Journal of Applied Phycology (2024) 36:1197–1208
https://doi.org/10.1007/s10811-023-03161-2

RESEARCH

Ecophysiological characterisation of a *Klebsormidium* strain isolated from a cave environment

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Received: 23 September 2023 / Revised: 3 December 2023 / Accepted: 5 December 2023 / Published online: 26 December 2023

Abstract

Members of the genus *Klebsormidium* are ubiquitously distributed over the Earth and are among the major biological soil crust (BSC) forming microalgae. Their representatives can be found in terrestrial, aquatic, polar, desert regions and have been investigated so far from various aspects. However, the available information about *Klebsormidium* isolates from lamp-flora is very limited. In our work, we examined a *Klebsormidium* strain isolated from a Hungarian cave. The temperature optimum of its photosynthetic performance was tested by oxygen yield measurements and pulse-amplitude-modulated fluorescence, which were completed by determination of specific growth rates at different temperatures, from 10 to 40 °C. In addition, we also evaluated the brassinosteroid (BR) content of these cultures. Our results indicated that the studied microalga is capable of growing from 10 to 40 °C, with a 20–25 °C temperature optimum; these findings were in accordance with the observed hormone levels. Regarding photosynthetic performance, the oxygen yield and chlorophyll fluorescence measurements showed maxima at 30–40 °C and 35–40 °C, respectively. Moreover, the examined *Klebsormidium* strain demonstrates traits associated with cave adaptation, i.e., by high light utilisation factor (α) and diminished light adaptation parameter (I_k) values.

Keywords Charophyta · Algal growth · Chlorophyll fluorescence · Ecotype · Oxygen evolution · Temperature optimum · Brassinosteroid

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Introduction

Oxygenic photoautotrophic microorganisms ("microalgae") are a very diverse group in terms of both phylogenetics and morphology. They include a large variety of pro- and eukaryotic photoautotrophs (i.e. cyanobacteria and various algal clades) which can be either unicellular, filamentous, or colony-forming. Regarding eukaryotic microalgae, the oldest fossils are dated to be 1.45–1.50 billion-year-old (Yoon et al. 2004; Moczydłowska et al. 2011), while oldest cyanobacterial fossils are thought to be much older: about 3.5 billion years old (Brasier et al. 2005). Both pro- and eukaryotic microalgae have the ability to adapt evolutionarily to various environments, which, in turn, entails the variability of their cellular composition. During the evolution, microalgae colonised besides many freshwater and marine habitats, almost all kinds of terrestrial biotopes including even deserts, polar regions and high mountains (Grama et al. 2022). In these habitats, they provide diverse ecosystem services contributing significantly to human well-being (recently reviewed by B-Béres et al. 2023; Lengyel et al. 2023; Naselli-Flores and Padisák 2023) and ecosystem diversity. Ecosystem services of algae include the production of valuable bio-based products, such as phytohormones, pigments, polysaccharides, vitamins, polyunsaturated fatty acids and lipids. The demand for these bio-products inspires the use of microalgae in various biotechnological applications (Khalid 2020). Microalgae have been used in food industry, agriculture, aquacultures, pharmaceutical, nutraceutical and cosmetic industry, biofuel production, wastewater treatment, as well as soil fertiliser and biostimulant (e.g. Pulz and Gross 2004; Odjadjare et al. 2017; Morais Junior et al. 2020; Yap et al. 2021). Nowadays, the number of biotechnologically relevant taxa as well as the size of this market is constantly increasing. The global microalgae market was estimated at US$ 3.4 billion in 2020 and is projected to reach US$ 4.6 billion by the year 2027 (Loke Show 2022).

Among microalgae, the genus *Klebsormidium* (Charophyta, Klebsormidiales) comprises a relatively small number of species (27 taxa and four variates, Guiry and Guiry 2023), being distributed worldwide. They occur both in cold and hot climate areas, populating both some aquatic and numerous terrestrial ecosystems (Smith et al. 2004; Rindi et al. 2011; Kaplan et al. 2012; Borchhardt et al. 2017; Donner et al. 2017). Many members of the genus are generalists (Borchhardt and Gründling-Pfaff 2020), but there are lineages that are so specialised to their habitat that they are unable to establish a subpopulation in other environments (Škaloud and Rindi 2013; Škaloud et al. 2014).

The soil-dwelling members of *Klebsormidium* have special ecological importance as they play an important role in the formation of biological soil crusts (BSCs) (Smith et al. 2004; Kitzing and Karsten 2015; Büdel et al. 2016; Samolov et al. 2019). The BSC developed on the soil surface significantly reduces soil erosion, improves the structure and hydrological properties of the soil, provides habitat for microorganisms and has role in cycling nutrients (Belnap et al. 2016; Antoninka et al. 2020; Baumann et al. 2021; Warren et al. 2021). Moreover, *Klebsormidium* species have a great potential for use in agriculture as biostimulants due to their high endogenous phytohormone content, such as brassinosteroids (BRs) (Futó et al. unpublished; Stirk et al. 2013). Brassinosteroids are polyhydroxylated steroidal phytohormones with multiple physiological effects on plant growth and development and involved also in the defence against both biotic and abiotic stress (Hayat and Ahmad 2011; Stirk et al. 2013; Nguyen et al 2020), mainly via cross-talk with other hormones (Krishna 2003; Oklestkova et al. 2015). The most biological active brassinosteroid is brassinolide (BL) having fivefold higher biological activity compared to its biosynthetic intermediate castasterone (Fujioka et al. 1995). The brassinosteroid levels in microalgae are influenced by different abiotic stresses (Stirk et al. 2018; Stirk and van Staden 2020).

Besides phytohormone production, algal colonisation of soil surfaces and development of BSC are also significantly influenced by a variety of abiotic and biotic parameters (e.g. trophic mode, soil moisture, nutrient content and chemical composition of the soil, temperature, irradiation, pH, the local CO_2 concentration, etc., Lepossa 2003; Chen et al. 2017; Choi et al. 2017; Piotrowska-Niczyporuk et al. 2018; Stirk et al. 2018; Beigbeder and Lavoie 2022). From these parameters, light intensity and temperature are considered as the main variables responsible for ecotype differentiation of the members of *Klebsormidium* (Karsten et al. 2016a; Míguez et al. 2020). Importantly, most of the available data are based on aquatic and light-exposed terrestrial *Klebsormidium* isolates, while data on *Klebsormidium* isolated from caves are inexistent.

The main goal of the present work is to characterise a thus far poorly investigated member of the *Klebsormidium* genus with specific attention on ecological aspects and biotechnological perspectives (BSC formation, high brassinosteroid content, culturing conditions). For this purpose, the temperature dependence of growth and photosynthetic performance of this strain isolated from a cave were thoroughly characterised. Based on literature data, we hypothesised that the studied strain (i) could grow efficiently under a broad temperature range, yet with a low temperature optimum, (ii) it favours low light intensities and consequently is photoinhibited by strong light, (iii) the BR content will change with the temperature.

Material and methods

Algal culturing

The biofilm was collected from a cave located in Northern Hungary (GPS coordinates: 47.90067, 20.37917) in 2012. During isolation, the biofilm was scraped off the cave wall with sterilised spatula and stored in a sterile test tube. The filamentous green alga *Klebsormidium* sp. was purified by streak plating (Fig. 1). The strain was patented as BEA_IDA_0061B and deposited at the Spanish Bank of Algae (BEA, Telde, Spain). The liquid culture of *Klebsormidium* was primarily maintained in modified BBM medium (Bold Basal Medium, Starr and Zeikus 1993) at 20 ± 2 °C. The applied light intensity was 35 µmol photons m^{-2} s^{-1} provided by 36 W warm white Polylux (Tungsram, Hungary) light tubes. The monocultures for the experiments were grown in an aerated cylindrical photobioreactor (0.3 vvm) of a volume of 8 L. During a 7-day long incubation period the cultures were kept at 22 °C under 20 µmol photons m^{-2} s^{-1} (36 W, natural white LED, V-TAC, Hungary) photosynthetically active radiation (PAR).

Fig. 1 Light micrograph of *Klebsormidium* BEA_IDA_0061B. Scale bar: 10 µm

DNA sequence analysis

For DNA-based taxonomic identification, the total genomic DNA was extracted from the strain with a DNeasy Plant Mini kit (QiagenGermany). PCR amplification and sequencing of the 18S rRNA gene and the ribosomal ITS region (containing ITS-1, the 5.8S rRNA gene, ITS-2 and a short region from the 28S rRNA gene) was performed with primers Euk328f and Chlo02R (Moon-Van der Staay et al. 2000; Zhu et al. 2005) and ITS_f and ITS_r (Liu et al. 2014), respectively, as described in detail by Somogyi et al. (2013) and Greipel et al. (2023). The obtained sequences were deposited in the GenBank database under the accession codes OR794009 (18S) and OR807367 (ITS), respectively. Similarity searches were performed with BLAST using the GenBank database (Sayers et al. 2022). In the case of the 18S rRNA gene, sequence alignment was conducted using the SINA Alignment Service (Pruesse et al. 2012), while in the case of the ITS region the ClustalW incorporated in the MEGA 11 software (Tamura et al. 2021) was used. Maximum likelihood analysis (including the search for the best-fit model) was conducted also with MEGA 11.

Oxygen yield measurements

The experiments were performed in a 9-cell photosynthetron described by Üveges et al. (2011) (Fig. 2). In this incubation system, 56 W Daylight fluorescent tubes (Tungsram, Hungary) provided the desired light intensities (0—5—18—40—100—200—450—900—1200 µmol photons m^{-2} s^{-1}) which were measured by a LI-1400 (LI-COR, USA) light meter equipped with a US-SQS/L (Walz, Germany) spherical (4π) microsensor (head diameter: 3.7 mm). The cells were connected to each other allowing water to circulate. A constant temperature was established and maintained with a Neslab RTE-211 thermostat.

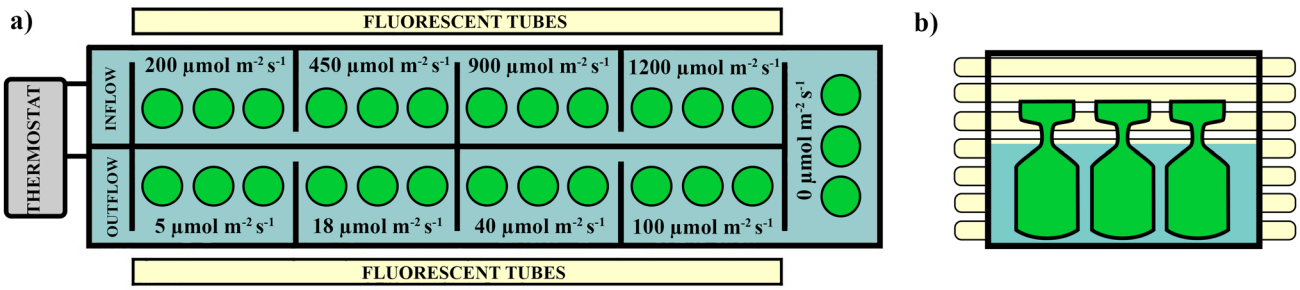

Fig. 2 Schematic illustration of the setup of the photosynthetron system used in present study from the top (**a**) and side view (**b**)

During the experiment, homogenised algal culture was filled into Karlsruhe flasks with a volume of 250 mL and three of them were placed into each cell of the photosynthetron. The cultures were preincubated in complete darkness for one hour, then the amount of dissolved oxygen in each flask was measured using an LDO sensor (HQ-20, Hach Lange, Germany). After determining the initial dissolved oxygen concentration, the flasks were placed back to the appropriate cells of the photosynthetron and illuminated for another one hour. Then the concentration of dissolved oxygen was measured again. We repeated these measurements at all studied temperatures (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C) using re-homogenized cultures. Furthermore, every time the chlorophyll *a* content of the algal samples was spectrophotometrically measured after an extraction with dimethyl sulfoxide (Caesar et al. 2018). Photosynthetic, as well as respiratory rates were biomass specifically calculated according to Wetzel and Likens (2000). The obtained photosynthesis-light curves (PI) were fitted either by according to Eilers and Peeters (1988) or Webb et al. (1974), from where the photosynthetic parameters (such as the light utilisation factor [α], the light adaptation parameter [I_k], and the maximal photosynthetic rate (P_{max}) were obtained.

Determination of the photosynthetic activity by chlorophyll fluorescence measurements

Simultaneously with the oxygen measurements, various photosynthetic parameters of the *Klebsormidium* cultures were also determined by chlorophyll fluorescence measurements. Here, similarly to the oxygen yield measurements, the cultures were filled into a Karlsruhe flask after homogenization and then incubated in the dark for 1 h at each temperature. During the measurements, both rapid light curves (RLCs) and fluorescence induction curves were recorded. At RLCs stepwise increased actinic light intensities from 11 to 830 µmol photons m^{-2} s^{-1} (logarithmic increment, 11 light intensities) with 30 s acclimation periods were applied, while the induction curves were recorded with 131 µmol photons m^{-2} s^{-1} actinic light intensities. In both cases 600 ms saturating pulses (SPs) with the intensity of 10,000 µmol photons m^{-2} s^{-1} were applied on top. Key parameters of the photosynthetic performance were determined by SP analysis of the instrumental software of the measuring system. Photosystem II (PSII) mediated relative electron transport rates (rETR) at various light intensities were determined using RLCs, while photochemical quenching (qP) and non-photochemical quenching (qN) were calculated by SP analysis of the induction curves. The light utilisation factor (α) and the light adaptation parameter (I_k) were calculated from the slope of the initial linear part of the PI graphs.

Determination of growth rates

Growth rates of *Klebsormidium* were determined at the following temperatures: 10, 15, 20, 25, 30, 35 and 40 °C. *Klebsormidium* cultures were grown in a Binder KBWF-720 growth chamber (Germany). Cool white fluorescent tubes (Narva Colorlux plus T8-865 LT 30W Cool White, Osram, Germany) provided the light with an intensity of 85 µmol photons m^{-2} s^{-1} under a 12:12 h light/dark cycle. 150 mL batch cultures of *Klebsormidium* were grown in Erlenmeyer flasks (n = 4) and were shaken once a day manually and studied for four days. The optical density at 750 nm (OD_{750}) of the cultures were determined daily using a Hitachi U2900 UV–Vis dual beam spectrophotometer (Japan) in standard 1 cm cuvettes. The specific growth rates (µ) were calculated according to Krzemińska et al. (2014).

Brassinolide (BL) determination

For LC–MS analysis, 600 mL of samples were filtered through Whatman GF/F filters. Solid phase extraction (SPE) of the filtrates was carried out using an AutoTrace 280 instrument (Thermo Scientific) equipped with polymeric weak cation cartridges (StrataTM-X-CW), 200 mg, 6 cm^3; Phenomenex, USA). Washing of the cartridges and the elution of BRs were performed following the protocol of Maasz et al. (2019). Finally, each sample was reconstituted with 0.5 mL pure acetonitrile (ACN) and were stored in amber-coloured glass vials at 4 °C until HPLC–MS analysis. BL standard (Sigma Aldrich) was dissolved in LC–MS grade methanol, which stock solution (0.1 mg mL^{-1}) was further used to prepare a calibration series: 5, 10, 100, 500 and 1000 ng L^{-1}. Chromatographic analysis was conducted using an Agilent HPLC system (Agilent) using Phenomenex Kinetex Polar C18 (50 × 4.6 mm, 2.6 µm and 100 Å pore size) reversed phase HPLC column. An aqueous solution of 0.1% formic acid and 10 mM ammonium formate was used as weak mobile phase (eluent A) while the corresponding ACN-based solution served as strong solvent (eluent B). 25 µL of sample was injected into the column and eluted with eluent B as the following gradient: 5% for 2 min, 60% for 3 min, 100% for 5 min, and 5% for 3 min. BL was detected with a Bruker amaZon SL ion trap mass spectrometer (Bruker, Germany). The flow rate of the drying gas with a temperature of 300 °C was adjusted to 10 L min^{-1}. Capillary voltage and the nebulizing gas pressure was 5000 V and 3.8 bar, respectively. Measurements were performed in ESI + mode. MS/MS scan with 0.4 m/z resolution was applied to identify BL using the m/z values of 315.56 and 479.68 for the fragment and precursor ion, respectively. Data evaluation and analysis was performed with a Bruker Compass DataAnalysis 4.4 SR1 module.

Statistical analysis

Welch tests were used to examine the differences between (i) the estimated values of the photosynthetic oxygen evolution and respiratory oxygen consumption ratio (P/R) and (ii) the brassinolide levels along the temperature gradient. Linear regression models were developed to reveal the changes in the photosynthetic parameters (α, I_k, qN, qP) along the studied temperature scale. Statistical analyses were conducted in R statistical software (4.2.1 version, R Core Team, 2021) using the 'vegan' (Oksanen et al. 2022) and 'nlme' packages (Pinheiro et al. 2022).

Results

Taxonomic identification of the new strain

Based on the obtained partial 18S rRNA gene sequence, strain BEA_IDA_0061B was indistinguishable (100% pairwise sequence similarity, excluding introns) from the authentic strains *Klebsormidium flaccidum* SAG 2307, *Interfilum paradoxum* SAG 338–1, and *Interfilum terricola* SAG 2100. However, based on the ITS region, the new strain showed 98.1–99.3% sequence similarity with other *Klebsormidium flaccidum* strains (including the authentic SAG 2307), while less than 95.4% to other *Klebsormidium* and *Interfilum* species. The taxonomic identification of the new strain as *Klebsormidium flaccidum* (Kützing) P.C.Silva, Mattox & W.H.Blackwell was also supported by phylogenetic analysis (Supplementary information S1).

Temperature dependence of the photosynthetic performance

The photosynthesis-irradiance (PI) curves Two types of PI curves were recorded: i.e., oxygen evolution *vs*. irradiance and rETR *vs*. irradiance curves. Using the latter approach, all PI curves followed a similar pattern at each tested temperature between 5–45 °C: after a linear, light limited phase each curve reached a maximum (photosynthesis limited phase) with increasing light intensities followed by a subsequent apparent decrease (Fig. 3a). The initial slope (light utilisation factor, α), the maximum rETR ($rETR_{max}$) and the minimum light saturation irradiance (= light adaptation parameter, I_k) are the main parameters derived from these PI curves (see next paragraph). The shape of the PI curves based on oxygen yield measurements are somewhat different: they showed a simple saturation curve with a light limited phase and a photosynthesis limited plateau with exception of the PI curve recorded at 45 °C which also shows some photoinhibition at high irradiances (Fig. 3b). No photosynthetic activity was observed at 50 °C with either method. In both cases the standard deviation was less than 10%.

Derived photosynthetic parameters Both $rETR_{max}$ and the maximum oxygen evolution rates largely depended on temperature (Fig. 4). Each of them increased gradually until a maximum at 35–40 °C ($rETR_{max}$) and 30–40 °C (P_{max}) with maximal values of 47 (relative units) and 272 µmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}, respectively. Above 40 °C, both parameters showed a sharp decrease with no detectable activity at 50 °C, in accordance with the PI curves.

The light utilisation factors values obtained via the chlorophyll fluorescence and oxygen yield measurements (α_{ETR}

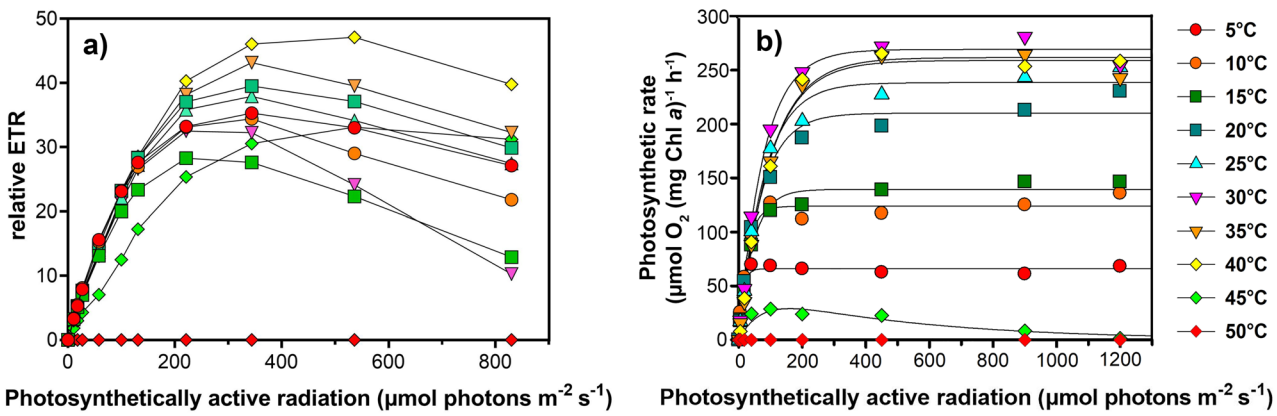

Fig. 3 PI curves of *Klebsormidium* at different temperatures. (**a**) rETR *vs*. irradiance curves, (**b**) oxygen evolution *vs*. irradiance curves (n = 3; mean values are indicated)

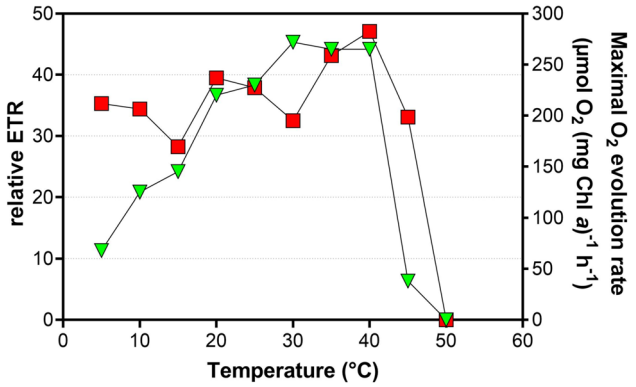

Fig. 4 Temperature dependence of rETR (red squares) and maximal oxygen evolution (P_{max}, green triangles). Values are derived from Fig. 3 (n = 3; mean values are indicated)

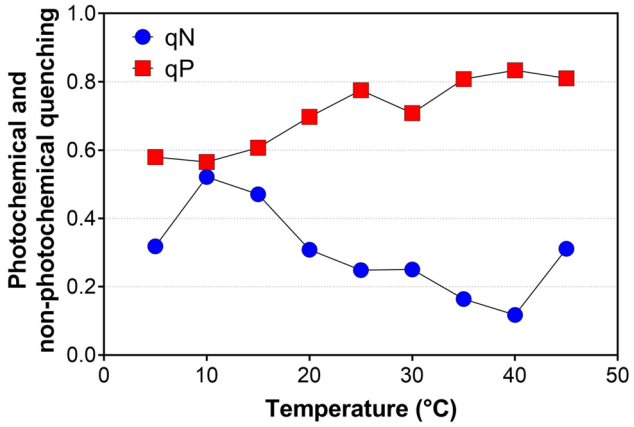

Fig. 6 Temperature dependence of photochemical (qP) and non-photochemical quenching (qN) of *Klebsormidium* cultures. qP and qN were determined via fluorescence induction curves with actinic light intensities of 131 µmol photons m^{-2} $s.^{-1}$ (n = 3, mean values are indicated)

and α_{PS}, respectively) showed very similar temperature dependencies: they were more or less constant between 5 and 40 °C (based on the linear regression $p > 0.05$), and dropped sharply above that temperature (Fig. 5a).

In contrast, the I_k values showed slowly increasing tendencies up to 45 °C and 40 °C based on chlorophyll fluorescence and oxygen yield measurements, respectively (linear regression $p < 0.001$; Fig. 5b). The I_k values derived via oxygen yield measurements showed a decline above 40 °C. Although the tendencies were similar, the light adaptation parameters derived either from rETR *vs*. irradiance or oxygen evolution *vs*. irradiance PI curves were remarkably different: they ranged between 104–204 µmol photons m^{-2} s^{-1} and 36–118 µmol photons m^{-2} s^{-1}, respectively.

The photochemical quenching (qP), determined via SP analysis of fluorescence induction curves with an actinic light intensity of 131 µmol photons m^{-2} s^{-1} ranged from 0.565 to 0.834, and showed an increasing trend with increasing temperatures (Fig. 6). The standard deviation values were less than 10%. The highest values were observed from 35 to 45 °C, in accordance with rETR and oxygen yield measurements. Concomitantly (and evidently), the non-photochemical quenching (qN) was generally lower than qP (ranging from 0.117 to 0.522) and essentially showed a decreasing tendency with increasing temperature (Fig. 6).

At oxygen yield measurements not only net oxygen evolution but oxygen consumption via respiration was determined, as well. The temperature dependence of oxygen evolution and respiratory oxygen consumption were plotted together on Fig. 7a. The mean net oxygen evolution (average of the 8 values on the oxygen evolution *vs*. irradiance PI curves, Fig. 3b) and respiratory oxygen consumption ranged from 13 to 179 µmol O_2 (mg Chl *a*)$^{-1}$ h^{-1} and -18 to -6 µmol O_2 (mg Chl *a*)$^{-1}$ h^{-1}, respectively (Fig. 7a).

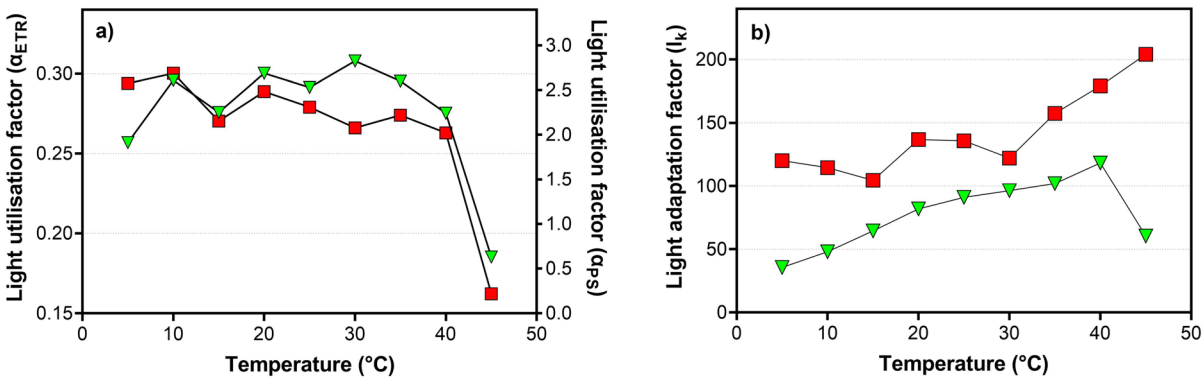

Fig. 5 Temperature dependence of light utilisation factor (α, a) and the light adaptation parameter (I_k, b) of *Klebsormidium* derived from oxygen evolution *vs*. irradiance (green triangles) and and rETR *vs*. irradiance PI curves (red squares) (n = 3; mean values are indicated, SD is lower than 10%)

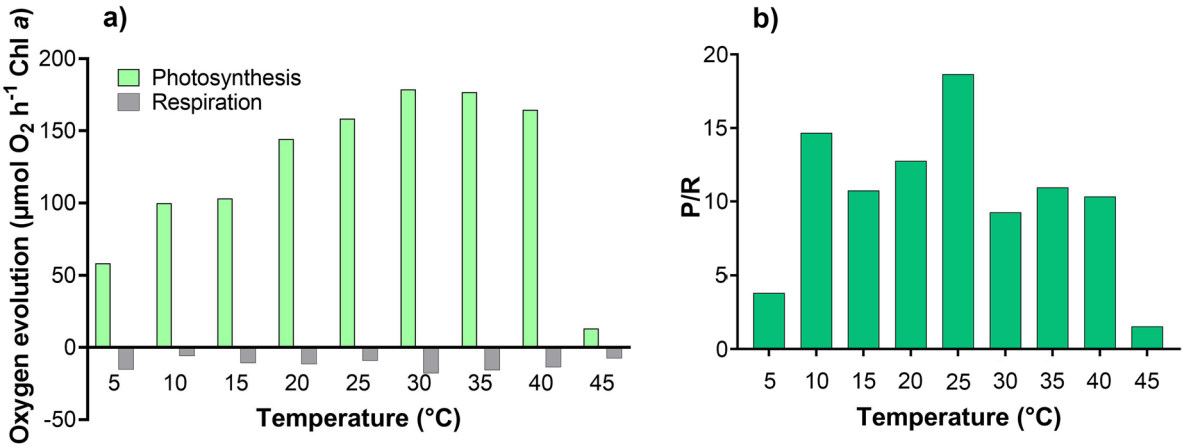

Fig. 7 Temperature dependence of the photosynthetic oxygen evolution and respiratory oxygen consumption (**a**), their ratio (P,R) (**b**)

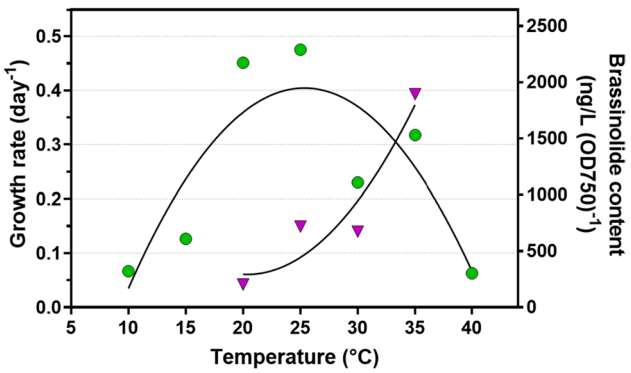

Fig. 8 Temperature dependence of the specific growth rates (green circles) and mean brassinolide content (purple triangles) of *Klebsormidium*. Lognormal fit was used for fitting the specific growth rate values ($r^2 = 0.72$), and second order polynomial fit for the mean brassinolide content values ($r^2 = 0.89$), respectively

The ratio of these two parameters showed strong temperature dependence. It ranged between 1.54 and 18.67 with the highest value at 25 °C, while the lowest values were observed at 5 (3.81) and 45 °C (1.54), respectively.

Temperature dependence of the growth rates

For a better interpretation of the data above we also tested the specific growth rates (μ) of *Klebsormidium* over the 10 °C to 40 °C temperature range. *Klebsormidium* was able to grow at each tested temperature (Fig. 8), however, with very different rates. The specific growth rates were the highest at 20 to 25 °C (0.451 ± 0.013 and 0.475 ± 0.011 day^{-1}, respectively) which were not statistically different (Welch-test, $p > 0.05$). Above the growth temperature of 25 °C the growth remarkably slowed, although it still remained significantly high even at 30 and 35 °C. In contrast, the culture growths at 10, 15 and 40 °C were minimal (0.067 ± 0.016, 0.126 ± 0.012, and 0.063 day^{-1}, respectively).

Bassinolide content

Next, we tested the BL content of *Klebsormidium* cultures over the temperature range of 20 to 35 °C (i.e., the temperature range with significantly high growth rates, see above) and normalised them to the biomass (i.e. OD_{750}) of the corresponding cultures. The BL content ranged between from 202 to 1892 ng L^{-1} with less than 10% of SD, and showed a strong temperature dependence on the basin of the Welch test: it was low at 20 °C, moderately high at 25 and 30 °C, and very high at 35 °C (Fig. 8).

Discussion

Members of the *Klebsormidium* genus can potentially be used in various biotechnological applications, thus their in-depth examination is of great importance. Nevertheless, as the adaptation to different habitats may result in major inter- and even intraspecific differences of ecophysiological traits, that should also be taken into consideration. Our work focussed on a poorly studied *Klebsormidium* strain isolated from a cave environment. Although *Klebsormidium* already has been found in such types of specific biotopes (Popović et al. 2023), the in-depth ecophysiological characterisation of such strain(s) has been missing. In order to fill this gap, we explored the ecophysiological performance of a cave-originated *Klebsormidium* strain using temperature and light as major variables. The studied ecotype showed a mainly generalist nature, and had ecophysiological overlaps with other *Klebsormidium*

strains isolated from polar, terrestrial, aquatic, desert, and alpine environmental conditions.

Light requirements

Light, providing energy for photosynthesis, is a key environmental factor for all kinds of photoautotrophs. The intensity and quality of the available light fundamentally determines the distribution of such species (see in Krebs 2013). The studied strain was isolated from a low-light habitat: from lamp-flora in a touristic cave. Photosynthetic microorganisms can respond to such circumstances by increased pigment content, photosystem abundance and/or antenna size (Masojídek et al. 2013). Usually, the rate of photosynthesis of low-light adapted photoautotrophs increases rapidly with increasing light intensities and reaches the maximal photosynthetic activity (i.e., electron transport rate, oxygen yield) at relatively low light intensities.

The adaptation of the studied *Klebsormidium* to low light was verified by our findings, i.e. by high light utilisation factors (α) and low light adaptation parameters (I_k). Regarding α's, their values were apparently temperature-independent between 5 and 40 °C (Fig. 5), which relationship is typical for most eukaryotic algae (Davison 1991); above that temperature a sharp drop was observed. In contrast, the I_k values significantly increased with temperature, yet with a considerable decline at 45 °C with the oxygen yield method. The values of both α and I_k concur with previous results obtained by *Klebsormidium* spp. isolated from high alpine soil crusts and freshwaters (Karsten et al. 2016a; Pierangelini et al. 2017). The light utilisation factor of terrestrial *Klebsormidium* isolate was lower, whilst their light adaptation parameter was much higher compared to the studied strain isolated from a cave environment (Karsten et al. 2016a). In contrast, opposing trends—higher α and lower I_k—were observed from isolate from former mining sites, *Klebsormidium dissectum* (F.Gay) H.Ettl & G.Gärtner from Ötztal Alps in Tyrol, Austria and from the isolate from urban walls (Karsten and Rindi 2010; Karsten and Holzinger 2011; Pierangelini et al. 2017).

Regarding the PI curves, we found similar patterns to those of previous studies (Karsten and Holzinger 2011; Pierangelini et al. 2017). The PI curves obtained by chlorophyll fluorescence measurements represent a rapid photosynthetic response ("rapid light curves") of the culture to increased light intensities (duration: 30 s), while the corresponding oxygen yield measurements provide data on light acclimation on a somewhat slower time scale (duration: 1 h). This latter time regime is long enough to induce photoinhibition at high light intensities. However, we observed photoinhibition only at 45 °C (Fig. 3b) using cultures with a very low P:R ratio. This suggests a broad temperature tolerance.

Temperature requirements

The effect of temperature on photosynthesis is very complex (see Berry and Bjorkman 1980; Allakhverdiev et al. 2008; Yamamoto et al. 2008). For example, enzyme-mediated biochemical reactions follow an exponential relationship with temperature, which, in combination with reversible or irreversible enzyme deactivation processes (i.e. conformational changes and degradation, respectively) at high temperatures, determines the optimum temperature of the organisms. According to the current view, the primary targets of thermal damage are the PSII recovery system, carbon fixation and ATP generation (Allakhverdiev et al. 2008). Secondary or indirect effects like decrease in P:R ratios (Bulthuis and Woelkerling 1983; Marsh et al. 1986; Raven and Geider 1988; Ralph 1998), changes in cellular pH (Beer and Waisel 1982; Beer et al. 2006), denaturation of chlorophyll-proteins (Briantais et al. 1986) and inactivation of photosynthetic oxygen-evolving complex at higher temperatures are also reported (Samson et al. 1999). The effect of temperature on growth is diverse: growth at non-optimal temperatures decreases the efficiency of carbon and nitrogen utilisation (Juneja et al. 2013), reduces protein synthesis rates (Rhee and Gotham 1981), can affect enzyme activity or modify proteins (Ras et al. 2013), and effect the biochemical composition of the cells (Nakamura and Miyachi 1982).

The investigated cave-originated *Klebsormidium* strain exhibited a broad temperature range in terms of both photosynthetic activity (5–45 °C) and capability to grow (10–40 °C). This observation is in accordance with the ubiquitous distribution of the *Klebsormidium* genus, indicating that its members are likely to be ecophysiological generalists with a broad thermal tolerance (Borchhardt and Gründling-Pfaff 2020).

Accordingly, the higher the temperature, the higher the rates of electron transport until reaching its optima, as manifested by both oxygen yield (30–40 °C) and chlorophyll fluorescence measurements (35–40 °C). In contrast, the growth response pattern as a function of temperature exhibited an optimum at 20–25 °C. These differences in temperature optima suggest that duration of the treatments at a selected temperature is an important factor. The shortest treatment belonged to the rETR measurements (1-h incubation) which resulted in relatively small differences over a broad temperature range. The overall incubation time during the oxygen yield measurements lasted longer (2–3 h), hence, allowed the activation of some long-term regulatory processes, involving some changes e.g. in macromolecular composition of the cells. Evidently, the results of the growth experiments (with a duration of a few days) reflecting, beside photosynthesis, the temperature dependence of all cellular processes, thus provide the sharpest temperature optimum.

Nevertheless, the temperature range of growth remained relatively wide, similar to other previously examined *Klebsormidium* strains including both terrestrial and some polar ones (Donner et al. 2017; Borchhardt and Gründling-Pfaff 2020).

Even though the short-term impact of temperature on photosynthesis has often been employed as an indicator of the long-term effect of temperature on algal growth, sometimes it does not provide corresponding information about growth (Davison 1991). For example, the optimal temperatures for photosynthesis among Arctic species were found to be significantly higher than the ambient water temperature and consistently exceeded the optimal temperatures for growth (Healey 1972; Drew 1977). This phenomenon indicates that the impact of temperature on a specific physiological process such as photosynthesis may not necessarily be in accordance with the temperature-growth relationship, as growth is influenced by the overall effect of temperature on metabolism. Therefore, this apparent inconsistency highlights the complex nature of temperature effects, which was also confirmed by our presented results.

Compared to other ecotypes of *Klebsormidium*, our strain shares similar growth properties to the isolates from BSCs in Central European grasslands and forests (Donner et al. 2017), and several strains from polar regions (Borchhardt and Gründling-Pfaff 2020). Despite the different nature of the habitats, similar growth patterns were observed, further supporting the generalist nature of the *Klebsormidium* genus. Regarding PI curves, we observed similarities to those obtained using other ecotypes, i.e. terrestrial (Pierangelini et al. 2017), aquatic (Karsten et al. 2016a), desert (Karsten et al. 2016b), alpine ones (Karsten and Holzinger 2011). On the other hand, the highest P:R ratios for terrestrial, mountainous, and desert *Klebsormidium* isolates were found at 5–10 °C, while for the studied cave isolate, the highest P:R ratios were observed from 10 to 25 °C. Its photosynthetic oxygen evolution rates were similar to the desert and alpine *Klebsormidium* strains, while its respiratory rates were among the lowest and showed the highest similarity to the alpine strains. Current model calculations revealed that P:R ratios could largely determine niche differentiation of photoautotrophic microorganisms (Masuda et al. 2023).

Phytohormones play an important role in alleviating the effects of various biotic and abiotic stress on plants and algae. In agreement with our expectations, the cellular BR levels in this *Klebsormidium* strain largely depended on the applied temperature and it was the highest under temperature stress, in accordance with the corresponding growth rates. As a possible explanation, BRs can alternate the HSP (heat shock protein) transcript levels and increase abscisic acid levels, which may result in an enhanced thermal tolerance (Bajguz 2009; Bajguz and Hayat 2009; Ahanger et al. 2018). This feature is beneficial for culturing microalgae in hot regions and also is of biotechnological relevance.

Conclusion

Our findings showed that the studied *Klebsormidium* strain is indeed capable of photosynthesis and growth over a broad temperature range; however, surprisingly, can also tolerate high irradiance without suffering from photoinhibition despite being isolated from a cave environment. These properties significantly may reduce the costs associated with their culturing, and make it a promising strain for biotechnological purposes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-023-03161-2.

Acknowledgements We express our special thanks to Máté Burányi for the technical support during the ecophysiological experiments referred to the growth investigation. Péter Futó was supported by the New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund [ÚNKP-22-3]. J.P. and E.L. were supported by the National Research, Development and Innovation Office of Hungary (KKP 144068) during manuscript preparation.

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Funding Open access funding provided by University of Pannonia. Open access funding provided by University of Pannonia. This research was financially supported by the National Research, Development and Innovation Office of Hungary [K 140351, RRF-2.3.1–21-2022–00014 (G.B)., MKI-2018–00034 (P.F., J.K., M.F.)].

Data availability All data generated or analysed during this study are included in this published article.

Declarations

Competing interests The authors declare no competing interests.

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