

## Temperature-dependent growth rate and photosynthetic performance of Antarctic symbiotic alga *Trebouxia* sp. cultivated in a bioreactor

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### Abstract

Optimum growth temperature of *Trebouxia* sp. (re-classified as *Asterochloris* sp. recently), a symbiotic lichenized alga was evaluated using a batch culture cultivated in a bioreactor. The algae were isolated from lichen thalli of *Usnea antarctica* collected at the James Ross Island, Antarctica in February 2012. The algae were isolated under laboratory conditions and then cultivated on agar medium at 5°C. When sufficiently developed, the algae were suspended in a BBM liquid medium and cultivated in a photobioreactor for 33 days at either 15, or 10°C. During cultivation, optical density (OD) characterizing culture growth, and effective quantum yield of photosystem II ( $\Phi_{\text{PSII}}$ ) characterizing photosynthetic performance were measured simultaneously. Thanks to higher  $\Phi_{\text{PSII}}$  values, faster growth was achieved at 10°C than 15°C indicating that *Trebouxia* sp. might be ranked among psychrotolerant species. Such conclusion is supported also by a higher specific growth rate found during exponential phase of culture growth. The results are discussed and compared to available data on temperature-dependent growth of polar microalgae.

**Key words:** *Usnea antarctica*, chlorophyll fluorescence, lichen, effective quantum yield, James Ross Island, psychrotolerance, *Asterochloris*

**Abbreviations:**  $\Phi_{\text{PSII}}$  - effective quantum yield of photosystem II,  $\mu$  - specific growth rate, OD - optical density, PBRr - photobioreactors

**DOI:** 10.5817/CPR2013-1-4

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Received January 5, 2013, accepted March 31, 2013.

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**Acknowledgement:** The authors thank the project CzechPolar for providing the infrastructure of the Johann Gregor Mendel station during sample collection in February 2012 as well as the EEL laboratory for the support provided during the cultivation of *Trebouxia* sp.

## Introduction

*Trebouxia* sp. is a green unicellular microalga. It belongs to division *Chlorophyta*, class *Trebouxiophyceae*, order *Trebouxiales*. Recently, it is re-classified as *Asterochloris* (Peksa et Škaloud 2011). Although there are various types of photobionts in the lichens, algae of genus *Trebouxia* sp. are well known as a photobionts presented in two thirds of all lichen species. It is found also in extremophilic lichens growing in Antarctica, including *Usnea antarctica*, *U. sphacelata* and *U. aurantiaco-atra*, most common fruticose species found at the islands on the west (South Shetlands, Argentine Islands) and east side of the Antarctic Peninsula (James Ross Island group). *Trebouxia* sp. is a typical photobiont for the lichens of genus *Usnea*, however, *Poterioochromonas* sp. is recently reported as rare co-occurring symbiont in *Usnea longissima* (He et Zhang 2012). In general, cells of *Trebouxia* sp. has about 10 – 15 µm in diameter. There is about 26 species in this genus.

*Trebouxia* sp. reproduces asexually by autospores or zoospores. Sexually reproductive stages have not been observed directly in any genus of class *Trebouxiophyceae* (Lewis et McCourt 2004). Species of genus *Trebouxia* differ in morphology of plastids and structure of pyrenoids (Friedl 1989). Many species of genus *Trebouxia* has been studied, but there is still little known about its physiological characteristics and photosynthetic parameters. However, there is a basic knowledge about its photosynthetic responses to heavy metal induced stress (*e.g.* Branquinho et al. 2011, Piovar et al. 2011), high light stress (Showman 1972), dehydration stress (Wieners et al. 2012), osmotic (Vácz et Barták 2006) and oxidative-induced stress (Del Hoyo et al. 2011). The alga is in many cases studied as a part of lichen thalli (*e.g.* Alvarez et al. 2012, Branquinho et al. 2011).

*Trebouxia* sp. algae are considered either psychrophilic or psychrotolerant. Psychrophilic algae grow at temperatures below 0 °C and typically have temperature growth maxima only a few degrees above zero. Typical psychrophilic algae, such as *e.g.* genera *Chlamydomonas* and *Chloromonas* are found in polar regions. They have growth optimum below 10°C. In spite of the fact that lichenized *Trebouxia* thrives well in cold polar environments, its growth optimum is reported above 15°C (Kvídová et al. 2010). Therefore, it may be ranked rather psychrotolerant than psychrophilic.

For long-term storage, symbiotic algae are mostly cultivated on agar plates in Petri dishes. Cultivation on agar plates is also used to develop cultures suitable for determination purposes (Gärtner et Inolčić 1998). Cultures on agars and/or nitrocellulose disks are frequently used in the experiments studying stress-induced physiological responses, such as *e.g.* *Trebouxia* sensitivity to heavy metals (Piovar et al. 2011), photosynthesis sensitivity to low temperature and drought stress (Sadowsky et Ott 2012). Another possibility is to cultivate the alga in a liquid medium. For cultivation of lichen symbiotic algae, a variety of liquid media and volumes of cell culture flasks is used. This approach was applied in the experiments studying dehydration (Wieners et al. 2012, Gasulla et al. 2013), and oxidative stress effects (Del Hoyo et al. 2011). Recently, photobioreactors (PBRs) are used in cultivation of numerous algal species. There are many types of photobioreactors – *e.g.* large open ponds, enclosed flat-plate PBRs, vertical column PBRs, panel PBRs and smaller indoor PBRs. Algae cultivated in large outdoor photobioreactors can be used for the production of some bioactive substances (Fu et al. 2012). Small indoor bioreactors are often used for laboratory experiments or growth analysis of algal

cultures using different indicators, such as *e.g.* changes in various physiological parameters caused by cultivation temperature and/or photosynthetic radiation. There are some advantages of using these bioreactors for algae cultivation such as *e.g.*

the possibility of automatic continual measurements of physiological parameters during the whole cultivation period. In our study, we used a PBR for long-term cultivation in liquid medium to evaluate temperature optimum.

## Material and Methods

*Trebouxia* sp. was isolated from an Antarctic lichen *Usnea antarctica* collected at the James Ross Island (63.81 S, 57.83 W). The isolation was done by a gradient centrifugation method according to Gasulla et al. (2010) using a Percoll®. After isolation, the alga was cultivated on agar medium at 5°C. When algal culture was sufficiently developed, it was collected from the surface of agar medium and suspended in a liquid medium (nitrogen-enriched anorganic Bold's Basal Medium, 3N-BBM, Ahmadjian 1993). Then, it was cultivated in a FMT-150/400 photobioreactor (PSI, Czech Republic) for 33 days. For cultivation, two different growth temperatures (15°C, 10°C) and 16/8 h light/dark period were used (70  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The photobioreactors had a flat - vessel design that enabled uniform

illumination within the whole volume of cultivated culture of *Trebouxia* sp. During cultivation, optical density (OD at 680 nm) of *Trebouxia* sp. culture, and effective quantum yield ( $\Phi_{\text{PSII}}$ ) of photosynthetic processes in photosystem II were measured repeatedly. This was possible thanks to integrated densitometer that measured light scattering at 680 nm and a fluorometer that measured fluorometric signals  $F_m'$  and  $F_s$  from which  $\Phi_{\text{PSII}}$  was calculated ( $\Phi_{\text{PSII}} = [F_m' - F_s] / F_m'$ , Genty et al. 1989). Finally, OD and  $\Phi_{\text{PSII}}$  were plotted against the time of cultivation, so that temperature-induced differences in growth rate and photosynthetic efficiency could be distinguished. The relations of OD and  $\Phi_{\text{PSII}}$  to the time of cultivation were fitted by S-curves using the below log-logistic model with five parameters:

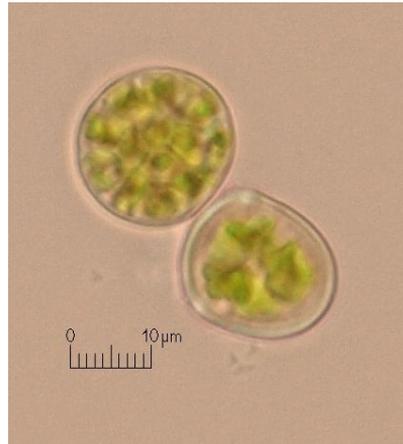
$$f(x) = C + \frac{D - C}{\left[1 + \left(\frac{x}{E}\right)^B\right]^F} \quad \text{Eqn. 1}$$

where B is Hill slope, C is minimum value, D is maximum value of dependent variable, E is  $C_{50}$ , and F is parameter describing asymmetry of S-curve.

Since the values of OD were measured repeatedly throughout cultivation time, specific growth rate ( $\mu$ ) was calculated for 50 h subperiods using the below equation:

$$\mu = (N_2 - N_1) / t_2 - t_1 \quad \text{Eqn. 2}$$

where  $t_2$ ,  $t_1$  are the times within the exponential phase of culture growth ( $t_2 > t_1$ ,  $t_2 - t_1 = 50$  h).  $N_2$  is optical density (OD) derived from the fitted S curve at  $t_2$ ,  $N_1$  is OD at  $t_1$ .



**Fig. 1.** *Trebouxia* sp. cells isolated from *Usnea antarctica* collected at the James Ross Island, Antarctica. © Photo: K. Trnková.

	Estimate	Std.Error	t-value	p-value
b:1	-0.43	0.13	-3.30	0.00
c:1	0.13	0.01	19.60	0.00
d:1	4.73	5.15	0.92	0.36
e:1	554.45			
f:1	4.91			
b:2	-46.91	24.74	-1.90	0.06
c:2	0.12	0.01	22.49	0.00
d:2	0.41	0.00	105.44	0.00
e:2	490.47	7.41	66.17	0.00
f:2	0.06	0.03	1.83	0.07

**Table 1.** Generalized log-logistic parameters of S-curve (see Eqn. 1) fitting data points of optical density (OD) of *Trebouxia* sp. culture as dependent on the time of cultivation.

	Estimate	Std.Error	t-value	p-value
b:1	15.85	16.45	0.96	0.34
c:1	0.55	0.01	88.03	0.00
d:1	0.61	0.01	74.38	0.00
e:1	439.89	254.17	1.73	0.09
f:1	21.87	168.99	0.13	0.90
b:2	-189.89	183.34	-1.04	0.30
c:2	0.68	0.01	97.31	0.00
d:2	0.85	0.01	126.09	0.00
e:2	429.84	4.31	99.69	0.00
f:2	0.22	0.26	0.84	0.40

**Table 2.** Generalized log-logistic parameters of S-curve (see Eqn. 1) fitting data points of effective quantum yield of PS II ( $\Phi_{PSII}$ ) of *Trebouxia* sp. culture as dependent on the time of cultivation.

## Results

Time courses of OD showed that faster growth of *Trebouxia* sp. was achieved at 10°C than 15°C. At 10°C, the culture OD showed typical S curve, with maximum growth rate found after 420 h of cultivation. At 15°C, however, the OD values exhibited rather constant growth rate (see Fig. 2, Table 3). Final culture density was

higher when cultivated at 10°C (OD = 0.45) than 15°C (OD = 0.35) indicating that optimum cultivation temperature for *Trebouxia* sp. is definitely below 15°C. Such conclusion is supported also by fluorometric data. While effective quantum yield ( $\Phi_{PSII}$ ) reached relatively lowered value ranging from 0.5 to 0.7 at

15°C, it was much higher at 10°C (0.6-0.8). Moreover,  $\Phi_{\text{PSII}}$  showed increasing values with cultivation time indicating progressive acclimation to cultivation conditions (10°C), highly effective performance of photosynthetic apparatus at such more favourable temperature. At

15°C, contrastingly,  $\Phi_{\text{PSII}}$  exhibited slightly decreasing trend with cultivation time. This finding supports the conclusion that 15°C is suboptimal cultivation temperature for *Trebouxia* sp. cultured in liquid medium.

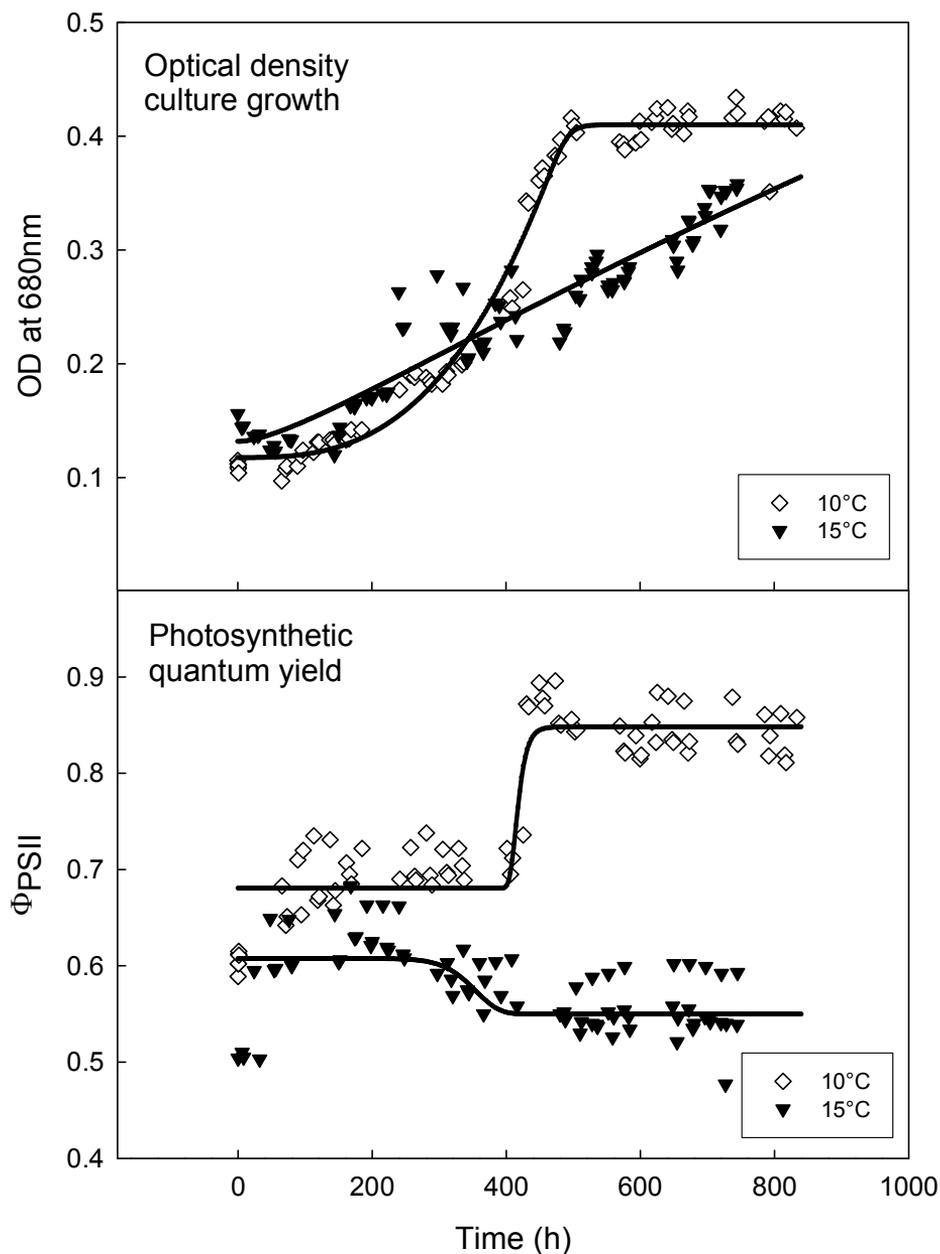
Days of cultivation	Cultivation temperature	
	15°C	10°C
Intervals (h)	$\mu_{15}$	$\mu_{10}$
0 - 50	0.0001	below 0.0001
50 - 100	0.0002	below 0.0001
100 - 150	0.0003	0.0001
150 - 200	0.0003	0.0003
200 - 250	0.0003	0.0004
250 - 300	0.0003	0.0006
300 - 350	0.0003	0.0008
350 - 400	0.0003	0.0010
400 - 450	0.0003	0.0013
450 - 500	0.0003	0.0012

**Table 3.** Specific growth rate ( $\mu$ ) derived from optical density (OD) measurements of *Trebouxia* sp. cultured in a photobioreactor at 10 and 15°C.

## Discussion

In this study, an optimum growth temperature of *Trebouxia* sp. cultivated in batch culture was studied using two cultivation temperatures (10 and 15°C). Faster growth found for *Trebouxia* sp. cultivated at 10°C is well comparable to the results of Teoh et al. (2004) who investigated growth of several Antarctic microalgae in response to temperature. The authors reported the highest specific growth rates for temperature ranging from 6°C to 14°C. Similarly, earlier study of Seaburg et al. (1981) showed that optimum growth temperature for 35 species of Antarctic microalgae ranged between 7.7 to 18°C. Fogliano et al. (2010) reported

that *Koliella antarctica*, an unicellular extremophilic green alga from the Ross Sea, Antarctica, exhibited higher growth rates and biomass production in 15°C than in 10°C of cultivation temperature. This seems to be contradictory to our findings since in *Trebouxia* sp., there was faster growth and OD values found in 10°C than in 15°C (see Fig. 2). However, Fogliano et al. (2010) measured only the initial phase of growth (10 d), while our results showed completed S-curves reaching maximum constant value of OD because of long-term cultivation (more than one month). During the first 7 day of cultivation *Trebouxia* sp., similarly to *K. antarctica* exhibited higher



**Fig. 2.** Time courses of optical density (OD, the upper panel), and effective quantum yield of photosystem II ( $\Phi_{PSII}$ , the lower panel) in the culture *Trebouxia* sp. cultivated in a photobioreactor at two growth temperatures: 10°C (open symbols), and 15°C (full symbols).

OD in 15°C, while further cultivation led to more rapid growth in 10 than 15°C. Some unicellular algae from Antarctica, however, exhibit higher growth rates in cultivation temperature above 20°C, as reported by Chen et al. (2012) for *Stichococcus* (*Trebouxiophyceae*) and Blanc et al. (2012) for polar alga *Coccomyxa subellipsoidea*.

In our experiment, *Trebouxia* sp. exhibited higher values of  $\Phi_{\text{PSII}}$  and resulting biomass at the temperature of 10°C throughout the cultivation. Therefore, the *Trebouxia* strain isolated from lichen thalli of *Usnea antarctica* could be ranked among psychrophilic algae because similar responses, *i.e.* optimum growth at temperature below 15°C is reported by Vona et al. (2004) for cryophilic alga *Koliella antarctica*. Such conclusion may be supported also by the study of Ocampo-Friedmann et al. (1988) who classified two species of genus *Trebouxia* from Antarctica as psychrophilic algae with temperature optimum around 17.5°C. The algae of genus *Trebouxia*, however, are present in many lichen species distributed worldwide. Across vegetation zones, chlorolichens that have *Trebouxia* sp. as photosynthetic partner occupy a wide range of ecosystems with the temperature regimen differing from polar region habitats. Therefore, the genus *Trebouxia* should be

classified rather psychrotolerant than psychrophilic.

Simultaneous measurements of optical density (OD) and  $\Phi_{\text{PSII}}$  during the cultivation of the culture allowed to analyze their relationship. When cultivated at 10°C, *Trebouxia* showed absolute maximum of growth rate at the same time, at which an increase in  $\Phi_{\text{PSII}}$  values was recorded (*see* Fig. 2, Table 3, 400-420 h of cultivation). Such increase in the effectivity of photosynthetic processes in photosystem II is thus responsible for an acceleration of *Trebouxia* culture growth. This conclusion can be supported by the two facts: (1) No such increase in  $\Phi_{\text{PSII}}$  was found at higher cultivation temperature (15°C) simultaneously with no change in OD, (2) whenever  $\Phi_{\text{PSII}}$  of *Trebouxia* cultured at 10°C reached constant value after the previous increase (*see* Fig. 2, 480 h), OD became constant indicating that the culture reached maximum density. Generally, the time required for reaching final density of *Trebouxia* culture was much longer (840 h) than reported for majority of temperate unicellular freshwater (*e.g.* *Chlamydomonas reinhardtii* in Morlon et al. 2005 – 120 h) and marine alga (*e.g.* *Chlorella minutissima* in Sankar et al. 2011 – 350 h).

## Concluding remarks

In this study, we support the idea that *Trebouxia* sp. isolated from Antarctic lichen *Usnea antarctica* could be ranked among psychrotolerant species. Recent knowledge on temperature dependence of particular photo- and biochemical processes of photosynthesis in *Trebouxia* from polar regions is, however, still inadequate. Therefore, axenic cultures of *Trebouxia* sp. grown in liquid medium represent an

excellent tool to study *Trebouxia* resistance to low temperature and freezing stress (Hájek et al. 2012). Our further study (Barták et al., MS in prep.) will be focused on inhibition of photochemical processes in photosystem II in isolated *Trebouxia* sp. in relation to gradual cooling of the culture from physiological to freezing temperature.

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