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Original Article

Effect of High Salinity on Mycosporine-Like Amino Acid Production

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HIGHLIGHTS

- Mycosporine like amino acids (MAAs) can be considered as valuable sunscreens.
- High salinity condition can induce MAAs production.
- *Desmodesmus* sp. in the medium with high salinity can produce MAA compound.

ABSTRACT

Mycosporine like amino acids (MAAs) are UV absorbing compounds which are produced by a variety of organisms such as algae and cyanobacteria, in order to protect themselves from harmful UV irradiation. Thus, they can potentially be used as sunscreens in pharmaceutical and cosmetic industry. Many abiotic factors are studied which induce the production of MAAs in algae and cyanobacteria. In this investigation, we have cultivated the green microalga *Desmodesmus* sp. under salt stress and studied the effect of high salinity on MAAs biosynthesis. MAAs was extracted and partially purified using HPLC method. One compound with similar properties to MAAs was detected from the biomass extract, having a maximum absorption at 320 nm. Accordingly, this genus of microalgae can produce MAA-like compound under this condition, whereas it was not capable to synthesize MAA in normal condition. In fact, salinity is a stressor which can lead to the induction of MAAs synthesis in this microalga. Moreover, this investigation supports that the production of MAAs in microalgae helps the organism to survive in harsh environments, such as high salinity conditions.

Introduction

UV irradiation is recognized as one of the major risk factor for skin cancers and many other skin disorders, although it has also some beneficial effects on human health such as the induction of vitamin D synthesis (D'Orazio et al., 2013). Sunscreens as UV filters play a key role in skin protection against the exposure to solar UV radiation and therefore, can be considered as an effective agent in the prevention of common types of skin carcinoma (Van Der Pols et al., 2006; Antoniou et al., 2008).

A variety of organisms including prokaryotes and eukaryotes are capable of protecting themselves from harmful UV irradiation by producing UV absorbing compounds. Some of these compounds such as

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Mycosporine like amino acids (MAAs) and Scytonemin are detected in cyanobacteria and algae. MAAs as bioactive metabolites from cyanobacteria and microalgae, are synthesized from different amino acids and like to the other protein-based UV absorbing pigments can reduce the harmful effect of UV irradiation (Rastogi et al., 2017).

Mycosporine like amino acids are water soluble secondary metabolites, which have a wide range of molecular weight (188-1050 Da). They are composed of cyclohexenone or cyclohexenimine chromophores, which are conjugated to a nitrogen substitute of amino acids or imine alcohols. Some of them contain sulfate esters or link through a glycoside bond to different oligosaccharides. Thanks to this structure, MAAs have the ability of absorbing UV radiation in harmful range including 309 nm to 362 nm with high molar extinction coefficients (e= 28,000–50,000 M^{-1} cm^{-1}) (Lawrence et al., 2017; Chrapusta et al., 2017).

Many studies have been also showed that MAAs could have radical scavenging activities. Thus, they can protect biomolecules including DNA and proteins from oxidative stress (Wada et al., 2015; Cheewinthamrongrod et al., 2016). Moreover, they have a protective effect against UV-induced photo-aging as well as have anti-inflammatory activity in response to skin aging (Ryu et al., 2014; Suh et al., 2014). Therefore, MAAs exhibit great potential as natural sunscreens and can be used in pharmaceutical and cosmetic industry.

The biosynthesis of MAAs can be influenced by many different abiotic factors. UV radiation is one of the major factor which can force the algae to produce MAAs in order to protect themselves from harmful irradiation (Rastogi and Incharoensakdi, 2013; Khanipour Roshan et al., 2015). Another important factor is nutrient. Adjusting the concentration of nutrients like nitrate would lead to the accumulation of MAAs in cells (Figueroa et al., 2010; Barufi et al., 2011; Vale, 2015b; Saadatmand and Zamani, 2015). Salinity is also another factor which showed a positive effect on MAA production (Portwich and Garcia-Pichel, 2007; Vale, 2015a). It has been also proved that these abiotic factors could have synergistic effect on MAAs production (Singh et al., 2008a). Hence, the production of MAAs can increase by the adjustment of these factors.

Desmodesmus sp. is a green microalga which has been well studied for the treatment of wastewater and biofuel production (Mandal and Mallick, 2011; Doria et al., 2012; Mata et al., 2012). In this research we have focused on *Desmodesmus* sp. MAA production and investigated the effect of salinity on MAA production in this genus.

Materials and Methods

Cell cultivation and salinity experiment

20 mL of stocked culture (approved by Department of Biotechnology, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran) were cultivated in 200 mL of BG-11 culture medium. Sodium chloride at the concentration of 513 mM was added to the culture medium. This concentration of NaCl has previously shown that could exert a positive effect on MAAs production (Portwich and Garcia-Pichel, 2007). A culture medium of *Desmodesmus* sp. under salinity stress and a culture medium of *Desmodesmus* sp. under normal conditions (without adding NaCl) were prepared and analyzed.

Molecular identification of microalga using polymerase chain reaction (PCR)

DNA of the studied microalga was extracted using the technique described by Wan *et al.* with minor modifications (Wan et al., 2011). One mL of cells was collected and centrifuged at 10000 ×g for 1 min. The biomass was then suspended in 100 µL EDTA 10 mM as an extraction buffer and well vortexed. The sample was preserved at -20°C overnight. Then, it was incubated at 100°C for 15 min and in following, it was cooled immediately by placing on ice for 5 min. The cells were vortexed again and centrifuged at 10000 ×g for 1 min. The supernatant was used as DNA template.

The DNA of samples were amplified using 18s rRNA. PCR was carried out by forward primer (GTCAGAGGTGAAATTCTTGGATTTA) and reverse primer (AGGGCAGGGACGTAATCAACG) of 18s rRNA, and in the presence of 5 µL of DNA template. The PCR was run using thermal cycler (T100™ Thermal Cycler, BIORAD, USA). The thermal program of PCR was as the following: 1-Denaturation: at 95°C, initially for 3 minutes and then, through cycles for each cycle, 30 seconds. 2-Annealing: at 57°C for 30 seconds. 3-Extension: at 72°C for 30 seconds and finally, for extra 1 min. The cycle was repeated 35 times. The PCR products were evaluated by gel electrophoresis on 1% agarose gel. Then, the related bands were extracted from gel using Expin® kit protocol (Roche, Germany). The extracted genes were precipitated using ethanol precipitation method. Finally, the amplified gene segment was sequenced.

MAA extraction and partially purification

MAA was extracted using the method of Rastogi *et al.* with mild modification (Rastogi and Incharoensakdi, 2013). The biomass was separated and centrifuged at

6800 × g 10 min. Cells were then lyophilized. Then, the dried biomass was slightly soaked in water and extracted in 100% HPLC grade methanol. The methanolic extracts were incubated overnight at 4°C and dark condition followed by sonication using ultrasound bath at 35°C for 15 minutes. The extract was then centrifuged at 6800 × g 10 min and the supernatant was transferred to a new tube. The methanolic extract was evaporated under nitrogen. One mL of deionized Mili-Q water was added to dried product and transferred to a new eppendorf tube. In order to separate water insoluble impurities, it was centrifuged at 13200× g for 10 min. The supernatant was transferred in to a new micro tube and 500 µL of chloroform were added, followed by shaking and vortexing vigorously. The tubes were again centrifuged at 13200× g (10 min) and the colorless supernatant was transferred to a new Eppendorf tube. This step was repeated until the chloroform layer did not turn green more. Before injecting to HPLC column, the samples were filtered through 0.22-µm syringe filter (MS® PTFE Syringe Filter).

Characterization of MAAs

HPLC was performed using a Shimadzu HPLC system equipped with a photodiode array (PDA) detector (SPD-M10A- Shimatzu, Japan). The samples were injected to the C_{18} HPLC column (5 µm, 25 × 4.6 mm) using the isocratic mobile phase of 25% aqueous methanol plus 0.1% acetic acid (HPLC grade) with flow rate of 1 mL/min, and the presence of MAA compounds were detected at 320 nm. The absorbance spectrum was scanned at the wavelength range of 250 to 450 nm.

Results and Discussion

Genus identification

According to the morphological assessment of this species, it was classified as *Chlorella* sp. However, for more specific classification, molecular genetic assessment was applied. As shown in Fig. 1, the result of NCBI blasting for 18srRNA sequences amplified from the studied microalga to the reported data in genebank confirmed about 98.68% and 98.54% similarity between the sequence obtained to the *Desmodesmus* sp. and *Scenedesmus* sp., respectively (Fig. 1a). This similarity value was about 98.13% for *Chlorella* sp. (Fig. 1b). Accordingly, this studied species can be more likely considered as *Desmodesmus* sp.

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

a

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Desmodesmus sp. YACCYB330 18S ribosomal RNA gene, partial sequence	1227	1227	96%	0.0	98.68%	MH683854.1
Desmodesmus sp. YACCYB297 18S ribosomal RNA gene, partial sequence	1227	1227	96%	0.0	98.68%	MH683841.1
Desmodesmus sp. YACCYB214 18S ribosomal RNA gene, partial sequence	1227	1227	96%	0.0	98.68%	MH651226.1
Desmodesmus communis strain CCAP 258/97 small subunit ribosomal RNA gene, partial sequence	1221	1221	96%	0.0	98.54%	MK541738.1
Desmodesmus armatus strain CCAP 258/87 small subunit ribosomal RNA gene, partial sequence	1221	1221	96%	0.0	98.54%	MK541735.1
Desmodesmus armatus strain CCAP 258/67 small subunit ribosomal RNA gene, partial sequence	1221	1221	96%	0.0	98.54%	MK541733.1
Desmodesmus pannonicus strain CCAP 258/90 small subunit ribosomal RNA gene, partial sequence; internal transcribed s	1221	1221	96%	0.0	98.54%	MK541797.1
Desmodesmus armatus strain CCAP 258/197 small subunit ribosomal RNA gene, partial sequence; internal transcribed spa	1221	1221	96%	0.0	98.54%	MK541796.1
Scenedesmus sp. UKM 9 18S ribosomal RNA gene, partial sequence	1221	1221	96%	0.0	98.54%	KU170547.1

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

b

	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Chlorella sp. AK-2013 clone CH9 18S ribosomal RNA gene, partial sequence	1027	1027	82%	0.0	98.13%	KC790435.1
Chlorella sp. YACCYB54 18S ribosomal RNA gene, partial sequence	1011	1011	96%	0.0	93.01%	KT279474.1
Chlorella sp. YACCYB105 18S ribosomal RNA gene, partial sequence	992	992	96%	0.0	92.57%	MH619552.1
Chlorella sp. YACCYB104 18S ribosomal RNA gene, partial sequence	992	992	96%	0.0	92.57%	MH619551.1
Chlorella sp. YACCYB103 18S ribosomal RNA gene, partial sequence	992	992	96%	0.0	92.57%	MH619550.1
Chlorella sp. YACCYB102 18S ribosomal RNA gene, partial sequence	992	992	96%	0.0	92.57%	MH619549.1
Chlorella sp. YACCYB101 18S ribosomal RNA gene, partial sequence	992	992	96%	0.0	92.57%	MH619548.1
Chlorella sp. YACCYB100 18S ribosomal RNA gene, partial sequence	992	992	96%	0.0	92.57%	MH619547.1
Chlorella sp. YACCYB196 18S ribosomal RNA gene, partial sequence	992	992	96%	0.0	92.57%	MH636653.1

Figure 1. BLAST analysis of 18srRNA amplified sequence from studies microalgae; BLAST with all data of genebank (a), BLAST with the gene data of *Cholorella* sp. (taxid. 3071)

MAA production under normal condition

This species of green microalga did not show significant absorption in the range of 309-365 nm under normal conditions and in the absence of high concentration of NaCl. The HPLC chromatogram for the methanolic extract of *Desmodesmus* sp. under normal condition did not similar to that was reported in hypersaline medium, as shown in Fig.2 a and Fig.2 b. As it was seen in the Fig. 2, the maximum absorbance of main compound detected in the methanolic extract of *Desmodesmus* sp. biomass in normal condition was recorded in 257 nm. It may be one of the precursors of MAAs compounds. Accordingly, under normal condition without the exertion of any induction strategies, this microalga was not capable to produce MAAs.

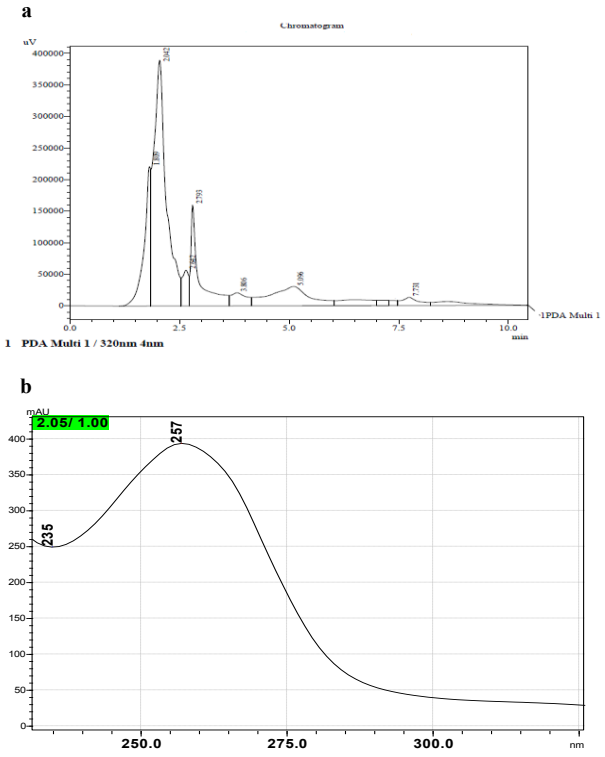

Figure 2. HPLC chromatogram for *Desmodesmus* sp. (a) and UV absorbance of the major peak of chromatogram (RT=2.05 min) for *Desmodesmus* sp (b). Under normal condition shows a maximum absorption at 275 nm which is not in the range of MAAs.

MAA production under saline-stress

The HPLC chromatogram of the methanolic extract of *Desmodesmus* sp. biomass under salinity stress had a main peak with the retention time of about 2.28 min (Fig. 3a) which showed a maximum absorbance at about 320 nm in the range of UV wavelength (Fig. 3b). Comparing to the chromatograms for *Desmodesmus* sp. under normal condition (without adding NaCl to the medium), these results indicate that this microalga can produce an UV absorbing compound in the presence of NaCl (hypersaline medium). Thus, increasing the salinity can be considered as an inducing factor for MAA production in *Desmodesmus* sp., similar to some previous reports indicated the effect of salinity condition on the MAAs production in other species of microalgae or cyanobacteria (Portwich and Garcia-Pichel, 2007; Kageyama et al., 2017).

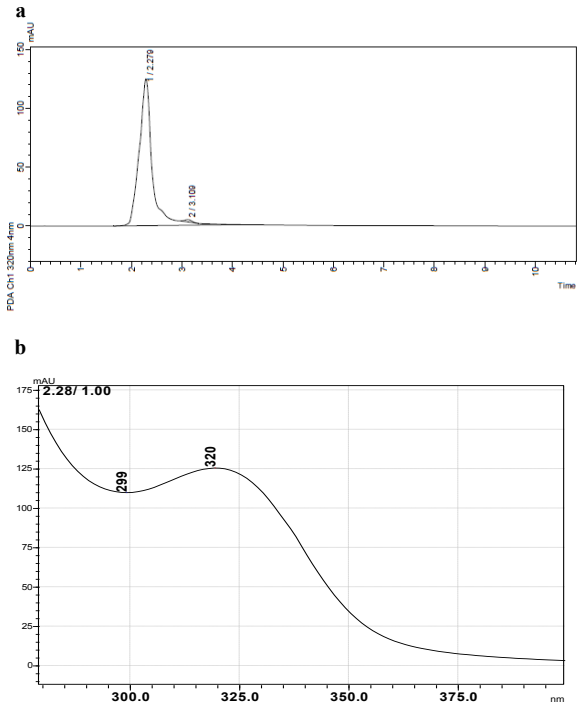

Figure 3. HPLC chromatogram for *Desmodesmus* sp. extract under salinity stress (a), and UV absorbance of the major peak of chromatogram (RT=2.28 min) for *Desmodesmus* sp. (b) under salinity stress, which shows an absorption at 320 nm in the UV absorbance range of MAAs.

Generally, MAAs production in algae can be influenced by many abiotic factors. UV irradiation as a major factor induced the production of MAA in cyanobacteria and algae. It has been confirmed that long-term exposure (up to 72 hours) to UV irradiation would lead to the induction of MAAs in many species of cyanobacteria and microalgae (Singh et al., 2008a; Rastogi and Incharoensakdi, 2013; Rastogi and Incharoensakdi, 2014; Roshan et al., 2015). Nutritional factors can be also considered as key factors for MAAs production. Increasing the concentration of nitrate and phosphate had a positive effect on the induction of MAAs in *Arthrospira* sp. (Saadatmand and Zamani, 2015). Barufi *et al.* had investigated that the elevation of nitrate concentration could lead to the accumulation of MAAs compounds in red alga, however, after a specific

concentration value was reached, the MAAs production would reduce (Barufi et al., 2011). The MAAs production may be also adjusted by the circadian rhythm. Rastogi *et al.* has studied the effect of light and dark periods on MAAs production in the species of *Fischerella muscicola* as a cyanobacterium. The results showed that some of MAAs increased in light periods and some of them in dark condition (Rastogi and Incharoensakdi, 2015). Desiccation was also investigated as a stressor for the induction of MAAs production. Joshi *et al.* examined the effect of desiccation and UV-B irradiation on MAAs production in marine cyanobacterium of *Leptolyngbya* sp. It was showed that these two factors could rise the concentration of MAAs and led to their accumulation in this cyanobacterium (Joshi et al., 2018). Salinity can be considered as another factor for the induction of MAAs production by cyanobacteria and algae, such as in *Anaebena variabilis*. UV radiation, heat, aluminum treatment and salinity were examined in this species. Results showed that heat had no effect on MAAs production, however the 72 hours exposure to UV irradiation, aluminum treatment and salinity induced the MAAs production, and also, these factors could show a synergistic effect on MAAs production (Singh et al., 2008b).

Conclusion

In this investigation, we have proved that salinity has a positive effect on MAAs production in *Desmodesmus* sp. while in normal condition, MAAs were not produced in this microalga. This study confirmed the previous results showed that several species of cyanobacteria and algae can thrive on high salinity environments by producing MAAs as an osmoprotectant. Therefore, MAAs appear to play a role not only in protecting against light stress, but also in salinity condition, maybe as an mechanism balanced the cellular osmotic pool. Additionally, this genus of *Desmodesmus* sp. could produce MAAs-like compound under high salinity without other inducers, such as the exposure to ultraviolet irradiation. Further studies are needed to optimize the MAAs production in this microalga.

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Competing Interests

Authors declare that there is no conflict of interest.

References

Antoniou, C., Kosmadaki, M.G., Stratigos, A.J. and A.D. Katsambas, (2008)."Sunscreens–what's important to know."*Journal of the European Academy of Dermatology and Venereology*, **22**(9): 1110-1119.

Barufi, J.B., Korbee, N., Oliveira, M.C. and F.L. Figueroa, (2011)." Effects of N supply on the accumulation of photosynthetic pigments and photoprotectors in *Gracilaria tenuistipitata* (Rhodophyta) cultured under UV radiation. " *Journal of Applied Phycology*, **23**(3): 457-466.

Saadatmand, S. and M. Zamani, (2015)." Investigating the effect of different concentrations of nitrate and phosphate on the quantity of mycosporine like amino acids production and growth in *Spirulina platensis*."*International Journal of Biosciences (IJB)*, **6**(5): 63-69.

Cheewinthamrongrod, V., Kageyama, H., Palaga, T., Takabe, T. and R. Waditee-Sirisattha, (2016)." DNA damage protecting and free radical scavenging properties of mycosporine-2-glycine from the Dead Sea cyanobacterium in A375 human melanoma cell lines."*Journal of Photochemistry and Photobiology B: Biology*, **164**: 289-295.

Chrapusta, E., Kaminski, A., Duchnik, K., Bober, B., Adamski, M. and J. Bialczyk, (2017)." Mycosporine-like amino acids: potential health and beauty ingredients."*Marine Drugs*, **15**(10): 326.

D'Orazio, J., Jarrett, S., Amaro-Ortiz, A. and T. Scott, (2013)."UV radiation and the skin."*International Journal of Molecular Sciences*, **14**(6): 12222-12248.

Doria, E., Longoni, P., Scibilia, L., Iazzi, N., Cella, R. and E. Nielsen, (2012)." Isolation and characterization of a *Scenedesmus acutus* strain to be used for bioremediation of urban wastewater."*Journal of Applied Phycology*, **24**(3): 375-383.

Figueroa, F.L., Israel, A., Neori, A., Martínez, B., Malta, E.J., Put, A., Inken, S., Marquardt, R., Abdala, R. and N. Korbee, (2010)."Effect of nutrient supply on photosynthesis and pigmentation to short-term stress (UV radiation) in *Gracilaria conferta* (Rhodophyta)." *Marine Pollution Bulletin*, **60**(10): 1768-1778.

Joshi, D., Mohandass, C. and M. Dhale, (2018)." Effect of UV-B radiation and desiccation stress on photoprotective compounds accumulation in marine *Leptolyngbya* sp." *Applied biochemistry and biotechnology*, *184*(1), pp.35-47.

Kageyama, H., Waditee-Sirisattha, R., Tanaka, Y. and T. Takabe, (2017)." Osmoprotectant and sunscreen molecules from halophilic algae and cyanobacteria." In: *Algal green chemistry,* Rastogi, R.P., Madamwar, D. and A. Pandey (Eds). Elsevier, Oxford, United Kingdom. pp. 1-16

Khanipour Roshan, S., Farhangi, M., Emtyazjoo, M. and M. Rabbani, (2015)." Effects of solar radiation on pigmentation and induction of a mycosporine-like amino acid in two cyanobacteria, *Anabaena* sp. and *Nostoc* sp. ISC26."*European Journal of Phycology*, **50**(2): 173-181.

Mandal, S. and N. Mallick, (2011)."Waste utilization and biodiesel production by the green microalga *Scenedesmus obliquus*." *Applied and Environmental Microbiology*, **77**(1): 374-377.

Mata, T.M., Melo, A.C., Simões, M. and N.S. Caetano, (2012). " Parametric study of a brewery effluent treatment by microalgae *Scenedesmus obliquus*."*Bioresource Technology*, **107:** 151-158.

Lawrence, K.P., Long, P.F. and A.R. Young, (2018). " Mycosporine-like amino acids for skin photoprotection." *Current Medicinal Chemistry*, **25**(40): 5512-5527.

Portwich, A. and F. Garcia Pichel, (2000)." A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912."*Photochemistry and Photobiology*, **71**(4): 493-498.

Rastogi, R.P. and A. Incharoensakdi, (2013)." UV radiation-induced accumulation of photoprotective compounds in the green alga *Tetraspora* sp. CU2551."*Plant Physiology and Biochemistry*, **70**: 7-13.

Rastogi, R.P. and A. Incharoensakdi, (2014)." Characterization of UV-screening compounds, mycosporine-like amino acids, and scytonemin in the cyanobacterium *Lyngbya* sp. CU2555." *FEMS Microbiology Ecology*, **87**(1): 244-256.

Rastogi, R.P. and A. Incharoensakdi, (2015)." Occurrence and induction of a ultraviolet absorbing substance in the cyanobacterium *Fischerella muscicola* TISTR8215."*Phycological Research*, **63**(1): 51-55.

Rastogi, R.P., Sonani, R.R. and D. Madamwar, (2017).″ UV Photoprotectants From Algae—Synthesis and Bio-Functionalities.″ In *Algal Green Chemistry*, Rastogi, R.P., Madamwar, D. and A. Pandey (Eds). Elsevier, Oxford, United Kingdom. pp. 17-38

Ryu, J., Park, S.J., Kim, I.H., Choi, Y.H. and T.J. Nam, (2014).″ Protective effect of porphyra-334 on UVA-induced photoaging in human skin fibroblasts.″ *International Journal of Molecular Medicine*, **34**(3): 796-803.

Singh, S.P., Klisch, M., Sinha, R.P. and D.P. Häder, (2008a).″Effects of abiotic stressors on synthesis of the mycosporine like amino acid shinorine in the cyanobacterium *Anabaena variabilis* PCC 7937.″ *Photochemistry and Photobiology*, **84**(6): 1500-1505.

Singh, S.P., Sinha, R.P., Klisch, M. and D.P. Häder, (2008b).″ Mycosporine-like amino acids (MAAs) profile of a rice-field cyanobacterium *Anabaena doliolum* as influenced by PAR and UVR.″ *Planta*, **229**(1): 225-233.

Suh, S.S., Hwang, J., Park, M., Seo, H.H., Kim, H.S., Lee, J.H., Moh, S.H. and T.K. Lee, (2014).″ Anti-inflammation activities of mycosporine-like amino acids (MAAs) in response to UV radiation suggest potential anti-skin aging activity.″ *Marine Drugs*, **12**(10): 5174-5187.

Vale, P. (2015a).″ Effects of light quality and nutrient availability on accumulation of mycosporine-like amino acids in *Gymnodinium catenatum* (Dinophycea).″ *Journal of Photochemistry and Photobiology B: Biology*, **143:** 20-29.

Vale, P. (2015b).″ Effects of light and salinity stresses in production of mycosporine like amino acids by *Gymnodinium catenatum* (Dinophyceae).″ *Photochemistry and Photobiology*, **91**(5): 1112-1122.

Van Der Pols, J.C., Williams, G.M., Pandeya, N., Logan, V. and A.C. Green, (2006).″ Prolonged prevention of squamous cell carcinoma of the skin by regular sunscreen use.″ *Cancer Epidemiology and Prevention Biomarkers*, **15**(12): 2546-2548.

Wada, N., Sakamoto, T. and S. Matsugo, (2015).″ Mycosporine-like amino acids and their derivatives as natural antioxidants.″ *Antioxidants*, **4**(3): 603-646.

Wan, M., Rosenberg, J.N., Faruq, J., Betenbaugh, M.J. and J. Xia, (2011).″ An improved colony PCR procedure for genetic screening of Chlorella and related microalgae.″ *Biotechnology Letters*, **33**(8): 1615-1619.

