Photosynthesis Research (2020) 144:341–347
https://doi.org/10.1007/s11120-020-00744-9

ORIGINAL ARTICLE

Photosynthetic complexes and light-dependant electron transport chain in the aerobic anoxygenic phototroph *Roseicyclus mahoneyensis* and its spontaneous mutants

Elizabeth Hughes[1] · Jean Alric[2] · Vladimir Yurkov[1]

Received: 17 December 2019 / Accepted: 30 March 2020 / Published online: 4 April 2020

Abstract
Spontaneous photosynthetic mutants of the aerobic anoxygenic phototrophic bacterium *Roseicyclus mahoneyensis*, strain ML6 have been identified based on phenotypic differences and spectrophotometric analysis. ML6 contains a reaction centre (RC) with absorption peaks at 755, 800, and 870 nm, light harvesting (LH) complex 1 at 870 nm, and monomodal LH2 at 805 nm; the mutant ML6(B) has only the LH2; ML6(DB) has also lost the LH1; in ML6(BN9O), the LH2 is absent and concentrations of LH1 and RC are much lower than in the wild type. RCs were isolated and purified from ML6 and ML6(BN9O); LH1-RC from ML6; and LH2 from ML6, ML6(B), and ML6(DB). All protein subunits composing the complexes were found to be of typical size. Flash-induced difference spectra revealed ML6 has a fully functional photosynthetic apparatus under aerobic and microaerophilic conditions, and as is typical for AAP, there is no photosynthetic activity anaerobically. ML6(BN9O), while also functional photosynthetically aerobically, showed lower rates due to the lack of LH2 and decreased concentrations of LH1 and RC. ML6(B) and ML6(DB) showed no photoinduced electron transport. Action spectra of light-mediated reactions were also performed on ML6 and ML6(BN9O) to reveal that the majority of carotenoids are not involved in light harvesting. Finally, redox titrations were carried out on membranes of ML6 and ML6(BN9O) to confirm that midpoint redox potentials of the Q_A, RC-bound cytochrome, and P^+ were similar in both strains. Q_A midpoint potential is + 65 mV, cytochrome is + 245 mV, and P^+ is + 430 mV.

Keywords *Roseicyclus mahoneyensis* · Aerobic anoxygenic phototroph · Photosynthetic electron transport · Carotenoids · Bacteriochlorophyll *a* · Spontaneous mutations

Introduction

The aerobic anoxygenic phototrophs (AAP) are a diverse group of heterotrophic bacteria that supplement energy generation through photosynthesis (Yurkov and Csotonyi 2009; Yurkov and Hughes 2013) that resemble the photosynthetic systems of the purple non-sulphur bacteria (PNSB). They have a reaction centre (RC) with absorption peaks at 750 nm, due to bacteriopheophytin (BPheo), 800 and 865 nm for bacteriochlorophyll (BChl) *a*, and a light harvesting (LH) complex 1, with a single BChl *a* peak at 870 nm. Some also have a peripheral LH2, which typically shows dual BChl *a* peaks at 805 and 850 nm (Yurkov and Beatty 1998).

The cyclic photosynthetic electron (e^-) transport chain (ETC) begins when BChl *a* of the P^+ is excited and energy is passed to auxiliary BChl *a* and BPheo (Yurkov and Csotonyi 2009). Electrons are next passed to the primary e^- acceptor (Q_A, a quinone), followed by the cytochrome (cyt) bc_1 complex and then the cyt*c* (can either be bound to the RC or soluble in the periplasmic space), and are finally cycled back to the beginning of the chain (Yurkov and Csotonyi 2009). The redox midpoint potential of the Q_A, which typically falls between + 5 and + 170 mV (Rathgeber et al. 2012), may be one of the main reasons why AAP carry out photosynthesis under obligately aerobic conditions. Under anoxic conditions, the Q_A may be over-reduced and therefore

✉ Vladimir Yurkov
vyurkov@umanitoba.ca

[1] Department of Microbiology, University of Manitoba, Winnipeg, Canada

[2] Photosynthesis and Environment, Aix Marseille Univ, CEA Cadarache, CNRS UMR7265, BIAM, 13108 Saint Paul-Lez-Durance, France

cannot accept additional e^- causing the ETC to come to a halt (Rathgeber et al. 2004).

There is speculation as to the role of the diverse and abundant carotenoids (crt) that give AAP their characteristic bright pigmentation (Yurkov and Csotonyi 2009; Yurkov and Hughes 2013). In some cases, they can be used as auxiliary light harvesting molecules to increase the range of light used for photosynthesis. In other cases, crt may play a role in photoprotection of the cells and to neutralize triplet BChl *a* and singlet oxygen formed during BChl *a* synthesis, as these reactive species can exert oxidative stress to the cells (Fraser et al. 2001; Yurkov and Hughes 2013).

Roseicyclus mahoneyensis, strain ML6, was isolated from the meromictic Mahoney Lake, Canada, in October 1997 (Yurkova et al. 2002; Rathgeber et al. 2005). It is a pink AAP that forms a typical LH1-RC complex and an unusual monomodal LH2 with a single peak at 805 nm. This type of LH2 has only ever been observed in two other AAP genera, *Roseobacter* and *Rubrimonas* (Shiba 1991; Suzuki et al. 1999; Rathgeber et al. 2005), as well as in the PNSB *Rhodopseudomonas palustris* (Hartigan et al. 2002). Interestingly, ML6 has been found to be photosynthetically most active under microaerophilic conditions (Rathgeber et al. 2012). This suggests that it may be an evolutionary link between the AAP and PNSB making it an important species for study.

This research investigates the photosynthetic complexes and the light-dependant e^- transport chain in ML6 and three stable spontaneous mutants with unique photosynthetic characteristics.

Materials and methods

Isolation of spontaneous mutants

R. mahoneyensis, wild-type strain ML6 was spread plated on Medium N1 (Yurkova et al. 2002) pH 7.8 for single colonies and incubated at 28 °C for 7 days. Colonies displaying different pigmentation from the pink wild type were purified for analysis. Frequency of mutation was subsequently calculated as number of mutated colonies divided by total number of colonies on a plate. This was then expressed as a percentage by multiplying by 100. Mutated colonies were picked and purified through further serial dilutions and subcultured a minimum of 10 times to ensure stability.

Spectroscopy

Full-wavelength scans of whole cells, membranes, and purified complexes were performed on a Hitachi U-2010 spectrophotometer as described previously (Rathgeber et al. 2012).

Purification of photosynthetic complexes

All strains were grown for 3 days in Medium N1 (Yurkova et al. 2002) at pH 7.8 and 28 °C. Cells were then collected using centrifugation at 10 K rpm for 20 min on a Beckman J2HS centrifuge with a JA-10 rotor. Cells were washed 3 times with 20 mM Tris–HCl pH 7.8, then broken using a French Pressure Cell at 20 K psi, and membrane fractions were isolated (Rathgeber et al. 2012). The soluble fraction was collected and spectrophotometrically tested for the presence of soluble cyt. Membrane pellets were homogenized in 20 mM Tris–HCl pH 7.8 buffer to make a thick suspension. Ice cold membranes were treated for 30 min with 8% Triton X-100 at a slow mixing speed and loaded onto sucrose density gradients (0.6, 0.9, 1.2, and 1.5 M sucrose, with 0.05% Triton X-100). Gradients were centrifuged in a Beckman Optima LE-80 K ultracentrifuge with a 70-Ti rotor for 15 h at 38 K rpm to separate photosynthetic complexes. Individual bands were removed, and full-wavelength scans were performed to determine which fractions contain photosynthetic units. Samples were dialyzed three times for 8 h each to remove sucrose and detergents and were concentrated by packing tubing in PEG6000. Further purification was carried out using a DEAE-Sephacel anion exchange column equilibrated with 20 mM Tris–HCl, pH 7.8 with 0.05% Triton X-100. Individual fractions were collected, dialyzed, and concentrated as described above.

Polyacrylamide gel electrophoresis

SDS-PAGE was performed with 10% acrylamide/bisacrylamide for separation of RC complex subunits and 14% acrylamide/bisacrylamide for LH complex subunits.

Biophysical analysis

Flash-induced difference spectra were carried out on whole cells of the wild-type and all mutants, as well as membrane fractions and pure pigment–protein complexes, using a Joliot-type spectrophotometer (Joliot et al. 1980) with an excitation by a xenon flash bulb. Absorbance was detected at 5, 30, and 60 ms after the flash. Whole cells were put in a closed cuvette and allowed to deplete the O_2 through respiration for a period of 2 h before flash-induced difference spectra were performed again for analysis of anaerobic photosynthesis. Action spectra were performed on membrane fractions and pure complexes. Monochromatic continuous illumination (10 nm bandwidth) was provided by a SPEX Fluorolog-2 illuminator (a xenon arc-lamp fitted to a Jobin Yvon 1681 Minimate f/4 monochromator with 2.5 mm slits). The

photochemical activity was determined by the accumulation of P^+ (measured at 605 nm) after the illumination was switched on by a computer-controlled mechanical shutter opening faster than 6 ms. Action spectra represent the photochemical rate corrected by the irradiance of the illuminator, measured with a Macam photometer.

Membrane fractions were used for redox titrations of the Q_A, RC-bound cyt*c* and P^+ in the presence of the redox mediators ferrocenecarboxylic acid, 1′,1′-dimethylferrocene, 1,2-naphthoquinone, menadione, p-benzoquinone, and 2,5-dimethyl-p-benzoquinone dissolved in 100% ethanol at a concentration of 10 µM as well as potassium ferricyanide, 2-hydroxy-1,4-naphthoquinone, anthraquinone-2-sulfonic acid, benzyl viologen dichloride, methyl viologen dichloride, and phenazine methylsulfate dissolved in water, also at 10 µM concentration. 100 mM KCl was added for conductivity.

Results and discussion

Description of mutants

Wild-type strain ML6 was pink in colour and cell suspensions were absorbed spectrophotometrically due to BChl *a* bound to protein complexes at 871 nm, representing the typical LH1 complex, and at 806 nm, which corresponds to the presence of the unusual monomodal LH2. Absorption peak at 750 nm shows the presence of BPheo typically bound to the RC (Fig. 1a). This strain often spontaneously mutates to form novel stable lines, three of which we found to be photosynthetically interesting. The first, ML6(B), appeared as light brown colonies and spectrophotometric analysis revealed that it had lost the RC-LH1 complexes, apparent from a sole peak at 805 nm, representing the LH2 (Fig. 1b). The second, ML6(DB), emerged from ML6(B) and was slightly darker brown in colour. It arose at a frequency of 2.97% of colonies, when grown under optimal conditions, or 2.1% at room temperature. The frequency

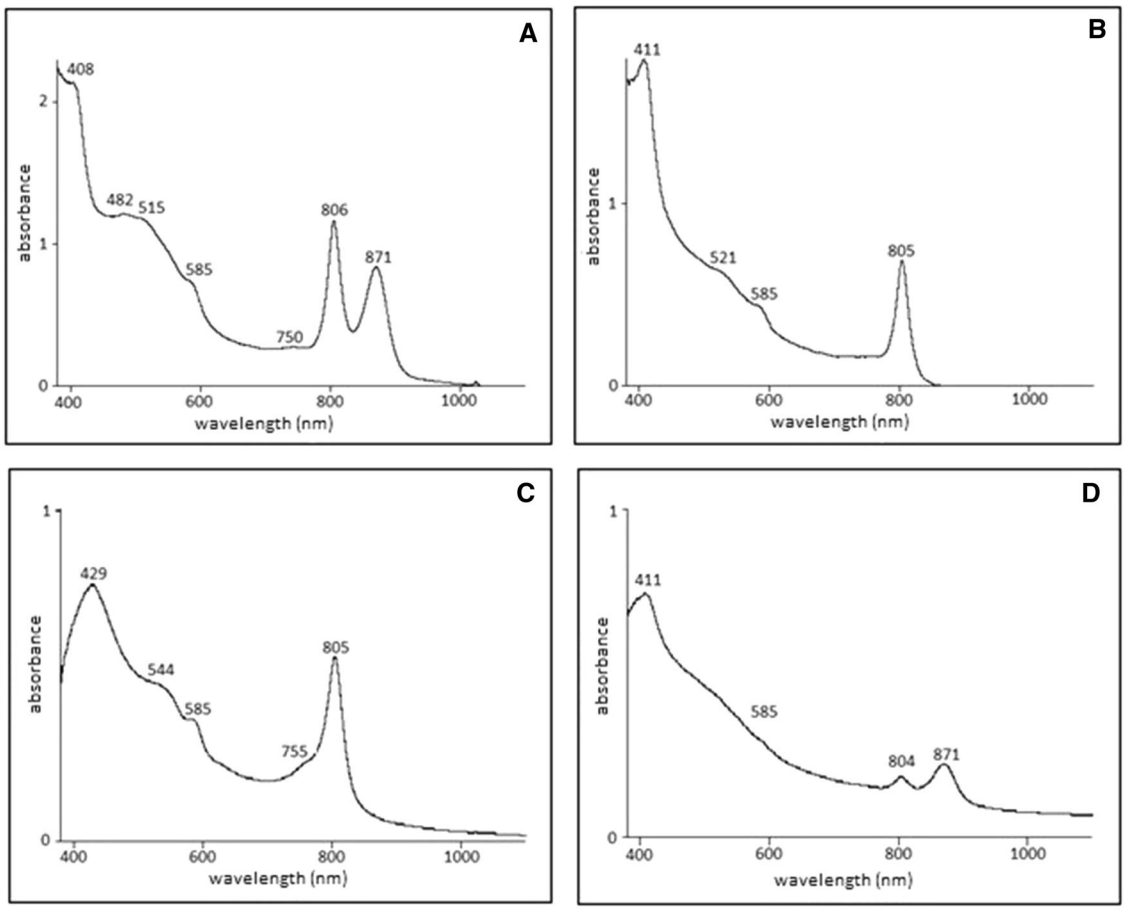

Fig. 1 Full-wavelength absorption spectrum of whole actively growing cells of *Roseicyclus mahoneyensis* strains: **a** ML6; **b** ML6(B); **c** ML6(DB); **d** ML6(BN9O)

climbed as high as 9.1% of colonies when plates were grown under a light/dark regimen. While it also did not contain the LH1 or BChl *a* peaks of the RC, it retained a small peak at 755 nm, indicating BPheo synthesis, in addition to the LH2 peak (Fig. 1c). This suggests ML6(DB) may be producing an incomplete RC or accumulates some BPheo as a precursor of BChl *a* synthesis.. Finally, the third stable spontaneous mutant is ML6(BN9O), which was a light brown similar to that of ML6(B); however, the LH2 complex was absent and the RC-LH1 complexes were produced, though in lower amounts than by the wild type (Fig. 1d). It is derived from another mutant ML6(N9O), which was found on plates of the wild-type ML6. ML6(BN9O) arises in only 0.05% of colonies when grown under optimal conditions, and 0.26% of colonies at room temperature. All of the spontaneous mutants were stable, able to be sub-cultured indefinitely, and no reversion to the wild type was observed, which supports that these are true mutations and not simply a change in gene expression or regulation. Future DNA sequence analysis of the photosynthetic gene cluster will be used to confirm this hypothesis.

Isolation and purification of photosynthetic complexes

Membrane and soluble fractions were collected for each strain as described above. Full-wavelength scans of membranes confirmed that photosynthetic pigment-protein complexes and cyt are membrane bound. Soluble fractions also showed the presence of cyt*c* (Fig. 2a), confirming that ML6 and its mutants all employ cytoplasm-soluble cyt in their ETC. Photosynthetic complexes were isolated and purified from membrane fractions of all four strains. The RC from ML6 (Fig. 2b) and ML6(BN9O) (not shown) had BChl *a* peaks at 868 nm and 800 nm and BPheo peak at 750 nm. The LH1-RC complex from ML6 (Fig. 2c) showed absorption peaks at 868 nm and 803 due to incorporated BChl *a*. The LH2 from ML6 (not shown), ML6(B) (Fig. 2d) and ML6(DB) (not shown) had a single BChl *a* peak at 803 nm. Additional peaks between 420 and 600 nm were indicative of crt associated with the photosynthetic complexes. The final peak at approximately 410 nm belonged to cyt*c*, which is tightly bound to the RC in addition to a cytoplasm-soluble form.

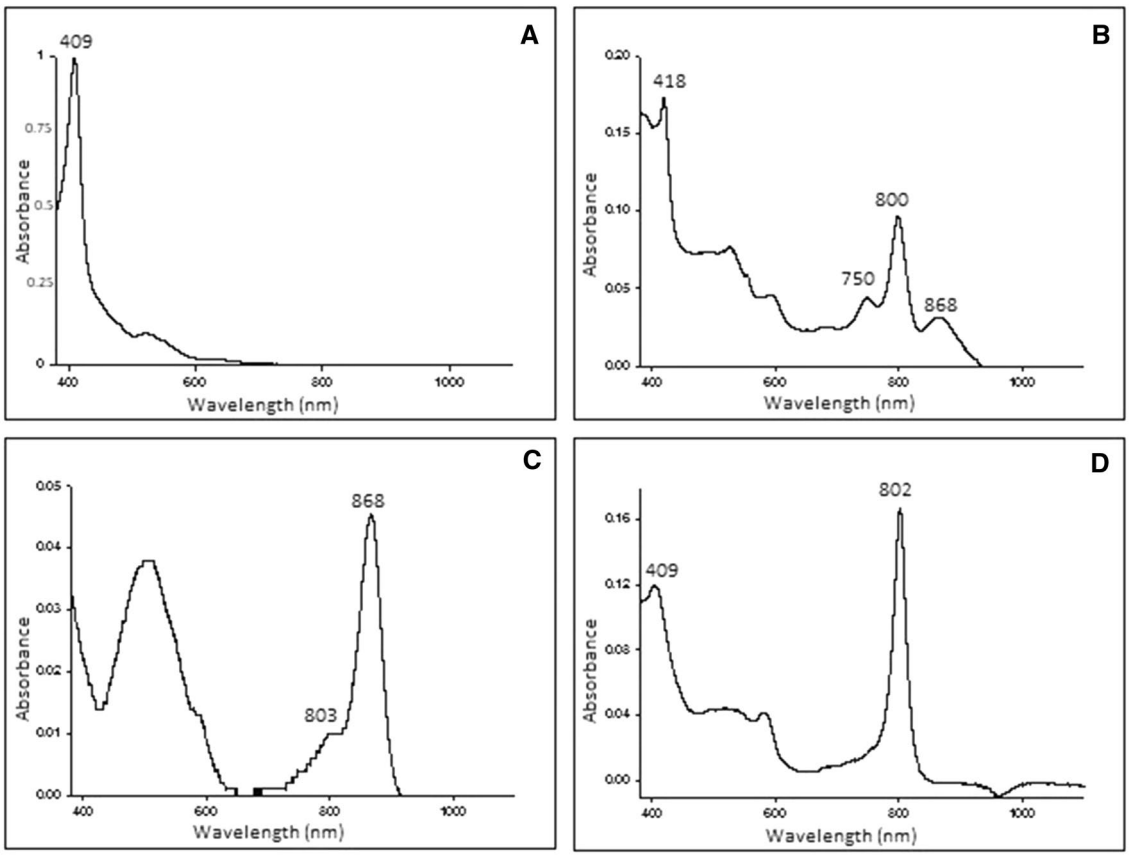

Fig. 2 Absorption spectrum of: **a** strain ML6-soluble fraction; **b** ML6-purified RC; **c** ML6-purified LH1-RC; **d** ML6(B)-purified LH2

SDS-PAGE

SDS-PAGE revealed bands at about 8 and 10 KDa, matching what is expected for subunits of the LH2 complex, and similar bands were present in ML6(B) and ML6(DB). Of note, the wild-type ML6 and mutant ML6(BN9O) had RC bands of 28, 31, and 33 KDa. ML6(B) had the same bands at 28 and 33 KDa and ML6(DB) had those at 28 and 31 KDa; hence, the RC was obviously partially formed in these mutants and not completely eliminated. This would result in the non-functional RC, which did not include bound BChl *a* and therefore was not detected spectrophotometrically in the 800–900 nm region. The LH1 complex subunits were also of typical size, with polypeptide bands found at approximately 7 and 9 KDa.

Photosynthetic activity

Flash-induced difference spectra were carried out in order to determine the levels and kinetic rate of photoinduced electron transfer. ML6 showed strong cyt*c* and P^+ signals in whole cells (Fig. 3a), membranes (Fig. 3b), RC, and LH1-RC complexes under aerobic and microaerophilic conditions. Under in vivo conditions, there was a very strong photo-oxidized cyt*c* signal 5 ms after the actinic flash (troughs at 420 and 552 nm), which, as time progresses, gradually decreased in size (Fig. 3a). This was because once excitation occurred, e^- were passed along the ETC, causing re-reduction of the cyt*c* and allowed us to see the rate of e^- transport. The same could also be observed under ambient oxidized conditions on membranes as a decrease in P^+ signal (peak at 430 nm and trough at 605 nm) (Fig. 3b). This P^+ signal decreased at a slower rate, indicating that a partial oxidation of the cyt*c* prior to the flash may have already occurred. ML6(BN9O) exhibited similar trends with cyt*c* and P^+ signals, though they were weaker in whole cells (Fig. 3c) than observed in the wild type, which was expected as concentrations of RC-LH1 complexes were lower in mutant ML6(BN9O). Additionally, ML6 had the LH2 complex, resulting in enhanced levels of light harvesting, and therefore more active photoinduced e^- transport. Despite the weaker signals, the rate of e^- transport in ML6(BN9O) remained rapid, particularly in purified RC, where the cyt*c* signals at 420 and 552 nm shrunk, while the P^+ signal at 605 nm increased in size (Fig. 3d). Obviously, photoinduced e^- transport was progressing unimpeded. As re-reduction of the cyt*c* occurred in whole cells of strain ML6(BN9O) (Fig. 3c), there was a shift in the trough from 420 to 430 nm, which is indicative of the oxidized cytbc_1 complex, that, like the cyt*c,* was subsequently re-reduced. As predicted, neither ML6 nor ML6(BN9O) showed any sign of light induced absorbance changes in whole cells under anaerobic conditions, strongly confirming again that they

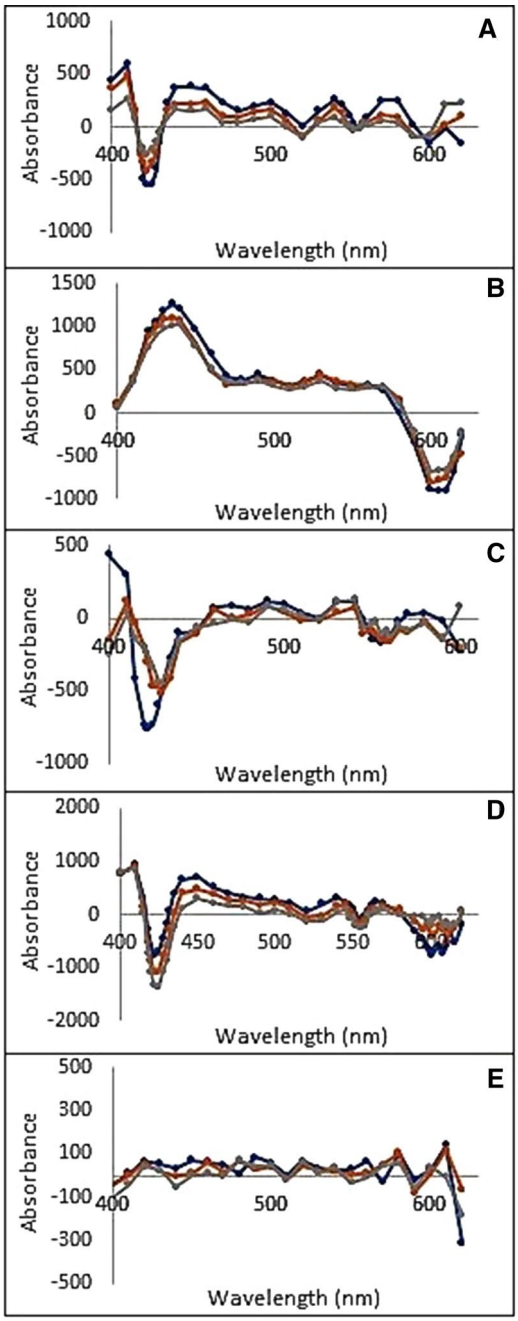

Fig. 3 Flash-induced difference spectra of *Roseicyclus mahoneyensis*: **a** strain ML6 whole cells; **b** ML6 membranes; **c** ML6(BN9O) whole cells; **d** ML6(BN9O)-purified RC; and **e** ML6(DB) whole cells. Blue: 5 ms after the actinic flash; orange: 30 ms after the actinic flash; grey: 60 ms after the actinic flash

are true obligately aerobic photosynthesizers and showing consistency with other previously studied AAP (Yurkov and Beatty 1998; Rathgeber et al. 2004). ML6(B) and ML6(DB) whole cells (Fig. 3e) and membranes were tested for light-induced electron transfer under aerobic, semi-aerobic, and anoxic conditions. Neither mutant produced any cyt*c* or P^+

signal, confirming that without a complete RC, the strains are not photosynthetically active. Subsequent experiments were therefore not performed on ML6(B) and ML6(DB).

Action spectra were carried out on membranes and purified complexes of ML6 and ML6(BN9O) to determine what wavelengths of light were harvested for photosynthesis. This allowed us to see what, if any, crt were involved in photoexcitation of the ETC. Absorption spectra of membranes and complexes with action spectra are shown in Fig. 4. Action spectra show P^+ accumulation at 605 nm, when samples were excited with each individual wavelength of light. In both strains, BChl *a* and BPheo harvested light, confirmed by action spectra peaks at 805 nm in ML6 (Fig. 4a) and ML6(BN9O) (Fig. 4c) membranes. The same is demonstrated with peaks at 750 and 800 nm in ML6 (Fig. 4b) and ML6(BN9O) (Fig. 4d), corresponding to absorption spectra peaks at the same wavelengths. Additional BChl *a* and BPheo peaks were present at 600 and 530 nm, respectively. Crt peaks present in absorption spectra of membranes as well as the shape of the spectrum between 450 and 600 nm were compared to that of the action spectrum, revealing that most crt are not actually involved in light harvesting (Fig. 4a, c). This implies that their main role may be in photoprotection and not in energy generation. Even crt associated with the purified RC complexes in both strains did not seem to harvest light (Fig. 4b, d). The lack of crt light harvesting has been previously observed in other AAP species, such as *Erythromicrobium ramosum* and *Roseococcus thiosulfatophilus* (Yurkov et al. 1994). In the case of *Erythrobacter longus,* more than 70% of crt did not participate in light collection (Noguchi et al. 1992). We also noted that there were much higher levels of crt in membrane fractions compared to pure complexes. This illustrated that while there were some crt associated with the RC and LH complexes, most were scattered elsewhere throughout the membranes.

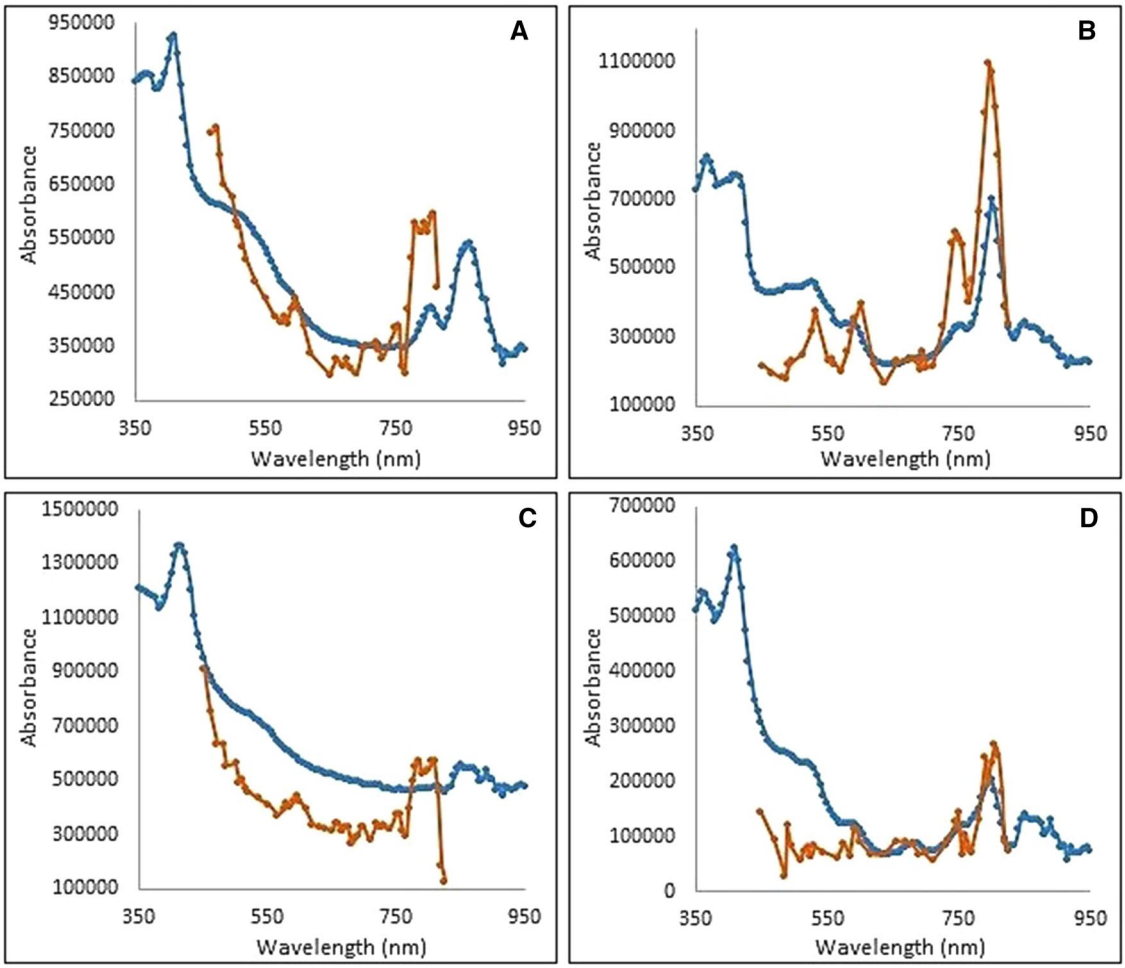

Fig. 4 Absorption spectrum (Blue) and action spectrum (Orange) where y-axis represents accumulation of P^+ measured at 605 nm and x-axis is the wavelength of light used to excite the sample of: **a** ML6 membranes; **b** ML6-purified RC; **c** ML6(BN9O) membranes; and **d** ML6(BN9O)-purified RC

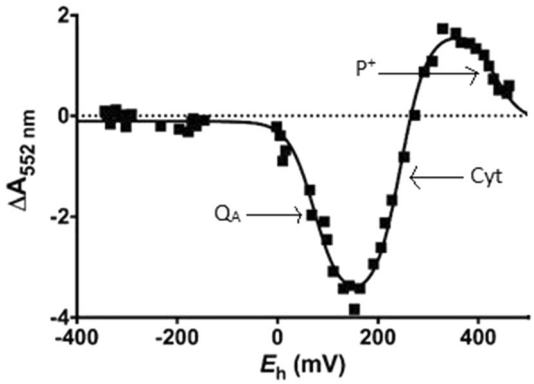

Fig. 5 Flash-induced redox titrations of strain ML6 membranes at 552 nm. Redox midpoint potentials of Q_A, RC-bound cyt*c,* and P^+ are indicated with arrows

Finally, the flash-induced redox titrations using membrane fractions determined that the redox midpoint potential of P^+ was + 430 ± 10 mV, of RC-bound cyt was + 245 ± 10 mV and Q_A was + 65 ± 10 mV (Fig. 5), which all fell into the expected range for typical AAP. These data supported that Q_A redox midpoint potential may contribute to the inability of ML6 and ML6(B) to perform anaerobic photosynthetic e^- transfer. In AAP, the redox potential of the Q_A typically falls between + 5 and + 150 mV (Rathgeber et al. 2012). Contrarily, the Q_A in PNSB has a redox midpoint potential that is generally much lower, in the significantly negative numbers (typically below -20 mV) (Prince and Dutton 1978; Yurkov and Csotonyi 2009).

In summary, *Roseicyclus mahoneyensis* wild-type strain ML6 spontaneously mutates frequently to produce the highly stable photosynthetically interesting strains ML6(B), ML6(DB), and ML6(BN9O). While photosynthetic e^- transfer in ML6 and ML6(BN9O) is very similar, the rates are somewhat lower in ML6(BN9O). This is likely due to the lack of LH2 as well as the lower concentration of photosynthetic apparatuses in ML6(BN9O), compared to the wild-type strain. As many of the crt found in the cells are not associated with the photosynthetic pigment–protein complexes and none participate in light harvesting, it is possible their primary role is to protect the cells from light damage or to neutralize singlet oxygen and triplet BChl to reduce oxidative stress (Yurkov and Hughes 2013). Redox midpoint potentials of the Q_A, RC-bound cyt*c,* and P^+ are very typical of what is expected for AAP. ML6(B) and ML6(DB) showed no signs of photosynthetic activity; therefore, it is unclear why the cells continue to produce the LH2 complex. Further research may help to resolve the puzzle.

Acknowledgements This research was supported by an NSERC Discovery Grant and GETS Funds from the University of Manitoba, both held by Vladimir Yurkov.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Fraser NJ, Hashimoto H, Cogdell RJ (2001) Carotenoids and bacterial photosynthesis: the story so far…. Photosynth Res 70:249–256

Hartigan N, Tharia HA, Sweeney F, Lawless AM, Papiz MZ (2002) The 7.5-A ° electron density and spectroscopic properties of a novel low-light B800 LH2 from *Rhodopseudomonas palustris*. Biophys J 82:963–977

Joliot P, Béal D, Frilley B (1980) Une nouvelle méthode spectrophotométrique destinée à l'étude des reactions photosynthétiques. J Chim Phys 77:209–216

Noguchi TH, Hayashi H, Shimada K, Takaichi S, Tasumi M (1992) In vivo states and function of carotenoids in an aerobic photosynthetic bacterium, *Erythrobacter longus*. Photosynth Res 31:21–30

Prince RC, Dutton PL (1978) Protonation and the reducing potential of the primary electron acceptor. In: Clayton RK, Sistrom WR (eds) The photosynthetic bacteria. Plenum Press, New York, pp 439–453

Rathgeber C, Beatty JT, Yurkov V (2004) Aerobic phototrophic bacteria: new evidence for the diversity, ecological importance and applied potential of this previously overlooked group. Photosynth Res 81:113–128

Rathgeber C, Yurkova N, Stackebrandt E, Schumann P, Beatty JT, Yurkov V (2005) *Rosiecyclus mahoneyensis* gen. nov., sp. nov., an aerobic phototrophic bacterium isolated from a meromictic lake. IJSEM 55:1597–1603

Rathgeber C, Alric J, Hughes E, Vermeglio A, Yurkov V (2012) The photosynthetic apparatus and photoinduced electron transfer in the aerobic phototrophic bacteria *Roseicyclus mahoneyensis* and *Porphyrobacter meromictius*. Photosynth Res 110(3):193–203

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

Suzuki T, Muroga Y, Takahama M, Nishimura Y (1999) *Roseivivax halodurans* gen. nov., sp. nov. and *Roseivivax halotolerans* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from a saline lake. Int J Syst Bacteriol 49:629–634

Yurkov V, Beatty JT (1998) Aerobic anoxygenic phototrophic bacteria. Microbiol Mol Biol Rev 62:695–724

Yurkov V, Csotonyi J (2009) New light on aerobic anoxygenic photosynthesis. In: Hunter CN, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria. Springer, New York, pp 31–55

Yurkov V, Hughes E (2013) Genes associated with the peculiar phenotypes of the aerobic anoxygenic phototrophs. In: Beatty JT, Jacquot JP, Gadal P (eds) Genome evolution of photosynthetic bacteria. Elsevier, Amsterdam, pp 327–358

Yurkov V, Gad'on N, Angerhofer A, Drews G (1994) Light harvesting complexes of aerobic bacteriochlorophyll-containing bacteria *Roseococcus thiosulfatophilus*, RB3 and *Erythromicrobium ramosum*, E5 and the transfer of excitation energy from carotenoids to bacteriochlorophyll. Z Naturforsch 49:579–586

Yurkova N, Rathgeber C, Swiderski J, Stackebrandt E, Beatty JT, Hall KJ, Yurkov V (2002) Diversity, distribution and physiology of the aerobic phototrophic bacteria in the mixolimnion of a meromictic lake. FEMS Microbiol Ecol 40:191–204