La mer 38 : 95-97, 2000
Société franco-japonaise d'océanographie, Tokyo

Short note

Pigment composition of *Pedinomonas noctilucae* (Pedinophyceae), an endosymbiont of green *Noctiluca* (Dinophyceae)

Ken FURUYA* and Thaithaworn LIRDWITAYAPRASIT**

Abstract : Pigment composition of *Pedinomonas noctilucae*, a green flagellated endosymbiont of the green *Noctiluca* was examined using a unialgal culture by a reverse-phase HPLC method. The eight pigments identified were neoxanthin, antheraxanthin, violaxanthin, zeaxanthin, lutein, chlorophyll *b*, chlorophyll *a* and *β*, *β*-carotene in the order of elution. Five major pigments except antheraxanthin, were quantified to determine their pigment ratios to chlorophyll *a* for use in the evaluation of group-specific algal abundance from pigment composition.

Key words: Pedinomonas notilucae, *green* Noctiluca, *plant pigment, HPLC*

Noctiluca scintillans (Macartney) Ehrenberg, a widely distributed non-photosynthetic dinoflagellate forms dense blooms in temperate, subtropical and tropical coastal waters. The blooms of *N. scintillans* produce a strong pinkish red discoloration of the water in temperate regions. However, in southeast Asian tropical waters, *N. scintillans* causes green discoloration, as it contains the green flagellated endosymbionts, *Pedinomonas noctilucae* (Subrahmanyan) Sweeney (OSTROUMOFF, 1924; SWEENEY, 1971). The endosymbiont containing *Noctiluca* is commonly called the green *Noctiluca*, and their growth is sustained by the symbiont (SWEENEY, 1971: OKAICHI *et al.*, 1991). The presence of *P. noctilucae* is considered to be of vital impportance for bloom formation of the host organism. Although various biological characteristics of *P. noctilucae* have been revealed (SWEENEY, 1971, 1976; OKAICHI *et al.*, 1991), pigment composition of this species has not examined yet. In this communication, we describe the pigment composition of *P. noctilucae* as a baseline study for biomarker pigment analysis of the phytoplankton community in the Gulf of Thailand (MACKEY *et al.*, 1996). We thank Prof. Tomotoshi OKAICHI for valualle suggestions, Dr. Neelam RAMAIAH and Ms. Haruna SAITOH for their cooperation in the laboratory work.

A unialgal culture of *P. noctilucae* associated with the green *Noctiluca* was isolated from the inner Gulf of Thailand and maintained in the modified ESM medium (OKAICHI *et al.*, 1991). During the course of maintenance, cell morphology changed due to some unknown reason, from the flagellated form to a coccoid one lacking the flagella. The HPLC analysis reported here was made on the coccoid forms. The cultures were incubated at 28℃ under cool white fluorescence light at 10 and 150 μ mol $m^{-2}s^{-1}$ intensities. Cells during the exponential and stationary phases were harvested on GF /F filters by gentle suction with a pressure difference of 150 mmHg and frozen at −85℃. The

* Department of Aquatic Bioresource, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657 Japan

** Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330

Corresponding author: Ken Furuya
furuya@fs.a.u-tokyo.ac.jp

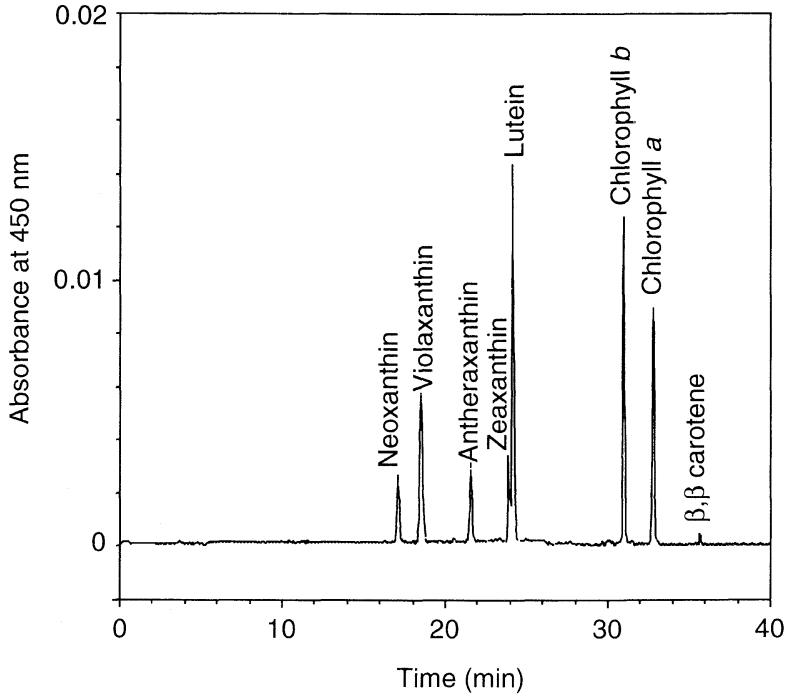

Fig. 1. Pigment composition of *Pedinomonas noctilucae* detected at 450nm.

filters were homogenized in 95% methanol with sonication and the extract passed through 0.2 μ m PTFE syringe filters was subjected to the HPLC analysis (ZAPATA *et al.*, 2000) using a reverse phase C8 column. Details of the HPLC equipment we used are given in FURUYA *et al.* (1998).

Pigments were identified and quantified based on the retention time and peak area of the online absorption spectrum measured by a photodiode array spectrophotometer (SPD-M10AV, Shimadzu; 1.2 nm otpical resolution). Pure standards used for the identification and quantification viz., chlorophyll *a* (Chl. *a*), chlorophyll *b* (Chl. *b*) and β, β-carotene were obtained form Sigma Chemicals, while those for fucoxanthin, 19'-butanoyloxyfucoxanthin, 19'-hexanoyloxyfucoxanthin, peridinin, diadinoxanthin, alloxanthin, neoxanthin, violaxanthin, prasinoxanthin, echinenone, lutein, zeaxanthin, chlorophyll c_1 and chlorophyll c_2 were from the International Agency for ^{14}C Determination. Purity of these standards was examined both by the HPLC system and Shimadzu MPS-2400 spectrophotometer.

Six pigments identified as major-peaks were the neoxanthin, violaxanthin, zeaxanthin, lutein, Chl. *b* and Chl. *a* (Fig. 1). β, β-caroten e was eluted as the most non-polar pigment and appeared small peak that was possibly not well resolved from β, ε-carotene, if existed. A peak that was observed between the violaxanthin and zeaxanthin, was provisionally identified as antheraxanthin according to ZAPATA *et al.* (2000). This was re-examined by an additional analysis following the protocol of MANTOURA and LLEWELLYN (1983), and was confirmed to be antheraxanthin according to its characteristic phtodiode array spectrum (JEFFREY *et al.*, 1997). Three trace peaks were found at 3.8, 20.3 and 30.0 min. However, their identity was unclear, being extremely low in concentration that did not allow a clear inference from the diode array spectrum. Prasinoxanthin expected to appear between neoxanthin and violaxanthin (ZAPATA *et al.*, 2000) was not detected at all. *Pedinomonas minor* is known to lack prasinoxanthin (RICKETTS, 1970). Although available information is limited, the absence of prasinoxanthin seems

Table 1. Pigment composition of *Pedinomonas noctilucae* and the relative amount of respective pigments to Chl. *a* (w/w). Cells were harvested during the exponential phase from the cultures grown at 10 μ mol $m^{-2}s^{-1}$(A) and 150 μ mol $m^{-2}s^{-1}$ (B) : "C" represents the cells harvested during the stationary phase from the culture grown at 150 μ mol $m^{-2}s^{-1}$.

	Relative amount to chlorophyll *a* (w/w)					
Growth	Neoxanthin	Violaxanthin	Zeaxanthin	Lutein	Chlorophyll *b*	Chlorophyll *a*
A	0.078	0.128	0.088	0.271	0.115	1
B	0.080	0.118	0.059	0.224	0.092	1
C	0.132	0.194	0.244	0.477	0.088	1

to be a common feature of species belonging to the Pedinophyceae (MOESTRUP,1991).

Chl. *a* was the most abundant light-harvesting pigment and the ratio of Chl. *a* to Chl. *b* (w/w) remained rather constant around 10 (Table 1). In contrast, the relative amount of carotenoids to Chl. *a* increased during the stationary phase compared to that observed during the exponential phase. This was a common trend in the concentrations of the carotenoids detected. Although antheraxanthin was not quantified, a similar trend was observed in its peak area. β,β-carotene was not evaluated owing to its trace amount and a possible overlapping of β,ε-carotene. Light intensity did not exerted a significant influence on the pigment composition, as the ratios of the respective pigments to Chl. *a* did not vary much between the low and high light conditions. Nonetheless, light intensities used in the present study were rather low due to instrumental constraints. Since the light intensity near the surface in the tropical waters is much higher than those we used in our laboratory experiment, further investigation is required to confirm the variations, if any, in the pigment ratios that govern the predictability of group-specific algal abundance from pigment analysis (MACKEY *et al.*, 1996).

Literature

FURUYA, K., M. HAYASHI and Y. YABUSHITA (1998): HPLC Determination of phytoplankton pigment using N, N-dimethylformamide. J. Oceanogr., **54,** 199–203.

JEFFREY, S. W., R. F. C. MANTOURA and S. W. WRIGHT (Eds.) (1997) : Phytoplankton pigments in oceanography, Monographs on oceanographic methodology 10, UNESCO, Paris, pp. 661.

MACKEY, M. D., D. J. MACKEY, H. W. HIGGINS and S. W. WRIGHT (1996) : CHEMTAX-a program for estimating class abundance from chemical markers: Application to HPLC measurements of phytoplankton. Mar. Ecol. Prog. Ser., **144,** 265–283.

MANTOURA, R. F. C. and C. A. LLEWELLYN(1983): The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse phase high performance liquid chromatography. Anal. Chim. Acta, **151,** 279–314.

MOESTRUP, ø., (1991): Further studies of presumedly primitive green algae, including the description Pedinophyceae class. nov. and *Resultor* gen. nov. J. Phycol, **27,** 119–133.

OKAICHI, T., T. OCHI, K. TADA, T. URAI, S. WISSESANG, T. ISHIMARU, and Y. FUKUYO (1991): Isolation and culture of *Pedinomonas noctilucae*, a symbiont of *Noctiluca scintillans* of Gulf of Thailand. Proc. Second WESTPAC Symp., 166–176.

OSTROUMOFF, A. (1924): *Noctiluca miliaris* in Symboise mit Grüunen Algen. Zool. Anz., **58,** 162.

RICKETTS, T. R.(1970): The pigments of the prasinophyceae and related organisms. Phytochemistry, **9,** 1835–1842.

SWEENEY, B. M. (1971): Laboratory studies of a green *Noctiluca* from New Guinea. J. Phycol., **7,** 53–58.

SWEENEY,B.M. (1976): *Pedinomonas noctilucae* (Prasinophyceae), the flagellate symbiotic in *Noctiluca* (Dinophyceae) in Southeast Asia. J. Phycol., **12,** 460–464.

ZAPATA, M. F. RODRIGUEZ and J. L. GARRIDO (2000) : Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed phase C_8 column and pyrydine-containing mobile phases. Mar. Ecol. Prog. Ser., **195,** 29–45.

Received Octover 10, 2000
Accepted November 6, 2000