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Temporal and Depth Variation of Photoprotective Mycosporine-Like Amino Acids in Soft Coral Species from the Eastern Red Sea Coast

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Abstract. Mycosporine-like amino acids (MAAs) were investigated in two soft coral species collected during summer and winter of 2003 and 2004 at 5, 10, 15, and 20 m depth. Four different MAAs were detected. Palythine was the dominant compound in *Sinularia polydactyla* and *Sarcophyton trocheliophorum.* The palythine concentration in *S. trocheliophorum* varied from 44 to 141 µg mg^{-1} protein in summer compared to 33 to 129 µg mg^{-1} protein in winter. The corresponding variation in *S. polydactyla* was 34 to 187 µg mg^{-1} in summer and 24 to 127 µg mg^{-1} protein in winter. Both the maxima and minima were higher in summer compared to winter and at all depths. Next to palythine; porphyra-334 was detected in *S. trocheliophorum.* It was one eigth compared to palythine. The concentration of asterina-330 in *S. trocheliophorum* was almost negligible. Mycosporine-glycine was not detected at any depth or season indicating that the organism has passed through the initial evolutionary process. Photosynthetically Active Radiation (PAR) was 67% more in summer compared to winter and hence explains the fact that the production of MAAs is more in summer compared to winter indicating that the photosynthetic zone favours the production of MAAs.

Keywords: UV-absorbing compounds, soft corals, Jeddah Coast, Red Sea, Saudi Arabia.

Introduction

Mycosporine-like amino acids are a family of water soluble, low molecular weight compounds and absorption maximum between 309-360 nm. They have been detected in terrestrial as well as marine organisms. The frequently observed correlation between MAAs concentration and solar exposure found in nature suggests that these compounds play an important role in the ultraviolet protection as this type of radiation has deleterious effect on the marine organisms, these include damage to DNA in the cells, oxidative stress, damage to lipids and proteins (Shick *et al.*, 1996). These MAAs have a cyclohexenone or cyclohexeneimine structure in common and were first detected by Wittenberg (1960). The presence of the compounds that absorb at 320 nm was observed in the zooxanthellae of scleractinian corals and a cyanobacterium of Great Barrier Reef (Shibata, 1969). The first evidence of their photoprotection properties, in the colonies of *Porites labata*, was provided by Maragos (1972). MAAs are almost ubiquitous ranging from tropical to polar regions among marine organisms (Davidson *et al*., 1994; and Zhang *et al*., 2005). It is postulated that these MAAs are initially synthesized in the zooxanthellae *via* shikimatic pathways that link the metabolism of carbohydrates to the synthesis of aromatic amino acids that are required to synthesize these compounds and that have been demonstrated in the zooxanthellae of the corals (Favre-Bonvin *et al*., 1987). There are now 25 MAAs identified (Karsten *et al.*, 1998); four of them are considered as primary MAAs, others being secondary MAAs derived from the former probably by processing in the host (Dunlap and Shick, 1998). The information about such compounds in the marine organisms in the Red Sea is scarce. Woesik, (2001) studied the bleaching phenomena of some coral along the coast of Eriteria and it was observed that photoprotection was achieved by increasing their level of MAAs in these corals. The effect of increased UV radiations was studied in 10 different coastal regions including Red Sea and it was observed that biomass was reduced to a large extent by UV-A and UV-B radiations while MAAs production was increased (Wahl *et al.*, 2004). However no work has been done on the MAAs in the corals along the Eastern coast of Red Sea. The present study was designed not only to identify and quantify the MAAs in various species of corals but also to investigate their seasonal variations (summer/winter); and depth profile.

Materials and Methods

Two collections of coral samples were made during the months of July 2003 and January 2004, near the coast of Jeddah, at 21º51′27″N and 39º07′64″N. Samples were collected at depths of 5, 10, 15, and 20 m. After collection, the specimens were brought to the laboratory and frozen at –20ºC. At the study site

the average temperature was 24.5ºC during the month of January and 30ºC during the month of July. There was little annual variations in the salinity during the study period; ranging from 39‰ to 41‰. The organisms were identified as *Sinularia polydactyla* and *Sarcophyton trocheliophorum*. These species are so-called soft corals and are very abundant in shallow, sheltered water, and also in turbid water. The substrate is mostly soft bottom. The colony form of *S. polydactyla* is a finger-like projection and the surface of the colony is stiff, due to the dense mass of large spindle shaped scelerites (spicules). *S. trocheliophorum* is very abundant in sheltered shallow waters with moderately turbid habitat near the sea shore reefs and soft bottom. The colony has a mushroom like shape and it has stout short trunk. The surface of the colony is soft with less density of small scelerites.

Chlorophyll *a* and protein contents were estimated using standard methods. The chlorophyll *a* was estimated by treating a measured amount of coral powder with 90% acetone over night at 4ºC and the determination was performed using a double beam spectrophotometer at 665 nm. The concentration was calculated according to the method of Lichtenthaler (1987).

$$\text{Chlorophyll "}a\text{"} = \frac{(11.2\ Abs.\ 665) - (2.04\ Abs.\ 645) \times volume}{1 \times 1000 \times wt.\ (g)} = \mu g\ g^{-1}$$

The protein content was determined using albumin as a standard and measuring the absorbance at 600 nm. Egg albumin solution (Sigma) was taken as standard; solution of Na, K-tartarate was mixed with alkaline solution of copper sulfate and then added to Folin-Phenol reagent. These reagents were added to the standard solutions of egg albumin and also to the samples and kept at room temperature for half an hour till the blue color has fully developed. The absorbance was recorded at 600 nm on spectrophotometer. Standard curve was plotted by calibrating the absorbance against the concentration (Lowry *et al*., 1951).

Extraction and Analysis of MAAs

Fresh samples of coral were cut into pieces (8 g) and treated with 90% aqueous methanol and shaken at 25ºC for 24 hours. They were then centrifuged, the supernatant was isolated, and the process was repeated twice. The bulk of the organic solvent was removed under vacuum at 40ºC and the crude extracts were finally freeze-dried and were stored at –8ºC.

The study of the role that these MAAs play in marine environment as photoprotective agents depends on our ability to identify and quantify these compounds. HPLC provides a quick clue to characterize these compounds in small quantities, therefore the separation and quantification of MAAs was

carried out using isocratic reverse-phase High Performance Liquid Chromatography (HPLC). The dried extracts were resuspended in 50 to 100 µl of 25% MeOH (v/v), and 20-70 µl aliquots were injected into a Phenosphere 5-µm pore size C_8 column (250 × 4.5 mm) protected with an RP-8 (Brownlee) guard column. During the analysis, samples in the autosamplers were kept at 15ºC, while the column was maintained at 20ºC. The mobile phase consisted of 0.1% acetic acid in 25% aqueous methanol (v/v) running at a flow rate of 0.79 ml min^{-1}. MAAs were detected in a diode array detector using four preselected channels (310, 320, 334, and 360 nm). MAAs were identified by comparison with published retention times and by co-chromatography with purified standards. Peak purity was checked by analyzing the absorption spectrum over the entire range of wavelengths. The total content of specific MAAs in each sample was calculated from the HPLC peak area and the concentrations were expressed in µg mg^{-1} of protein (Tartarotti and Sommaruga, 2002). MAAs found in trace amounts was not included.

Results

An average of two samples was taken for chlorophyll, proteins and MAAs analysis. Chlorophyll *a* and protein contents are presented in Table 1. The average (of two samples) of chlorophyll *a* in *S. polydactyla* was generally higher in

Table 1. Chlorophyll a (µg g^{-1}) and protein content (mg g^{-1}) in two soft corals (*Sinularia polydactyla* and *Sarcophyton trocheliophorum*) collected from the Eastern Red Sea coast.

Month	Depth (m)	*Sinularia polydactyla*		*Sarcophyton trocheliophorum*	
		Chlorophyll *a* µg g^{-1}	Protein mg g^{-1}	Chlorophyll *a* µ g^{-1}	Protein mg g^{-1}
July 2003	**5**	1.69	1.17	5.51	2.39
	10	3.01	2.77	4.79	4.33
	15	3.13	1.26	5.65	5.40
	20	2.05	1.57	9.02	2.38
	S.D	± 0.70	± 0.73	± 1.88	± 1.49
	Average	**2.47**	**1.69**	**6.24**	**3.62**
Jan. 2004	**5**	7.03	4.42	2.10	1.66
	10	4.50	7.18	2.80	3.63
	15	5.15	3.76	4.13	3.37
	20	8.02	6.05	5.14	5.25
	S.D	± 1.63	± 1.55	± 1.35	±1.46
	Average	**6.17**	**5.35**	**3.54**	**3.47**

winter (6.17 µg g^{-1} dry wt. coral) compared to summer (2.25 µg g^{-1} dry wt. coral). Similar pattern of the protein average in *S. polydactyla* was also observed in both seasons. A different pattern was observed in *S. trocheliophorum* where the average of chlorophyll *a* was 3.54 mg g^{-1} in winter and 6.24 mg g^{-1} in summer, whereas the protein showed not much differences in both seasons, on the contrararily in *S. polydactyla* the protein content was approximately three times higher in winter compared to summer.

Four MAAs were identified as palythine, porphyra-334, asterina -330 and shinorine. Palythine was the major compound in the two species, regardless of season or depth (Tables 2 and 3). Shinorine, porphyrine-334 and asterina-330 were detected in very low concentrations. There was no correlation between the protein content and the MAAs content e.g. *S. polydactyla* at 10 m depth in winter had a protein content of 7.18 mg g^{-1} dry wt. coral and the total of MAAs was 88.5 µg mg^{-1} protein, whereas the protein content in the same coral at depth 5 m in summer was 1.17 mg g^{-1} dry wt. coral and the total of MAAs content was 193.9 µg mg^{-1} protein.

Table 2. Concentration of MAAs (µg g mg^{-1} protein) and percentage given in parenthesis in *Sarcophyton trocheliophorum* at different depths and seasons from Eastern Red Sea coast.

Month	Depth (m)	MAAs				ΣMAAs
		SH	PR	PI	AS	
July 2003	5	2.051 (1.3)	14.84 (9.1)	141.31 (86.9)	4.50 (2.8)	162.69
	10	0.69 (1.1)	3.35 (5.2)	58.63 (91.7)	1.26 (2.0)	63.93
	15	1.23 (2.5)	3.24 (6.5)	44.11 (88.8)	1.10 (2.2)	49.68
	20	1.15 (2.1)	5.09 (6.9)	65.63 (88.6)	1.81 (2.4)	74.07
Jan. 2004	5	1.84 (1.3)	6.61 (4.7)	129.99 (92.7)	1.77 (1.3)	140.21
	10	2.20 (3.3)	5.02 (7.5)	57.77 (86.8)	1.55 (2.30)	66.55
	15	0.70 (1.9)	2.74 (7.3)	33.10 (88.2)	0.98 (2.6)	37.52
	20	0.53 (0.9	4.42 (7.5)	52.13 (89.0)	1.51 (2.6)	58.59

SH = Shinorine; PR = Prophyra-334; PL = Palythine; AS = Asterina-330.

Table 3. Concentration of MAAs (μg g mg^{-1} protein) and percentage given in parenthesis in *Sinularia polydactyla* at different depths and seasons from Eastern Red Sea coast.

Month	Depth (m)	MAAs				ΣMAAs
		SH	PR	PI	AS	
July 2003	5	0.02 (0.00)	1.18 (0.6)	187.10 (96.5)	5.60 (2.9)	193.91
	10	0.03 (0.00)	0.61 (0.7)	83.34 (96.7)	2.24 (2.6)	86.23
	15	0.025 (0.00)	0.94 (1.10)	86.30 (96.8)	1.83 (2.1)	89.16
	20	0.092 (0.3)	0.65 (1.8)	34.35 (95.7)	0.79 (2.2)	35.89
Jan. 2004	5	0.20 (0.1)	2.03 (1.5)	127.81 (95.5)	3.74 (2.8)	133.74
	10	0.91 (1.0)	0.001 (0.0)	76.81 (98.0)	0.86 (1.0)	78.60
	15	1.07 (0.7)	0.0 (0.0)	143.36 (99.0)	0.43 (0.3)	144.85
	20	0.26 (1.0)	0.0 (0.0)	24.60 (96.7)	0.57 (2.3)	25.43

SH = Shinorine; PR = Prophyra-334; PL = Palythine; AS = Asterina-330.

Palythine constituted the bulk of MAAs in both the species and at two seasons. The palythine concentration in *S. trocheliophorum* varied from 44 to 141 μg mg^{-1} protein in summer compared to 33 to 129 μg mg^{-1} protein in winter. The corresponding variation in *S. polydactyla* was 34 to 187 μg mg^{-1} in summer and 24 to 127 μg mg^{-1} in winter. The production was slightly more in summer compared to winter, it may be due to the fact that spawning season in such corals is mostly in summer. Porphyra-334 was also negligible in both the species, however, the quantity was comparatively more in *S. trocheliophorum.*

Mycosporine-glycine was not detectable in both species in winter and summer. There was a distinct decrease in shinorine in *S. polydactyla*, the average of this MAAs was 0.05 μg mg^{-1} in summer and 0.60 μg mg^{-1} protein in winter. Asterina-330 was the second highest MAAs in *S. polydactyla* (after palythine). It was observed that asterina-330 was highest at 5 m both in summer and winter (5.60 μg mg^{-1} and 3.74 μg mg^{-1} respectively), while this MAA was comparatively low in all stations at all depths in *S. trocheliophorum* with an average of 2.62 μg mg^{-1} in summer and 1.4 μg mg^{-1} in winter.

The solar radiation flux reached a maximum of 10.5 hours in January and had a value of 727 $mE.m^{-2}.S^{-1}$, while, in July, it was 13.75 hours and led to a value of 1212 $mE.m^{-2}.S^{-1}$ and this explains why that the production of MAAs is higher in summer, as they produce more MAAs against UV radiations in self defence.

Discussion

The plant pigment plays a vital role in the rate of photosynthesis. The expansion of chloroplast cell size reversibly correlates with light intensity. It has been found that during summer chloroplast is shrunken as heaps of light are available whereas in winter they expand in size so as to accumulate more energy (Dubinsky *et al*., 1984). Photoinhibition is a function of intensity and duration of the exposure to light. The present study reveals that Chlorophyll *a* increases with depth as light transmission decreases. Dubinsky, (1999) found that Chlorophyll *a* per unit biomass increases with decreasing irradiations. Similarly protein content was more in summer in *S. trocheliophorum* than in winter, contrarily in *S. polydactyla* it was more in winter compared to summer. The variations in the protein content may be possibility due to the different phlyotype clades of zooxanthellae *symbiodinium*, they are identified as A, B, C, and D, during the summer they have different genotype zooxanthellae and after the exodus of the symbiont algae the next strain during the winter may be the same species but different strains (Banaszak *et al.*, 2000). This has also been further confirmed by Davidson *et al*., (1994) that the relationship between MAAs and protein was not straightforward, for instance in some cases of corals with smaller protein content more MAAs were detected and the reverse could be true in some other coral species.

The four MAAs, shinorine, asterina-330, porphyra-334; and palythine were detectable in all the samples studied. Palythine constituted the bulk of the MAAs, ranging from 95-99% of total amino acids in *S. polydactyla* and 86-92% in *S. trocheliophorum.* It has also been found to be most prevalent in other coral species as well (Shick *et al.*, 1996). It is worth mentioning that Wagner (2001) found that palythine was the major MAAs in *Sinularia flexibilis* and *Lobophytum compactum*, 93% and 92% respectively. Other corals e.g. *Lobophyllia corymbosa* and *Acropora danai* revealed this palythine to be 72% and 77% of total MAAs (Teai *et al*., 1998). Yokovleva *et al*., (2004) noticed, that this palythine in *Platygyra ruykuensis* coral was the major component although the percentage was comparatively low *i.e.* 67%. It presumably provides certain level of protection against high irradiance stress during progeny (Wagner, 2001). A minimal quantity of shinorine was observed in both species. One thing was common in all the samples studied that maximum MAAs were present nearest to the surface *i.e.* 5m depth. It has also been found that the concentration

of MAAs decreases with depth. This finding is in good agreement with the study of Banaszak *et al*., (1998). It appears that the concentration of MAAs produced by the corals can be specific to that coral whether this difference was due to environmental or genetic factor or was only a consequence of normalization to coral mass, since tissue, calcium carbonate ratios can vary between species, it can give variations in the MAAs content. The capacity of zooxanthellae also changes with environment and seasons (Zhang *et al*., 2005).

The explanation of the absence of mycosporine-glycine is that it is consistently being consumed in the biosynthesis of other MAAs. It is converted into palythine by reductive transamination with metabolic ammonium to form unsubstituted amine. Perhaps there are other possible explanations such as a dietary origin for the MAAs or the organism has achieved the required target for the production of MAAs sufficient for its defense against photoradiation. The other secondary MAAs are probably derived from shinorine, porphyra-334, and mycosporine-2 glycine (Whitehead *et al*., 2001). Acclimation of corals to MAAs production is a function of PAR, UV-R, depth, season and phylogenetic affiliation (Shick, 2004). The amount of MAAs was also distinctly related to solar flux. Helbling *et al*., (1996) observed that the quantity of the MAAs was slightly less when the marine organisms were exposed only to PAR than when exposed to ultra violet radiations in addition to phtosynthetically active radiations.

Conclusions

The present study revealed the presence of four UV-absorbing compounds in these corals. A consistency was observed while investigating the presence of these compounds *i.e.* palythine was found to be the major MAA during summer and winter at all depths. Other MAAs that included were (although in small quantity) shinorine, porphyra-334, and asterina-330. Prophyra-334 was the second highest in quantity in *S. trocheliophorum* both in summer and winter; while *S. polydactyla* indicated asterina-330 to be the next highest (after palythine) in both seasons. The remaining three MAAs were in negligible amount. It was interesting to see that in both the corals MAAs were less in winter compared to summer. There was no correlation between the quantity of protein and the amount of MAAs. It was distinctly noticeable that mycosporine-glycine was not detectable. The UV radiations play a vital role in the MAAs formation in addition to the PAR.

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اختلافات محتوى الأحماض الأمينية شبيهة – المايكوسبورين الواقية ضوئيًا تبعًا للعمق والفصل في بعض أنواع المراجين الرخوة من الساحل الشرقي للبحر الأحمر

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المستخلص. جرى دراسة الأحماض الأمينية شبيهة – المايكوسبورين في نوعين من المرجان الرخوة التي تم جمعها خلال فصلي الصيف والشتاء من أعماق ٥، ١٠، ١٥و ٢٠م. حيث أمكن الكشف عن وجود ٤ أنواع مختلفة من هذه الأحماض. وقد وجد أن الباليثين هو الحامض المهيمن في نوعي *Sinularia polydactyla* و *Sarcophyton trochelioph-orum* وقد تفاوت تركيز الباليثين في *S. trocheliophorum* من ٤٤ إلى ١٤١ ميكروجرام ملجم$^{-1}$ بروتين في الصيف مقارنة بـ ٣٣ إلى ١٢٩ ميكروجرام ملجم$^{-1}$ بروتين في الشتاء. وتراوح التفاوت الممثل في *S. polydactyla* ما بين ٣٤ إلى ١٨٧ ميكروجرام ملجم$^{-1}$ بروتين في الصيف و ٢٤ إلى ١٢٧ ميكروجرام ملجم$^{-1}$ بروتين في الشتاء. التراكيز القصوى والدنيا كانت أعلى في الصيف مقارنة بالشتاء في جميع الأعماق. وجد أن حامض البروفيرا-٣٣٤ يأتي في المرتبة الثانية بعد الباليثين من حيث التركيز في نوع *S. trocheliophorum* ، حيث يمثل حوالي ٨٪ مقارنة بالباليثين. تركيز حامض أسترينا -٣٣٠ في *S. trochel-iophorum* أثر لا يذكر. لم يتم الكشف عن وجود أثر لحامض مايكوسبورين جلاييسين في أي عمق أو فصل مما يدل على أن الكائن قد تخطى مرحلة التطور الأولى. وجد أن شدة الإشعاع المتوفر للتمثيل الضوئي في الصيف يفوق وصيفه في الشتاء بحوالي ٦٧٪ وهذا يفسر

زيادة إنتاج الأحماض الأمينية – شبيهة المايكوسبورين في الصيف مقارنة بالشتاء.

الكلمات الدالة: مركبات ماصة لأشعة فوق البنفسجية، مراجين رخوة، ساحل جدة، البحر الأحمر، المملكة العربية السعودية.