SỰ BIẾN ĐỘNG HÀM LƯỢNG LECTIN THEO MÙA CỦA RONG ĐỎ, *KAPPAPHYCUS ALVAREZII* (DOTY) DOTY EX SILVA

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Tóm tắt Hàm lượng lectin của ba giống màu của tảo đỏ, *Kappaphycus alvarezii* được nuôi trồng qua một giai đoạn 6 tháng đến 1 năm đã được xác định bằng phương pháp cố định đáy. Nhiệt độ nước biển và độ muối ở khu vực nghiên cứu tương ứng là từ 24,6 đến 31,5^{0}C và từ 29,3 đến 34,6 ‰. Cường độ chiếu sáng trong quá trình nghiên cứu dao động khá mạnh nằm trong phạm vi từ 340,7 đến 864,5 µmol photon m^{-2} s^{-1}. Amon được đánh giá từ 60,6 đến 80,1% của nitơ hòa tan tổng số và phụ thuộc theo mùa. Nhiệt độ và cường độ ánh sáng là hai yếu tố chính tạo nên sự thay đổi của hàm lượng lectin với các giá trị 91% và 94% (R^2= 0,91 và R^2= 0,94). Hàm lượng và hoạt tính của lectin đều được phát hiện trong suốt một năm nghiên cứu với các giá trị lectin cao nhất đạt được là 338 ± 19 µg/g tảo khô cho giống đỏ, 302 ± 15 µg/g tảo khô cho giống nâu và 259 ± 14 µg/g tảo khô cho giống xanh trong tháng mười hai, và giá trị thấp nhất là 23 ± 6 và 27 ± 4 µg/g tảo khô cho giống nâu trong tháng năm và tháng sáu. Kết quả đã cho thấy rằng hàm lượng lectin có thể đạt giá trị cao hoặc thấp tùy thuộc vào những giai đoạn khác nhau của năm.

SEASONAL VARIATION IN LECTIN CONTENT OF THE RED ALGA, *KAPPAPHYCUS ALVAREZII* (DOTY) DOTY EX SILVA

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Abstract Three color strains of the red alga *Kappaphycus alvarezii* were cultivated in the period of six months to one year using the fixed off bottom monoline culture method to determine on the lectin content of these commercial strains. The seawater temperature and salinity at the studied site ranged from 24.6 to 31.5^{0}C and 29.3 to 34.6 ‰, respectively. Irradiance showing a strong variation in the studied period ranged from 340.7 to 864.5 µmol photon m^{-2} s^{-1}. Ammonium accounted for 60.6 – 80.1% of total DIN depending on the season. Temperature and irradiance were two main environmental factors that explained 91% and 94% of the obtained variations in lectin content (R^2= 0.91 and R^2= 0.94, respectively). The lectin contents were detected during the studied period of one year with the highest of 338 ± 19 µg. g^{-1} dried alga for red

strain, 302 ± 15 μg. g^{-1} dried alga for brown strain, and 259 ± 14 μg. g^{-1} dried alga for green strain during December and the lowest ones were obtained during May and June (23 ± 6 and 27 ± 4 μg. g^{-1} dried alga for brown strain), respectively. These results showed that lectin content may be presented at high or low level during different time of the year.

I. INTRODUCTION

Lectins are carbohydrate-binding proteins or glycoproteins of non-immune origin and plays important roles as recognition molecules in cell-cell or cell-matrix interaction. Some of lectins are useful as convenient tools in glycomics and medical fields because they can discriminate the difference in carbohydrate structures and reveal various biological activities via binding to carbohydrates (Goldstein and Poretz, 1986; Sharon and Lis, 2003). Lectins are present in a wide range of organisms from virus to humans. With respect to lectins from marine algae, there are conflicting results about lectin presence and these may be due to difference of the time of the year in which the algal sample was collected. The red marine algae, *Gracilaria verrucosa*, and *G. domigensis*, *Gelidium pusillum* have been shown to different molecular mass and lectin presence (Takahashi and Katagiri, 1987; Benevides *et al.*, 1999), respectively. The green marine alga, *Ulva lactuca* was observed marked variation in the presence of hemagglutinating activity in different months of the year (Sampaio *et al.*, 1996).

The existence of color strains of *K. alvarezii* with varied morphologies and pigmentations (brown, red and green) has been extensively reported for decades. Differences in their physiological characteristics including growth performance and photosynthesis have been reported from laboratory studies (Dawes *et al.*, 1994; Aguirre von Wobeser *et al.*, 2001).

In view of these observations we carried out to investigate on the seasonal variation in lectin content of *K. alvarezii* using three color strains over a 6 to 12 month cultivated period.

II. MATERIALS AND METHODS

Kappaphycus alvarezii was cultivated at a well-defined area ($109^0$12'16.2" N, $11^0$59'21.12" S) in CamRanh Bay, KhanhHoa, Viet Nam from September 2005 to August 2006. The collected material was cleaned of epiphytes, transported rapidly to the laboratory on ice. Sample of rabbit blood cell was obtained from Institute of Vaccine- Nhatrang, Vietnam. Lectin KAA-2 was purified from *Kappaphycus alvarezii* used as a standard. Goat antibodies against rabbit IgG, conjugated with alkaline phosphatase, were purchased from Sigma Chemical Co. Rabbit antisera against the lectin KAA-2 was obtained from laboratory of

Marine Bioresource Chemistry, Hiroshima University. Other reagents were of analytical grade.

1. Environmental factors

In the cultivation area, seawater temperature was registered over the study period at regular intervals (2 and 1 h, respectively). Mean monthly temperature (±S.D.) was obtained by averaging all the values obtained for a single month. Light readings during the sunlight period (9 and 13h, respectively) were converted from Lux (Custom lux Meter Lx 1332, Japan) to photon flux density (photosynthetic active radiation, PAR, 400 – 700 nm) in µmol photons m^{-2} s^{-1}. At each sampling time, salinity and pH were recorded in situ. Two separate seawater samples were collected in the cultivation area fortnightly for nutrient analysis. Dissolved inorganic nitrogen (DIN= sum of $N–NO_3$ + $N–NO_2$ + $N–NH_4$) and dissolved reactive phosphate (DRP= $P–PO_4$) were determined according to the method described by Parsons *et al.* (1989).

2. Cultivation of seaweed:

In Cam Ranh Bay, the hot season occurs from April to September, while the cool season occurs, from October to next March. The brown strain of *K. alvarezii* was cultivated during one year from September of 2005 to August of 2006 at Cam Ranh Bay, coinciding with both hot and cool seasons. Two red and green strains were cultivated over a 6 month period from September of 2005 to February of 2006 because they did not tolerate the environmental factors in the hot season. The fixed off bottom monoline culture method (Trono, 1992) was employed and the monolines were positioned parallel to the direction of the prevailing current. The cultivation area was confined with fishing net to avoid fragment dispersion. All the specimens were harvested at 15 day intervals, washed with sea water, freeze dried and stored at – 20 ^{0}C until used.

3. Preparations of algal extracts

The dried alga (3 g) was prepared by homogenisation with 10 volumes of 20% ethanol by stirring at 4 ^{0}C overnight. After filtration through a cheese cloth, the residues were extracted once again with 20% cold ethanol in a similar manner. The filtrates were combined and centrifuged at 3000 rpm for 20 min. The supernatant was collected and stored at -20^{0}C for examination of hemagglutinating activity and lectin content.

4. Preparation of a 2% suspension of enzyme-treated erythrocytes

Blood sample was washed three to five times with 50 volumes of saline. After washing, one tenth volume of 0.5% (w/v) trypsin solution was added to erythrocyte suspension and the mixture was incubated at 37 ^{0}C for 60 min. After incubation, the erythrocytes were washed three to five times with saline and a 2% suspension (v/v) of trypsin-treated erythrocyte was prepared in saline.

5. Hemagglutination assay

Hemagglutinating assay was carried out by a microtiter method using a 96-well microtiter V-plate (Hori *et al.*, 1986). First, 25 µl each of the serially two-fold dilutions of a test solution were prepared in saline on a microtiter V-plate. To each well, 25 µl of a 2 % erythrocyte suspension were added and the mixtures gently shaken and incubated at room temperature for 2 h. Hemagglutination was observed macroscopically and judged as positive in the case that more than 50 % of erythrocytes in well are agglutinated. Hemagglutinating activity was given as a titer which was a reciprocal of the highest two-fold dilution exhibiting positive agglutination. The hemagglutinating titer was defined as containing one hemagglutinating unit per ml (H.U. mL^{-1}). The assay was carried out in triplicate per a test solution.

6. Protein contents

Protein contents were determined by the method of Lowry (1951) using bovine serum albumin as a standard.

7. Enzyme linked immunosorbant assay

The wells of flat-bottomed titer plates were incubated with solutions of standard lectin KAA-2 (1.95 ng to 250 ng per 50µL), and crude extracts of alga (~1µg protein per 50µL) in 50 mM carbonate buffer, pH 9.6 overnight at 4 ^{0}C followed by washing three times with 100 mM phosphate buffer saline containing 0.05% Tween 20 (PBS-T). After washing, 250 µL of 0.05% of Tween-20 and 1% of skim milk in PBS (PBS-T-S) was added to each well and incubated for 90 min at 37 ^{0}C. The plates were emptied again and washed with PBS-T three times. The antiserum against lectin KAA-2 was diluted 2000-fold with PBS-T-S, and wells were incubated with 50 µL of the diluted solution of antiserum for 60 min at 37 ^{0}C. The plates were emptied again and washed twice with PBS-T. The wells were further incubated with 50µL of the second antibody (antibodies raised in goat against rabbit IgG, conjugated with alkaline phosphatase and diluted 5000-fold with PBS-T-S) for 60 min at 37 ^{0}C. The

wells were washed twice with PBS-T in order to remove unbound conjugates. The phosphatase reaction was initiated by the addition of 100µL of 2.7mM p-nitrophenyl phosphate in 1M diethanolamine – HCl buffer, pH 9.8 to each well, and the reaction was quenched by the addition of 50µl of 1M NaOH after 30 min. The developed colour of liberated *p*-nitrophenol was monitored at 405 nm with a model 450 microplate reader (BioRad, Richmond, CA, USA). Control reactions (blank) were performed with carbonate buffer. The assay was carried out in duplicate per a test solution and the optical densities of blanks were subtracted from the respective test values to obtain corrected absorbance values. Mean monthly lectin content was obtained by averaging all the values obtained for a single month.

8. Statistical analysis

Data were presented as means ±SD for at least three independent measurements. Pearson's correlation analysis was used to determine the correlation coefficient between environmental factors with lectin content, hemagglutinating activity and protein yield. Stepwise multiple regression was used to determine the individual contribution of the environmental factors (independent variables) to the total variance of lectin contents, hemagglutinating activities (dependent variable).

III. RESULTS AND DISCUSSION

1. Environmental factors

Monthly changes of environmental factors at the experimental site were shown in Fig. 1 (A, B and C). Seawater temperature around the study area ranged from 24.6±0.6 ^{0}C to 31.5±0.3^{0}C. Salinity ranged from 29.3±0.9 ‰ to 34.6±1.2 ‰ in May. pH did not change significantly from 7.83 ± 0.12 to 8.26 ± 0.04. Irradiance showed a strong fluctuation along study period with maximum recorded value of 864.5 µmol photon m^{-2} s^{-1} during May and the lowest one was 340.7 µmol photon m^{-2} s^{-1} during December. Nutrients ranged from 5.31 ± 0.09 µmol.L^{-1} to 10.35 ± 0.2 µmol.L^{-1} for dissolved inorganic nitrogen (DIN) and from 0.43 ± 0.1 µmol.L^{-1} to 0.93 ± 0.2 µmol.L^{-1} for dissolved reactive phosphate (DRP). Ammonium accounted for 60.6 – 80.1% of total DIN depending on the season. The most abundant form of nitrogen in the sea is nitrate.. Table 1 showed the positive correlations between DIN and DRP with lectin content, hemagglutinating activity ($P<0.05$).

Temperature, irradiance and nutrients were believed to be the most important factors affecting successful cultivation of *K. alvarezii* (Glenn and

Doty, 1990). In the present study, the temperature and irradiance were the main two factors affecting the lectin content and hemagglutinating activity of *K. alvarezii*. The variation of seawater temperature and irradiance at CamRanh Bay, also explained 91% and 94% of variation in lectin content, respectively, and exhibited negatively significant correlation (Table 1). This may be attributed transcripts of genes whose protein products are involved in photosynthesis during low and high irradiant cycles (Jacobsen *et al.*, 2003; Schubert *et al.*, 2004).

Tab. 1. Correlation coefficients between lectin content, hemagglutinating activity of *K. alvarezii* and environmental factors

	Lectin	H.U	Temp	Sal	Irradiance	pH	DIN	DRP
Lectin	1							
H.U	0.88	1						
Temperature	-0.88	-0.92	1					
Salinity	-0.68	-0.77	0.61	1				
Irradiance	-0.94	-0.92	0.85	0.78	1			
pH	0.01	-0.02	0.25	-0.38	-0.14	1		
DIN	0.31	0.60	-0.33	-0.69	-0.53	0.51	1	
DRP	0.34	0.07	0.07	-0.28	-0.36	0.57	0.03	1

*Temperature (^{0}C), Salinity (‰), Irradiance (µmol photon m^{-2} s^{-1}) indicate significant at P>0.05 and nutrients: DIN and DRP (µmol.L^{-1}) indicate significant at P<0.05.

2. Lectin content and hemagglutinating activity

The lectin contents and hemagglutinating activities of three color strains of *K. alvarezii* shown in Fig. 2 (A, B and C, respectively) were almost detected over a 6 to 12 month study period with the highest values obtained were 338 ± 19 µg. g^{-1} dried alga and 1365 H.U. mL^{-1} for red strain, respectively; 302 ± 15 µg. g^{-1} dried alga and 1365 H.U. ml^{-1} for brown strain, respectively; and 259 ± 14 µg. g^{-1} dried alga and 1024 H.U. mL^{-1} for green strain, respectively, during December, and the lowest values in May and June for brown strain, were 23 ± 6 and 27 ± 4 µg. g^{-1} dried alga and 28 and 48 H.U.mL^{-1}, respectively. The highest values of lectin contents and activities were observed coinciding with the period of the high growth rates of *K. alvarezii* during the cool season from October to March (5.73 to 6.25 % day^{-1}). Similar results have been found in previous papers which demonstrated that temperature was the main environmental factor affecting growth rates of *K. alvarezii* and showed an inverse significant relationship between maximum temperature and growth rate (Glenn and Doty, 1992; Muñoz *et al.*, 2004). This may explain the differences in growth between subtropical and tropical areas such as in lectin content and hemagglutinating activity.

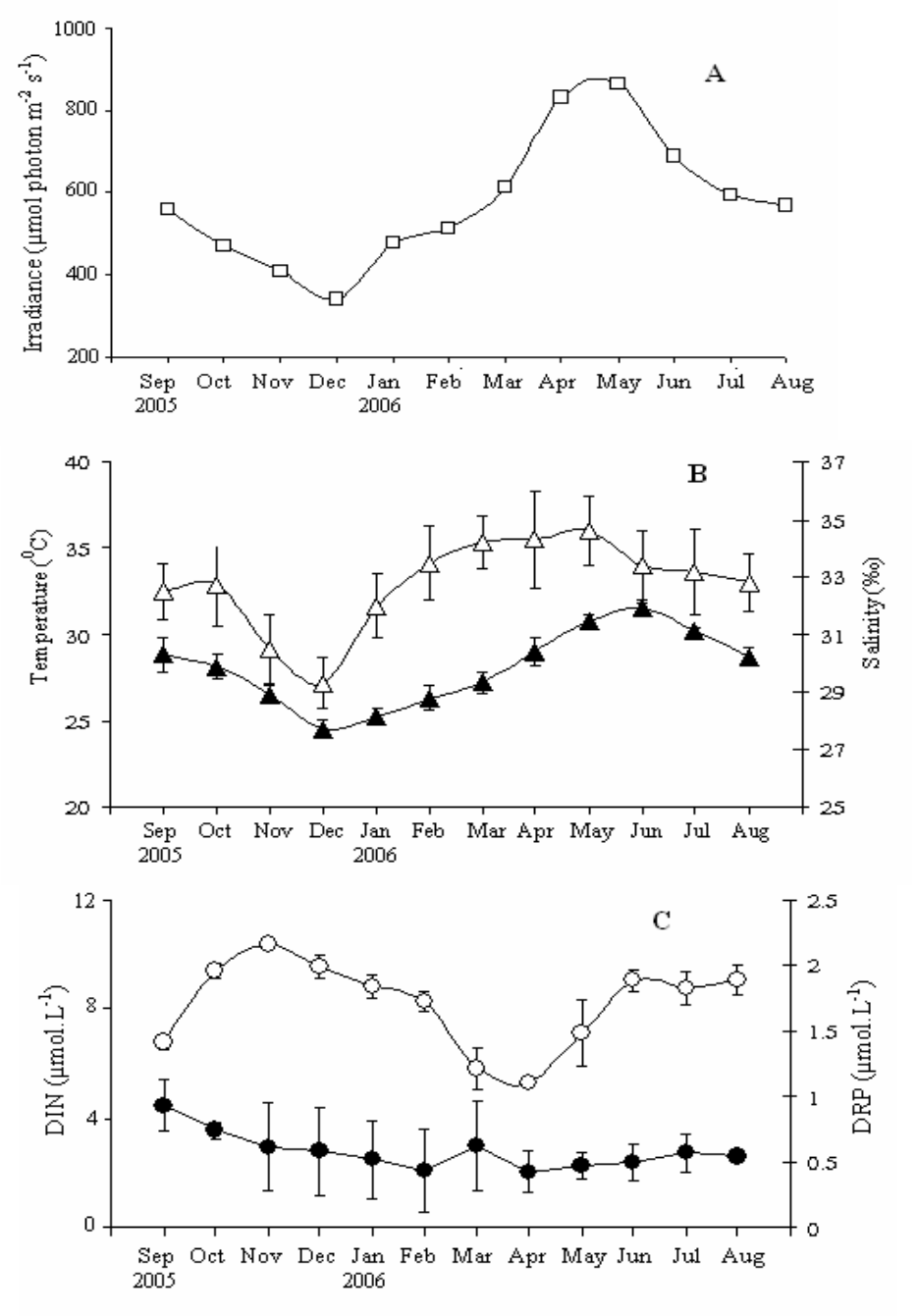

Fig. 1. Monthly variation of environmental factors over the cultivation period in CamRanh Bay. (A) Irradiance (—□—), (B) temperature (—▲—) and salinity (—△—), and (C) DIN (—○—) and DRP (—●—). Standard deviation indicated (±S.D.)

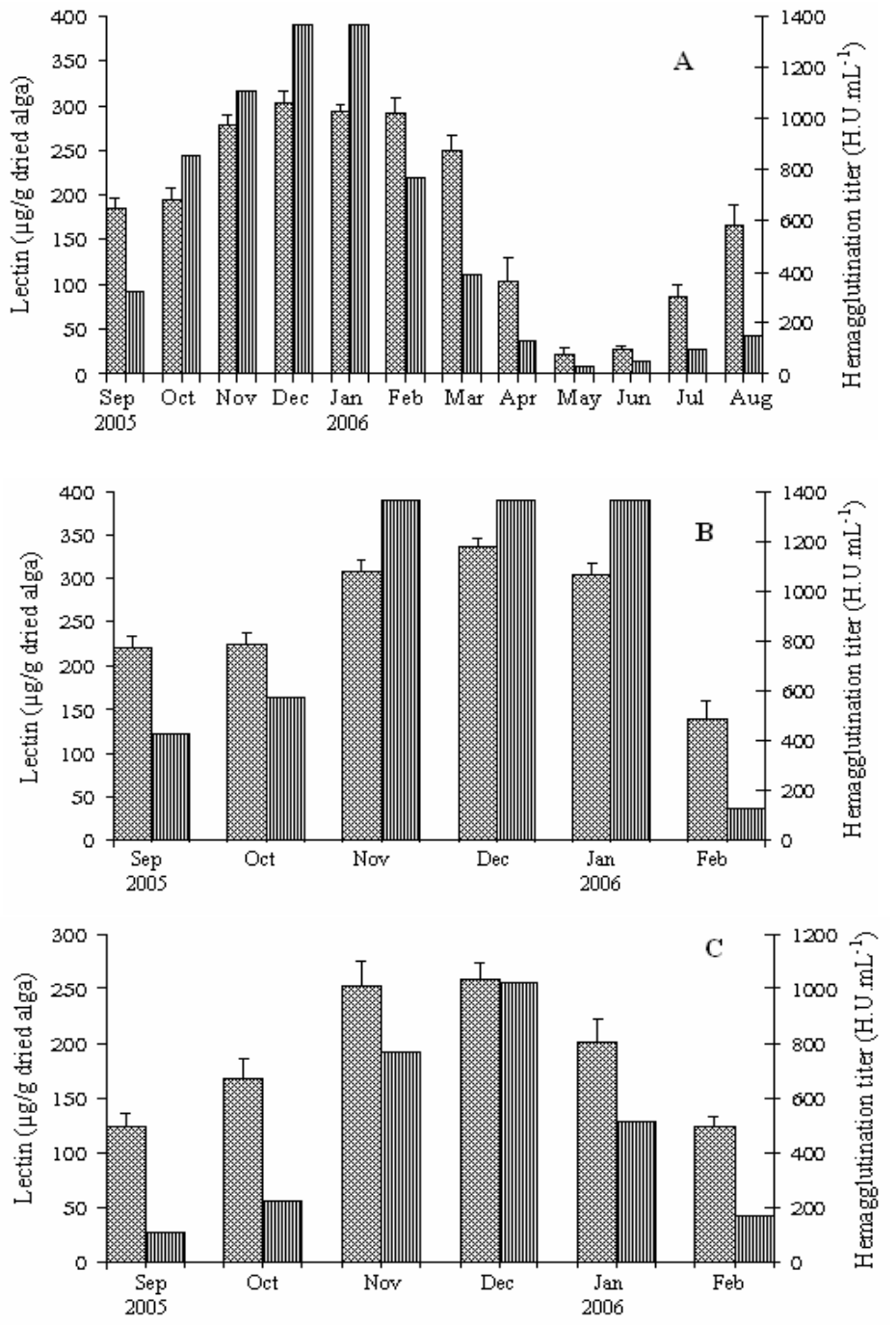

Fig. 2. Lectin content and hemagglutinating activity of *K.alvarezii* over the cultivation period in CamRanh Bay. (A) Brown, (B) red, and (C) green color strain. Lectin content (▨), and hemagglutinating activity (▥). Standard error indicated (±S.E.)

In general, reduction in growth rate, lectin content and hemagglutinating activity of *K. alvarezii* during the hot season could be attributed to increased seawater temperature and high irradiance. The effect of these two factors could be compensated by increasing water motion or water depth to avoid any detrimental effect on growth rate, lectin content and hemagglutinating activity.

In addition, the lectin content could be related to the time of the year in which the alga presented the maximum production of lectins as the life cycle of the alga and/or their morphological features. The results presented here agree with the idea that the presence of lectins in marine algae was related to seasonal variation as those reported for *Gracilaria verrucosa* (Takahashi and Katagiri, 1987), *Ulva lactuca* (Sampaio *et al.*, 1996) and *Gracilaria domigensis* and *Gelidium pusillum* (Benevides *et al.*, 1999). Therefore, attention should be paid to the time of the year in which the alga would be collected and prepared for studies of lectins.

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