing encouragement, helpful suggestions, and considerable support of this research. I would like to acknowledge constant help and suggestions from late Prof. Sueharu Horinouchi and Prof. Yasuo Ohnishi (The University of Tokyo). I am deeply indebted to Prof. Haruo Ikeda (Kitasato University), Dr. Akeo Shinkai, Prof. Hideaki Nojiri, and Prof. Akira Nakamura for helpful discussions and for teaching me many valuable experimental methods. I would like to express my appreciation for support during this work to all members of the Laboratory of Biotechnology, Life Science Research Center, College of Bioresource Sciences, Nihon University. I thank H. Takano and S. Sumi for construction of the phylogenetic tree.

Disclosure statement

No potential conflict of interest was reported by the author.

Funding

This study was partly supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology and by various other grants mentioned in the related publications.

References

[1] Herrou J, Crosson S. Function, structure and mechanism of bacterial photosensory LOV proteins. Nat. Rev. Microbiol. 2011;9:713–723.
[2] Purcell EB, Crosson S. Photoregulation in prokaryotes. Curr. Opin. Microbiol. 2008;11:168–178.
[3] Van der Horst MA, Hellingwerf KJ. Photoreceptor proteins, "star actors of modern times": a review of the functional dynamics in the structure of representative members of six different photoreceptor families. Acc. Chem. Res. 2004;37:13–20.
[4] Falciatore A, Bowler C. The evolution and function of blue and red light photoreceptors. Curr. Top. Dev. Biol. 2005;68:317–350.
[5] Herrera-Estrella A, Horwitz BA. Looking through the eyes of fungi: molecular genetics of photoreception. Mol. Microbiol. 2007;64:5–15.
[6] Nisar N, Li L, Lu S, et al. Carotenoid metabolism in plants. Mol. Plant. 2015;8:68–82.
[7] Avalos J. Biological roles of fungal carotenoids. Curr. Genet. 2015;61:309–324.
[8] Takaichi S. Carotenoids in algae: distributions, biosyntheses and functions. Mar. Drugs. 2011;9:1101–1118.
[9] Vachali P, Bhosale P, Bernstein PS. Microbial carotenoids. Methods Mol. Biol. 2012;898:41–59.
[10] Edge R, McGarvey DJ, Truscott TG. The carotenoids as anti-oxidants–a review. J. Photochem. Photobiol. B. 1997;41:189–200.
[11] Takano H, Asker D, Beppu T, et al. Genetic control for light-induced carotenoid production in non-phototrophic bacteria. J. Ind. Microbiol. Biotechnol. 2006;33:88–93.
[12] Koyama Y, Kato F, Yazawa Y. Effect of light on the pigmentation of bacteria in Actinomycetales. In: Arai T, editor. Actinomycetales, the boundary microorganisms. Tokyo: Toppan; 1976. p. 65–85.

[13] Netzer R, Stafsnes MH, Andreassen T, et al. Biosynthetic pathway for γ-cyclic sarcinaxanthin in *Micrococcus luteus*: heterologous expression and evidence for diverse and multiple catalytic functions of C(50) carotenoid cyclases. J. Bacteriol. 2010;192:5688–5699.

[14] Lee HS, Ohnishi Y, Horinouchi S. A sigmaB-like factor responsible for carotenoid biosynthesis in *Streptomyces griseus*. J. Mol. Microbiol. Biotechnol. 2001;3:95–101.

[15] Kato F, Hino T, Nakaji A, et al. Carotenoid synthesis in *Streptomyces setonii* ISP5395 is induced by the gene *crtS*, whose product is similar to a sigma factor. Mol. Gen. Genet. 1995;247:387–390.

[16] Ramakrishnan L, Tran H, Federspiel N, et al. A *crtB* homolog essential for photochromogenicity in *Mycobacterium marinum*: isolation, characterization, and gene disruption via homologous recombination. J. Bacteriol. 1997;179:5862–5868.

[17] Gao L, Groger R, Cox J, et al. Transposon mutagenesis of Mycobacterium marinum identifies a locus linking pigmentation and intracellular survival. Infect. Immun. 2003;71:922–929.

[18] López-Rubio JJ, Padmanabhan S, Lázaro JM, et al. Operator design and mechanism for CarA repressor-mediated down-regulation of the photoinducible CarB operon in Myxococcus xanthus. J. Biol. Chem. 2004;279:28945–28953.

[19] Pérez-Marín MC, López-Rubio JJ, Murillo FJ, et al. The N terminus of Myxococcus xanthus CarA repressor is an autonomously folding domain that mediates physical and functional interactions with both operator dna and antirepressor protein. J. Biol. Chem. 2004;279:33093–33103.

[20] León E, Navarro-Avilés G, Santiveri CM, et al. A bacterial antirepressor with SH3 domain topology mimics operator DNA in sequestering the repressor DNA recognition helix. Nucleic Acids Res. 2010;38:5226–5241.

[21] Whitworth DE, Bryan SJ, Berry AE, et al. Genetic dissection of the light-inducible carqrs promoter region of Myxococcus xanthus. J. Bacteriol. 2004;186:7836–7846.

[22] Browning DF, Whitworth DE, Hodgson DA. Light-induced carotenogenesis in *Myxococcus xanthus*: functional characterization of the ECF sigma factor CarQ and antisigma factor CarR. Mol. Microbiol. 2003;48:237–251.

[23] Gorham HC, McGowan SJ, Robson PR, et al. Light-induced carotenogenesis in *Myxococcus xanthus*: light-dependent membrane sequestration of ECF sigma factor CarQ by anti-sigma factor CarR. Mol. Microbiol. 1996;19:171–186.

[24] Chandra G, Chater KF. Developmental biology of Streptomyces from the perspective of 100 actinobacterial genome sequences. FEMS Microbiol. Rev. 2013;38:345–379.

[25] Liu G, Chater KF, Chandra G, et al. Molecular regulation of antibiotic biosynthesis in streptomyces. Microbiol. Mol. Biol. Rev. 2013;77:112–143.

[26] Houssaini-Iraqui M, Lazraq MH, Clavel-Sérès S, et al. Cloning and expression of *Mycobacterium aurum* carotenogenesis genes in *Mycobacterium smegmatis*. FEMS Microbiol. Lett. 1992;90:239–244.

[27] Krügel H, Krubasik P, Weber K, et al. Functional analysis of genes from *Streptomyces griseus* involved in the synthesis of isorenieratene, a carotenoid with aromatic end groups, revealed a novel type of carotenoid desaturase. Biochim. Biophys. Acta. 1999;1439:57–64.

[28] Bentley SD, Chater KF, Cerdeno-Tarraga AM, et al. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). Nature. 2002;417:141–147.

[29] Takano H, Obitsu S, Beppu T, et al. Light-induced carotenogenesis in Streptomyces coelicolor a3(2): identification of an extracytoplasmic function sigma factor that directs photodependent transcription of the carotenoid biosynthesis gene cluster. J. Bacteriol. 2005;187:1825–1832.

[30] Brown NL, Stoyanov JV, Kidd SP, et al. The MerR family of transcriptional regulators. FEMS Microbiol. Rev. 2003;27:145–163.

[31] Hobman JL. MerR family transcription activators: similar designs, different specificities. Mol. Microbiol. 2007;63: 1275–1278.

[32] Takano H, Beppu T, Ueda K. The CarA/LitR-family transcriptional regulator: Its possible role as a photosensor and wide distribution in non-phototrophic bacteria. Biosci. Biotechnol. Biochem. 2006;70:2320–2324.

[33] Takano H, Hagiwara K, Ueda K. Fundamental role of cobalamin biosynthesis in the developmental growth of *Streptomyces coelicolor* A3 (2). Appl. Microbiol. Biotechnol. 2015;99:2329–2337.

[34] Takano H, Nishiyama T, Amano SI, et al. *Streptomyces* metabolites in divergent microbial interactions. J. Ind. Microbiol. Biotechnol. 2016;43:143–148.

[35] Medema MH, Fischbach MA. Computational approaches to natural product discovery. Nat. Chem. Biol. 2015;11:639–648.

[36] Teh BS, Lau NS, Ng FL, et al. Complete genome sequence of the thermophilic *Thermus* sp. CCB_US3_UF1 from a hot spring in Malaysia. Stand. Genomic Sci. 2015;10:289.

[37] Jentzsch K, Wirtz A, Circolone F, et al. Mutual exchange of kinetic properties by extended mutagenesis in two short LOV domain proteins from Pseudomonas putida. Biochemistry. 2009;48:10321–10333.

[38] Bamann C, Bamberg E, Wachtveitl J, et al. Proteorhodopsin. Biochim. Biophys. Acta. 1837;2014:614–625.

[39] Brown LS. Eubacterial rhodopsins — unique photosensors and diverse ion pumps. Biochim. Biophys. Acta. 2014;1837:553–561.

[40] Land M, Lapidus A, Mayilraj S, et al. Complete genome sequence of Actinosynnema mirum type strain (101T). Stand. Genomic Sci. 2009;1:46–53.

[41] Ichikawa N, Oguchi A, Ikeda H, et al. Genome sequence of Kitasatospora setae nbrc 14216t: an evolutionary snapshot of the family Streptomycetaceae. DNA Res. 2010;17:393–406.

[42] Zhao W, Zhong Y, Yuan H, et al. Complete genome sequence of the rifamycin SV-producing *Amycolatopsis mediterranei* U32 revealed its genetic characteristics in phylogeny and metabolism. Cell Res. 2010;20:1096–1108.

[43] Henne A, Brüggemann H, Raasch C, et al. The genome sequence of the extreme thermophile *Thermus thermophilus*. Nat. Biotechnol. 2004;22:547–553.

[44] Brüggemann H, Chen C. Comparative genomics of *Thermus thermophilus*: plasticity of the megaplasmid and its contribution to a thermophilic lifestyle. J. Biotechnol. 2006;124:654–661.

[45] Eppinger M, Bunk B, Johns MA, et al. Genome sequences of the biotechnologically important Bacillus megaterium strains QM B1551 and DSM319. J. Bacteriol. 2011;193:4199–4213.

[46] Bunk B, Schulz A, Stammen S, et al. A short story about a big magic bug. Bioeng Bugs. 2010;1:85–91.

[47] Takano H, Kondo M, Usui N, et al. Involvement of CarA/LitR and CRP/FNR family transcriptional regulators in light-induced carotenoid production in Thermus thermophilus. J. Bacteriol. 2011;193:2451–2459.

[48] Green J, Scott C, Guest JR. Functional versatility in the CRP-FNR superfamily of transcription factors: FNR and FLP. Adv. Microb. Physiol. 2001;44:1–34.

[49] Yokoyama A, Shizuri Y, Hoshino T, et al. Thermocryptoxanthins: novel intermediates in the carotenoid biosynthetic pathway of *Thermus thermophilus*. Arch. Microbiol. 1996;165:342–345.

[50] Agari Y, Kuramitsu S, Shinkai A. X-ray crystal structure of TTHB099, a CRP/FNR superfamily transcriptional regulator from Thermus thermophilus HB8, reveals a DNA-binding protein with no required allosteric effector molecule. Proteins. 2012;80:1490–1494.

[51] Popovych N, Tzeng SR, Tonelli M, et al. Structural basis for cAMP-mediated allosteric control of the catabolite activator protein. Proc. Natl. Acad. Sci. USA. 2009;106:6927–6932.

[52] Ortiz-Guerrero JM, Polanco MC, Murillo FJ, et al. Light-dependent gene regulation by a coenzyme B12-based photoreceptor. Proc. Natl. Acad. Sci. USA. 2011;108:7565–7570.

[53] Díez AI, Ortiz-Guerrero JM, Ortega A, et al. Analytical ultracentrifugation studies of oligomerization and DNA-binding of TtCarH, a *Thermus thermophilus* coenzyme B12-based photosensory regulator. Eur. Biophys. J. 2013;42:463–476.

[54] Mondol MA, Shin HJ, Islam MT. Diversity of secondary metabolites from marine bacillus species: chemistry and biological activity. Mar. Drugs. 2013;11:2846–2872.

[55] Maeda I. Genetic modification in *Bacillus subtilis* for production of C30 carotenoids. Methods Mol. Biol. 2012;892:197–205.

[56] Steiger S, Perez-Fons L, Fraser PD, et al. Biosynthesis of a novel C30 carotenoid in *Bacillus firmus* isolates. J. Appl. Microbiol. 2012;113:888–895.

[57] Khaneja R, Perez-Fons L, Fakhry S, et al. Carotenoids found in *Bacillus*. J. Appl .Microbiol. 2010;108:1889–1902.

[58] Takano H, Mise K, Hagiwara K, et al. Role and function of LitR, an Adenosyl B 12 -bound light-sensitive regulator of bacillus megaterium QM b1551, in regulation of carotenoid production. J. Bacteriol. 2015;197:2301–2315.

[59] Takano H, Agari Y, Hagiwara K, et al. LdrP, a cAMP receptor protein/FNR family transcriptional regulator, serves as a positive regulator for the light-inducible gene cluster in the megaplasmid of *Thermus thermophilus*. Microbiology. 2014;160: 2650–2660.

[60] Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 2011;28:2731–2739.