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Research Article

Isolation, cultivation and biochemical characterization of the symbiotic dinoflagellate, *Symbiodinium* sp., from the sea anemone, *Stichodactyla haddoni* (Saville-Kent, 1893), from the Strait of Hormuz

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Abstract

In the present work, extraction of an endosymbiont from a host of *Stichodactyla haddoni* collected from the coast of Hormuz Island (the Strait of Hormuz) and afterwards identification, determination of the optimal cultivation conditions and then finding out the parameters and how they affect its bioactive compounds were conducted. The sequence of ITS2 region from the endosymbiont of *S. haddoni* clustered with 17 sequences representing clade C. The sequence was deposited to the GenBank under accession number MT448855. The study results showed that ASP_{12} medium and a temperature of 23°C were optimal to cultivate *Symbiodinium* sp. outside its host. Under these conditions, the highest cell density was 12×10^5 cell ml^{-1} and the maximal amount of chlorophyll-a and peridinin were 22.81 and 47.86 mgL^{-1}, respectively. Further cultivation of the obtained strain in a Twin-Layer photobioreactor at light intensities of 50, 100 and 250 µmol photons m^{-2} s^{-1} for 16 days demonstrated that the biomass content was observed to be 35.72, 54.77 and 57.12 g m^{-2} at 50, 100 and 250 µmol photons m^{-2} s^{-1}; the peridinin content was 0.26, 0.63 and 0.79 g m^{-2}, respectively; total lipid content was 19.5 and 27.77% of dry weight at 50 and 250 µmol photons m^{-2} s^{-1}.

Keywords: Endosymbiont, Peridinin, Protein, Chlorophyll-a, Total lipid, Growth condition

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Introduction

The genus *Symbiodinium* is the most important and abundant symbiont; especially in corals, sea anemones and some other invertebrates (LaJeunesse *et al.*, 2018). *Symbiodinium* thrive within the gastrodermal cells of corals and fixe 95% of photosynthetic carbon into the coral hosts (Stambler, 2011). Zooxanthellae also produce unique and complex bioactive secondary metabolites (Onodera *et al.*, 2014). Moreover, they attract many wavelengths of light to their hosts (Jeffrey and Wright, 2006) and can protect host cells from oxidative stress during exposure to temperatures of over 30°C and active radiation thanks to a unique photosynthetic antenna system incorporating peridinin–chlorophyll *a*–protein (PCPs), called light-harvesting complex (LHC) (Abrego *et al*., 2008; Kanazawa *et al*., 2014).

One of the most valuable compounds, which only occur in dinoflagellates, especially in symbiotic ones in large quantity, located in PCPs, is peridinin and its derivatives (Wakahama *et al*., 2012; Jiang *et al.*, 2014). In general, the value of peridinin is 10 times more than β-carotene because peridinin has strong antioxidant properties and it is applied in medicine to prevent tumor formation or induction of apoptosis in tumor cells; inhibit effect on primary antigen activation of Human Herpes viruses and improve allergic reactions (Sugawara *et al*., 2009; Takaichi, 2011). First researchers, who determined the ratio protein of PCP complex contained in symbiotic alga, measured that in the range of 4:1:1 to 8:2:1 depending on the species of *Symbiodinium* (Haxo *et al*., 1976; Prézelin and Haxo, 1976).

It should also be noted that symbiotic algae are a fairly good source of lipids. Among the investigated symbiotic algae, *Symbiodinium microadriaticum* had the highest percentage of total fatty acid content (Yeesang and Cheirsilp, 2011). The predominant lipids produced by *Symbiodinium* are palmitic (C16) and stearic (C18) saturated fatty acids and their unsaturated analogs, docosahexaenoic (C22:6, n-3) polyunsaturated fatty acid (PUFA) and a variety of sterols (Díaz-Almeyda *et al*., 2011; Kneeland *et al*., 2013).

Temperature and light are the most important environmental variables that affect biochemical compositions of *Symbiodinium* and anemone tissues in nature (Klueter *et al*., 2015). Their effect causes changes in growth dynamics, ultrastructure, biophysics and physiology of algae to adapt them to the changing environmental conditions (Schlüter *et al*., 2000; Grégoire *et al*., 2017). Various studies showed that algal bioactive compounds such as pigments, lipids and fatty acids respond differently on light intensity changes (Treignier *et al*., 2008; Valenzuela-Espinoza *et al*., 2011). For example, increased light intensity can inhibit algae growth (Lim *et al*., 2019) or can increase the quality of bioactive compounds such as polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in dinoflagellates (Fuentes-Grünewald *et al*., 2009; Fuentes-Grünewald *et al*., 2013). Lipid metabolism and lipid profile are also

altered by different light intensities (Treignier *et al*., 2008; Yeesang and Cheirsilp, 2011).

Evidently, value of the symbiotic algae is beyond doubt. There was only a problem: a poor growth of endosymbionts at a technical scale because the symbiotic species are not able to produce secondary metabolites independently of their host. Fortunately, this is solved by German scientists who developed a phototrophic biofilm based on Twin Layer (TL) immobilization technology (Shi *et al.*, 2007; Benstein *et al.*, 2014). Taking into account it, the aim of the present work was to isolate symbiotic algae from *Stichodactyla haddoni* Saville-Kent, which is one of the common soft corals in intertidal zones of the Strait of Hormuz along coast of Hormuz Island; identify the endosymbiont and determine optimal cultivation conditions and impact on the content of peridinin and other compounds interesting to biotechnology.

Materials and methods

Isolation and determination of optimum growth conditions and cultivation

For isolation of *Symbiodinium* cells, several pieces of the oral disc from the sea anemone *Stichodactyla haddoni*, which was collected from the coast of Hormuz Island (the Strait of Hormuz) at low tide, were cut off, filtered, mixed with sea water and were then vortexed. The remaining sediment was diluted and centrifuged at a temperature of 4°C. This procedure was repeated several times in order to obtain a sufficient amount of algae (Jeffrey and Haxo, 1968). Then, 10 antibiotic stock solutions were added to the samples to combat bacterial contamination according to the recipe published by Polne-Fuller (1991); and algal samples were kept in a refrigerator.

After washing algae from antibiotics, different culture media like ASP_8, ASP_{12}, and F2 were used in order to adapt symbiotic algae to them (Polne-Fuller, 1991; Rogers and Marcovich, 2007; Benstein *et al.*, 2014). Further *Symbiodinium* cultivation, which was carried out in several stages, took place in ASP_{12} culture medium to study its biochemical composition. At first, *Symbiodinium* sp. was cultured at the flask, containing liquid medium, at temperatures of 17, 23, 29°C (as low, average and high temperatures in which this species is found in nature) and the light intensity of 100 µmol m^{-2} s^{-1}. Photoperiod was adjusted to a dark/light cycle of 8/16 h. Then, the suspension culture was performed in a 50 ml Erlenmeyer flask with initial cell number of 20×10^4 cells ml^{-1} at a light intensity of 100 µmol m^{-2} s^{-1} and at a temperature of 23±1°C. Finally, after reaching 2 liters of suspension, the biomass was centrifuged at 4°C and 500 rounds per minutes (RPM). Then, microalga biomass was transferred onto a Twin-Layer photobioreactor culture disc and cultured at 23±1°C and at light intensities of 50, 100 and 250 µmol m^{-2} s^{-1} (as low, average and high values of light intensities) for 16 days with the addition of a fresh medium every 4 days (Gibson and Thomas, 1995; Benstein *et al.*, 2014). The vertical Twin-Layer photobioreactor used in the present study was based on the design of (Shi *et al*., 2007) and described by (Schultze *et al.*, 2015).

Identification of microalga

Initially the cells were examined with a light microscope, (LM) Zeiss Axiostar (Germany), equipped with both differential interference contrast (DIC) and fluorescence emission. Micrographs were taken using a Zeiss, Axiocam HRc, digital camera. Morphological identification and microscopic observations were based on Trench and Blank (1987) identification key.

DNA was extracted from enriched *Symbiodinium* sediment according to the modified method of Echt *et al*. (1992) (Kiselev *et al*., 2013; 2015). A pair of primers and the amplification protocol used to amplify ITS2 rRNA are given in the Table 1 (Hume *et al*., 2013; 2015). The PCR products were sequenced using an ABI 3130 genetic analyzer (Applied Biosystems, USA) with a BigDye terminator v. 3.1 sequencing kit (Applied Biosystems, USA) and the same primers. The sequence was deposited to the GenBank under accession number MT448855. Thirty-five ITS rDNA sequences representing major clades of *Symbiodinium* (Coffroth and Santos, 2005) were retrieved from the GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The ITS2 sequences were aligned manually in the SeaView program (Galtier *et al*., 1996**)**. Phylogenetic tree was inferred with ML-optimality criteria using PAUP 4.0b10 (Swofford, 2002). Robustness of the tree was estimated by bootstrap percentages **(**Felsenstein, 1985). Bootstrap percentages <50% were not taken into account.

Table 1: Primers and PCR conditions used to amplify what DNA marker.

Primer pair	Sequence 5'-3'	Aplicon size bp	Protocol
SYM_VAR_5.8S2	GAATTGCAGAACTCCGTGAACC	~234 – 266	98°C for 2 min (98°C for 10 s, 56°C for 30 s, 72°C for 30 s)×35 times, 72°C for 5 min
SYM_VAR_REV	CGGGTTCWCTTGTYTGACTTCATGC		

Determination of growth rate and cell biomass

The initial cell number was considered about 20×10^4 cell mL^{-1}. Algal cells were counted with a hemocytometer and LM with lens of 20. The following equation was used for cell counting (Guillard and Ryther, 1962):

Cell density /per milliliter = total counted cells × 10^4 × dilution factor

To investigate the growth of microalga in terms of dry weight in a Twin-Layer photobioreactor, specimens were sampled once every four days in triplicates and dried in freeze drier (Model FD-5010-BT) for 2 hours. The specific growth rate was calculated using Guillard (1973) equation:

$\mu = (\ln X_t - \ln X_0)/t$, day^{-1}

Where X_t, is cell density after t days; X_0, initial cell density.

The dry weight of biomass (B) was calculated using the following equation:

$B = Wt - Wm/a$, g m^{-2}

Where Wt and Wm are total (biomass + membrane) and membrane weights; a, inoculation area in m^2.

Determination of pigments, total protein and lipids

Determination of concentration of total carotenoid content

For carotenoid determination, initially, 5 ml of 90% acetone was added to the frozen algal specimens and cells were vortexed for 2 min. Then, the pellets were placed in an ultrasonic bath at 4°C for 15 minutes. Next, the material was kept in an ice bath under dark conditions for 2 hours. The specimens were then vortexed for 4 seconds over four 30-min periods and stored at -20°C overnight. Finally, the samples were centrifuged and the acetone extract was measured with a spectrophotometer (model Vis 5100) at 470 wave length and calculated using the equation presented by Lira *et al*. (2017) (Jeffrey *et al*., 1975), all sample analyses were performed in triplicate:

$$\text{Carotenoids (mg)} = \frac{A \times vol \times 1000}{A_{1\ cm}^{1\%}}$$

Where "A" is absorbance at 470 nm, "vol" is the volume (mL) used in extraction of carotenoids and "A1%" is absorption coefficient for 1% of the mixture of unknown carotenes at 2500.

Determination of chlorophyll-a and peridinin

To measure the concentration of peridinin and chlorophyll-a, algal cells were extracted with solvent of pure methanol: 2% ammonium acetate. Initially, 4.5 ml of pure methanol was added to the removed biomass and the material was vortexed for 120 seconds. Next, the supernatant was placed in an ice bath under dark condition for 2 hours. 0.5 ml 0.5 M ammonium acetate with 7.2 acidity was added and the mixture was vortexed again. The material was stored at -20°C overnight. Samples were vortexed for 10 seconds over three 30-min periods. In the next stage, the insoluble impurities were removed by double centrifugation at 3500 RPM at 4°C for 10 min.

The concentrations of peridinin and chlorophyll-a were measured with a spectrophotometer (model Vis 5100) at 469 and 665 nm wavelengths (Jeffrey *et al*., 1975; Porra *et al*., 1989). Peridinin content was calculated using the equations $A = E_{\lambda}.c.d$ and the volumetric absorption coefficient was $E_{1cm}^{1\%} = 1360$ determined by Jeffery and Haxo (1968). All sample analyses were performed in triplicate.

Determination of total protein and lipid content

The symbiotic alga was homogenized and total protein was measured by Lowry's method (López *et al*., 2010). The total lipid content was estimated according to the protocol of Bligh and Dyer (1959). For lipid extraction, a methanol-chloroform-water mixture (MCWM) was used. The lipid fractions were separated in a clean pre-weighed vial (first wt) and the solvent was evaporated using a rotary evaporator. Finally, the containers having sediment were weighed and calculated in proportion to their initial weight (Nigam *et al*., 2011). All sample analyses were performed in triplicate.

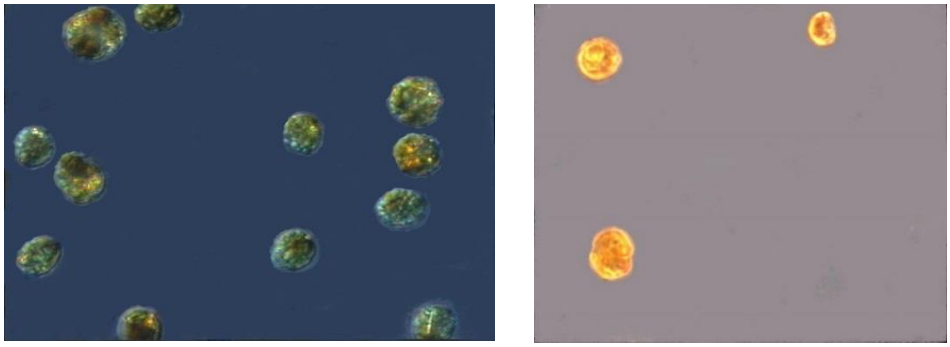
Statistical analysis

Data analyses were done using SPSS 16 and Excel 2010 software. The data normality was evaluated by Kolmogorov-Smirnov test. ANOVA test, Tukey's test

and Duncan's test were also used to compare significant differences between the data obtained at different light intensities and temperatures.

Results

Under the light microscope, the spherical and egg-shaped endosymbiont cells from 6 to 13 µm in diameter were recorded (Fig. 1a). For size, approximately 50 cells were measured under the light microscope in phase contrast, and DIC revealed plastids in the cells (Fig. 1b).



(a) (b)

Figure 1: Light micrographs of the investigated *Symbiodinium* sp. illustrating its gross morphology, (a): cells in DIC regime x40; (b) cells in the contrast phase showing alga mitosis.

The sequence of ITS2 region from endosymbiont of *S. haddoni* was clustered with 17 sequences representing clade C (Fig. 2). In our tree this lineage attained no support and formed a polytomy with clades F and B.

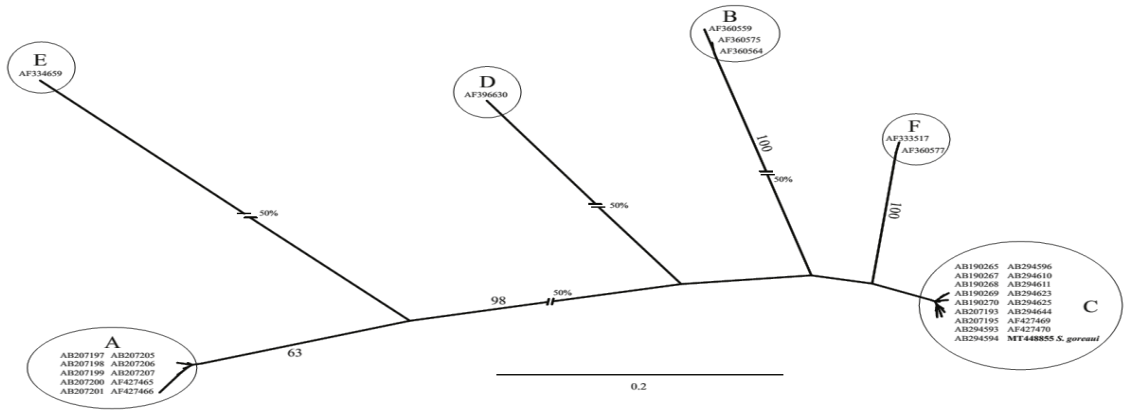


Figure 2: ML-phylogenetic tree inferred in PAUP from 35 ITS2 sequences of *Symbiodinium*. Bootstrap percentages >50% are shown above/below major branches of the tree. Some branches are graphically 50% reduced. Gene sequence of *Symbiodinium* sp. registered in GenBank database No SUB7406661. The newly obtained sequence of *Symbiodinium goreaui* is given in bold.

The results of the preliminary cultivation showed that the highest cell number of about 4×10^5 cells ml^{-1}were obtained in the culture medium of ASP_{12}, whereas these parameters in the culture media including F_2 and ASP_8 were much lower (Fig. 3a).

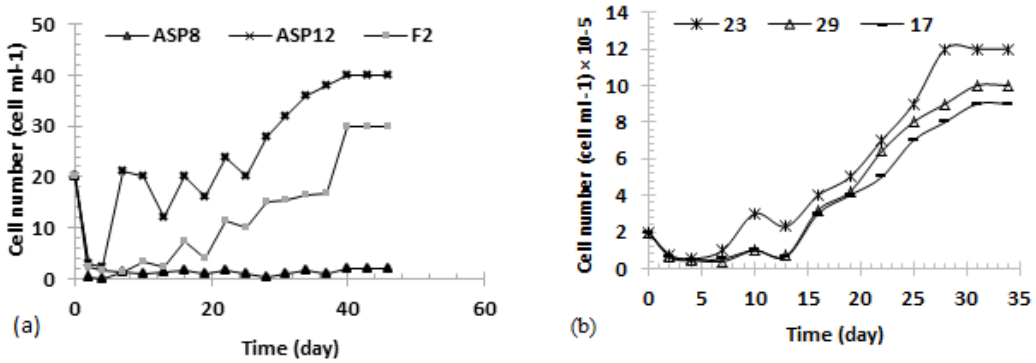


Figure 3: Growth curves of *Symbiodinium* sp. at the flask containing liquid medium, (a) in different culture media including ASP_8, ASP_{12}, and F2, (b) at different temperatures in suspension medium ASP_{12}.

First of all, this study investigated the influence of three temperatures on the growth of *Symbiodinium* sp. that was cultured at the flask, containing liquid medium, at temperature of 17, 23, and 29°C to determine the optimum growth temperature for further cultivation onto a Twin-Layer photobioreactor. The highest cell number of 1.2×10^5 cell ml^{-1} was observed at the temperature of 23±1°C, and on the 27th day, the alga reached the stationary growth phase. A minimal cell density was obtained 0.9×10^5 cell mL^{-1} at a temperature of 17±1°C and the stationary growth phase was on the 31st day (Fig. 3b). There was significant difference in cell number among all three temperatures (p<0.05).

Secondly, the effect of light intensity on the productivity of *Symbiodinium* sp. biomass in a Twin-Layer system was measured at three light intensities of 50, 100 and 250 µmol m^{-2} s^{-1} on days 4, 8, 12 and 16 at a temperature of 23±1°C (Figure 4a). The results showed that the dry biomass content increased linearly over time after a short lag phase (until day 4) and there was a significant difference in biomass content among all three light intensities (p<0.05). By the 16th day, the biomass was observed 35.7, 54.8 and 72.0 g m^{-2} at 50, 100 and 250 µmol photons m^{-2} s^{-1}. The specific growth rate values (µ) of *Symbiodinium* sp. are presented in Figure 4b.

The acquired concentration of chlorophyll-a at temperatures of 17, 23 and 29°C is presented in Figure 5a while the amount of peridinin is illustrated in Figure 5b for suspension cultures. It was found that the temperature of 23°C provided the highest amount of these pigments as 22.8 and 47.9 mgL^{-1}, respectively. At temperatures of 17 and 29°C, chlorophyll-a concentrations were approximately the same 17.61 and 17.44 mg l^{-1} whereas the peridinin amount of cells grown at higher temperature (from 17 to 23°C) increased to 1.27 times higher. The protein content amounted to 27.6, 26.3 and 43.4 % at the temperatures of 17, 23 and 29°C, respectively (Fig. 5c). Statistical results showed a significant difference among the amount of peridinin and protein at different temperatures (p<0.05).

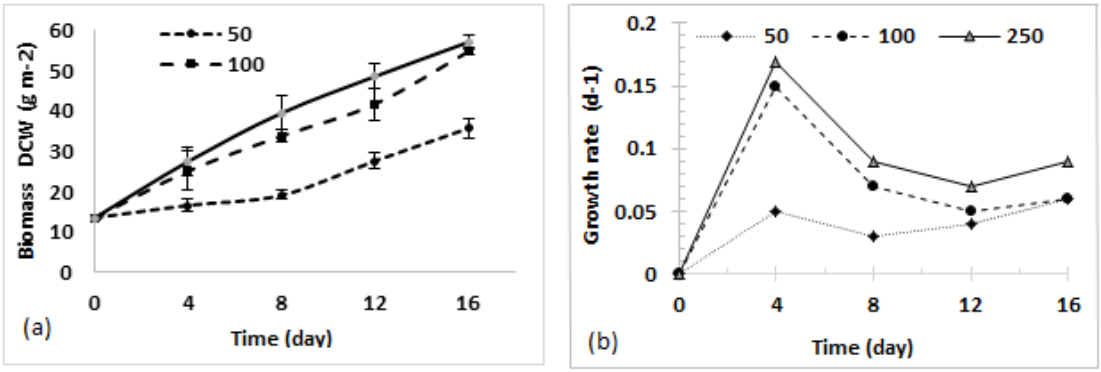


Figure 4: Effect of different light intensity (50, 100 and 250 µmol m^{-2} s^{-1}) on growth of *Symbiodinium* sp. in a Twin-Layer, (a) on dry biomass, (b) on growth rate (n = 3, three replicate filters). Error bars represent standard deviation.

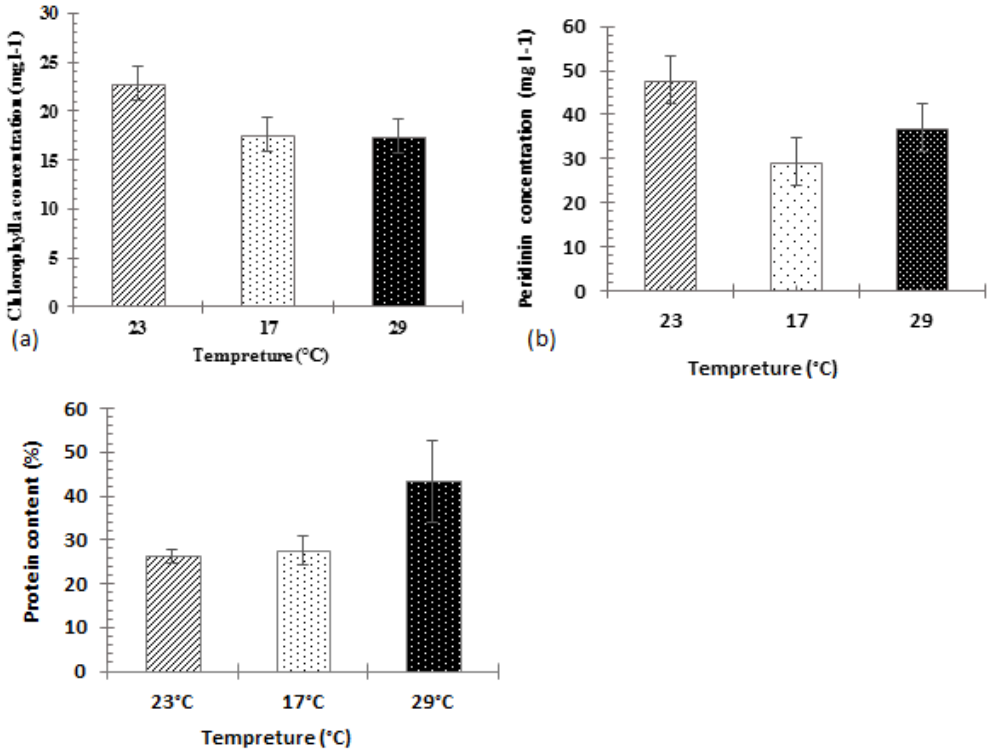


Figure 5: Chlorophyll-a (a), peridinin (b) concentration, and protein content (c), obtained from *Symbiodinium* cells cultured in suspension at temperatures of 17, 23 and 29 C by the 35th day (n=3, three replicates). Error bars represent standard deviation.

Investigation on *Symbiodinium* sp. indicated that the bioactive compounds produced by the symbiotic alga in the Twin-Layer system under different light intensities also had different concentrations (Fig. 6). The highest and the lowest chlorophyll-a production per square meter were 0.96 and 0.69 g m^{-2} at light intensities of 250 and 50, respectively (Fig. 6a). The total carotenoid concentrations were obtained as 0.36, 0.61 and 0.85 g m^{-2} by the 16th day at light intensities of 50, 100 and 250 µmol m^{-2} s^{-1}, respectively (Fig. 6c). Results related to the concentration of peridinin pigment at 50, 100 and 250 µmol m^{-2} s^{-1} at day 16 were 0.26, 0.47 and 0.62 g

m^{-2}, respectively (Fig. 6b). Statistical results (one-way analysis of variance) showed that there were significant differences among the concentration of total carotenoid, peridinin and chlorophyll-a at the investigated light intensities by the 16th day (p<0.05). It was also revealed that the lowest and highest percent of total lipid were 19.5 and 27.8% of dry weight at 50 and 250 µmol m^{-2} s^{-1} light intensities, respectively; and with increasing light intensity the amount of total lipid production increased (Fig. 6d). The percentage of total lipid in the dinoflagellate *Symbiodinium* sp., had significant differences (p<0.05) among the above-mentioned light intensities by the 16th day.

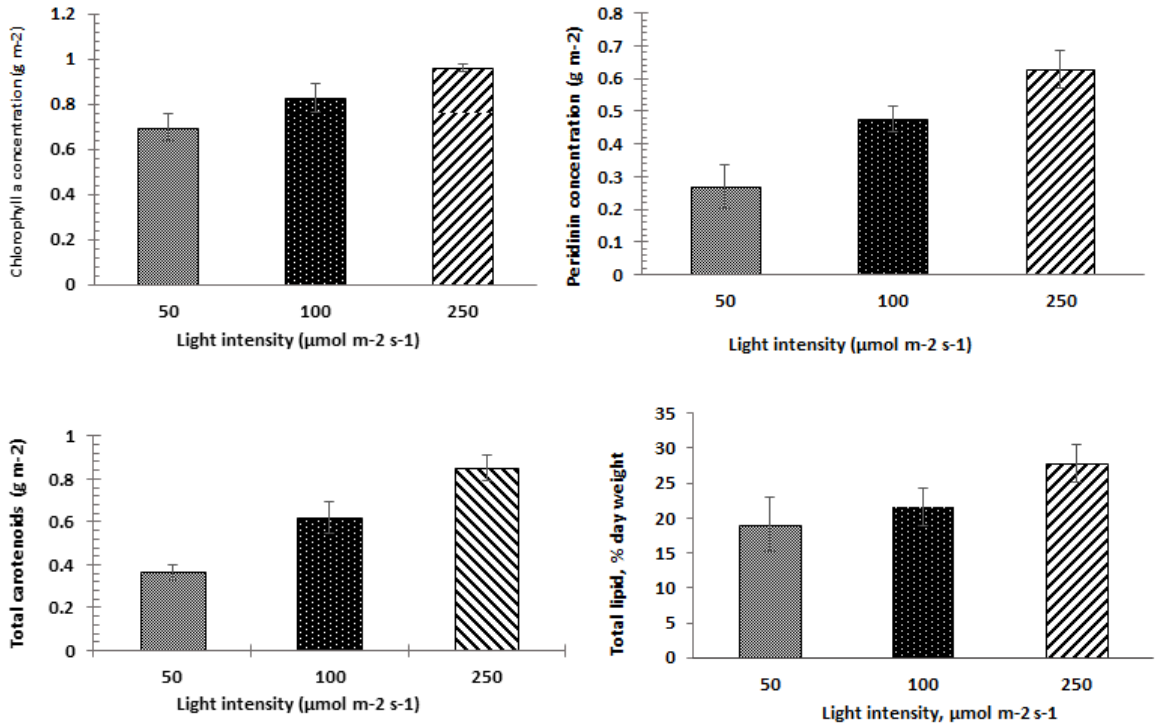


Figure 6: Chlorophyll-a (a), peridinin (b) concentration, total carotenoid content (c) and total lipid (d) in *Symbiodinium* sp. in a Twin-Layer photobioreactor at 50, 100 and 250 µmol photons m^{-2} s^{-1} by the 16th day (n = 3, three replicate filters). Error bars represent standard deviation.

Discussion

The present study is the first report of detailed biotechnological features of the *Symbiodinium* species coexisting with the sea anemone *S. haddoni* in Persian Gulf, specifically in the Strait of Hormuz. Conducted molecular studies showed that the isolated species belonged to clade C. On Iranian coast of Persian Gulf, the molecular investigations of algal symbionts in hard and soft corals were carried out in northern and southern parts of Persian Gulf (Baker *et al.*, 2004; Moghaddam *et al.*, 2018). They also revealed that all studied coral hosts contained C or D clades of *Symbiodinium* spp. that are resistant to harsh environmental conditions of Persian Gulf.

The optimal medium selection was the first important step to obtain the biomass and high pigment concentrations. The

experiments showed that isolated alga had better growth in ASP_{12} than in F_2 and ASP_8 media. To obtain higher growth rate of the studied symbiotic dinoflagellate, and higher concentration of peridinin and chlorophyll-a, temperature of 23°C was recommended to use as the culture item. Temperature is thus the second important factor which controls cell growth, photosynthesis process, amount of chlorophyll-a and peridinin and biochemical composition as a whole in species of *Symbiodinium* (Benstein *et al*., 2014). A temperature of 25±3°C can also highly provide the amount of peridinin as 25 mg m^{-2} day^{-1} (Benstein *et al.*, 2014). However, the investigated species such as *S. microadriaticum* CCAC 2475 B and *S. voratum* CCAC 3869 B enhanced growth at low temperature of 17°C (Tsirigoti *et al.*, 2020). Apparently, it depends from which region of the world the species was isolated, that is, under what conditions it grew in nature. Even it is concluded that although *Symbiodinium* from different hosts were morphologically the same, they did differ biochemically (Trench and Blank, 1987). Increasing temperature to 29°C and higher inhibits the measured values. Nevertheless, the investigated clade C may provide thermotolerance to the sea anemone. In nature, temperatures as high as 30°C can even cause the phenomenon of coral bleaching in long-term (Dutra *et al*., 2018). Extensive pigment damage without significant reduction of zooxanthellae cells number was observed in a coral species of *Agaricia* sp. bearing clade C algae under thermal stress. However, *Montastraea cavernosa* with clade C bleached by loss of 50-80% of their algal cells, with no significant impact to chlorophyll-a or peridinin (Venn *et al*., 2006). Peridinin amount reported here (29.19 - 47.86 mg l^{-1} depending on the temperature) was not in the same range as that reported for other cultured *Symbiodinium* (Benstein *et al.*, 2014). Chlorophyll-a concentration amounts were 17.61, 22.81, and 17.44 mg l^{-1} so the ratio of peridinin to chlorophyll-a (PC) concentration was 1.66, 2.10 and 2.13. That is higher than that in work of Zapata *et al.* (2004), who indicated that the PC concentration can change from 0.5 to 1.5. Pigments in photosynthetic dinoflagellates bind in protein complexes that are essential for light-harvesting. The percentages of amount of crude protein relative to dry weight (PPD) of *Symbiodinium* sp. tested in the present study fell within the range of 27.6-43.4 % at temperatures of 17, 23 and 29°C, respectively. In other words, when temperature rises, the protein content also increases, presumably due to shifts in the "stress proteins", called heat-shock protein and HSP that are specific proteins that are made when cells are exposed to temperatures above their normal growth temperature (which was revealed for the investigated species as 23°C). They are necessary to repair or stabilize impacted physiological processes driving some of these energetic costs, and enabling the organism to acclimatize to the changing conditions (Edmund and Gates, 2008).

Growth efficiency of symbiotic dinoflagellates in vivo is, firstly, influenced by light effect that was the third growth controlling parameter (Fuentes-Grünewald *et al.*, 2013; Langenbach and Melkonian, 2019). According to the present study results, biomass content increased linearly

under all three light intensities. Biomass productivity of investigated *Symbiodinium* sp. in general, was found to be in the same range as that of other microalgae immobilized on Twin-Layers under comparable conditions (Schnurr *et al.*, 2013; Benstein *et al*., 2014; Podola *et al.*, 2017). Moreover, the obtained carotenoid pattern was that increasing light intensity led to an increase in total carotenoids including peridinin production. Under low light illumination (50 µmol m^{-2} s^{-1}), total carotenoid and peridinin contents were 0.36 and 0.26 g m^{-2}, respectively, whereas under higher photon fluency (250 µmol m^{-2} s^{-1}), immobilized cells accumulated up to 0.85 of total carotenoids and 0.62 g m^{-2} of peridinin. The peridinin content reported here was higher than that reported by Rogers and Marcovich (2007) who cultivated *Symbiodinium* spp. in L_1 medium at 100 µmol photons m^{-2} s^{-1}. Furthermore, it was in the same range as that reported for *Symbiodinium voratum* cultured using the printing paper TL-PBR at a light intensity of 73 µmol m^{-2} s^{-1} and supplementary CO_2 (Benstein *et al.*, 2014). Schlüter *et al*. (2000) also measured peridinin concentration in two dinoflagellate species (*Scrippsiella acuminata* and *Prorocentrum micans*) under different light intensities (50, 150 and 429 µmol m^{-2} s^{-1}) and reported its amount in ranges of 0.619 - 0.711 and 0.375- 0.442 mg. The highest peridinin productivity reported to date was obtained as 51.4 mg peridinin m^{-2} day^{-1} by Langenbach and Melkonian (2019), who conducted the cultivation in a two-phase approach.

Algae are highly adaptable to changing environmental conditions by altering lipid metabolism. In general, microalgae increase lipid content under unfavorable and stressful conditions (Nigam *et al*., 2011). In comparison, the biomass and total lipid values of some dinoflagellates cultured in a photobioreactor are quite similar or even higher than those of some green algae such as *Mychonastes homosphaera*, *Tetradesmus obliquus* and different species of *Botryococcus* that are often used to produce biodiesel (Yeesang and Cheirsilp, 2011; Fuentes-Grünewald *et al*., 2013). Moreover, Yeesang and Cheirsilp (2011) showed that lipid production increased with increasing light intensity. The results of the present study regarding the effect of light intensities on total lipid content of *Symbiodinium* sp. microalga also proved that with increasing light intensity, total lipid amount increased and the highest and lowest total lipid content at 250 and 50 µmol m^{-2} s^{-1}, were 27.83% and 19.31% of the dry weight, respectively. Comparing obtained total lipid content of the investigated *Symbiodinium* sp. to total lipid content of other dinoflagellates, (*Alexandrium catenella*, 27%, *Amphidinium* sp., 18.9%, *Scrippsiella* sp., 16%, *Symbiodinium microadriaticum*, 10 and 15%, *S. voratum* 12.8%, *Gymnodinium* sp., 22.6%, *Akashiwo sanguinea* and *Fragilidium* sp. 13% of the dry weight), the alga can be presented as a prospective species with a fairly high lipid content (Schlüter *et al*., 2000; Islam *et al.*, 2013; Tsirigoti *et al.*, 2020).

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