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Short Communication

Effects of light and oxygen on the enlargement of *Erythrobacter litoralis* spheroplasts

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Photosynthetic purple bacteria contain inner and outer membranes. Photosynthesis-related proteins are present within the inner membrane (Niwa et al., 2014; Roszak et al., 2003). The anoxygenic photosynthetic apparatus of purple bacteria transforms light energy into an electrochemical gradient of H^+ ions across the inner membrane (Yurkov and Beatty, 1998). Thus, photosynthesis in purple bacteria occurs in a manner similar to respiration. *Erythrobacter litoralis*, an aerobic anoxygenic photosynthetic marine bacterium, belongs to Alphaproteobacteria (purple bacteria), which produce bacteriochlorophyll *a* and carotenoids (Yurkov and Beatty, 1998).

In the presence of penicillin, *E. litoralis* spheroplasts do not divide but grow and enlarge in marine broth under aerobic and dark conditions (Takayanagi et al., 2016). Continuous light inhibits the enlargement of *E. litoralis* spheroplasts (Takayangi et al., 2016). Enlarged spheroplasts show upregulation of the Mg^{2+} influx gene and downregulation of the Mg^{2+} efflux gene (Takahashi et al., 2016). Bacteriochlorophyll requires Mg^{2+} (Kirmaier and Holten, 1987), suggesting the occurrence of photosynthesis in enlarged spheroplasts.

In this study, we have examined the effects of light and oxygen on the enlargement of *E. litoralis* spheroplasts and we discuss the photosynthetic ability of enlarged spheroplasts.

E. litoralis spheroplasts were prepared using a method described by Takayanagi et al. (2016). Briefly, *E. litoralis* spheroplasts were incubated at 25°C in dark, light-dark (12 h each), and light conditions. A fluorescent lamp was used for maintaining the light condition. Spheroplasts isolated from three growth time points (48, 72, and 144 h) were used for morphological evaluation. BIONIX2 (Sugiyama-Gen, Tokyo, Japan) was used for culturing spheroplasts under oxygen-free conditions. Culture dishes were placed in a plastic bag, and a capsule containing a deoxidizing agent was opened and placed in the plastic bag along with an oxygen meter.

We observed that *E. litoralis* spheroplasts did not enlarge under anaerobic dark conditions, but enlarged under aerobic dark conditions (Figs. 1 and 2), indicating that *E. litoralis* spheroplasts required oxygen in the dark for enlargement. This result strongly suggests that ATP generation through respiration is required for the enlargement of *E. litoralis* spheroplasts in the dark.

In intact *Erythrobacter* cells, ATP is synthesized through photosynthesis under aerobic conditions, but not under anaerobic conditions (Okamura et al., 1986). Furthermore, *E. litoralis* spheroplasts enlarged under both anaerobic and aerobic light-dark (each for 12 h) conditions (Figs. 1 and 2). Enlargement of *E. litoralis* spheroplasts under anaerobic light-dark conditions (Figs. 1 and 2) suggests that these spheroplasts synthesized ATP through photosynthesis under anaerobic conditions. At least, the light-dark conditions lead to the enlargement of *E. litoralis* spheroplasts under anaerobic condition. However, at 144 h of growth, the number of enlarged spheroplasts under anaerobic light-dark conditions was less than that under aerobic dark conditions (Fig. 2), which suggests that the light-dark effect on spheroplast enlargement is weaker than the respiration effect.

The photosynthetic apparatus of purple bacteria is synthesized in the dark (Harashima et al., 1987; Yurkov and Beatty, 1998), indicating that dark conditions are essential for photosynthesis. Under continuous light, enlarged *E. litoralis* spheroplasts were observed at 48 and 72 h of growth, but were not observed at 144 h of growth, under both anaerobic and aerobic conditions (Figs. 1 and 2). This result is consistent with that of a previous study (Takayanagi et al., 2016). Continuous light damages the photosynthetic system. However, inhibition of spheroplast enlargement by continuous light under aerobic conditions

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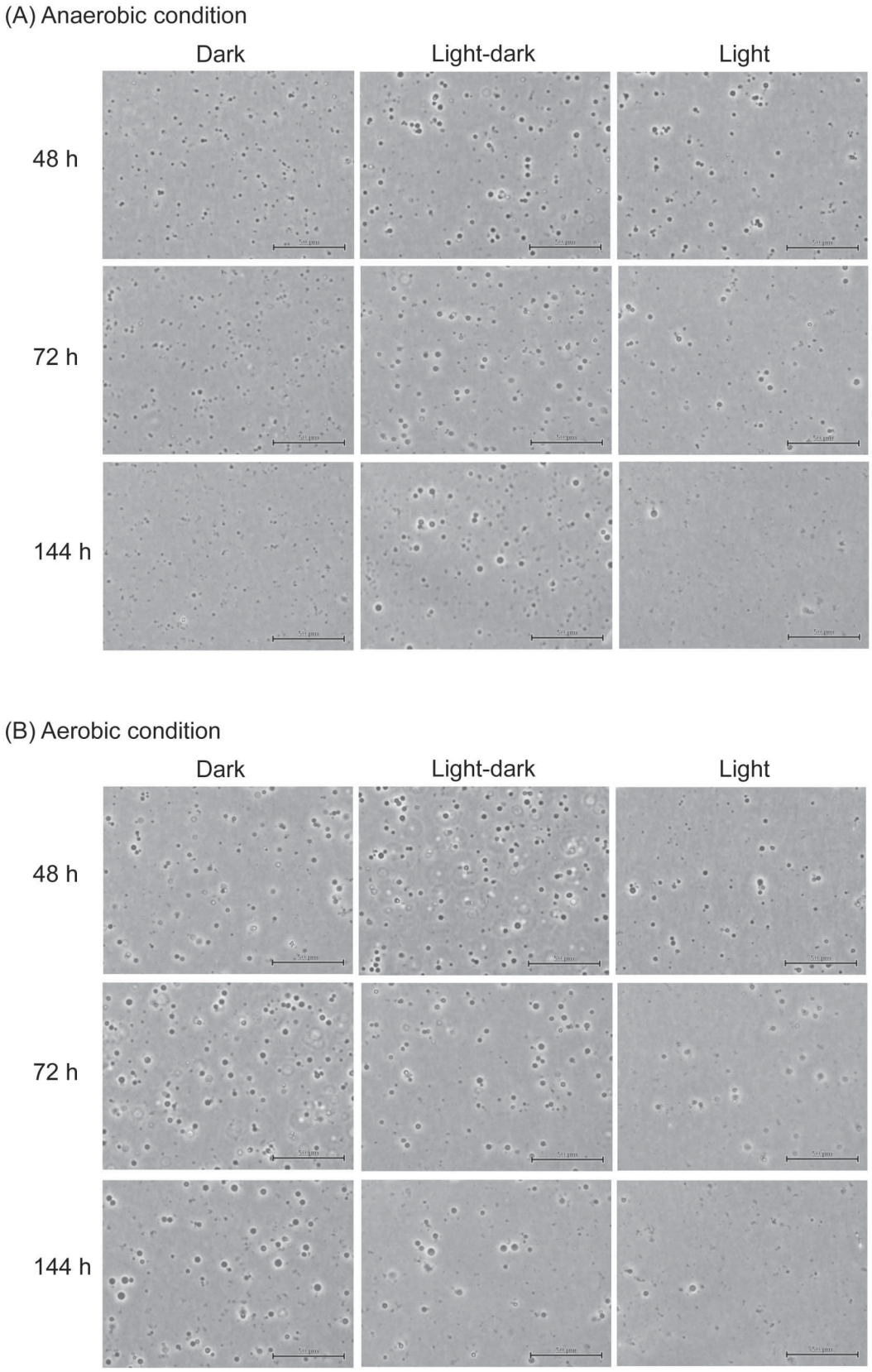

Fig. 1. Phase-contrast micrographs of *Erythrobacter litoralis* cells.

(A) Cells under anaerobic (oxygen free) conditions. (B) Cells under aerobic (20.5% oxygen) conditions. Phase-contrast microscopy images were obtained using Olympus CKK41; scale bar = 50 μm.

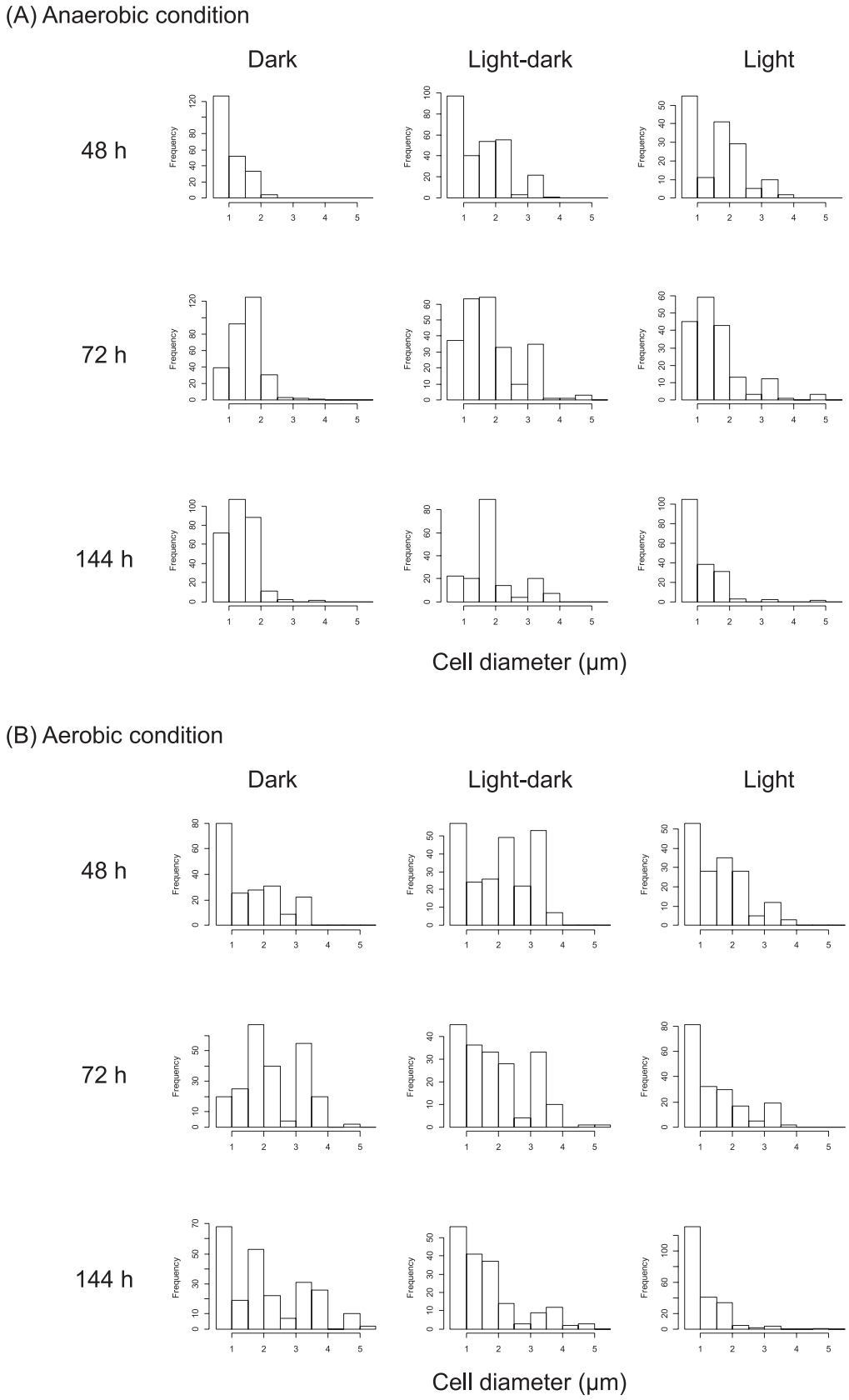

Fig. 2. Histograms showing the diameters of *Erythrobacter litoralis* cells.
(A) Cells under anaerobic (oxygen free) conditions. (B) Cells under aerobic (20.5% oxygen) conditions.

is unclear. This may be because of the following two reasons: (1) In *E. litoralis*, the photosynthetic system may share components with the respiration system (Harashima et al., 1982, 1987; Shiba, 1984) because of which continuous light may not only affect photosynthesis, but may also affect respiration. (2) Continuous light may generate reactive oxygen species (Schmitt et al., 2014), which leads to cell damage.

Acknowledgments

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