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Effects of rare earth elements on growth rate, lipids, fatty acids and pigments in microalgae

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SUMMARY

The extensive exploitation of rare earth elements (REEs), particularly in electronic technologies and agriculture has concomitantly raised the environmental load. Their resulting effects on primary producers such as microalgae are, however, poorly understood. We have studied these effects on two microalgae of biotechnological interest. The yellow-green alga *Trachydiscus minutus* (Eustigmatophyceae, Ochrophyta), and the green alga *Parachlorella kessleri* (Trebouxiophyceae, Chlorophyta) were cultivated in mineral medium supplemented with 10 μmol L^{-1} chlorides of REEs: cerium, gadolinium, lanthanum, lutetium, praseodymium, scandium, and with monazite, which is a mineral rich in those elements. We observed growth rates at different mean light intensities (20, 50, 150 and 300 μmol m^{-2} s^{-1}). The high growth rate of *P. kessleri* was not affected by the presence of any lanthanide, and decreased proportionally with light intensity (from 0.2 to 0.04 doublings per hour). In contrast, the growth rate of *T. minutus* was about three times lower compared with *P. kessleri*, with an optimum at 50 μmol m^{-2} s^{-1} and decreased at higher or lower light intensities. In the presence of Ce^{3+}, La^{3+} and Sc^{3+}, the growth rate markedly increased to a value that corresponded to the growth rate in *P. kessleri* at the same light intensity. The composition and content of pigments and lipids were followed at the optimum light intensity for both species. The lipid content (percentage of dry weight) varied only slightly in the presence of individual rare earths. There was, however, an increase in saturated fatty acids at the expense of polyunsaturated fatty acids. The effect of REEs on pigments was variable: the presence of Ce^{3+}, Gd^{3+}, La^{3+} and Sc^{3+} caused an increase in the concentrations of major pigments such as lutein, violaxanthin, β-carotene or chlorophylls, while Pr^{3+} and Lu^{3+} reduced them.

Key words: carotenoids, chlorophylls, lanthanides, monazite, *Parachlorella kessleri*, *Trachydiscus minutus*.

INTRODUCTION

Algae are both an important part of the ecosystem and a target of biotechnology (Alexandrov *et al.* 2012). Due to their extraordinary and diverse biosynthetic potential, microalgae are increasingly being used as sources of biomass and a vast range of valuable products of nutritional and pharmaceutical interest (Gigova *et al.* 2012). Nevertheless, there are various technological and economic obstacles that must be overcome before industrial-scale production of microalgal products can take place, for example, strain selection, optimum product content (lipids and pigments), elevated growth rate, and resistance to diseases (Halim *et al.* 2012).

Rare earth elements (REEs), which are known as non-essential elements, include scandium, yttrium, lanthanum and a series of 14 lanthanides with atomic numbers 58–71. They are members of Group IIIb and the f-block elements in the periodic table and thus exhibit very similar physical and chemical properties due to the lanthanide contraction. These elements are widely dispersed and are generally common in nature, in both terrestrial and marine environments, although they rarely occur in concentrated forms (Brown *et al.* 1990). Nowadays, and because of their unique physical and chemical properties, they are used in a growing number of applications (Du & Graedel 2011).

The potential importance of lanthanides in biology and agriculture is a matter of conflicting evidence and opinions (Tyler 2004), although mixtures of lanthanide compounds have, for instance, been used extensively for more than two decades to increase crop yields in Chinese agriculture (Pang *et al.* 2002; Xu *et al.* 2002). Similar to other trace elements, lanthanides exhibit both positive and negative effects on plant growth and development at low and high concentrations, respectively (Goecke *et al.* 2015).

Although it has not been confirmed that lanthanides are essential for plant growth, studies on different agricultural products (e.g., tobacco, tomato and wheat) have demonstrated positive effects of REEs on growth and product quality. These effects have been linked to the stimulation of nutrient absorption, transfer and assimilation, alleviation of metal deficiencies, enhancement of metabolism (by enzymes), increases in oxygen evolution, an influence on photosynthesis, and effects on stress resistance (Pang *et al.* 2002; Hu *et al.* 2004; Tyler 2004; Volland *et al.* 2014; Řezanka *et al.* 2016). Consequently, REEs affect the chemical composition of living organisms, such as their lipid and pigment profiles. It has already been demonstrated that cerium and lanthanum enhanced the concentrations of polar and non-polar lipids in cell membranes. In the case of pigments, lanthanides (e.g. lanthanum) have been shown to enhance chlorophyll *a*, chlorophyll *b*, and carotenoids in different crops and terrestrial plants (Hu *et al.* 2004). The only

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evidence for algae came from Li *et al.* (2008), who showed that low concentrations of Ce^{3+} could improve growth and astaxanthin production in *Haematococcus pluvialis* Flotow, and lanthanides, when added to the green alga *Desmodesmus quadricauda* (Turp.) Bréb. led to an increase in growth and total chlorophylls (Řezanka *et al.* 2016). At low light intensities, photosynthetic rate increased markedly in the presence of Nd, and in the presence of REEs, the chlorophyll *a*/*b* ratio changed from 4.58 to 1.05.

The effects of REEs on algae and cyanobacteria remain particularly poorly understood (Goecke *et al.* 2015). In general, such effects have been studied rather to determine toxic concentrations and accumulation in biomass, with regard to runoff from fields into streams during their use in agronomy or from their production and processing in REEs mines (Evseeva *et al.* 2010; Das & Das 2013). There is also evidence that salts of REEs, at certain concentrations, stimulate the growth of some algae; nevertheless, the specific mechanisms of action of such growth-promoting factors are still unknown. Currently there are almost no published data available to describe the effect of REEs on pigments and the fatty acid profiles of algae. The aim of this study is to investigate the effect of some REEs on growth, pigment composition, production of lipids and fatty acids in microalgae, and to determine whether, by addition of these metals, the chemical profile of the algae is modified.

Two, taxonomically distant species have recently been studied extensively for biotechnological purposes. The yellow alga, *Trachydiscus minutus* (Bourelly) Ettl, is an interesting organism from a biochemical point of view, because it contains high levels of polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) (Iliev *et al.* 2010; Řezanka *et al.* 2010, 2011; Lukavský 2012; Alexandrov *et al.* 2012; Cepák *et al.* 2014). The green alga *Parachlorella kessleri* (Fott & Nováková) Krienitz, E.H.Hegewald, Hepperle, V.Huss, T.Rohr & M.Wolf has a rapid growth rate and the potential for mass production of lipids, and thus is a candidate taxon for algal biofuel production (Přibyl *et al.* 2012a; Mizuno *et al.* 2013; Li *et al.* 2013).

MATERIAL AND METHODS

Microalgal strains and culture conditions

The Culture Collection of Autotrophic Organisms at the Institute of Botany, CAS in Třeboň, Czech Republic (CCALA) provided *Trachydiscus minutus* (Eustigmatophyceae, Ochrophyta) strain CCALA 931 and *Parachlorella kessleri* (Trebouxiophyceae, Chlorophyta) strain CCALA 255.

Laboratory cultivation units consisted of glass cylinders (inner diameter 36 mm, height 500 mm). They were placed in a thermostatic bath 23 ± 0.5°C for *T. minutus* and (30 ± 0.5°C for *P. kessleri*), and continuously illuminated by a panel of dimmable fluorescent lamps (OSRAM DULUX L55W/950 Daylight, Milano, Italy) with a mean light intensity of 50 μmol m^{-2} s^{-1} for *T. minutus* and 300 μmol m^{-2} s^{-1} for *P. kessleri*. The volume of algal suspension in each cylinder was 300 mL, aerated with air containing 2% carbon dioxide (v/v). For the mineral medium used in experimental cultures, see Přibyl *et al.* (2012b) for *T. minutus* and Brányiková *et al.* (2011) for *P. kessleri*.

We tested the following REEs as chlorides: cerium (Ce^{3+}), gadolinium (Gd^{3+}), lanthanum (La^{3+}), praseodymium (Pr^{3+}) and scandium (Sc^{3+}) that belong to the group of light rare earth elements; plus lutetium (Lu^{3+}), which is the only one belonging to the heavy rare earth elements. For that purpose, we used analytical grade chlorides (99%; Sigma-Aldrich, Steinhelm, Germany) dissolved in sterile distilled water. We also tested monazite (Mon), a mineral that contains more than 50% of rare earth elements in the form of phosphates, especially yttrium, cerium, gadolinium and lanthanum and a lower content of neodymium, samarium and praseodymium (Table 1).

For the latter case, 5 g of Mon (originally from Vietnam) was ground into a very fine powder and boiled for 20 min with 25 mL of concentrated H_2SO_4. The extract was cooled, centrifuged and the supernatant was used for experiments. In order to determine optimal concentrations, preliminary tests were performed and a concentration 10 μmol L^{-1} was found to be optimum.

Table 1. ICP-MS analysis of monazite extract, values are given in mg L^{-1}

Y	Fe	Th	Gd	Ce	La	Nd	Sm	Pr
276	139	26.8	26.6	24.5	23.4	15.9	8.16	3.80

Measurements

A quantum/radiometer-photometer (LI-COR, Inc., Lincoln, NE, USA) was used to measure irradiances. In the culture unit, dimmable fluorescent tubes were used for adjustment of irradiance. To obtain a measure of light energy absorbed by a layer of cell suspension grown at different incident light intensities and different absorbances (A_{750}) (equivalent to cell concentration), the mean light intensity (I) was calculated according to the Lambert–Beer formula: $I = (I_i - I_t)/\ln(I_i/I_t)$, where I_i is the incident light intensity measured at the surface of the culture vessel, and I_t is the transmitted light intensity measured at the rear side of the culture vessel.

Cell suspensions were diluted before measurements to an A_{750} lower than 0.2 (in 0.5 cm measuring cuvettes) to avoid any distortion of measured values due to pigment absorption.

Samples were centrifuged, the supernatant was discarded, and the resulting pellet was frozen at –80°C. Prior to analysis, frozen samples were lyophilized and weighed on an analytical 1601 MPB balance (Sartorius, Austin, TX, USA).

Cultures were grown in the presence of lanthanides (Ce^{3+}, Gd^{3+}, La^{3+}, Lu^{3+}, Pr^{3+}, Sc^{3+} and Mon), at four mean light intensities (20, 50, 150, and 300 μmol m^{-2} s^{-1}), and growth rates were compared with growth of a control culture (REE free). To keep growth in the exponential phase, cultures were diluted to maintain an A_{750} between 0.05 and 0.9. Four such growth steps were performed for each of the light intensities tested to calculate a mean growth rate. Growth rate (μ) (expressed in doublings of dry weight per hour) was then calculated as $\mu = (\log_2 dw_t - \log_2 dw_0)/(t - t_0)$, where dw_t was dry weight at the end (t) and dw_0 at the beginning (t_0) of each growth step.

Lipid analyses

For lipid analysis, frozen samples were lyophilized and weighed. The extraction procedure was based on the method of Bligh and Dyer (1959); the sample was vortexed with $CHCl_3$: MetOH 1:2 (v/v), water and $CHCl_3$ were added in equal volumes, and the $CHCl_3$ bottom phase was separated. The chloroform phase was washed three times with two parts of 1 mol/L KCl. The resulting chloroform phase was evaporated to dryness under reduced pressure and weighed.

Total lipid extracts were applied to a Cartridge Vac 35 cc (Sep-Pak, Milford, MA, USA) with 10 g of aminopropylsilica-based polar bonded phase), and neutral lipids (see Table 2) were eluted with 40 mL of chloroform-methanol (7:3, v/v).

Neutral lipids (approximately 5 mg) were saponified overnight in 10% KOH-methanol at room temperature. A fatty acid fraction obtained from the saponification was partitioned between an alkaline solution (pH 9) and diethyl-ether to remove basic and neutral components. The aqueous phase containing fatty acids was acidified to pH 2 and extracted with hexane. The fatty acid fraction was methylated using BF_3/MetOH (Řezanka & Podojil 1984).

The analysis of fatty acid methyl esters (FAMEs) was carried out on a gas chromatography–mass spectrometry (GC/MS) system consisting of a Varian 450-GC (Varian BV, now Agilent Technologies, Santa Clara, CA, USA), Varian 240-MS ion trap detector with electron impact ionization (EI), and CombiPal autosampler (Mitchell Drive Walnut Creek, CA, USA). Splitless injection was at 100°C, and a fused silica capillary column (Supelcowax 10; 60 m × 0.25 mm, i.e. 0.25 mm film thickness; Supelco, Prague, Czech Republic) was used. The temperature program was as follows: 100°C for 1 min, subsequently increasing at 20°C min^{-1} to 180°C and at 2°C min^{-1} to 280°C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm s^{-1}. FAMEs were identified according to their mass spectra and using a mixture of chemical standards obtained from Sigma-Aldrich (Dembitsky *et al.* 1992).

Table 2. Lipid production in *Trachydiscus minutus* and *Parachlorella kessleri*, grown at optimal mean light intensities 50 and 300 μmol m^{-2} s^{-1}, respectively and exposed to different rare earth elements (REEs) and monazite (Mon) extract

REE	*T. minutus* % of dw	*P. kessleri* % of dw
Control	**27.3 ± 1.2**	**15.7 ± 0.7**
Ce	27.0 ± 0.6	13.9 ± 0.6
Gd	21.1 ± 1.1	14.0 ± 0.9
La	27.1 ± 0.6	16.4 ± 0.8
Lu	23.4 ± 0.2	15.3 ± 0.8
Sc	21.3 ± 0.8	15.3 ± 0.2
Pr	26.9 ± 1.0	14.6 ± 0.3
Mon	28.1 ± 0.8	13.4 ± 0.4
R.V.	26.0	10.0

Results are expressed as percentage of neutral lipids per dry weight of biomass. Control values (no REEs) are given in bold, reference value (R.V.) for the strains *P. kessleri* and *T. minutus* corresponds to Li *et al.* (2013) and Iliev *et al.* (2010).

Pigment analyses

Pigments were extracted from the biomass with an excess of 90% methanol, 10% water, and measured as described previously (Hollingshead *et al.* 2012). Briefly, the extracts were injected onto an Agilent-1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with two Agilent 1100/1200 fluorescence detectors and a Agilent 1200 diode array detector (Hollingshead *et al.* 2012). Separation of pigments was performed on a reverse-phase column (Kinetex C8, 2.6 μm particle size, 3.9 × 150 mm (Phenomenex, Torrance, CA, USA) with 35% methanol, 15% acetonitrile, and 50% pyridine (solvent A); and 20% methanol, 60% acetonitrile, and 20% acetone as solvent B. Pigments were eluted with a linear gradient of solvent B (30–95% in 25 min) followed by 95% of solvent B at a flow rate of 0.8 mL min^{-1} at 40°C. The pigments were estimated using published extinction coefficients for chlorophyll and carotenes (Chidgey *et al.* 2014).

All analyses were carried out in triplicate and mean values and standard deviations were used for the construction of figures.

RESULTS

Growth rate of algal cultures grown under different light intensities

Cultures of the microalgae *T. minutus* and *P. kessleri* were grown in the presence of 10 μmol L^{-1} of Ce^{3+}, Gd^{3+}, La^{3+}, Lu^{3+}, Pr^{3+}, Sc^{3+} and Mon at a low cell concentration (A_{750} = 0.01) to ensure that a mean light intensity was nearly equal to incident light intensities of 20, 50, 150 and 300 μmol m^{-2} s^{-1}. Control cultures were grown in the absence of any lanthanides in the nutrient medium. To measure exponential growth, cultures were regularly diluted to maintain a mean light intensity within the range where substantial shadowing of cells, which would lead to a decreased mean light intensity, could not occur. Exponential growth was proven by linear lines if plotted on a log y scale.

No significant differences in growth rates between controls and cultures of *P. kessleri* grown in the presence of individual lanthanides were found at any light intensity used, (Fig. 1a). Growth rate was the highest at the highest mean light intensity (300 μmol m^{-2} s^{-1}) and decreased with decreasing light intensity (Fig. 1a).

The growth rate of *T. minutus* at all light intensities was substantially lower (about three times) than that of *P. kessleri* (Fig. 1a,b). Maximum growth rate was attained at a mean light intensity of 50 μmol m^{-2} s^{-1}. An increase or decrease in light intensity from optimum caused a decrease in growth rate (Fig. 1b). In contrast to *P. kessleri,* cultures of *T. minutus,* in the presence of Ce^{3+}, La^{3+} and Sc^{3+} at the optimum light intensity, had a significantly higher growth rate than the control culture (Fig. 1b).

Lipid composition

The relative neutral lipid content (% DW) in the presence of REEs did not vary markedly in comparison with the control culture (REE free), with a tendency to be slightly lower in the

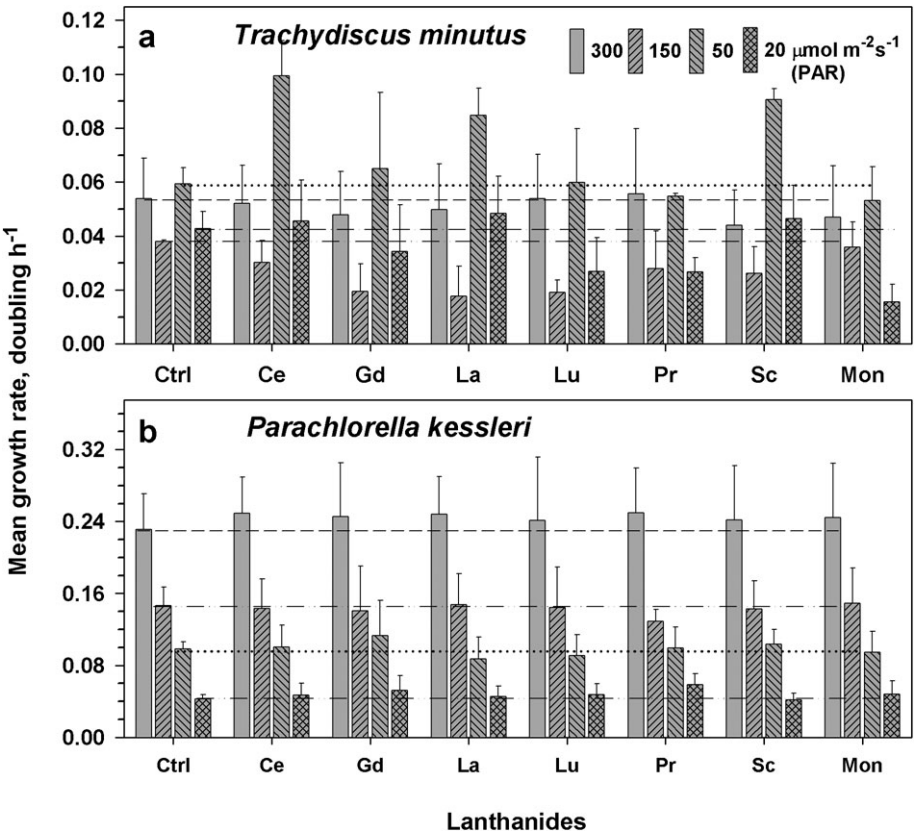

Fig. 1. Growth rate (in doublings of dry weight per hour) of (a) *Trachydiscus minutus* and (b) *Parachlorella kessleri* grown at different mean light intensities (20, 50, 150, 300 μmol m^{-2} s^{-1}) without (Ctrl) or with 10 μmol L^{-1} lanthanide chlorides (Ce^{3+}, Gd^{3+}, La^{3+}, Lu^{3+}, Pr^{3+} and Sc^{3+}) or monazite (Mon). Horizontal lines indicate growth rate in control culture (no REEs) grown at corresponding light intensity.

case of Gd^{3+}, Lu^{3+} and Sc^{3+} in *T. minutus,* and Ce^{3+}, Gd^{3+} and Mon in the case of *P. kessleri* (Table 2).

However, the presence of REEs strongly affected the composition of neutral lipids produced in both algae. All REEs applied caused an increase in saturated fatty acids (SFAs) at the expense of polyunsaturated fatty acids (PUFAs). In both species, Sc^{3+} was the most effective element (Table 3, Fig. 2).

Trachydiscus minutus growing under control conditions produced neutral lipids comprising SFAs, MUFAs and PUFAs (Table 3); however, after exposure to REEs, SFAs increased, varying from the lowest value by 4.9% with Ce^{3+} to the highest by 12.7% with Sc^{3+} treatments (mean value of all REEs was an increase in SFAs by 7.7%). Total PUFAs decreased in this alga by 3.9% in Gd^{3+} or Lu^{3+} or by 8.5% in Sc^{3+} (mean value was a decrease by 5.9%). MUFAs values were more constant and the mean decrease with treatments was by 1.1% of the total MUFAs (Table 3).

Parachlorella kessleri produced SFAs, MUFAs and PUFAs under standard conditions (Table 3), but total SFAs increased to an extent varying from the lowest value by 1.6% in the presence of Pr^{3+} to the highest by 7.6% with Sc^{3+} (mean increase of 4.4%). Total MUFAs were more stable (mean increase of 1.1%); and PUFAs decreased, varying from the lowest value by 3% with Gd^{3+} to the highest one by 8% with Sc^{3+} (mean decrease by 5.5%) (Table 3).

Saturated fatty acids with C14:0 (myristic acid) and C16:0 (palmitic acid) aliphatic chains were found in *T. minutus* and

Table 3. Content of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) expressed as a percentage of total neutral lipids in the microalgae *T. minutus* and *P. kessleri* grown at their optimum light intensities and exposed to six different REEs and Mon

T. minutus				*P. kessleri*		
REE	SFA %	MUFA %	PUFA %	SFA %	MUFA %	PUFA %
Control	**40.4 ± 0.7**	**13.2 ± 0.7**	**46.4 ± 0.7**	**34.3 ± 0.8**	**25.2 ± 0.7**	**40.5 ± 0.8**
Ce	45.3 ± 0.9	12.8 ± 1.2	41.9 ± 1.1	41.4 ± 1.0	25.8 ± 0.7	32.8 ± 1.0
Gd	45.3 ± 0.8	12.2 ± 0.8	42.5 ± 0.8	36.8 ± 1.1	25.7 ± 1.1	37.5 ± 0.9
La	47.5 ± 0.8	13.6 ± 1.1	38.9 ± 0.9	38.4 ± 0.9	24.6 ± 0.6	37.0 ± 0.5
Lu	49.1 ± 0.6	12.7 ± 0.6	38.2 ± 0.9	37.5 ± 0.7	26.9 ± 0.5	35.6 ± 0.4
Sc	53.1 ± 0.5	09.0 ± 0.9	37.9 ± 0.8	41.9 ± 0.7	25.6 ± 0.9	32.5 ± 0.9
Pr	48.1 ± 1.0	12.4 ± 0.7	39.5 ± 0.5	35.9 ± 1.2	29.4 ± 1.0	34.7 ± 1.0
Mon	43.6 ± 0.4	13.0 ± 0.9	43.4 ± 0.7	37.7 ± 0.5	27.0 ± 0.4	35.3 ± 0.9

See the caption of Table 2 for other information.

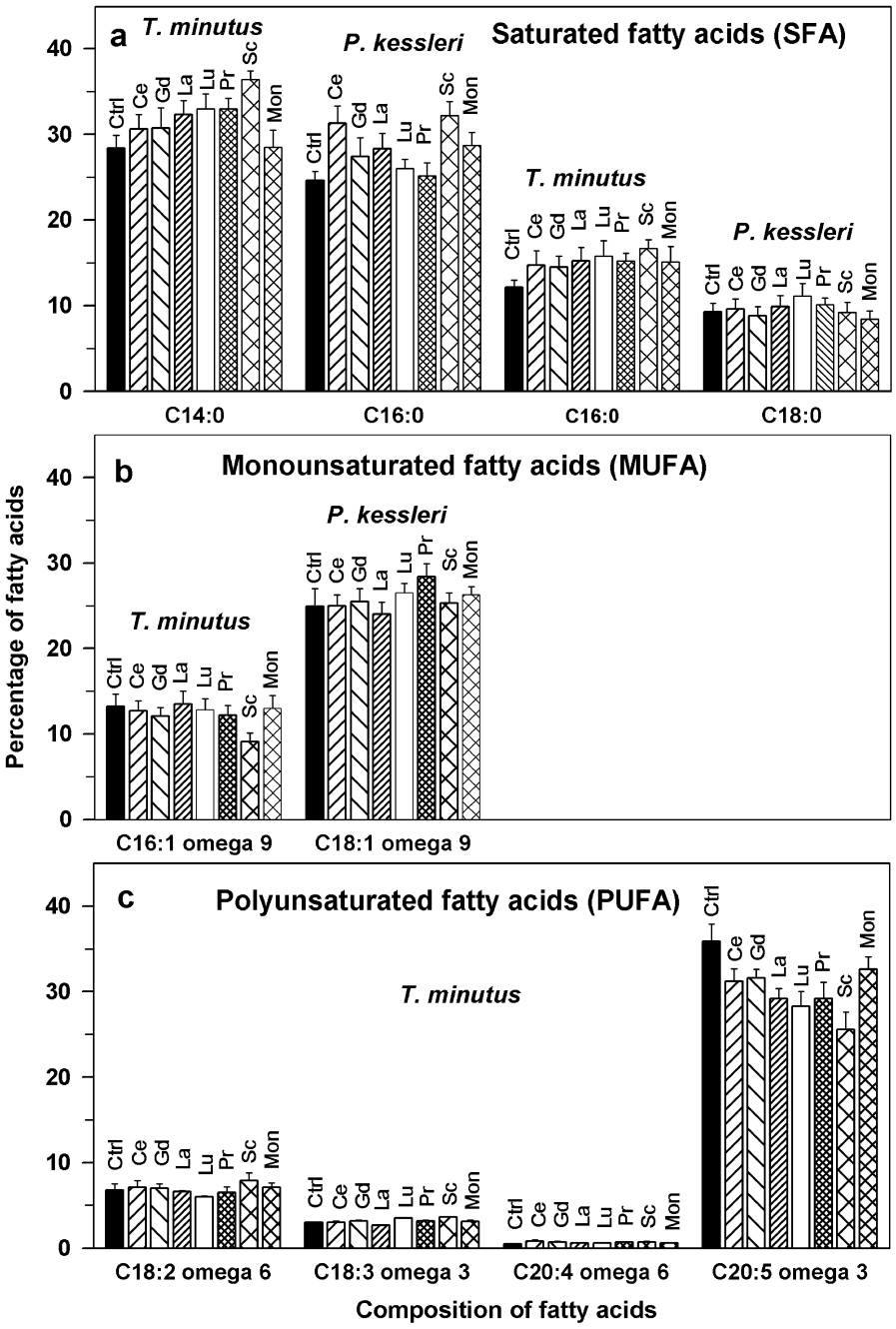

Fig. 2. The proportion of (a) SFA, (b) MUFA and (c) PUFA in neutral lipids of *T. minutus* and *P. kessleri* grown at their optimum light intensities 50 and 300 μmol m^{-2} s^{-1}, respectively. See the caption of Figure 1 for other information.

C16:0 and C18:0 (stearic acid) aliphatic chains were found in *P. kessleri* (Fig. 2a).

The monounsaturated fatty acid (MUFA), palmitoleic acid (C16:1ω9), was found in *T. minutus* and oleic acid (C18:1ω9) in *P. kessleri* (Fig. 2b). The di-unsaturated fatty acid, linoleic acid (C18:2ω6), was found at less than 10% w/w only in *T. minutus* (Fig. 2c). Fatty acids with a higher number of bonds (3–5), such as linolenic acid (C18:3ω3) and eicosapentaenoic acid (EPA) (C20:5ω3) were found only in *T. minutus* (Fig. 2c). The arachidonic acid (C20:4ω6) content was less than 1% w/w, and the fatty acid EPA (C20:5ω3) was the major fatty acid in *T. minutus*, (not present in *P. kessleri*). Its concentration ranged from 25 to 36% of total fatty acids in neutral lipids, and varied according to the REE species used (Fig. 2c). Other fatty acids, in decreasing order, were myristic acid (28–36%), palmitic acid (12–16%), palmitoleic acid, and the PUFAs linoleic acid (C18:2ω6), γ-linolenic acid (C18:3ω6) (Fig. 2) and arachidonic acid (C20:4ω6).

In contrast, in *P. kessleri* neutral lipids, linoleic acid was the major fatty acid, ranging from 32 to 40% of total fatty acids. Other fatty acids, in decreasing order, were palmitic acid (24–32%) (Fig. 2), oleic acid (24–28%) stearic acid, myristic acid and palmitoleic acid (Fig. 2). Although the neutral lipids were mainly composed of C16 and C18 fatty acids under all conditions tested, the ratio of saturated and unsaturated fatty acids was altered by culture conditions. In the presence of REEs, the total SFAs increased, while PUFAs decreased (Table 3, Fig. 2).

Pigment composition

Trachydiscus minutus contained violaxanthin as the main pigment, followed by vaucheriaxanthin, (not present in *P. kessleri*) and chlorophyll *a*. *P. kessleri* contained lutein (not present in *T. minutus*) as the dominant pigment, followed by chlorophylls *b,* and *a*. The composition of algal pigments varied according to algal species and individual REEs (Table 4).

Pigment content of *T. minutus* in the presence of REEs was not substantially affected in comparison to control (REE free) cultures (Fig. 3a,b). In contrast, significant changes in *P. kessleri* pigment content were found (Fig. 3c,d). The main pigments of this alga were clearly decreased by some REEs, such as lutein (by Lu^{3+} and Pr^{3+}), chlorophyll *b* (by Pr^{3+} and Mon), chlorophyll *a* (by Lu^{3+}, Pr^{3+} and Mon) and β-carotene (by all REEs except Ce^{3+}). However, an important increase in chlorophylls and carotenoids compared to controls was also demonstrated for some REEs, especially for Ce^{3+}, Gd^{3+}, La^{3+}, and Sc^{3+}. Mon produced an increase in violaxanthin (and related compounds) and lutein (Fig. 3c).

DISCUSSION

A comparison of growth rates of the selected species showed that the green alga *P. kessleri* grew much faster at high light intensities than the yellow-green alga *T. minutus.* The main difference between these two species, however, was in their response to the presence of different REEs.

The presence of REEs had practically no effect on the growth rate of *P. kessleri* cells (Fig. 1a). It could be assumed that any increase or decrease in the presence of lanthanides would not affect rapid growth processes such as photosynthetic rate. No effect of lanthanides on photosynthetic rate at high irradiances was observed in *Desmodesmus quadricauda*, and there were no significant differences in growth rates in the presence or absence of lanthanides (Řezanka *et al.* 2016). In contrast to *P. kessleri*, the growth rate of *T. minutus* in the presence of individual lanthanides was affected differently, both increasing and decreasing. The most remarkable was an increase in growth rate in the presence of Ce^{3+}, La^{3+}, or Sc^{3+} at the optimum mean light intensity (50 μmol m^{-2} s^{-1}). Growth rate increased from 0.06 to nearly 0.1 doublings per hour in the presence of Ce^{3+}, which corresponds to the growth rate of *P. kessleri* at the same mean light intensity.

This finding could be important for increasing the relatively slow growth rate of *T. minutus,* which is valuable for its high content of PUFAs such as linolenic acid and EPA, fatty acids that are not commonly found in other algae, particularly *P. kessleri*. As has been shown in earlier published papers (Řezanka *et al.* 2010, 2011; Lukavský 2012), *T. minutus* accumulates EPA-enriched triacrylglycerols (TAG). The authors described the lipid composition, particularly EPA, under either standard growth conditions or under N, S or P limitation. They found that only P limitation increased EPA production compared with control cultures. We are not aware of any paper that reports on the effect of lanthanides on the composition of algal fatty acids in neutral lipids.

There is evidence of a relationship between changes in lipid and pigment contents in microalgae, depending on the environmental conditions. In another Eustigmatophyceae microalga, *Nannochloropsis* sp., under stress conditions (high salinity and light), the content of the major PUFA, EPA, has

Table 4. Effect of selected concentrations of six different REEs and Mon on the pigment content of *T. minutus* and *P. kessleri* grown at their optimum light intensities

T. minutus

REE	BCA	VIO	VIO-like	Chl *a*	VAX-esters	ZEX	CAX-like	VAX
Control	**23 ± 3**	**627 ± 15**	**38 ± 2**	**147 ± 2**	**60 ± 0.7**	**57 ± 0.3**	**92 ± 6**	**248 ± 12**
Ce	26 ± 2	559 ± 18	36 ± 0.4	160 ± 3	52 ± 0.5	49 ± 0.6	85 ± 2	255 ± 10
Gd	26 ± 3	583 ± 16	36 ± 0.8	155 ± 2	54 ± 0.4	52 ± 0.2	84 ± 1	232 ± 9
La	38 ± 4	620 ± 20	41 ± 0.7	182 ± 6	56 ± 1	51 ± 0.4	89 ± 4	n.d.
Lu	26 ± 4	579 ± 13	35 ± 0.9	153 ± 3	58 ± 1.2	55 ± 0.9	86 ± 3	227 ± 9
Pr	30 ± 2	529 ± 13	34 ± 0.6	169 ± 4	46 ± 0.8	46 ± 0.4	82 ± 1	240 ± 7
Sc	33 ± 0.8	579 ± 7	37 ± 0.4	170 ± 4	54 ± 3	47 ± 2	80 ± 0.9	259 ± 4
Mon	29 ± 0.6	634 ± 14	38 ± 1	163 ± 5	58 ± 0.9	56 ± 3	96 ± 6	244 ± 4

P. kessleri

REE	BCA	VIO	VIO-like	Chl *a*	Chl *b*	NEX-like	LUT	unid
Control	**629 ± 12**	**175 ± 65**	**440 ± 14**	**1087 ± 24**	**2035 ± 303**	**242 ± 18**	**2768 ± 28**	**33 ± 2**
Ce	638 ± 18	19 ± 15	709 ± 9	1384 ± 19	184 ± 40	585 ± 19	3933 ± 37	106 ± 5
Gd	465 ± 17	527 ± 9	688 ± 8	1201 ± 16	2222 ± 25	360 ± 23	3863 ± 40	103 ± 12
La	462 ± 12	421 ± 25	578 ± 11	1106 ± 19	2610 ± 28	479 ± 25	3334 ± 38	910 ± 10
Lu	136 ± 10	348 ± 6	560 ± 7	564 ± 7	2076 ± 26	398 ± 22	2076 ± 15	150 ± 15
Pr	184 ± 12	264 ± 8	204 ± 6	430 ± 10	1114 ± 20	283 ± 20	1820 ± 15	9 ± 1
Sc	558 ± 11	275 ± 10	598 ± 10	1279 ± 22	2663 ± 32	378 ± 21	3745 ± 42	163 ± 4
Mon	126 ± 9	628 ± 11	601 ± 12	603 ± 12	1276 ± 18	437 ± 18	3214 ± 20	57 ± 6

The pigment concentrations are expressed as relative absorbance at 450 nm. BCA, β-carotene; CAX-like, canthaxanthin-like; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; LUT, lutein; NEX, neoxanthin; unid, unidentified pigment; VAX, vaucheriaxanthin; VAX-esters, vaucheriaxanthin-esters; VIO, violaxanthin; VIO-like, violaxanthin-like; ZEX, zeaxanthin. See the caption of Table 2 for other information.

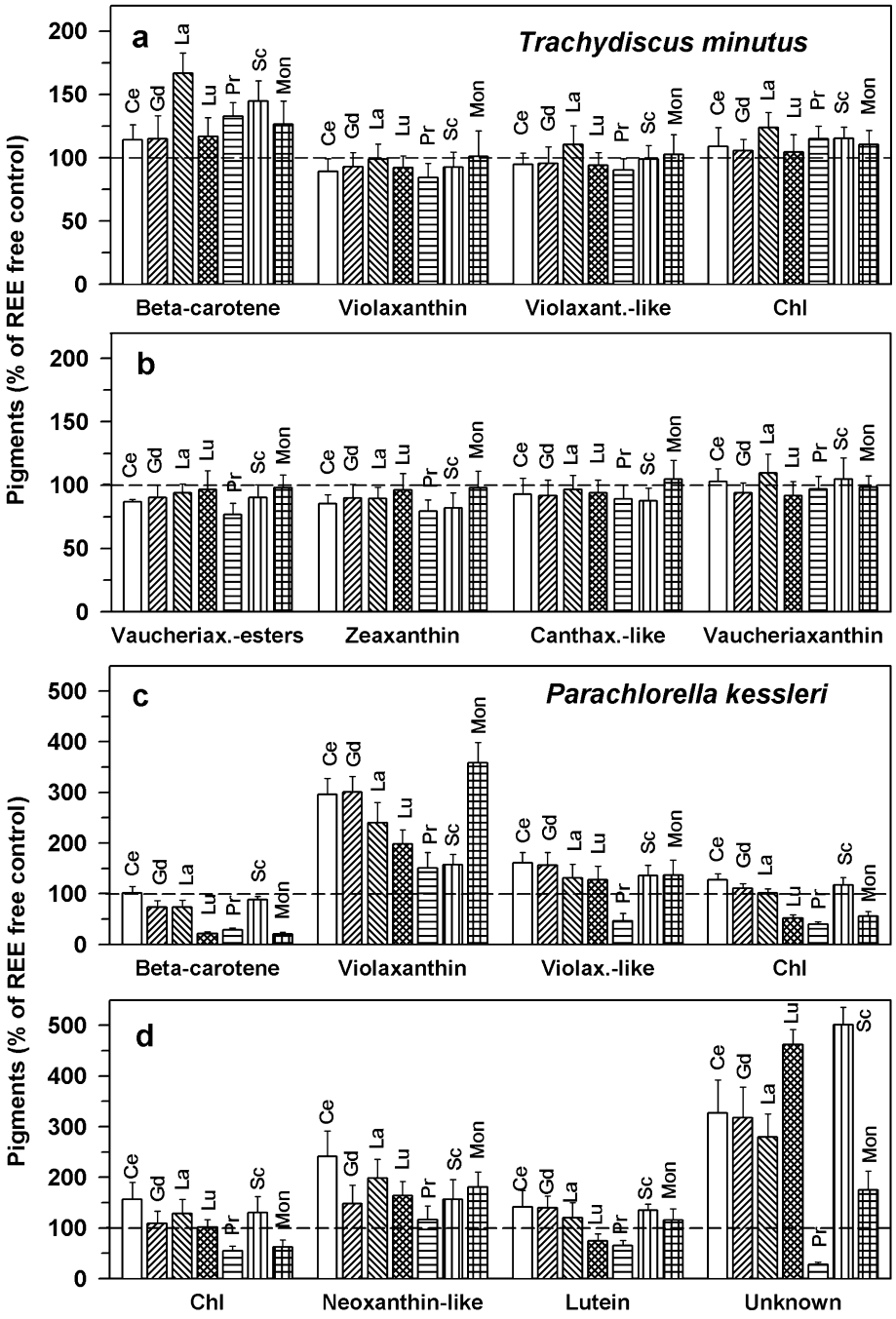

Fig. 3. Content of different pigments after exposure to six different REEs and monazite in *T. minutus* (a, b) and *P. kessleri* (c, d) grown at their optimum light intensities. Mean values with standard deviations are compared with those in controls (no REEs), which are taken as 100% (horizontal line). For abbreviations of pigments, see Table 4.

been shown to be reduced (Pal *et al.* 2011). It seems that if all elements required for growth are available in the mineral medium, microalgae divide rapidly and synthesize polar lipids. When any growth factor becomes limiting but photosynthetic carbon dioxide fixation continues, there is a reduction in cell division, frequently followed by a reduction in the photosynthetic apparatus and by accumulation of neutral lipids (Li *et al.* 2013), in particular TAG, and nonpolar carotenoids in carotenogenic species (Solovchenko 2012).

The present experiments provide evidence that in the presence of REEs, the proportion of total SFAs increases and total PUFAs decrease. The fatty acids were mostly reduced in their production of EPA, in the case of *T. minutus* and linoleic acid in *P. kessleri*. A similar effect was also found in other algal species such as in *Nannochloropsis* sp. (Pal *et al.* 2011) and *Euglena gracilis* Klebs (Rocchetta *et al.* 2006) but more surprisingly, in 3T3-L1 animal cell lines, where supplementation of La^{3+} (5 μM), Ce^{3+} (5 μM), or their combination (at 15 μM) increased SFAs (He *et al.* 2006). From the biotechnological point of view, increased SFA in the presence of REEs could be advantageous in *P. kessleri* as a potential producer of lipids for biofuels. On the other hand, a decrease in PUFAs, particularly EPA, in *T. minutus,* which is a potential source of PUFAs as food and feed additives, is counter-productive. It is

debatable if this negative effect can be compensated by an increase in growth rate (see above) in the presence of REEs.

From a metabolic point of view, accumulation of TAGs comprising long-chain PUFAs has proved to be less beneficial and therefore is less common in nature. Additionally, the accumulation of TAGs enriched in SFA and MUFA should be advantageous, because less energy is required for the synthesis of PUFAs (in comparison to starting *de novo*) and more energy is released during oxidation of these compounds (Solovchenko 2012). There are many papers reporting recent achievements in the improvement of microalgal oil production (see Přibyl *et al.* 2012a and references therein). A few of them have studied the effect of different factors on the chemical profile and growth kinetics of *T. minutus* and *P. kessleri* (Dembitsky *et al.* 1992; Řezanka *et al.* 2011; Gigova *et al.* 2012; Li *et al.* 2013; Cepák *et al.* 2014). We are not aware of any study dealing with the effect of REEs. It is well known that microalgae alter their fatty acid composition in response to various culture conditions; however, the mechanisms regulating lipid accumulation in response to altered growth environments remain unclear (Li *et al.* 2013). Especially, the effect of REEs on algae and cyanobacteria remains particularly poorly understood (Goecke *et al.* 2015).

The effects of REEs on pigment content were very variable depending on the element and species to which it was applied (Fig. 3). Practically no marked effect was found in *T. minutus* for β-carotene, violaxanthin, and chlorophyll *a*. In the presence of all REEs, a neoxanthin-like pigment was increased, while for other pigments such as chlorophyll *b* or lutein, the lanthanide lutetium caused a decrease compared with a culture without REEs. In *P. kessleri*, Lu^{3+} also caused a decrease in all pigments. Lutein β-carotene, and chlorophyll *a* also decreased in the presence of Sc^{3+}. Other REEs caused mostly slight changes in pigment content, although a marked increase in a violaxanthin-like pigment occurred. The specific mechanisms of action of such promoting factors are still unknown and require further investigation.

In *P. kessleri*, increased chlorophyll is related to more lutein (treatments with Ce^{3+}, Gd^{3+}, La^{3+}, Sc^{3+}). Only Mon did not follow this pattern. Less chlorophyll is associated with less lutein (treatments with Lu^{3+} and Pr^{3+}). From these results, we hypothesize that Ce^{3+}, Gd^{3+}, La^{3+}, Sc^{3+} (positively), and Lu^{3+} (negatively) affect chlorophyll synthesis, with other responses on pigment profiles; this needs to be corroborated.

In relation to other pigments, the specific functions of secondary carotenoids in carotenogenic microalgae are being vigorously debated. However, the mechanisms of their induction and regulation under stress are poorly understood.

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