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Research article

# Enhanced photosynthetic linear electron flow in mixotrophic green microalga *Ettlia oleoabundans* UTEX 1185

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ABSTRACT

Basic understanding of the photosynthetic physiology of the oleaginous green microalga *Ettlia oleoabundans* is still very limited, including the modulation of the photosynthetic membrane upon metabolism conversion from autotrophy to mixotrophy. It was previously reported that, upon glucose supply in the culture medium, *E. oleoabundans* preserves photosystem II (PSII) from degradation by virtue of a higher packing of thylakoid complexes. In this work, it was investigated whether in the mixotrophic exponential growth phase the PSII activity is merely preserved or even enhanced. Modulated fluorescence parameters were then recorded under short-term treatments with increasing irradiance values of white light. It was found that the mixotrophic microalga down-regulated the chlororespiratory electron recycling from photosystem I (PSI), but enhanced the linear electron flow from PSII to PSI. Ability to keep PSII more open than in autotrophic growth conditions indicated that the respiration of the glucose taken up from the medium fed the carbon fixing reactions with $CO_2$. The overall electron poise was indeed well regulated, with a lesser need for thermal dissipation of excess absorbed energy. It is proposed that the significant, though small, increase in PSII maximum quantum yield in mixotrophic cells just reflects an improved light energy use and an increased photochemical capacity as compared to the autotrophic cells.

## 1. Introduction

Starting from a seminal paper by Tornabene et al. (1983), for 35 years the unicellular green microalga *Ettlia oleoabundans* (synonym of *Neochloris oleoabundans*) has become the subject of several studies aimed at understanding and improving the capacity of this organism to accumulate large amounts of intracellular neutral lipids (reviewed by Abu Hajar et al., 2017). In fact, research has primarily been focused on the alga as a potential source of biofuels, in particular biodiesel (among others, Hegel et al., 2017; Levine et al., 2011; Popovich et al., 2012; Pruvost et al., 2009; Santos et al., 2012; Yoon et al., 2015). Among the diverse modes of *E. oleoabundans* cultivation, mixotrophy has gained some special attention. Mixotrophy is defined as "the physiological feature of an organism whose cells use both photosynthesis and external organic matter as a source of carbon and/or non-carbon elements" (Selosse et al., 2017). In *E. oleoabundans* the mixotrophic mode of growth can lead to high yields of lipid-rich biomass (Baldisserotto et al., 2014; Giovanardi et al., 2013, 2014; Sabia et al., 2015; Silva et al., 2016).

A batch culture of *E. oleoabundans* supplied with glucose grows through two steps (Giovanardi et al., 2014). The exponential growth phase is characterized by rapid cell divisions, with an increase in the maximum quantum yield ($F_V/F_M$) of photosystem II (PSII), i.e., the complex machinery initiating the electron transport chain in the photosynthetic membrane. During the subsequent stationary phase, cell divisions slow down until they stop, cell volume increases and neutral lipid droplets accumulate in the cytoplasm, while a progressive decline in $F_V/F_M$ occurs (Baldisserotto et al., 2016; Giovanardi et al., 2014). The increase in $F_V/F_M$ of *E. oleoabundans* during the exponential phase is interesting, because mixotrophy often leads to an early down-regulation of photosynthesis in green algae, with a decrease in PSII photochemical yield (Giovanardi et al., 2016; Martinez and Orus, 1991; Valverde et al., 2005). The reduced activity of PSII caused by the mixotrophic growth can indeed be interpreted in terms of a feedback inhibition loop on the photosynthetic machinery by the supplied organic carbon (Burch et al., 2015; Demmig-Adams et al., 2014b). Giovanardi et al. (2017) discovered that the preservation of PSII activity in mixotrophic *E. oleoabundans* was related to a modified supramolecular organization of the

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thylakoid complexes. In particular, PSII tended to organize with photosystem I (PSI) and the light harvesting complex II (LHCII) in large megacomplexes, which were instead not resolved in the autotrophic cells. However, it was also found that the higher $F_V/F_M$ ratio of mixotrophic cells actually resulted from a down-regulated chlororespiration (Giovanardi et al., 2017). This last process depends on alternative photosynthetic electron pathways, which recycle electrons from stromal reducing equivalents to the plastoquinone pool in darkness (Bennoun, 1983; Feild et al., 1998). Operation of such electron recycling route is deemed to be important as a safety valve against an excess of electron pressure in the light-exposed samples (reviewed by Alric and Johnson, 2017). In fact, electrons recycled from NAD(P)H to the plastoquinone pool can then be passed to molecular oxygen through a reaction catalysed by a plastoquinol terminal oxidase, PTOX; this pathway also contributes to the generation of the trans-thylakoidal ΔpH (Alric and Johnson, 2017). Electron recycling routes can be quite active in green algae (Peltier et al., 2010). In darkness, an effect of an operative electron recycling is an increased basal fluorescence of PSII ($F_0$), because plastoquinone is not fully re-oxidized and a population of PSII remains closed, i.e., with reduced $Q_A$ (Plyusnina et al., 2013). Moreover, another effect is a decreased maximum PSII fluorescence ($F_M$) in darkness, because protons are accumulated in the lumen, leading to activation of thermal de-excitation as non-photochemical quenching (*NPQ*; Cruz et al., 2011). Consequently, the $F_V/F_M$ calculated in a dark-acclimated sample is underestimated. In *E. oleoabundas*, a correction of $F_V/F_M$ taking into account the occurrence of chlororespiration led to comparable values between autotrophic and mixotrophic cells (Giovanardi et al., 2017). However, the impact of mixotrophy on the photosynthetic electron flow in *E. oleoabundans* remains elusive.

Using a formalism based on energy partitioning models (reviewed by Lazár, 2015), *Y(NO)* is a parameter related to the non-regulatory dissipation of absorbed energy. Importantly, *Y(NO)* gives information on the state of the electron flow between PSII and PSI (Grieco et al., 2012; Tikkanen et al., 2017). An efficient regulation of the electron flow depends on the capacity of PSI to receive the electrons that then feed the dark-reactions of photosynthesis and, at the same time, on the capacity to alleviate the electron pressure on PSI by recirculating excess electrons from the stromal acceptors to the Cytochrome $b_6f$ complex, which is interposed between PSII and PSI in the electron transport chain (Chaux et al., 2015). A less fluent electron flow towards PSI causes the accumulation of reduced plastoquinone at the acceptor side of PSII, with an increase in *Y(NO)*. With respect to *Y(NO)*, a comparison between autotrophic and mixotrophic *E. oleoabundans* cells is presently not conclusive about electron transport efficiency. In fact, in previous works probing *E. oleoabundans* with high irradiance, it was found either a decrease, or no variation, or an increase in *Y(NO)* (Baldisserotto et al., 2014, 2016; Giovanardi et al., 2017, respectively).

A plant organism can preserve the photosystems against photodamage through concerted types of short-term photoregulation, i.e., the regulation of the electron transport to reduce the electron pressure on PSI (Chaux et al., 2015) and the regulation of light energy harvesting and funnelling to photosystems, including the thermal dissipation of excess absorbed energy meant by *NPQ* (Demmig-Adams et al., 2014a). *NPQ* is activated upon the creation of a trans-thylakoidal ΔpH during the electron flow in the membrane and basically depends on the balance between the proton pumping into the thylakoid lumen during the electron flow and the proton use for the ATP synthesis catalysed by the chloroplastic ATP synthase. Ability to generate *NPQ* also depends on structural changes and specific modulators, including regulatory thylakoid proteins and carotenoids (reviewed by Goss and Lepetit, 2015; Ruban, 2016).

In spite of an ever increasing interest in *E. oleabundans* for its biotechnological potential as a source of lipids, the basic understanding of the microalgal photosynthetic physiology is still very limited. In particular, the modulation of the photosynthetic function upon metabolism conversion from autotrophy to mixotrophy is obscure in many instances. In particular, we consider of outmost importance to understand whether under mixotrophy the PSII activity is enhanced (Baldisserotto et al., 2016) or not (Giovanardi et al., 2017). To this aim, we recorded fluorescence parameters under short-term treatments with increasing irradiance values of white light, taking into due account the impact of chlororespiration as a non-negligible variable for a correct comparison of fluorescence parameters between autotrophic and mixotrophic cells.

## 2. Material and methods

*Ettlia oleoabundans* (syn. *Neochloris oleoabundans*) (S. Chantanachat & Bold) J. Komárek, strain UTEX 1185 (Trebouxiophyceae, Chlorellales - taxonomic position according to Garibay-Hernández et al., 2017) was maintained in static liquid culture in BM medium (Baldisserotto et al., 2012) in a growth chamber at 24 ± 1 °C, 80 μmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR), 16:8 h light:darkness photoperiod. For experiments, the microalgal coltures were set up according to Giovanardi et al. (2017), so as to have an initial cell density of 0.6 ± 0.1 × $10^6$ cells $mL^{-1}$ in fresh BM medium for autotrophic cultures, or in fresh BM medium supplied with 2.5 g $l^{-1}$ glucose for mixotrophic cultures. Experimental cultures were prepared in 500 mL-Erlenmeyer flasks with a culture volume of 300 mL and maintained under constant shacking at 80 rpm, the other culture conditions described above being the same. During the growth of cultures, cell density was checked by both cell density counts with a Thoma haemocytometer and optical density with a Pharmacia Ultrospec spectrophotometer (Baldisserotto et al., 2012). In order to have an experimental material fully comparable to previous reports, cultures were sampled in the late exponential phase at the fifth or sixth day of growth, i.e., when the $F_V/F_M$ showed the maximum difference between autotrophic and mixotrophic cultures (Baldisserotto et al., 2016; Giovanardi et al., 2014, 2017).

For fluorescence analysis, cells were collected by centrifugation at 8000*g* for 10 min. The resulting cell pellet was deposited as a single drop onto a small strip of filter paper soaked with BM medium (Ferroni et al., 2011). The strips were kept in darkness for 10 min for dark acclimation of the cells. Subsequently, a pulse amplitude modulated fluorimeter (ADS-OS1-FL, ADC Bioscientific Ltd., Herts, UK) was used for fluorescence analysis. The fluorimeter fibre optics was driven to the algal pellet spot for determination of the basal fluorescence $F_0$ and then a saturation pulse (0.6 s) was applied for determination of the maximum fluorescence $F_M$. The values were used to calculate the PSII fluorescence yield in the dark-acclimated state as $F_V/F_M = (F_M - F_0)/F_M$. For the induction curves, a halogen lamp was used as the source of white actinic light, which was driven to the sample through fibre optics. The PAR irradiance reaching the sample was accurately set as uniform as possible in the measuring spot of the sample clip and its precise value was checked with a quanto-photoradiometer (Delta Ohm HD9021). Actinic light was driven to the sample at the irradiance of 12.5, 25, 50, 100, 200, 400 and 800 μmol $m^{-2}$ $s^{-1}$. After turning on the actinic light, the sample was probed applying a saturation pulse every min for 10 min. The fluorescence value at each time $F_t$ and the maximum value $F_M'$ in the light-acclimated state were measured. Then, each sample was analysed for dark relaxation: actinic light was turned off and a saturation pulse was applied after 1, 2, 5, 10 and 20 min of darkness. The values obtained at the end of the induction phase ($10^{th}$ min) were used as an approximation of steady state fluorescence values to build light-response curves.

The formalism of the energy partitioning proposed by Hendrickson et al. (2004) was used to combine the fluorescence values into informative parameters. The photochemical yield of PSII was calculated as $Y(PSII) = (F_M' - F_t)/F_M'$ (Genty et al., 1989). $Y(NO) = F_t/F_M$ was used to evaluate the electron poise of the plastoquinone pool (Grieco et al., 2012). $Y(NPQ) = F_t/F_M' - F_t/F_M$ was used to evaluate the regulatory thermal dissipation. In the formalism of Hendrickson et al.