

## Short Communication

### Blue light inhibits the enlargement of *Erythrobacter litoralis* spheroplasts

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The aerobic anoxygenic photosynthetic marine bacterium *Erythrobacter litoralis* belongs to the class Alphaproteobacteria (purple bacteria), which contains bacteriochlorophyll *a* and carotenoids (Yurkov and Beatty, 1998). The anoxygenic photosynthetic apparatus functions to transform light energy into an electrochemical gradient of H<sup>+</sup> across the inner membrane (Niwa et al., 2014; Roszak et al., 2003; Yurkov and Beatty, 1998). The purple bacterial photosynthetic electron transport system might share components with the cell respiratory system (Harashima et al., 1982, 1987; Yurkov and Beatty, 1998).

In the presence of penicillin, *E. litoralis* spheroplasts did not divide but grew and enlarged in marine broth under aerobic and dark conditions (Takayanagi et al., 2016). We have shown the spheroplast growth at 24 h, 48 h, and 96 h, at 25°C (Takayanagi et al., 2016). The optimal culture conditions and gene expression patterns for spheroplast enlargement differed from those required for cell division (Takahashi et al., 2016; Takayanagi et al., 2016). Although continuous light inhibited their enlargement (Takayanagi et al., 2016), the spheroplasts enlarged under not only aerobic but also anaerobic light-dark (12 h each) conditions (Nakazawa and Nishida, 2017). Thus, light-dark signaling leads to the enlargement of *E. litoralis* spheroplasts under anaerobic conditions.

In this study, we estimated the effects of the color of light on *E. litoralis* spheroplast enlargement.

*E. litoralis* spheroplasts were prepared using a method described by Takayanagi et al. (2016). The spheroplasts were incubated at 25°C in aerobic and anaerobic light-dark (12 h each) conditions. A cold cathode fluorescent lamp LH-30-8CT (Nippon Medical & Chemical Instruments, Osaka, Japan) was used for the light condition. Three different lights, blue (400–500 nm, 25  $\mu\text{mol}/\text{m}^2/\text{s}$ ), green (500–600 nm, 30  $\mu\text{mol}/\text{m}^2/\text{s}$ ), and red (600–700 nm, 27  $\mu\text{mol}/\text{m}^2/\text{s}$ ), were used. Spheroplasts isolated from two

different growth time points (48 h and 144 h) were used for the morphological observation. We used BIONIX2 (Sugiyama-Gen, Tokyo, Japan) 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.

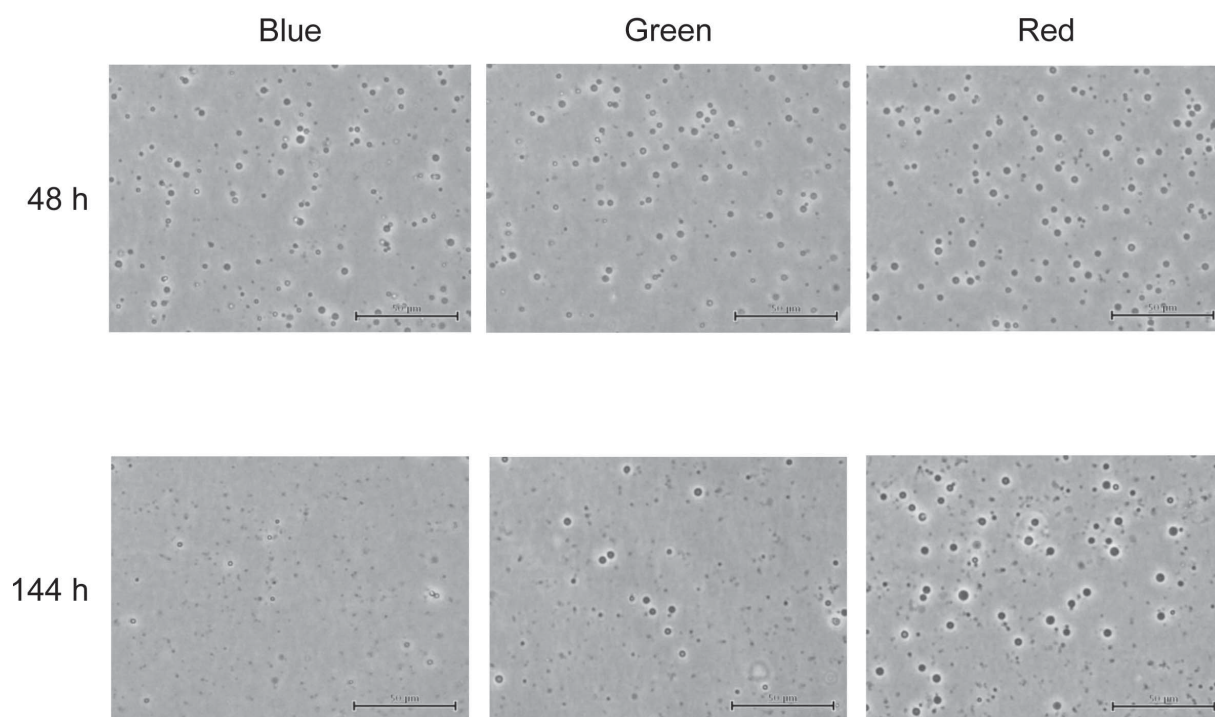
Although we observed that some of *E. litoralis* spheroplasts enlarged at 48 h of growth under aerobic conditions, they did not enlarge under blue light but enlarged under green and red lights at 144 h of growth (Figs. 1A and 2A), suggesting that the enlarged spheroplasts at 48 h shrunk or disrupted after 48 h of growth under blue light. On the other hand, under anaerobic conditions, *E. litoralis* spheroplasts did not enlarge under blue light but enlarged under green and red lights (Figs. 1B and 2B). Blue light represses photosynthesis gene expression in the purple bacterium *Rhodobacter sphaeroides*, which is controlled by the proteins AppA and PpsR (Masuda and Bauer, 2002). A protein sequence similarity search (e.g., Blastp at the National Center for Biotechnology Information) indicated that *E. litoralis* has homologs of both AppA and PpsR. These are WP\_051697460.1 (EH32\_04550) and WP\_034904402.1 (EH32\_13755), respectively. This suggests that blue light may also inhibit photosynthesis gene expression in *E. litoralis* spheroplasts. In addition, blue-light-activated histidine kinases of *E. litoralis* have been identified (Swartz et al., 2007), indicating that *E. litoralis* has a system to respond to blue light signals.

In a comparison of the enlarged spheroplasts of *E. litoralis* exposure to green and red lights, those cultured under red light were larger than those cultured under green light (Figs. 1 and 2). This result indicates that red light does not inhibit the enlargement of *E. litoralis* spheroplasts. In a comparison of green and red lights, the enlargement effect under green light was weaker than that under red light. It is uncertain whether small black parti-

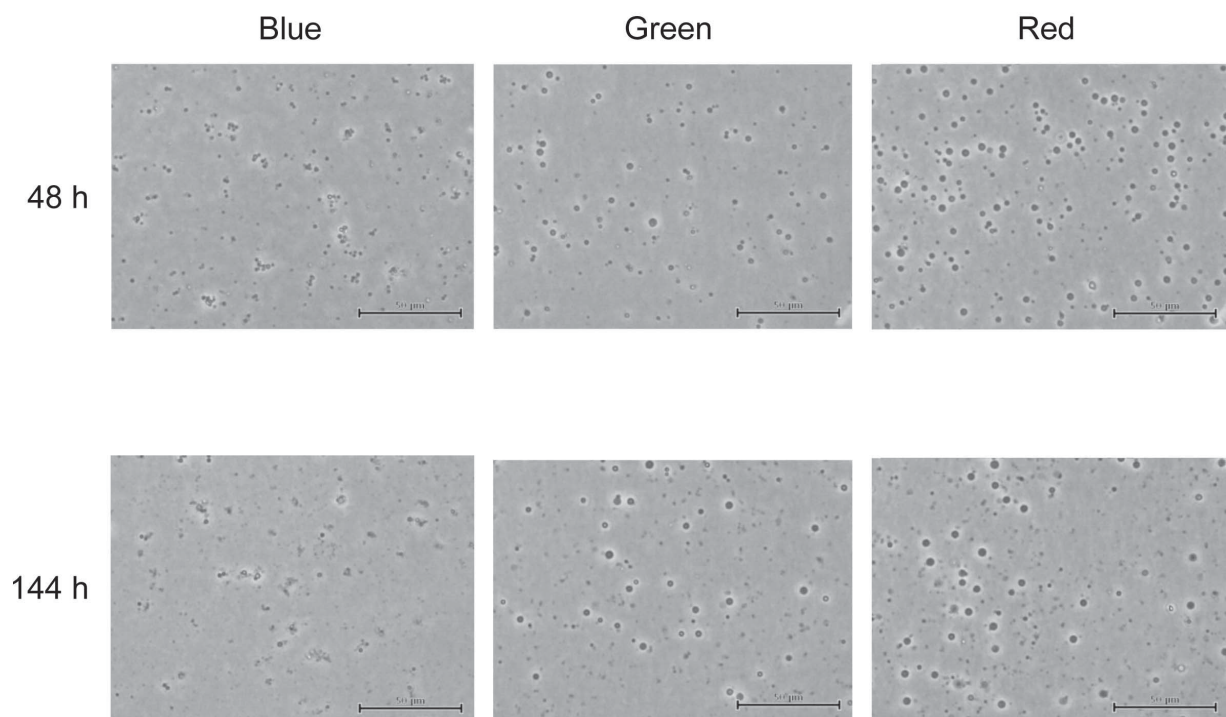
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## (A) Aerobic condition



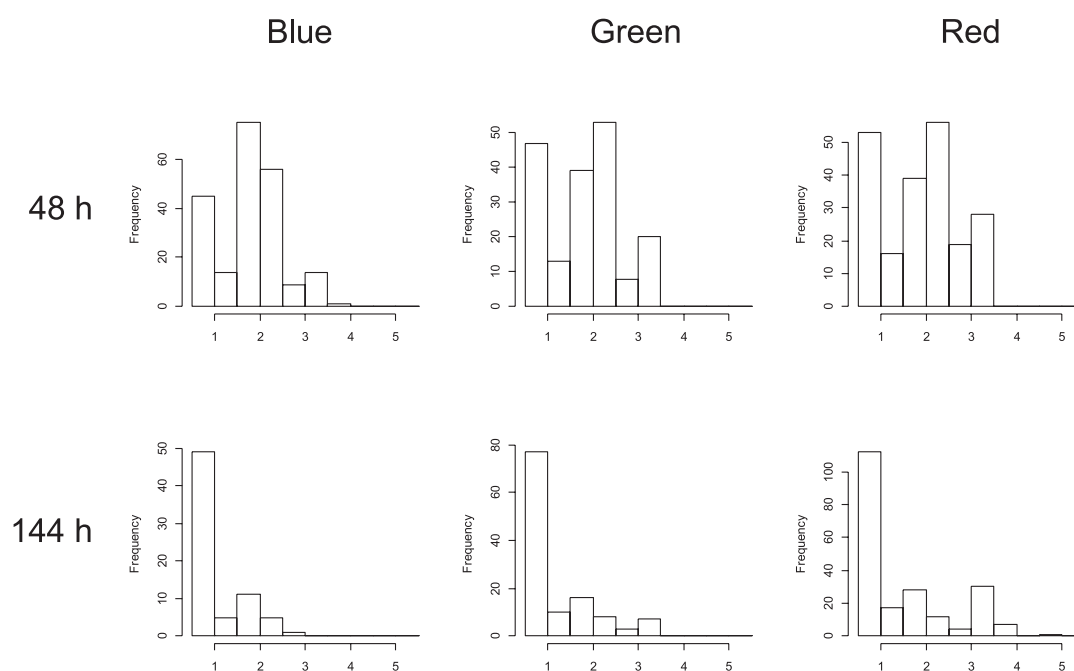
## (B) Anaerobic condition



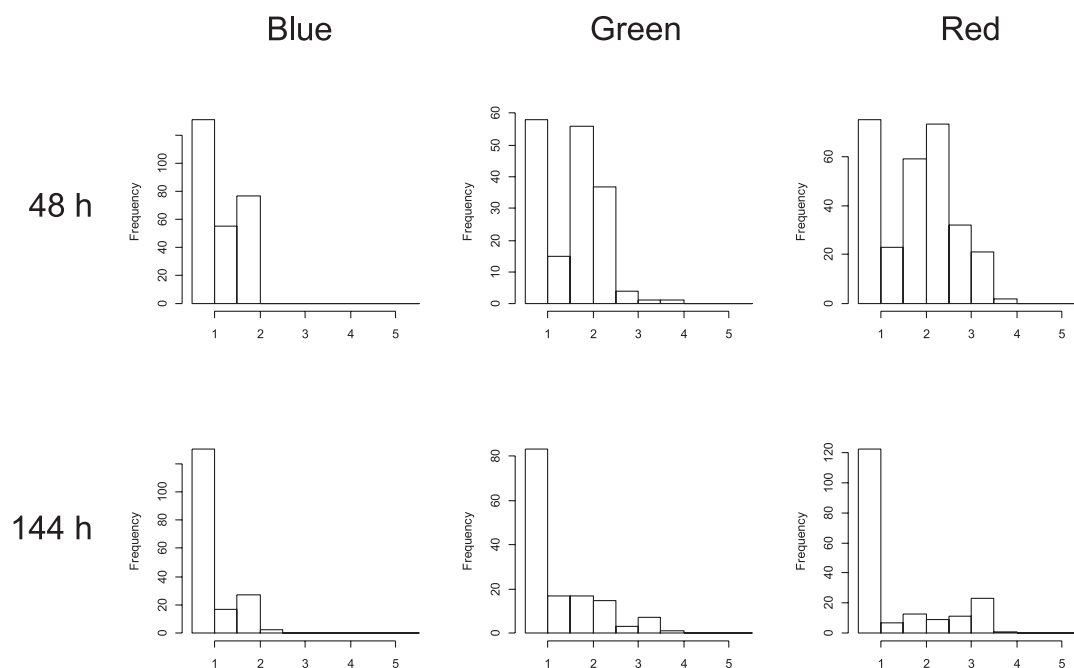
**Fig. 1.** Phase-contrast micrographs of *Erythrobacter litoralis* cells.

(A) Cells under aerobic conditions. (B) Cells under anaerobic (oxygen-free) conditions. Phase-contrast images were obtained using an Olympus CKK41 microscope; scale bar = 50  $\mu\text{m}$ .

## (A) Aerobic condition



## (B) Anaerobic condition

**Fig. 2.** Histograms showing the diameters of *Erythrobacter litoralis* cells.

(A) Cells under aerobic conditions. (B) Cells under anaerobic (oxygen-free) conditions. Cells with  $0.8 \mu\text{m}$  or less in diameter were not counted.

cles observed in micrographs (Fig. 1) were dead or alive.

Our findings strongly suggest that *E. litoralis* spheroplasts respond to light signals and may perform photosynthesis.

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