Full Paper

Comparison of gene expression levels of *appA*, *ppsR*, and *EL368* in *Erythrobacter litoralis* spheroplasts under aerobic and anaerobic conditions, and under blue light, red light, and dark conditions

(Received July 3, 2017; Accepted September 29, 2017; J-STAGE Advance publication date: March 31, 2018)

Koki Nishino, Sawako Takahashi, and Hiromi Nishida*

Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University,
5180 Kurokawa, Imizu, Toyama 939-0398, Japan

We compared the gene expression levels of the blue-light-responsive genes, *appA* (encoding photosynthesis promoting protein AppA), *ppsR* (encoding photosynthesis suppressing protein PpsR), and *EL368* (encoding a blue-light-activated histidine kinase with a light, oxygen, or voltage domain) between aerobic and anaerobic conditions in spheroplasts of the aerobic photosynthetic bacterium *Erythrobacter litoralis*. The spheroplasts conducted photosynthesis under red light but not under blue light. All three blue-light-responsive genes showed higher expression under aerobic conditions than under anaerobic conditions under blue light. In contrast, under red light, although the expression level of *appA* was higher in the presence of oxygen than in the absence of oxygen, the expression levels of *ppsR* and *EL368* were similar in the presence and absence of oxygen. Our findings demonstrate that the expression of blue-light-responsive genes is strongly affected by oxygen in *E. litoralis* spheroplasts.

Key Words: blue light; *Erythrobacter litoralis*; oxygen; photosynthesis; spheroplast enlargement

Introduction

Purple bacteria regulate their photosynthesis genes according to light and oxygen conditions. PpsR and AppA play a central role in the regulation of photosynthesis genes (Elsen et al., 2005; Gomelsky and Kaplan, 1995; Masuda and Bauer, 2002). In *Rhodobacter sphaeroides*, a facultative

anaerobic anoxygenic purple bacterium, PpsR binds to the promoters of photosynthesis genes to repress their expression. When AppA undergoes structural alterations to bind to PpsR, the AppA-PpsR complex is formed and PpsR leaves the promoter (Masuda and Bauer, 2002). Interestingly, blue light disrupts the AppA-PpsR complex, leading to repression of photosynthesis gene expression (Masuda and Bauer, 2002).

In contrast, *Erythrobacter litoralis*, an aerobic anoxygenic purple bacterium, contains blue-light-activated histidine kinases, which contain a light, oxygen, or voltage (LOV) domain at the N-terminus and histidine kinase at the C-terminus (Swartz et al., 2007). Blue light regulates kinase activity and subsequent downstream effects (Correa et al., 2013). In this study, we selected *EL368* as a gene encoding a blue-light-activated histidine kinase with an LOV domain. *EL368* is a stress response gene, which may be not related to photosynthesis (Correa et al., 2013).

Although continuous light inhibited the enlargement of *E. litoralis* spheroplasts (Takayanagi et al., 2016), these cells are enlarged under both anaerobic and aerobic light-dark (12 h each) conditions (Nakazawa and Nishida, 2017). In addition, *E. litoralis* spheroplasts did not enlarge under blue light but were enlarged under green and red lights at 144 h of growth (under light-dark conditions, each 12 h) (Nishino and Nishida, 2017).

Comparing the gene expression levels between spheroplasts of *E. litoralis* at the beginning of growth and enlarged spheroplasts at 96 h of growth under aerobic and dark conditions, although *appA* and *EL368* were not significantly different, *ppsR* was significantly upregulated in enlarged spheroplasts (Takahashi et al., 2016).

In this study, we compared the expression levels of homologs of *appA* and *ppsR*, as well as *EL368*, between

*Corresponding author: Hiromi Nishida, Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan.

Tel: (+81)-766567500 Fax: (+81)-766562498 E-mail: hnishida@pu-toyama.ac.jp

None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

Table 1. Quantification cycle (Cq) values.

Condition	Gene	Cq value	Condition	Gene	Cq value
Aerobic, Blue	<i>appA</i>	27.02	Anaerobic, Blue	<i>appA</i>	31.59
Aerobic, Blue	<i>appA</i>	27.42	Anaerobic, Blue	<i>appA</i>	31.48
Aerobic, Blue	<i>appA</i>	28.17	Anaerobic, Blue	<i>appA</i>	34.21
Aerobic, Blue	<i>ppsR</i>	27.21	Anaerobic, Blue	<i>ppsR</i>	33.60
Aerobic, Blue	<i>ppsR</i>	29.33	Anaerobic, Blue	<i>ppsR</i>	31.48
Aerobic, Blue	<i>ppsR</i>	28.11	Anaerobic, Blue	<i>ppsR</i>	31.79
Aerobic, Blue	<i>EL368</i>	33.26	Anaerobic, Blue	<i>EL368</i>	36.27
Aerobic, Blue	<i>EL368</i>	28.99	Anaerobic, Blue	<i>EL368</i>	36.78
Aerobic, Blue	<i>EL368</i>	32.57	Anaerobic, Blue	<i>EL368</i>	36.94
Aerobic, Red	<i>appA</i>	26.30	Anaerobic, Red	<i>appA</i>	28.61
Aerobic, Red	<i>appA</i>	26.08	Anaerobic, Red	<i>appA</i>	28.62
Aerobic, Red	<i>appA</i>	26.32	Anaerobic, Red	<i>appA</i>	28.84
Aerobic, Red	<i>ppsR</i>	25.91	Anaerobic, Red	<i>ppsR</i>	27.32
Aerobic, Red	<i>ppsR</i>	26.86	Anaerobic, Red	<i>ppsR</i>	28.27
Aerobic, Red	<i>ppsR</i>	26.52	Anaerobic, Red	<i>ppsR</i>	27.02
Aerobic, Red	<i>EL368</i>	30.88	Anaerobic, Red	<i>EL368</i>	33.50
Aerobic, Red	<i>EL368</i>	31.68	Anaerobic, Red	<i>EL368</i>	32.73
Aerobic, Red	<i>EL368</i>	31.72	Anaerobic, Red	<i>EL368</i>	32.04
Aerobic, Dark	<i>appA</i>	27.73	Anaerobic, Dark	<i>appA</i>	28.76
Aerobic, Dark	<i>appA</i>	27.82	Anaerobic, Dark	<i>appA</i>	29.29
Aerobic, Dark	<i>appA</i>	28.38	Anaerobic, Dark	<i>appA</i>	29.77
Aerobic, Dark	<i>ppsR</i>	29.84	Anaerobic, Dark	<i>ppsR</i>	27.86
Aerobic, Dark	<i>ppsR</i>	32.18	Anaerobic, Dark	<i>ppsR</i>	ND
Aerobic, Dark	<i>ppsR</i>	30.91	Anaerobic, Dark	<i>ppsR</i>	28.48
Aerobic, Dark	<i>EL368</i>	32.66	Anaerobic, Dark	<i>EL368</i>	30.93
Aerobic, Dark	<i>EL368</i>	35.62	Anaerobic, Dark	<i>EL368</i>	31.12
Aerobic, Dark	<i>EL368</i>	29.52	Anaerobic, Dark	<i>EL368</i>	31.61

Table 2. *P* values in pairwise *t* test adjusted by Bonferroni method.

	1	2	3	4	5	6	7	8
2	3.8×10^{-2}							
3	1.3×10^{-4}	1						
4	1	9.6×10^{-1}	8.3×10^{-3}					
5	1.3×10^{-3}	1	1	4.3×10^{-2}				
6	4.9×10^{-12}	7.3×10^{-6}	3.3×10^{-3}	6.2×10^{-10}	1.0×10^{-2}			
7	1	1.9×10^{-2}	5.6×10^{-5}	1	6.5×10^{-4}	1.9×10^{-12}		
8	1.6×10^{-4}	1	1	9.8×10^{-3}	1	2.8×10^{-3}	6.7×10^{-5}	
9	2.8×10^{-11}	3.7×10^{-5}	1.3×10^{-2}	3.5×10^{-9}	3.5×10^{-2}	1	1.1×10^{-11}	1.1×10^{-2}

1, *appA* under blue light; 2, *appA* under red light; 3, *appA* in the dark; 4, *ppsR* under blue light; 5, *ppsR* under red light; 6, *ppsR* in the dark; 7, *EL368* under blue light; 8, *EL368* under red light; and 9, *EL368* in the dark.

anaerobic and aerobic conditions under blue, red, or dark conditions.

Materials and Methods

Cultivation of *E. litoralis* spheroplasts. Cells of *E. litoralis* NBRC 102620 were grown on marine broth agar (Difco, Detroit, MI, USA). The harvested cells (approximately 3 mg) were suspended in a buffer (1 mL) consisting of 0.1 M Tris-HCl (pH 7.4) and 0.3 M sucrose. Lysozyme (Wako Co., Osaka, Japan) (200 μ g/mL) was added to the cell suspension, which was then incubated at 25°C for 15 min. After centrifugation for 5 min at 3000 rpm, the cells were suspended in marine broth (1 mL) containing 600 μ g/mL penicillin G (Serva, Heidelberg, Germany). The suspension was then diluted by adding 160 μ L of the suspension to marine broth (80 mL) containing penicillin G. The spheroplasts were incubated at 25°C under aero-

bic and anaerobic light-dark (12 h each) conditions. BMS-PS08RGB3 (Bio Medical Science, Tokyo, Japan) was used for the light conditions. Two different lights, blue (400–500 nm) and red (600–700 nm), were used. The spheroplasts were cultured for 36 h (light-dark-light conditions, each 12 h). Blue and red light intensities were 25 μ mol/m²/s and 27 μ mol/m²/s, respectively. The cells were sampled immediately at 36 h of growth. BIONIX2 (Sugiyama-Gen, Tokyo, Japan) was used for culture under oxygen-free conditions. The culture dish was placed in a plastic bag, and a capsule of deoxidizing agent was opened and placed in the plastic bag with an oxygen meter.

RNA isolation. Total RNA was isolated using the NucleoSpin RNA kit (Macherey-Nagel, Düren, Germany). RA1 buffer (1000 μ L) was added to the culture dish and 350 μ L of the total RA1 buffer (1000 μ L) was used. After

Fig. 1. Boxplots of delta Cq values.

We calculated the delta Cq value as follows: (Cq value under anaerobic conditions – Cq value under aerobic conditions). The p values in the pairwise t test among delta Cq values under each condition with Bonferroni adjustment are shown in Table 2. X-axis: 1, *appA* under blue light; 2, *appA* under red light; 3, *appA* in the dark; 4, *ppsR* under blue light; 5, *ppsR* under red light; 6, *ppsR* in the dark; 7, *EL368* under blue light; 8, *EL368* under red light; and 9, *EL368* in the dark. Y-axis indicates delta Cq values.

Fig. 2. Comparison of gene expression levels between aerobic and anaerobic conditions.

Large slope, median of delta Cq values > 4; small slope, 4 > median of delta Cq values > 1.5 or -1.5 > median of delta Cq values > -4; equilibrium, 1.5 > median of delta Cq values > -1.5.

removing rRNA using the RiboMinus Transcriptome Isolation Kit for bacteria (Thermo Fisher Science, Waltham, MA, USA), the RNA was purified and concentrated with the NucleoSpin RNA Clean-up XS kit (Macherey-Nagel).

Quantitative RT-PCR. Of the RNA solution, 2 μ L (0.1 ng/ μ L) was used for quantitative real-time PCR. Thus, based on the genome DNA sequence of *E. litoralis* NBRC 102620, we designed 3 primer sets to detect the mRNA expression of 3 genes. The mRNAs were amplified with the One Step SYBR PrimeScript PLUS RT-PCR Kit (TaKaRa, Shiga, Japan) using the LightCycler Nano system (Roche, Basel, Switzerland). PCR was performed using the following cycling conditions: 1 cycle of reverse transcription at 42°C for 300 s and 95°C for 10 s, 40 cycles of denaturation (95°C for 5 s), and annealing and extension (60°C for 30 s). After extension, a melting curve cycle was performed from 60°C to 95°C at 0.1°C/s to confirm the absence of non-specific products. Quantification cycle (Cq) values were obtained using LightCycler Nano Software (Roche). The primers 5'-TGTGCTCGCTCAGTATCTCG-3' and 5'-GGCGGTTCTTTCAGACACTC-3' were used for *appA*; the primers 5'-GATGAAGCTTGCCATGGTG-3' and 5'-CCTTCGAAAGAGGGGAAATC-3' were used for *ppsR*; and the primers 5'-TGCGATAATTGAGCAGTTCG-3' and 5'-CAGGGACCAAAGACAGATCC-3' were used for *EL368*. DNA contamination was checked by PCR without reverse transcription.

Statistical analysis. We calculated delta Cq values as follows: (Cq value under anaerobic conditions – Cq value under aerobic conditions). We performed a pairwise *t* test for delta Cq values under each condition with Bonferroni adjustment using the statistical software R (<http://www.r-project.org/>).

Results and Discussion

The Cq values from quantitative RT-PCR analysis are shown in Table 1. The *p* values determined by the pairwise *t* test and adjusted by Bonferroni correction are shown in Table 2.

Typically, *E. litoralis* conducts photosynthesis in the presence of oxygen, while spheroplasts conduct photosynthesis in both the presence and absence of oxygen (Nakazawa and Nishida, 2017). In this study, *E. litoralis* spheroplasts were enlarged under red light. The expression level of the photosynthesis-promoting gene *appA* was higher in the presence of oxygen than in the absence of oxygen (Fig. 1). In contrast, the expression level of the photosynthesis-suppressor gene *ppsR* was similar in the presence and absence of oxygen (Fig. 1). These results suggest that although *E. litoralis* spheroplasts photosynthesize in the presence and absence of oxygen, this activity may be stronger in the presence than in the absence of oxygen.

In the dark, *appA* showed no difference in expression level, regardless of the presence or absence of oxygen. *ppsR* expression was lower in the presence of oxygen than in the absence of oxygen (Fig. 1).

In addition, blue light irradiation inhibits the enlargement of *E. litoralis* spheroplasts, suggesting that photosynthesis did not occur under blue light (Nishino and Nishida, 2017). Under blue light, the gene expression of both *appA* and *ppsR* were higher in the presence of oxygen. Although blue light inhibits photosynthesis (Masuda and Bauer, 2002), the photosynthesis-promoting gene *appA* was more strongly expressed in the presence of oxygen (Figs. 1 and 2). Blue light irradiation causes cell death of *E. litoralis* spheroplasts (Nishino and Nishida, 2017). Thus, the regulation of these two genes may be abnormal under blue light.

Although the gene expression level of the blue-light-activated histidine kinase coding for *EL368* was nearly the same under red light in the presence and absence of oxygen, expression was higher under blue light in the presence of oxygen (Figs. 1 and 2). Under dark conditions, the gene expression level of *EL368* was higher in the absence of oxygen (Fig. 1), suggesting that the stress level may be higher in the absence of oxygen for *E. litoralis* spheroplasts. Thus, the stress level may be highest under blue light in the presence of oxygen. This is consistent with the fact that blue light irradiation inhibits enlargement of *E. litoralis* spheroplasts even in the presence of oxygen.

Acknowledgments

We would like to thank Editage for English language editing. This work was supported by a grant from The Cannon Foundation (to HN) and JSPS KAKENHI Grant Number 16K14891 (to HN).

References

- Correa, F., Ko, W. H., Ocasio, V., Bogomolni, R. A., and Grardner, K. H. (2013) Blue light regulated two-component systems: enzymatic and functional analyses of light-oxygen-voltage (LOV)-histidine kinases and downstream response regulators. *Biochemistry*, **52**, 4656–4666.
- Elsen, S., Jaubert, M., Pignol, D., and Giraud, E. (2005) PpsR: a multifaceted regulator of photosynthesis gene expression in purple bacteria. *Mol. Microbiol.*, **57**, 17–26.
- Gomelsky, M. and Kaplan, S. (1995) *appA*, a novel gene encoding a *trans*-acting factor involved in the regulation of photosynthesis gene expression in *Rhodobacter sphaeroides* 2.4.1. *J. Bacteriol.*, **177**, 4609–4618.
- Masuda, S. and Bauer, C. E. (2002) AppA is a blue light photoreceptor that antirepresses photosynthesis gene expression in *Rhodobacter sphaeroides*. *Cell*, **110**, 613–623.
- Nakazawa, M. and Nishida, H. (2017) Effects of light and oxygen on the enlargement of *Erythrobacter litoralis* spheroplasts. *J. Gen. Appl. Microbiol.*, **63**, 58–61.
- Nishino, K. and Nishida, H. (2017) Blue light inhibits the enlargement of *Erythrobacter litoralis* spheroplasts. *J. Gen. Appl. Microbiol.*, **63**, 203–206.
- Swartz, T. E., Tseng, T. S., Frederickson, M. A., Paris, G., Comerchi, D. J. et al. (2007) Blue-light-activated histidine kinases: two-component sensors in bacteria. *Science*, **317**, 1090–1093.
- Takayanagi, S., Takayanagi, A., Takahashi, Y., Oshima, T., and Nishida, H. (2016) Comparison of transcriptomes of enlarged spheroplasts of *Erythrobacter litoralis* and *Lelliottia amnigena*. *AIMS Microbiol.*, **2**, 152–189.
- Takayanagi, A., Takahashi, S., and Nishida, H. (2016) Requirement of dark culture condition for enlargement of spheroplasts of the aerobic anoxygenic photosynthetic marine bacterium *Erythrobacter litoralis*. *J. Gen. Appl. Microbiol.*, **62**, 14–17.