Real-time photoprotective response of xanthophyll pigments and Mycosporine-like amino acids of *Porosira glacialis* (Bacillariophyceae)

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Abstract

This study is investigation of the newly synthesis of photoprotective compounds by *Porosira glacialis* in real time using a ^{13}C tracer. Our results showed the relationship between the production rates of mycosporine-like amino acids (MAAs) and photoprotective pigment, such as diadinoxanthin (DD). We were experimented during May 2011 for use in the current experiment on indoor exposure to artificial UV radiation. Our results show that UV tolerance of *P. glacialis* was evidenced by the growth rate and chlorophyll *a* (Chl *a*) concentration under the UV conditions. The carbon uptake rate indicated that was continuously exposed to photosynthetically active radiation (PAR) for 24 hours, which was higher than that of one exposed to ultraviolet (UV) radiation (UVR). However, it indicated when the exposure time was 72 hours, which was higher than the initial value of *P. glacialis* exposed to UV radiation and also was higher than that of *P. glacialis* exposed to PAR. The time difference between the productions of these two compounds clearly reveals the defense strategy used by *P. glacialis* to synthesize photoprotective compounds (MAAs and DD). The results of this study appear to reflect the synthetic pathways of photoprotective compounds and the carbon cycle within the cell in contrasting patterns over time that are defined by the production of photoprotective pigments (DD) and MAAs.

Materials and Methods

***Porosira glacialis* (Bacillariophyceae)**

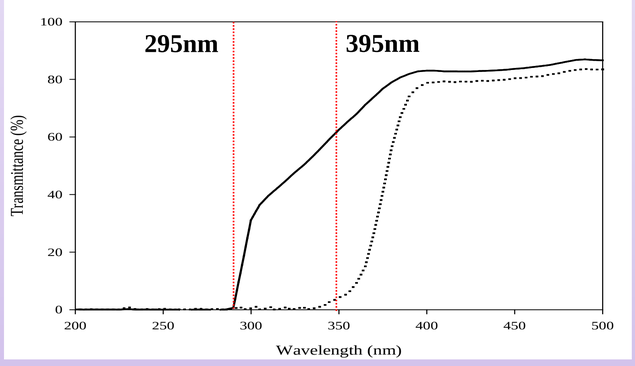

Fig. 1. Transmittance spectra of the quartz bottles sued for growing the algae and cutoff filters (solid line = 295 nm cutoff filter and broken line = 395 nm cutoff filter), used to cover respectively the PAR, UV-A and the UV-B lamps.

- ✓ UV-B: 5.2μW/m^2, UB-A: 12.3μW/m^2, PAR: 1.8μW/m^2
- ✓ Total irradiation 14.02UM Photons m^{-2}S^{-1},
- ✓ Culture temperature: 4℃

HPLC system – Agilent Technologies 1200 series
Mobile phase – Water with 0.1% acetic acid
Column – Waters 120DS-AP (5μm) 250mm x 4.6
Detecter –Agilent DAD (G1315D) 310nm (250~750nm Scan)
Fraction collector – Agilent analyt FC (G1364C)

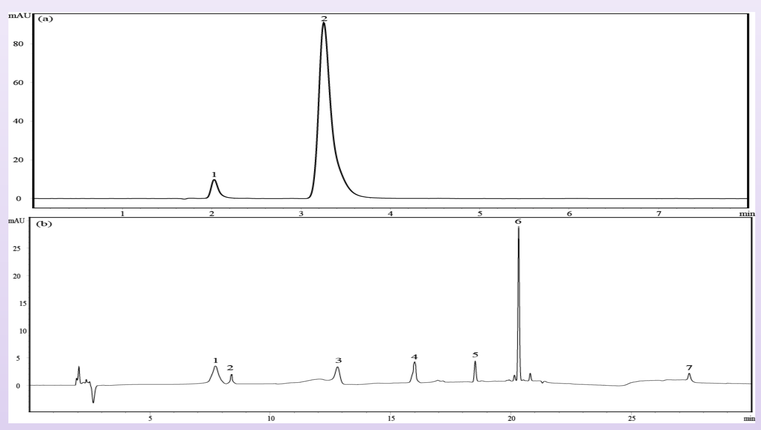

Fig. 2. HPLC chromatogram showing MAAs (a) and pigment composition (b) of cells of *P. glacialis.* (a) 1 = shinorine, 2 = porphyra-334; (b) 1 = chlorophyll c3; 2 = chlorophyll c2; 3 = fucoxanthin; 4 = diadinoxanthin; 5 = cantaxnathin; 6 = chlorophyll *a*; 7 = β – carotein.

Research purposes
The synthesis rates of photoprotective UV-absorbing compounds (MAAs) and xanthophyll-cycle compounds (DD) of diatoms were determined as a result of exposure to artificial UV radiation. These compounds were expected to be complementary to each other in the photoprotective mechanism of phytoplankton and were found to show a close correlation in this study.

Results and Discussion

➢ Total carbon uptake rate and production rate of diadinoxanthin in *P. glacialis*

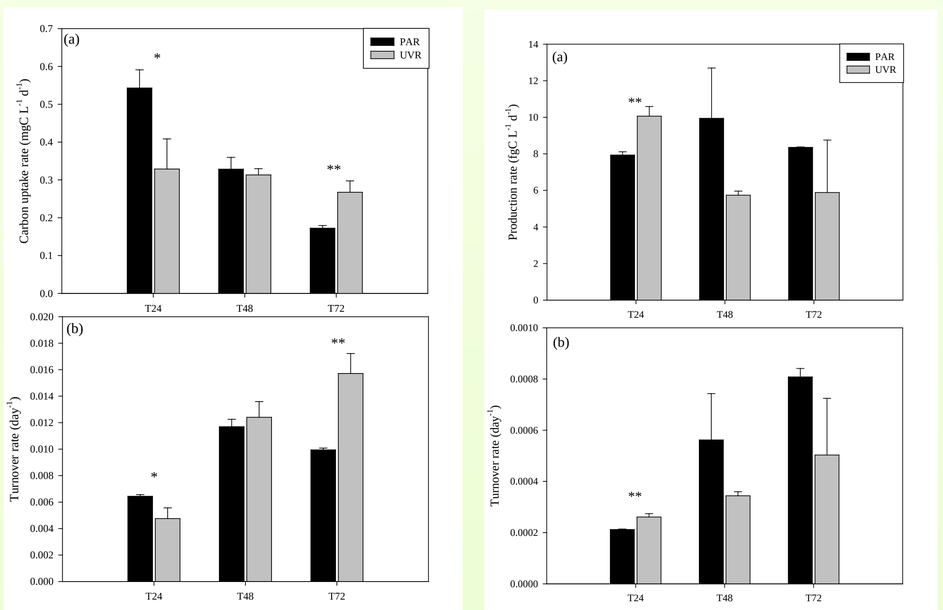

Fig. 3. Production rate (a) and turnover rate (b) of diadinoxanthin of *P. glacialis* according to exposed PAR and UVR conditions. Back bar indicate the exposed PAR and Gray bar is the UVR conditions. *P* value indicated **<0.01.

The production rate of DD, one of photoprotective pigments, was higher in *P. glacialis* exposed to UVR than in ones to PAR. But after 24-hour exposure, it was higher in *P. glacialis* exposed to PAR (5.29 (±0.15) fgC L^{-1} d^{-1}) than in one to UVR (6.63 (±0.44) fgC L^{-1} d^{-1}) (Fig. 3). But in *P. glacialis* exposed to PAR, the production rate showed an increasing trend with time and thus reached its maximum with 7.97 (±0.07) fgC L^{-1} d^{-1} 72 hours later. In *P. glacialis* exposed to UVR on the other hand, it showed a decreasing trend with time and thus indicated 5.74 (±2.7) fgC L^{-1} d^{-1} 72 hours later. The values of the production rate of DD were not significantly different (F=12.24, P>0.05) within interaction time and light condition. In both two light conditions, the turnover rate of DD tended to be faster with the passage of exposure time. The minimum values were 0.0002 (±0.000002) day^{-1} and 0.0003 (±0.00001) day^{-1} in *P. glacialis* exposed to PAR and ones to UVR respectively, and the maximum values were 0.0008 (±0.00003) day^{-1} and 0.0005 (±0.0002) day^{-1} respectively. The turnover rate of DD were observed the no significant in both treatments (F= 6.73, P>0.05).

➢ Production rate of UV-absorbing compounds in *P. glacialis*

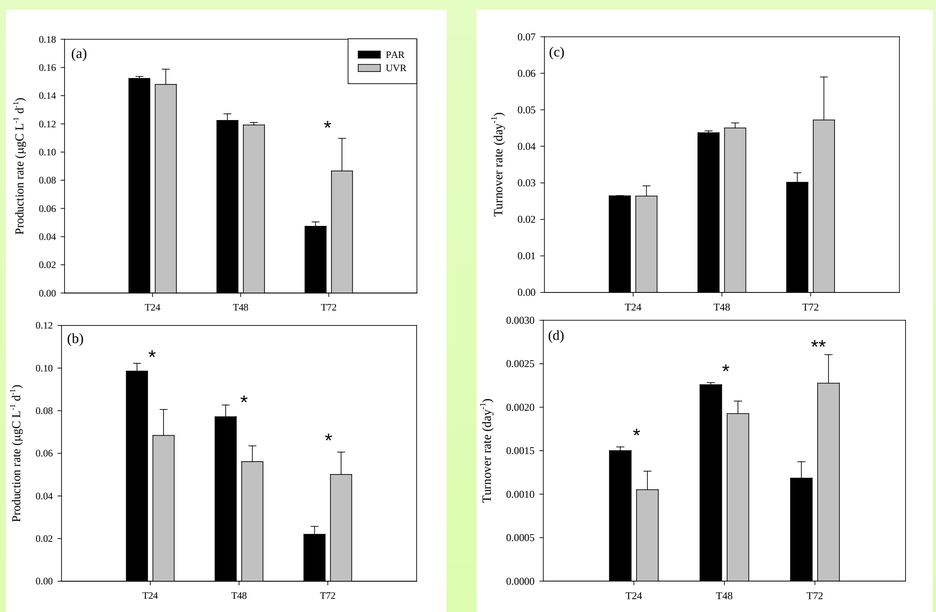

Fig. 4. Production rate and turnover rate of individual MAAs compounds of *Porosira* sp. under exposed PAR and UVR conditions. (a) production rate of shinorine and (b) porphyra-334; (c) turnover rate of shinorine and (d) porphyra-334. *P* value indicated * < 0.05 and **<0.01.

In the production rate of shinorine, *P. glacialis* exposed to PAR for 24 hours (0.15(±0.001) μgC L^{-1} d^{-1}) was similar to one exposed to UVR (0.15 (±0.01) μgC L^{-1} d^{-1}). But after 72-hour exposure, it was higher in *P. glacialis* exposed to UVR (0.09 (±0.02) μgC L^{-1} d^{-1}) than in ones to PAR (0.05 (±0.003) μgC L^{-1} d^{-1}). The production rate of shinorine was not significant in interaction between exposure time and light condition (F=6.9, P=0.05). However, the production rate of porphyra-334 was observed the significant different in exposure time and light condition (F=11.31, P<0.05). The production rate of porphyra-334 showed similar results, but was lower relatively to that of shinorien. In the case of shinorine in *P. glacialis* exposed to UVR, carbon uptake rate tended to be faster in proportion to the exposure time. In *P. glacialis* exposed to PAR, it showed festination 72 hours after culture (Fig. 4). However, the turnover rate in porphyra-334 was less than a tenth that in shinorine. Also, porphyra-334 was markedly inferior to shinorine in turnover rate and production rate, notwithstanding higher concentration (Fig. 4).

Table 1. Results of repeated-measures analysis of variance of the effects of the UVR treatments.

	UV treatment	time	UVR × time
Growth rate	486.99 **	2.162	28.45 **
Chl a concentration	150.72 **	21.03 **	13.42 *
Concentration of DD	88.74 *	522.56 **	4.44
Concentration of SH	1.19	75.45 **	3.1
Concentration of PR	1.74	9.13 *	1.78
Carbon uptake rate	3.98	25.79 **	1096.82 **
PP of DD	1.39	0.38	12.24 *
Turnover rate of DD	3.63	14.13 *	6.74
PP of SH	3.00	284.27 **	6.90
PP of PR	5.49	226.21 **	11.32 *
Turnover rate of SH	3.01	54.67 **	5.25
Turnover rate of PR	0.82	78.51 **	18.60 **

There were significant differences (by RM-ANOVA) an UV treatment effect for growth rate, concentration of chl a, and DD. And newly synthesized the specific compounds (carbon uptake rate, production rate of DD, SH, and PR) showed a significant interaction between treatments and times (Table 1). No UVR treatment effect and no interaction effect were obtained the concentration of SH, PR, and production rate of SH. Not statistically significant of production rate of SH suggested the actively stimulation in cell by wide wavelength. Significant UVR treatment–by-times interactions were found production rate of DD and PR, because trends between treatments varied over time (Table 1).

Conclusion

- ✓ The results of the study showed that organic carbon is initially fixed to produce photoprotective pigments and that organic carbon (^{13}C) is fixed to produce MAAs within the cell.
- ✓ This study shows that a discrepancy in selective strategies is reflected by the time at which different compounds are produced in response to hazardous stress at the stationary phase of phytoplankton.
- ✓ *P. glacialis* produced the between photoprotective pigment and UV-absorbing compounds at time gap so that they may be complementary to each other in the photoprotectie mechanism.