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Full length Research Paper

Influences of light and temperature on membrane potential and respiratory viability of an aerobic anoxygenic phototrophic bacterium *Erythrobacter sp.* JL475

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Flow cytometry's (FCM) measurement of membrane potential (MP) and cell respiration viability based on continuous culture was used to investigate the responses of aerobic anoxygenic phototrophic bacteria (AAPB) in the heterotrophic growth and regulation mechanism of photosynthesis to environmental changes. An AAPB strain *Erythrobacter sp.* JL475 and a non-AAPB strain *Erythrobacter sp.* JL316 were used as the experimental bacteria, both of which were isolated from the South China Sea. The results showed that light-cultured AAPB showed higher MP and biomass at 10°C, suggesting an obvious stimulation of light on AAPB growth. By contrast, dark-cultivated JL475 had higher MP and biomass at higher temperature (20, 30 and 40°C). The rate of heterotrophic respiration at different temperature environment ranked as follows: dark-cultivated JL316 > dark-cultivated JL475 > light/dark cycling cultivated JL475. Light undoubtedly increased the cell viability of AAPB, especially of apoptosis cells. The CTC^+% at different carbon concentration ranked as follows: light/dark cycling cultivated JL475 > dark-cultivated JL316 > dark-cultivated JL475. It was concluded that the heterotrophic respiration would played a key role in energy metabolism of AAPB, photosynthesis may provide an advantage for AAPB to survive in a variety of diverse environments.

Key words: Flow cytometry, aerobic anoxygenic phototrophic bacteria, membrane potential, respiration viability, biomass.

INTRODUCTION

Aerobic anoxygenic phototrophic bacteria (AAPB) are group of heterotrophic bacteria with capability of photosynthesis. They represent a newly recognized energy flow pathway and appear to be critical to carbon cycling in the ocean (Kolber et al., 2000, 2001). AAPB also would be of great value in understanding the evolution of photosynthesis and the structure and function of marine ecosystems (Jiao et al., 2003). Great progress has been made on AAPB's ecological study (Shiba et al., 1991; Kolber et al., 2001; Cottrell et al., 2006; Jiao et al., 2007; Zhang and Jiao, 2007). Dissolved organic carbon (DOC), particularly from phytoplankton may have great effects on the dynamics of AAPB (Jiao et al., 2007). To date, physiological studies of AAPB strains mainly focus on their specific photochemical properties. Carbon source, oxygen concentration and light are the most important factors in regulating the photochemical expression and synthesis. Although AAPB do not grow on light as a sole energy source (Harashima et al., 1982; Shimada 1995; Takamiya and Okamura 1984; Wakao et al., 1996), stimulation of growth in the presence of light has been reported (Harashima et al., 1987; Yurkov and Gemerden 1993; Hiraishi et al., 2000). It has also been suggested that the viability of AAPB is stimulated by the light in the deficiency of nutrients (Fleischman et al., 1995; Hiraishi et al., 2000; Shiba 1984; Suyama et al., 2002). *BChla* expression was induced by the decline of carbon sources (Suyama et al., 2002). However, current knowledge of physiology of AAPB is still far from enough for understanding

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of controlling mechanisms of AAPB in marine ecosystems. Jiao et al. (2004) established a simplified protocol for determination of bacterial MP by flow cytometry with 3, 3'-dihexyloxacarbocyanine iodide ($DiOC_6(3)$) as the MP probe, which was capable of precise indication of the physiological states of the cells as well as cellular responses to environmental factors.

The abundance of 5-cyano-2, 3-ditolyl tetrazolium chloride (CTC)-active cells and CTC reduction were highly correlated with respiration rates or metabolic activity or enzyme redox activity (Smith, 1998; Sherr et al., 1999; Iturriaga et al., 2001). In this study, we monitored by FCM the MP indicated by $DiOC_6(3)$ and respiratory viability indicated by CTC of a new AAPB strain of *Erythorobactor longus* under different light level and temperature during continuous culture courses, aiming at a better understanding of physiological responses of AAPB to variable environmental conditions.

MATERIALS AND METHODS

Experimental organisms

JL475 was isolated from water of 75 m depth in the South China Sea (location: 112.00ºE, 20.00ºN) using RO medium. JL475 was grown in Erlenmeyer flasks shaken aerobically in natural light rhythm at 25°C and 150 rpm in liquid RO medium. The same medium with addition of 1.5% agar was used for routine cultivation on agar plates. JL475, belonging to *Erythrobacter sp* as seen from the 16S rDNA sequence, was identified to be an AAPB strain based on the following examinations (Yurkov and Beatty, 1998; Kolber et al., 2001).

The absorption spectra of cell suspensions of the pigmented isolate (prepared according to Shiba and Simidu, 1982) gave a major peak in the 863 - 867 nm region, indicating the presence of bacterial chlorophyll incorporated into light-harvesting complex 1 (LH1) and a small peak in the 800 - 805 nm region indi-cating the presence of the photosynthetic reaction center (RC). The reference non-AAPB strain *Erythrobacter sp* JL316 was isolated from the surface water in the South China Sea (location: 114.50ºE, 21.50ºN; salinity: 34.38; temperature: 21.18℃) using RO medium. The culture conditions for JL316 were the same as that of JL475.

Continuous culture

Continuous culture was carried out in a 3.5 L cylindrical bioreactor KLF2000 (Bioengineering AG, Switzerland) with working volume of 2 L. The JL475 strain was grown in a modified RO medium (tryptone 0.1 g/L; vitamin B_{12}, 20 ug/l trace element solution 1 ml/l and 0.22 um-filtered old sea water). Trace metal solutions was autoclaved before added to the 2 L feeding carboy. After the cells were grown exponentially in batch mode at 28°C for about 20 h, continuous mode of operation was initiated by constantly pumping fresh medium into and removing excess volume from the bioreactor. The culture was stirred at 300 rpm and aerated at 1 v/v per minute to provide sufficient oxygen supply.

The continuous culture was run at the dilution rate of 0.05 L/h in darkness or light/dark cycling. Light intensity was 5000 Lux when JL475 was at light-cultivated and light/dark cycle was 12:12. The JL475 and JL316 strains were cultivated at pH 7.8. A temperature gradient of 10, 20, 30 and 40℃, were employed to detect the responses in MP, respiration election transport system (ETS) dehydrogenase and cell viability (CTC%).

FCM measurements

An Epics Altra II (Beckman Coulter, USA) FCM, equipped with an argon laser (306C-5, Coherent Inc., USA) as excitation source, a constant temperature apparatus (Model 911 Polyscience, USA) and an external quantitative sample injector (Harvard PHD2000, USA) was used for bacterial enumeration and MP measurement with the software ExpoTM32 MultiCOMP supplied by the manufacture. When measuring the MP, the basic FCM settings used in the analysis were: Laser Power, 60 mW; excitation wave length, 488 nm; green fluorescence filter, 503DF19; red fluorescence filter, 610BP12 (Omega, USA).

When measuring the respiration and viability, the basic FCM settings used in analysis were: Laser Power, 100 mW; excitation wave length, 488 nm; green fluorescence filter, 525BP; red fluorescence filter, 675BP (Omega, USA). When bacterial counting, the basic FCM settings used in analysis were: Laser Power, 100 mW; excitation wave length, 488 nm; green fluorescence filter, 525BP; red fluorescence filter, 675BP (Omega, USA). When analyzing, sample were run at a flow rate of about 100 events sec^{-1} and 1.0 me fluorescent beads (Polysciences, USA) were used as the internal reference.

Measurement of mp

$DiOC_6(3)$ (Molecular Probes, USA) was used as the MP probe for flow cytometry-based MP measurement. Bacterial suspensions for MP measurement were diluted with the filtrate of the corresponding culture to cell concentration of approximately 5×10^5 cells ml^{-1} and then added with $DiOC_6(3)$ at the final concentration of 10 µM and incubated for about 20 - 25 mins before FCM analysis (Jiao et al., 2004). Data acquisition was controlled by running time of 100 s, about 10000 events were collected for statistics. Cells were kept in darkness and at culture temperature during the staining and determination processes. The ratio metric method (red to green fluorescence readings) was used for the MP calculation (Novo et al., 1999).

Measurement and calculation of ETS dehydrogenase and cell viability

CTC (Flucka, USA) was used to quantify the ETS dehydrogenase and cell viability (Zheng and Yao, 2005). Bacterial suspensions for measurement were diluted with the filtrate of the corresponding culture to cell concentration of approximately 1×10^6 cells ml^{-1} and then added with CTC at the final concentration of 5 mM and incubated for about 60 min before FCM analysis. Data acquisition was controlled by running time of 100 s, approximately 10000 events were collected for statistics. Cells were kept in darkness and at culture temperature during the staining and determination processes.

As CTC was reduced by ETS into a fluorescent CTC-formazan (CTF) in the respiring cells, the CTF red fluorescence was taken as the measurement of ETS dehydrogenase which can be an indication of cell respiration.

Bacterial count

Aliquots of one milliliter of sample were taken from cultures for MP analysis and bacterial counting. Samples for the enumeration analysis were fixed with 0.1% glutaraldehyde and stained with SYBR Green I (molecular probes) (final concentration 10^{-4} the commercial

solution) (Marie et al., 1996) and then counted by FCM.

RESULTS

Variation of mp of JL475 and JL316 with temperature and light availability

As shown in Figure 1, the ratiometric MP values of JL475 and JL316 in all of the treatments ascended with increasing temperature (except 40℃), but to different extents at different levels of temperature and light availability. The MP value of JL475 light-cultivated was distinctly higher than those of both strains in the dark at 10℃. The MP values of non-AAPB strain JL316 dark-cultivated was still higher than those of JL475, however, was lower than AAPB strain JL475 light-cultivated at 10℃. The MP values of the JL475 light-cultivated were 70.11 - 5.28% higher than that of JL475 dark-cultivated at 10 and 20℃, while 12.95 -1 1.5% lower at 30 and 40℃.

Variation of respiration activity of JL475 and JL316 with temperature and light availability

CTF measurements during the incubation course showed likely pattern from that of MP. The non-AAPB strain JL316 showed significantly higher CTF values than the AAPB strain JL475 in all the treatments. By contrast, the AAPB strain JL475 light-cultivated showed lower CTF values than JL475 dark-cultivated regardless of temperature (Figure 2). The two strains' CTF continuously increased with increasing temperature (except 40℃).

Cell concentration changes at different temperature

The cell concentration continuously increased with increasing temperature (except 40℃). The non-AAPB strain JL316 yielded higher cell concentration than the AAPB strain JL475 at all the treatments. Within the AAPB strain, cell concentrations of the light-cultivated JL475 at 10 and 20℃ were 27.58 - 3.08% higher than those of the dark-cultivated ones, respectively. In contrast, cell concentrations of dark-cultivated JL475 at 30 and 40℃ were 13.35 - 16.15% higher than that of light-cultivated ones.

Cell viability at different temperature

The CTC^{+}% can directly characterize the presence of living cells. As shown in Figure 4, it increased with increasing temperature (except 40℃). The non-AAPB strain JL316 appeared higher CTC+% than that of the AAPB strain JL475 dark-cultivated at all the treatments. The AAPB strain JL475 light-cultivated also showed higher CTC+% than JL316 dark-cultivated at all the treatments (Figure 3).

DISCUSSION

Marine microbe show different responses to temperature. Some AAPB can grow in a temperature range as wide as 5 to 42℃ (Yurkov, 1998). In this study, we found that the abundance of JL475 cultivated in a chemostat was greatly influenced by temperature (Figure 3). Both strains grew more slowly at 5 than 30℃. Moreover, the membrane potential and respiratory rate of JL475 and JL316 were raised in accordance with the increasing of temperature from 5 to 30℃. Whereas at a higher temperature of 40℃, the abundance membrane potential and respiratory rate of JL475 and JL316 decreased remarkably. We suppose that 30℃ is likely the optimum temperature for the growth of the strains.

Interestingly, we also found that JL475's MP was higher in darkness than in light when cultivated at the optimum temperature of 30℃ in autoclaved seawater containing 0.1 g/L peptone. It revealed that JL475 mainly perform heterotrophic metabolism under this condition, meanwhile, it could also perform some photorespiration. Beatty (2002) argued that both photosynthesis and respiration of AAPB use the very composite Q10 of the electron transport chain. Hence, the two processes tend to repress each other. On the other hand, Koblizek et al. (2003) showed that the respiration of AAPB (*E. longus* clone NJ3Y) is restrained when stimulated by light, which means that when AAPB increases the viability of light, part of its respiration is repressed. The redox state of electronic carriers (such as quinone and cytochrome) which participates in the process of both photosynthesis and respiration in bacteria affected the transfer efficiency of light-driven electron and proton (Yurkov et al., 1998). When quinine and cytochrome are at their reduction state, the electron transport will be greatly reduced even if *Bchla* are stimulated by light (Beatty, 2002), thus will partially influence the efficiency of heterotrophic respiration. It was reflected with a lower MP value of JL475 cultivated in light than that in darkness. Zhang (2006) found that 1. Seasonal changes of AAPB are smaller than that of non-AAPB; 2. The biggest abundance of AAPB (11.6% of the total microbial community) usually appears in winter. It was also found that the abundance of non-AAPB could increase in accordance with the rising of temperature, whereas AAPB keep unchanged. It reveals that temperature has smaller effects on AAPB. It appears that less dependence on temperature is likely a unique feature of AAPB, which is correlated with photosynthesis. In other words, the energy required for bacterial metabolism of non-AAPB was merely produced by organic carbon respiration. While to AAPB, besides from the organic carbon respiration, photosynthesis can bring some additional energy, thus can somewhat offset the negative effect of low temperature on cell metabolism. Previous studies had revealed that light can prolong the living time of AAPB when it was transferred from a nutrient-rich medium to a carbon-free medium (Koblizek et al., 2003). Therefore, photosynthesis may provide an

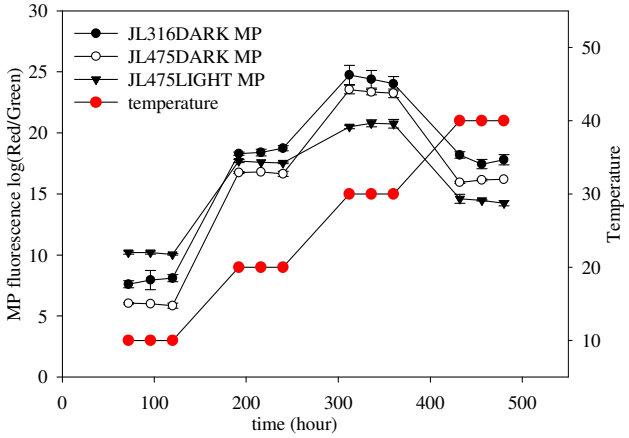

Figure 1. Responses of MP of dark-cultivated JL316, dark-cultivated JL475 and light/dark cultivated JL475 to a variation of temperature in the continuous culture.

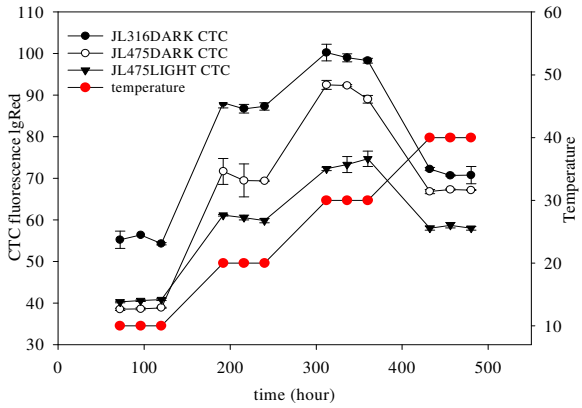

Figure 2. Responses of respiration (quantified by CTF red fluoresecence) of dark-cultivated JL316, dark-cultivated JL475 and light/dark cultivated JL475 to a variation of temperature in the continuous culture.

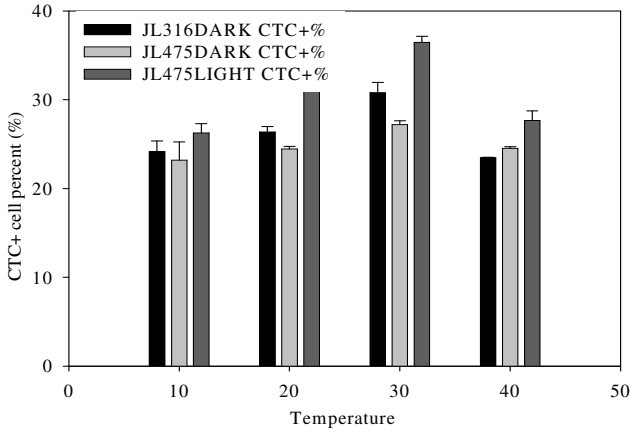

Figure 4. Responses of cell viability (CTC$^+$%) of dark-cultivated JL316, dark-cultivated JL475 and light/dark cultivated JL475 to a variation of temperature in the continuous culture.

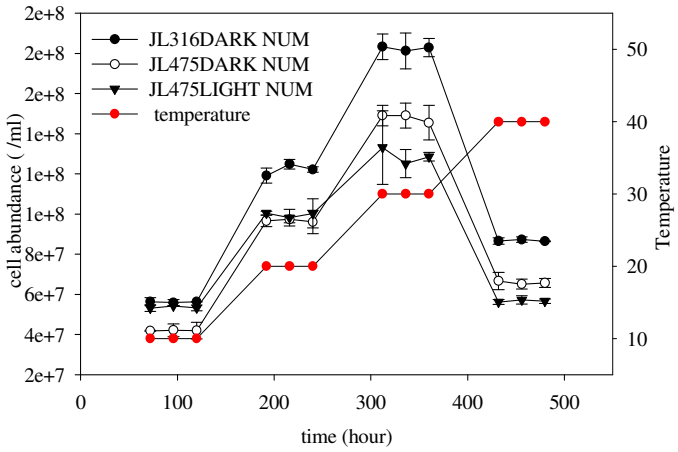

Figure 3. Responses of the cell concentration of dark-cultivated JL316, dark-cultivated JL475 and light/dark cultivated JL475 to a variation of temperature in the continuous culture.

advantage for AAPB to survive in a variety of diverse environments.

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REFERENCES

Beatty JT (2002). On the natural selection and evolution of the aerobic phototrophic bacteria. Photosynth. Res., 73: 109-114.

Cottrell MT, Mannino AD, Kirchman L (2006). Aerobic anoxygenic phototrophic bacteria in the Mid-Atlantic Bight and the North Pacific Gyre. Appl. Environ. Microbiol., 72(1): 557-564.

Fleischman DE, Evans WR, Miller IM (1995). acteriochlorophyll-containing *Rhizobium* species, In Blankenship, R. E., M. T. Madigan, and C. E. Bauer (ed.), Anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands pp. 123-136.

Harashima K, Kawazoe K, Yoshida I, Kamata H (1987). Light-stimulated aerobic growth of *Erythrobacter* species OCh 114. Plant Cell Physiol., 28: 365-374.

Harashima K, Nakagawa M, Murata N (1982). Photochemical activity of bacteriochlorophyll in aerobically grown cells of aerobic heterotrophs *Erythrobacter* species (OCh 114) and *Erythrobacter longus* (OCh 101). Plant Cell Physiol., 23: 185-193.

Hiraishi A, Matsuzawa Y, Kanbe T, Wakao N (2000). *Acidisphaera rubrifaciens* gen. nov., sp. nov., an aerobic bacteriochlorophyll-containing bacterium isolated from acidic environments. Int. J. Syst. Evol. Microbiol., 50: 1539-1546.

Iturriaga R, Zhang S, Sonek GJ, Stibbs H (2001). Detection of respiratory enzyme activity in *Giardia* cysts and *Cryptosporidium* oocysts using redox dyes and immunofluoresce techniques. J. Microb. Meth., 46: 19-28.

Jiao NZ, Sieracki ME, Zhang Y, Du HL (2003). Aerobic anoxygenic phototrophic bacteria and their roles in marine ecosystems. Chinese Sci. Bull., 48(11): 1064-1068.

Jiao NZ, Yang YJ, Luo TW (2004). Membrane potential based characterization of physiological states of an Aerobic Anoxygenic Phototrophic Bacterium by flow cytometry, Aquat. Microb. Ecol., 37(2): 149-158.

Jiao NZ, Zhang Y, Zeng YH, Hong N, Liu RL, Chen F, Wang PX (2007). Distinct distribution pattern of abundance and diversity of aerobic anoxygenic phototrophic bacteria in the global ocean. Environ. Microbiol., 9: 3091-3099.

Koblizek M, Beja O, Bidigare RR, Christensen S, Benitez-Nelson B, Vetriani C, Kolber MK, Falkowski PG, Kolber ZS (2003). Isolation and characterization of *Erythrobacter* sp. Strains from Upper Ocean. Arch. Microbiol., 180: 327-338.

Kolber ZS, Plumley FG, Lang AS, Bertty JT, Blankenship RE, Van Dover CL, Vetriani C, Koblizek M, Rathgeber C, Falkowski PG (2001). Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. Science, 292: 2492-2495.

Kolber ZS, Van Dover CL, Niederman RA, Falkowski PG (2000). Bacterial photosyntheses in surface waters of the open ocean. Nature, 407: 177-179.

Marie D, Vaulot D, Partensky F (1996). Application of the novel nucleic acid dyes YOYO-1, YO-PRO-1, and Pico Green for flow cytometry analysis of marine prokaryotes. Appl. Environ. Microbiol., 62: 1649-1655.

Shiba T (1984). Utilization of light energy by the strictly aerobic bacterium *Erythrobacter* sp. OCh 114. J. Gen. Appl. Microbiol., 30: 239-244.

Shiba T (1991). *Roseobacter litoralis* gen nov, sp nov and *Roseobacter denitrificans* sp nov, aerobic pink-pigmented bacteria which contain bacteriochlorophyll *a*. Syst. Appl. Microbiol., 14: 140 -145.

Shiba T, Shimidu U (1982). *Erythrobacter longus* gen. nov., an aerobic bacterium which contains bacteriochlorophyll *a*. Int. J. Syst. Bacteriol., 32: 211-217.

Shimada K (1995). Aerobic anoxygenic phototrophs, In Blankenship, R.E., M.T. Madigan, and C.E. Bauer (ed.), Anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands pp. 105-122.

Smith EM (1998). Coherence of microbial respiration rate and cell-specific bacterial cativity in a coastal planktonic community. Aquat. Microb. Ecol., 16: 27-35.

Takamiya K, Okamura K (1984). Photochemical activities and hotosynthetic ATP formation in membrane preparation from a facultative methylotroph, *Protaminobacter* rubbers strain NR-1. Arch. Microbiol., 140: 21-26.

Wakao N, Yokoi N, Isoyama N, Hiraishi A, Shimada K, Kobayashi M, Kise H, Iwaki M, Ito S, Takaichi S, Sakurai Y (1996). Discovery of natural photosynthesis using Zn-containing bacteriochlorophyll in an aerobic bacterium *Acidiphilium rubrum*. Plant Cell Physiol., 37: 889-893.

Yurkov VV Beatty JT (1998). Aerobic anoxygenic photo-trophic bacteria. Microbiol. Mol. Biol. Rev., 62: 695-724.

Yurkov VV, van Gemerden H (1993). Impact of light/dark regimen on growth rate, biomass formation and bacteriochlorophyll synthesis in *Erythromicrobium hydrolyticum*. Arch. Microbiol., 159: 84-89.

Zhang Y, Jiao NZ (2007). Dynamics of Aerobic Anoxygenic Phototrophic Bacteria in the East China Sea. FEMS Microbiol. Ecol., 61(3): 459-469.

Zheng Z, Yao S (2005). Colorimetric assay for respiratory chain dehydrogenase activity in *Escherichia coli*. J. Zhejiang Univ. Engine. Sci., 39(3): 433-436.