Chapter

The Solar Saltern of Sfax: Diversity of Hyperhalophilic Microalgae Species as a Promising Naturel Source of Biomolecules

Sana Gammoudi, Neila Annabi-Trabelsi, Mariem Bidhi, Nouha Diwani, Amira Rekik, Hajer Khmekhem, Habib Ayadi, Wassim Guermazi and Jannet Elloumi

Abstract

The saltern of Sfax is a thalasso haline paralic ecosystem were the salinity ranged from 45 to 450 PSU. The microalgae distribution of saltern showed a spatial ecological succession. The specific richness of microalgae decreased with the salinity, accounting 37, 17 and 5 species at three level of salinity from 40 to 80, 80 to 200 and 200 to 450 PSU, respectively. To better understand the behavior of the hyper-halo tolerant microalgae, three autotrophic species *Halamphora* sp. SB1 MK575516 (Diatom), *Phormidium versicolor NCC-466* (Cyanophyceae) and *Dunaliella salina* (Chlorophyceae) were isolated from each level of salinity and they are grown in batch in artificial seawater at laboratory scale. Growth and metabolites synthesized by these microalgae were assessed. Salinity reacts on the physiology of these three species which possess mechanisms of resistance to more or less effective stresses and generally by the synthesis of different biomolecules such as pigments, sugars, proteins and fatty acids.

Keywords: solar saltern, *Halamphora* sp., *P. versicolor*, *D. Salina*, culture, metabolites

1. Introduction

An ecosystem is qualified as extreme when it's physicochemical parameters are most often hostile to life Grégoire, Fardeau, Guasco, Bouanane, Michotey and Bonin [1]. Indeed, any biotope characterized by a very low or very high value of the main parameters that influence their life cycle can be characterized as an extreme environment [2]. These parameters are essentially temperature, salinity, pH, pressure, radiation, desiccation, and oxygenation. Organisms with the ability to live in extreme environments are called "extremophiles". And as a result, several groups have been described taking into account the extreme conditions they can tolerate [1]. These are essentially prokaryotic microorganisms, mostly belonging to the Archaea

group. Eukaryotes can also be recorded in extreme environments. They are essentially unicellular algal or fungal organisms [3].

Among these environments, hypersaline ecosystems are very widespread and they can be classified into natural and artificial biotopes. While the natural environments are essentially represented by salt lakes, lagoon and, Sabkhas, the artificial hypersaline environments are represented by saltworks. These latter are transitional ecosystems between the marine and the continental domain [4], consisting of shallow ponds used for the production of halite (NaCl) from seawater which is pumped to the first series of ponds. After an evaporation cause a sufficient increase in salinity, the water is transferred to the next series of ponds, and so on, until brine saturated with NaCl is obtained, from which the halite precipitates in the last series of ponds recognized the crystallization ponds. The salinity in each of the ponds is thus maintained more or less constant over time [5]. This process leads to the selection of the variety of microbial heterotrophs and autotrophs and ciliated protozoa [4]. Species were adapted to different salinity variations [6]. The Sfax solar saltern (Tunisia) is an artificial paralic ecosystem characterized by its floristic and faunal richness [7], as well as by its microalgae richness [8, 9]. This biotope has been the site of several studies since 1998: (i) microalgae [10], (ii) ciliates [11] and (iii) zooplankton [12–14] especially the branchiopod crustacean *Artemia salina* [15, 16]. The cultures of microalgae sampled from the Sfax solar saltern have been the subject of several studies [17–20].

Microalgae are very rarely grouped according to their energy metabolism or even according to their ability to synthesize the necessary metabolites, but rather according to their morphological properties [21]. There are therefore different taxonomic classes of microalgae, the main ones being Rhodophyceae, Chlorophyceae, Bacillariophyceae, Euglenophyceae, Dinophyceae and Cyanobacteria. Microalgae occupy a very important place in nature since they are at the base of a long food chain and contain impressive nutritional proerties [22]. Moreover, they have various fields of exploitation, due to the value-added molecules. The biochemical composition of microalgae proves that they contain high value natural fatty acids (omega-3), which can produce a high value dietary supplement [23]. Furthermore, microalgae contain a high amount of proteins reaching up to 70% of the dry matter for *Spirulina* and also producing mineral elements such as calcium and magnesium [24]. Also, extremophile microalgae have many substances recognized by their bioactive properties such as antiviral, antiproliferative and anticancer properties [25]. These biomolecule possess a powerful antioxidant effect as determined by several authors [26, 27]. Finally, microalgae are largely used in wastewater treatment [28].

In this chapter we will present the biodiversity of the halophilic microalgae of the Sfax solar saltern and the different techniques used for the isolation and valorization of culture or metabolites extracted from three microalgal species.

2. Biodiversity of halophilic microalgae in solar saltern of Sfax

The Sfax solar salternor the Thyna salt works (**Figure 1**) is an artificial system located in the Gulf of Gabes in an arid climate (34° 39′N and 10° 42′E). This system is composed of several interconnected shallow ponds (20 to 70 cm deep) with increasing salinities from the water intake (40 PSU) to the salt Tables (450 PSU) [9]. The saline is separated from the sea by a dam of red silt about 4 m high running along the southern coast of the city of Sfax for about 13 km (**Figure 1**), from the port area to the village of Gargour, occupying an area of 1500 ha [29]. It is one of the most

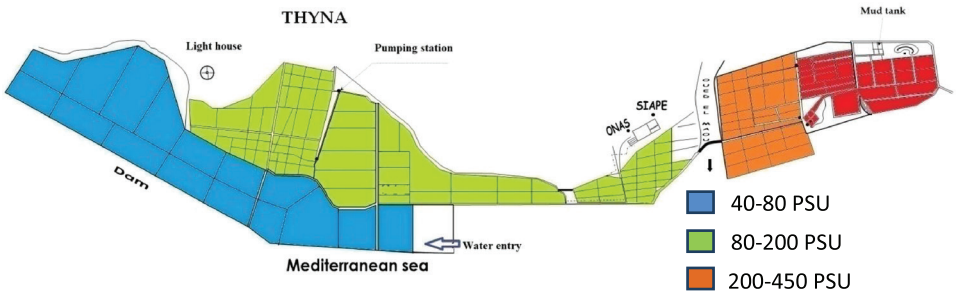


Figure 1.
General map of the Sfax Saltern showing the three levels of increasing salinity.

Salinity (PSU)	Microalgae	Species
40–80	Pennate diatoms	*Achnanthes brevipes*
		Achnanthes sp.
		Cocconeis sp.
		Cylindrotheca closterium
		Diatomella sp.
		Diploneis sp.
		Epithemia sp.
		Lichomphora sp.
		Nitzschia longissima
		Nitzschia ventricosa
		Navicula sp.
		Navicula elegans
		Navicula neoventricosa
		Pinnularia sp.
		Pleurosigma
		Stenopterobia sp.
		Surirella sp.
		Synedra longissima
	Centric diatoms	*Biddulphia* sp.
		Chaetoceros sp.
		Coscinodiscus sp.
		Halamphora sp. SB1 MK 575516*
		Thalassiosira mendiolana
	Dinoflagellates	*Akashiwo sanguinea*
		Gymnodinium sp.
		Mesoporos sp.
		Oxyrrhis marina
		Peridinium afrinacum
		Peridium sp.
		Prorocentrum hipes
		Prorocentrum gracile
		Protoperidinium micans
		Protoperidinium mite
		Protoperidinium pellucidum
		Scrippsiella gregaria
		Scrippsiella trochoïdea
	Euglenophyceae	*Euglena* sp.

Salinity (PSU)	Microalgae	Species
80–200	Pennate diatoms	*Cylindrotheca closterium* *Cymbella* sp. *Epithemia* sp. *Gyrosigma attenuatum* *Nitzschia longissima* *Pleurosigma*
	Dinoflagellates	*Gymnodinium* sp. *Oxyrrhis marina* *Peridium* sp. *Polykrikos* sp. *Protoperidinium micans* *Protoperidinium* sp.
	Cyanobacteria	*Aphanothece* sp. *Phormidium versicolor NCC 466** *Spirulina subsalsa*
	Chlorophyceae	*Chlamydomonas rubrifilum* *Dunaliella salina*
200–450	Cyanobacteria	*Aphanothece* sp. *Phormidium versicolor* *Spirulina subsalsa*
	Chlorophyceae	*Chlamydomonas rubrifilum* *Dunaliella salina**

**Species isolated and cultured in laboratory.*

Table 1.
Classification of microalgae species recorded in Sfax solar saltern according to the salinity gradient [9].

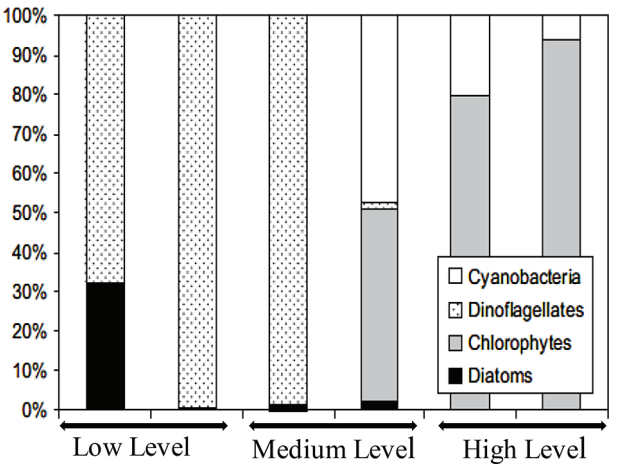


Figure 2.
Mean relative contribution of microalgae groups to total microalgae biomass in six ponds of increasing salinity in the saline of Sfax Tunisia [30].

important salt production areas in Tunisia (300,000 T of salt per year). A total of 45 microalgae taxa were recorded from the Sfax solar saltern and identified belonging to five groups: diatoms, dinoflagellates, Chlorophyceae, Euglenophyceae and

Cyanobacteria. For each group, we clearly observed a marked decrease in the number of taxa with the increase of salinity (**Table 1**, **Figure 2**). Diatoms were dominant in ponds that have salinity ranges from 40 to 80 PSU (67.95% of the microalgae total abundance), whereas the dinoflagellates represented only 22.19% and Euglenophyceae were poorly represented in this pond (1.2%) (**Figure 2**). Dinoflagellates dominated the densities and biomasses of microalgae in the ponds of 80–200 PSU, contributing to 56.7% and 34.4% of the microalgae total abundance, respectively. Chlorophytes largely dominated in the crystallization ponds>200 salinity which accounting for 69.1% of the total microalgae. While Cyanobacteria were relatively abundant in ponds of medium salinity (19.9%) they were rare in hypersaline ponds (0.5%) (**Figure 2**, **Table 1**).

3. Valorization of three algal species

Three different microalgae species were isolated from the three level of salinity of Sfax solar saltern: the diatom *Halamphora* sp. (45–80 PSU) cyanobacterium *Phormidium versicolor* (80–200 PSU) and the chlorophyceae *Dunaliella salina* (200–450 PSU) (**Figures 1** and **3**). These species were cultured in the laboratory. The extraction of the various pigments and biomolecules contained in these species was carried out.

3.1 Isolation and culture conditions

Halamphora sp. was isolated via micromanipulation and serial dilution from a collected water sample. Both *D. salina* and *P. versicolor* were isolated on agar medium. Several antibiotic and antifungal treatments were performed in order to obtain monoclonal and axenic cultures. These three algal species were batch cultured in flasks 500 ml Erlenmeyer flask with artificial sea water of 80 PSU. The cultures were initiated with cell densities of 10^6 cells ml^{-1} for *D. salina* [31], 50,000 cells ml^{-1} for *Halamphora* sp. [19] and an initial concentration of chlorophyll *a* of 0.005 μg ml^{-1} for *P. versicolor* because the numeration was impossible (the filaments intertwine) [9]. *P. versicolor* was cultivated using BG-11 medium as culture medium [32], *D. salina* was grown in Walne's growth medium as modified by Guermazi, Elloumi, Ayadi, Bouain and Aleya [17] and for *Halamphora* sp., the culture was carried out in F/2 Provasoli medium [33]. Cultures were maintained in incubator (FRIOCELL) and incubated in 24-light cycles at (25°C). *Phormidium* and *Halamphora* were reared under light intensity of 130 μmol photons m^{-2} s^{-1}, while Dunalielal was cultured under low irradiance of 27 μmol photons m^{-2} s^{-1}.

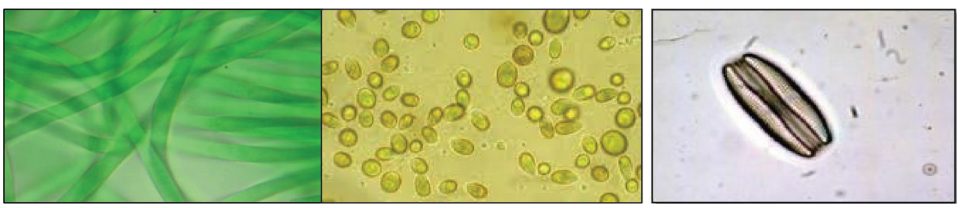


Figure 3.
Microscopic observation (G ×100) of (a) P. versicolor, (b) D.salina and (c) Halamphora sp. isolated from the Sfax solar salternand cultured in the laboratory.

The biomass of each microalga was separated from the culture media by centrifugation (4500 × g, 10 min), and the pellet was washed with distilled water and centrifuged again at 4500 × g for 10 min (the washing was repeated twice). The pellet was freeze-dried and stored at −70°C.

3.2 Growth tracking

The growth of *D. salina* and *Halamphora* sp. was determined by a daily cell count using a Malassez hemocytometer under a light microscope.

The cyanobacterium *P. versicolor* being filamentous, the chlorophyll *a* was determined to assess the growth of this cyanobacterium according to the equation of Speziale, Schreiner, Giammatteo and Schindler [34]:

$$\text{Chl } a\left(\mu g\, l^{-1}\right) = [11.47\,(OD_{664nm} - OD_{750nm}) - 0.4\,(OD_{664nm} - OD_{750nm})]\,\upsilon / V$$

with.

OD: Optical density.

υ: volume of the acetone extract (ml).

V: volume of the algae suspension (ml).

3.3 Determination of pigments content: chlorophyll *a*, carotenoids and phycocyanin

The dosage of photosynthetic pigments of each microalgal species was carried out after extraction in 90% acetone. The concentration of Chlorophyll *a* was calculated according to the equation of Speziale, Schreiner, Giammatteo and Schindler [34] for the cyanobacterium and conforming to the equations of Jeffrey and Humphrey [35] for Chlorophyceae and the diatom.

Equation of Jeffrey and Humphrey [36] for Chlorophyceae:

$$\text{Chl } a\left(\mu g\, l^{-1}\right) = 11.93OD_{664nm} - 1.93OD_{647nm}$$

Equation of Jeffrey and Humphrey [35] for Diatom

$$\text{Chl } a\left(\mu g\, l^{-1}\right) = 11.47OD_{664nm} - 0.40OD_{630nm}$$

Carotenoid concentrations were calculated according to the equation of Chamovitz, Sandmann and Hirschberg [36] for cyanobacterium and according to Salguero et al. [37] for Chlorophyceae.

Cyanobacteria carotenoid ($\mu g\, l^{-1}$) = ($OD_{461nm} - 0.046OD_{664nm}$) × 4

Chlorophyceae Carotenoid ($\mu g\, l^{-1}$) = $0.0045\,(3000OD_{470nm} - 1.63OD_{750nm}\text{Chl } a)$.

The phycocyanin (C-PC) pigment was isolated from *P. versicolor* using the method developed by Silveira et al. [38] With light modification. The C-PC contents were quantified according the following equation:

$$C - PC\left(mg\, ml^{-1}\right) = \left(OD_{615nm} - 0.474OD_{652nm}\right) / 5.43$$

3.4 Determination of dry matter, proteins, lipids, Total sugars and phenolic compounds

The dry matter of microalgae was determined according to the AOAC standard methods [39]. The protein assay method of Lowry [40] was used by the combination of Folin with Biuret's reagents. The Lipids content was determined gravimetrically after the Soxhlet extraction of dried samples with hexane for 2 hours using Nahita Model 655 (Navarra, Spain). The sugars were estimated by phenol-sulfuric acid method [41] using glucose as a standard. The total phenol content of the *P. versicolor* extract was determined by the method of Singleton and Rossi [42].

3.5 Determination of mineral content of *Halamphora* sp.

The analyses of sodium, potassium, calcium, magnesium, iron, copper, and zinc contents in *Halamphora* sp. were carried out using the inductively coupled plasma optical emission spectrophotometer (ICP-OES) Model 4300 DV, PerkinElmer, Shelton, CT, USA, according to the method of AOAC 1999 [43]. Measurements were done in triplicates.

3.6 Determination of fatty acids profile of *Halamphora* sp. and *D. salina*

For fatty acids analyses, cultures were harvested at the end of the log phase. All lipids were evaporated to dryness with nitrogen and concentrated with hexane. Fatty acids methyl esters (FAMEs) were prepared from the lipid extract by transesterification using a direct transmethylation method according to Lepage and Roy [44]. The FAMEs were then extracted with hexane and determined quantitatively by capillary gas chromatography. We used a Chromopack, CP 9001 gas chromatograph, HPS 5890 series II chromatograph, equipped with a polar 25-m capillary column CP wax 58 (Varian SA, France) (0.32 mm diameter and a layer thickness of 0.52 mm), and a flame ionization detector (FID). We used a splitless injection system with nitrogen as the carrier gas. The oven was programmed to rise from an initial temperature of 180–250°C at rates of

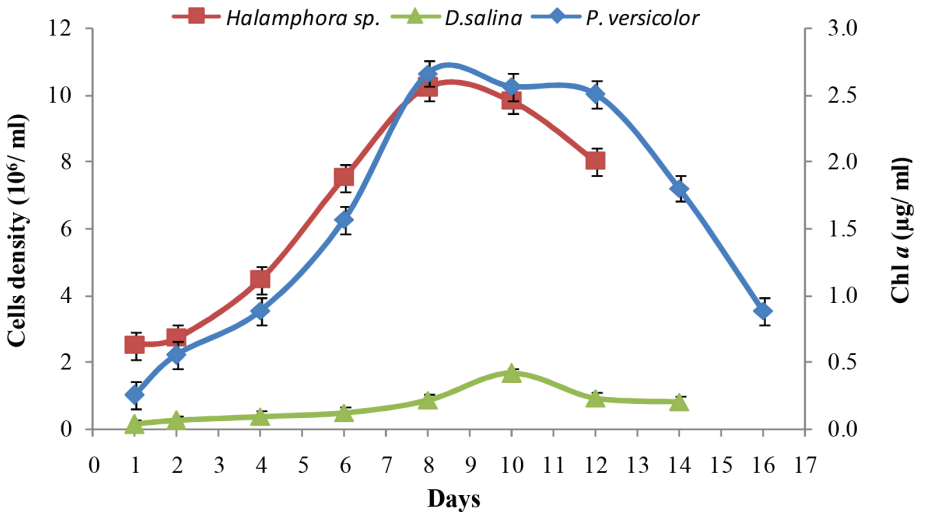


Figure 4.
Growth curves of Halamphora sp., D. salina and P. versicolor cultured batchwise.

10°C min^{-1} (from 180 to 220), 2°C min^{-1} (from 220 to 240), and 5°C min^{-1} (from 240 to 250). Individual FAMEs were identified by comparing retention times with those obtained with laboratory standards and the manufacturer's instructions (Supelco).

3.7 Growth kinetics of three microalgae

The four growth phases—lag, exponential, stationary and decline growth phases—are only observed on growth curves of *Halamphora* sp. (**Figure 4**). While *Halamphora* showed a short lag phase of 2 days, this phase was absent for *Phormidium* and *Dunaliella*. The exponential growth phase was observed for all the microalgae under continuous light but with different slopes. *Phormidium* and *Halamphora* grew faster with similar exponential phase about 5–6 days and reached maximum yield of 2.66 μg. ml^{-1} and 10.22 × 10^6 cells. ml^{-1}, respectively at 8th day. During the exponential phase, the specific growth rate (μ) was about 2.40 and 2.15 day^{-1} for both *Phormidium* and *Halamphora*, respectively. However, it did not exceed 0.7 day^{-1} for *Dunaliella salina*. The growth rate of microalgae is very sensitive to culture conditions, such as irradiance and photoperiod limitation [45]. The density of *Halamphora* sp. is higher than those of *Halamphora acutiuscula* (5.91 × 10^5 cells. ml^{-1}) and *Halamphora coffeaeformis* (6.17 × 10^5 cells. ml^{-1}) which they are cultured in artificial sea water under light-dark (14/10h) cycles at a photon flux density of 300 μmol photons m^{-2} s^{-1} [46].

For *Dunaliella salina*, the maximum cell density was recorded at 10th and did not exceed 1.65 × 10^6 cells. ml^{-1} (**Figure 4**). This value is higher than that reported in solar saltern by Elloumi et al. [47]. Guermazi et al. [31] stated that *D. salina* reached 6 × 10^6 cells. ml^{-1} when reard under 12h/12h light dark regime. Moreover, all microalgae curves are characterized by a short stationary phase (**Figure 4**). It seems that nutrients composition of the culture medium need to be optimized in order to maintain the cells at stationary phase.

3.8 Physicochemical characterization of three microalgal species

Microalgae could be easily grown in a laboratory and used for large-scale cultivation in bioreactors with the ability to control the quality of the cultures by providing purified culture medium that is free of toxic substances. Therefore, microalgae provide a more accessible way to produce qualitative biomolecules of interest [48–50]. Physicochemical characteristics of *D. salina*, *Halamphora* sp. and *P. versicolor* are presented in **Table 2**. The biomass of these microalgae contains moderate amounts of lipids, proteins, carbohydrates and an important percentage of chlorophyll a and carotenoids. The 7% dry matter content of *Halamphora* sp. is close to that found for other strains: 8% for *Halamphora* sp. [51], and for *P. versicolor* content 13% similarly with Singh, Parmar and Madamwar [52], who showed that the dry matter content of *Phormidium ceylanicum* is 10%. However, the lipids and proteins content of *Halamphora* sp. were relatively lower than the values published for other strains of *Halamphora* [53], and it was higher than that of *Amphora coffeaformis* [54]. For *D. salina*, the total lipids increased during growth hereas the amounts of proteins and sugars decreased, while for *P. versicolor*, it recorded a high level of protein (45%). The total sugars content of *Halamphora* sp. was 12.60% DW, which is consistent with that of some microalgae (5–23% DW) [55] and of *P. versicolor* was 21.56%. Moreover, these three algal species were found to be rich in chlorophyll, mainly chlorophyll a, and carotenoids. Continuous illumination favored also the synthesis of these pigments in *D. salina.* In fact, the synthesis of pigments was also stimulated under the

Component	*Halamphora* sp.	*Phormidium versicolor*	*Dunaliella salina*
Dry matter (%Fw)	7 ± 0.45	10 ± 0.66	—
Proteins (%Dw)	27.62 ± 0.33	42 ± 0.78	41.39 ± 6.40
Lipids (%Dw)	11.14 ± 0.19	15.7	27.04 ± 19.74
Total sugars (%Dw)	12.60 ± 0.76	21.56 ± 0.99	13.33 ± 8.06
Aches(% Dw)	37.78 ± 0.43	—	—
Chlorophyll a (%Dw)	4.94 ± 0.27	7.05 ± 0.81	17.02 ± 7.78
Phycocyanin (%Dw)	—	13 ± 0.47	—
Cartenoids(%DW)	1.083 ± 0.05	1.79 ± 0.08	1.22 ± 0.39
Polyphenols(mgGAE g^{-1})	38.27 ± 2.21	408.5 ± 18,18	—
Flavonoides(mgGAE g^{-1})	17.69 ± 0.70	13.67 ± 0.78	—

Data are expressed as mean ± standard deviation of triplicates. FW: fresh weight; DW: dry weight; GAE: gallic acid equivalent; – not realized.

Table 2.
Physicochemical characteristics of three microalgae species.

effect of light and allowing sufficient photosynthetic activity to be maintained for the synthesis of glycerol [56]. Our results also show that the 80% ethanolic extract of Bacillariophyceae and cyanobacterium the highest phenols and flavonoids contents. These high levels may be due to the culture conditions under the high salinity of 80 psu and the extraction conditions. Additionally, a high production of phycocyanin has been proven by the blue microalgae *P. versicolor* content 13%. These results are consisting with the observation by Singh, Parmar and Madamwar [52]. With respect to the ash content of *Halamphora* sp. (37.78% DW) (**Table 2**), it is in line with that found for another *Amphora* strain. Ash content exceeds 50% (55.8 to 67.9%) of the dry weight for some diatoms [54]. *Halamphora* sp. From Sfax solar salter has moderate amounts of sodium, potassium, calcium, and magnesium (**Table 3**). According to Boulay, Abasova, Six, Vass and Kirilovsky [57], the different species of microalgae do not develop the same strategies in order to survive under stressful conditions. We can assume that the species we studied might be a potential candidate for the production of biomolecules for pharmacological purposes.

Mineral	*Halamphora* sp.
Sodium (g Kg^{-1}DW)	1.125 ± 0.2
Potassium (g Kg^{-1}DW)	0.485 ± 0.05
Calcium (g Kg^{-1}DW)	0.584 ± 0.05
Magnesium (g Kg^{-1}DW)	0.747 ± 0.1
Iron (g Kg^{-1}DW)	0.016 ± 0.002
Copper (g Kg^{-1}DW)	0.008 ± 0.001
Zinc (g Kg^{-1}DW)	0.008 ± 0.001

Table 3.
Mineral content of Halamphora sp. [19].

Fatty acids	*Halamphora* sp.	*D. salina*
C14:0	3.623 ± 0.3	2.8 ± 1.2
C15:0	3.418 ± 0.3	—
C16:0	27.427 ± 0.5	21.0 ± 3.5
C17:0	1.664 ± 0.4	—
C18:0	1.974 ± 0.3	9.05 ± 1.0
C20:0	0.734 ± 0.2	—
C24:0	2.468 ± 0.2	—
SFA	**41.308 ± 0.8**	**35.9 ± 0.6**
C14:1	3.386 ± 0.3	9.6 ± 1.1
C16:1	45.089 ± 0.8	2.2 ± 1.2
C17:1	0.521 ± 0.1	—
C18:1	3.658 ± 0.3	14.9 ± 1.2
MUFA	**52.654 ± 1.2**	**30.2 ± 0.8**
C16:2	1.603 ± 0.2	—
C16:3	0.924 ± 0.3	0.1 ± 0.1
C16:4	—	14.0 ± 2.3
C18:2	0.432 ± 0.1	0.9 ± 0.2
C18:3	—	0.8 ± 0.3
C18:4	—	0.6 ± 0.2
C20:4	0.712 ± 0.2	—
C20:5 (EPA)	2.367 ± 0.3	0.1 ± 0.1
C22:6 (DHA)	—	4.3 ± 1.3
PUFA	**6.038 ± 0.5**	**28.66 ± 0.8**

(–): not detected.

Table 4.
Percentage of fatty acids composition of Halamphora sp. and D. salina reared in laboratory [19, 31].

3.9 Fatty acids composition of *Halamphora* sp. and *D. salina*

The fatty acid profile of *Halamphora* sp. and *Dunaliella* sp. was composed of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) which differed significantly from an alga to another (**Table 4**). The level of SFA recorded in *Halamphora* sp. and *Dunaliella* sp. is high, averaging 41.308 and 35.90% of total fatty acid, respectively. The pattern of SFA show that *Halamphora* is richer in SFA than *Dunaliella*. However, *Dunaliella* and *Halamphora* recorded a high level of palmitic acid (16:0) which accounted 21.0 and 27.42%, respectively. Hence, *Halamphora* sp. could be a suitable producer of SFA, which are easily convertible to biodiesel [58].

Moreover, *Halamphora* exhibited a high amount of palmitoleic acid (C16:1) which reached 45.089%, while that of *Dunaliella* did not exceed 2.2% of total FA. High levels of palmitoleic acid and other bioactive fatty acids were also detected in the fusi form morphotype of the Bacillariophyceae [59]. For *D. salina*, MUFAs were represented by 14:1 (n-5), 16:1 (n-7) and 18:1 (n-9). *D. salina* is an important source of 18:1 (n-9), whichreached14.9 ± 1.2%.

Dunaliella is rich in PUFAs with a percentage of 28.66%, while those of *Halamphora* did not exceed 6% of total fatty acid (**Table 4**). While *Halamphora* produced a noticeable level of EPA (2.367%), *Dunalila* is an important source of DHA reaching 4.3% of total fatty acid. Indeed, it is known that EPA is an important PUFA for health protection from many pathologies, including cardiovascular diseases [60] and cancer [61]. These PUFAs are known to have a number of important nutritional and pharmaceutical applications [62, 63]. They are also known to have beneficial effects on the health of human beings and to play a major role in the prevention of medical disorders in three areas: the heart and the circulation [64], inflammatory conditions and cancers, in particular colon tumorigenesis [65].

4. Conclusion

In conclusion, the saline of Sfax presents a high microalga diversified. Three species *Halamphora* sp., *P. versicolor* and *D. salina* are interesting for biomolecules production such as cartenoid, protein, sugar, fatty acids which they could be valorized in several fields.

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References

[1] Grégoire P, Fardeau M, Guasco S, Bouanane A, Michotey V, Bonin P. Les micro-organismes de l'extrême. La Presse Thermale et Climatique. 2009;**146**:49-61

[2] Oarga A. Life in extreme environments, Revista de Biologia e ciencias da. Terrain. 2009;**9**(1):1-10

[3] Cantrell SA, Casillas-Martinez L, Molina M. Characterization of fungi from hypersaline environments of solar salterns using morphological and molecular techniques. Mycological Research. 2006;**110**(8):962-970

[4] Elloumi J, Guermazi W, Ayadi H, Bouain A, Aleya L. Abundance and biomass of prokaryotic and eukaryotic microorganisms coupled with environmental factors in an arid multi-pond solar saltern (Sfax, Tunisia). Journal of the Marine Biological Association of the United Kingdom. 2009;**89**(2):243-253

[5] Oren A. Thoughts on the "missing link" between saltworks biology and solar salt quality. Global NEST Journal. 2010;**12**(4):417-425

[6] Baati H, Amdouni R, Gharsallah N, Sghir A, Ammar E. Isolation and characterization of moderately halophilic bacteria from Tunisian solar saltern. Current Microbiology. 2010;**60**(3):157-161

[7] Chokri MA, Sadoul N, Medhioub K, et al. Analyse comparative de la richesse avifaunistique du salin de Sfax dans le contexte tunisien et méditerranéen. Revue d'Ecologie, Terre et Vie. 2008;**63**(4):351-369

[8] Ayadi H, Abid O, Elloumi J, Bouaïn A, Sime-Ngando T. Structure of the phytoplankton communities in two lagoons of different salinity in the Sfax saltern (Tunisia). Journal of Plankton Research. 2004;**26**(6):669-679

[9] Masmoudi S, Tastard E, Guermazi W, Caruso A, Morant-Manceau A, Ayadi H. Salinity gradient and nutrients as major structuring factors of the phytoplankton communities in salt marshes. Aquatic Ecology. 2014;**49**(1):1-19

[10] Khemakhem H, Elloumi J, Ayadi H, Aleya L, Moussa M. Modelling the phytoplankton dynamics in a nutrient-rich solar saltern pond: Predicting the impact of restoration and climate change. Environmental Science and Pollution Research. 2013;**20**(12):9057-9065

[11] Kchaou N, Elloumi J, Drira Z, Hamza A, Ayadi H, Bouain A, et al. Distribution of ciliates in relation to environmental factors along the coastline of the Gulf of Gabes, Tunisia. Estuarine, Coastal and Shelf Science. 2009;**83**(4):414-424

[12] Toumi N, Ayadi H, Abid O, Carrias J-F, Sime-Ngando T, Boukhris M, et al. Zooplankton distribution in four ponds of different salinity: A seasonal study in the solar salterns of Sfax (Tunisia). Hydrobiologia. 2005;**534**(1):1-9

[13] Thabet R, Leignel V, Ayadi H, Tastard E. Interannual and seasonal effects of environmental factors on the zooplankton distribution in the solar saltern of Sfax (South-Western Mediterranean Sea). Continental Shelf Research. 2018;**165**:1-11

[14] Ladhar C, Tastard E, Casse N, Denis F, Ayadi H. Strong and stable environmental structuring of the zooplankton communities in interconnected salt ponds. Hydrobiologia. 2015;**743**(1):1-13

[15] Guermazi W, Elloumi J, Ayadi H, Bouain A, Aleya L. Coupling changes in fatty acid and protein composition of Artemia salina with environmental factors in the Sfax solar saltern (Tunisia). Aquatic Living Resources. 2008;**21**(1):63-73

[16] Guermazi W, Ayadi H, Aleya L. Correspondence of the seasonal patterns of the brine shrimp, Artemia salina (LEACH, 1819) (Anostraca) with several environmental factors in an arid solar saltern (Sfax, southern Tunisia). Crustaceana. 2009;**82**(3):327-348

[17] Guermazi W, Elloumi J, Ayadi H, Bouain A, Aleya L. Rearing of Fabrea salina Henneguy (Ciliophora, Heterotrichida) with three unicellular feeds. Comptes Rendus Biologies. 2008;**331**(1):56-63

[18] Gammoudi S, Athmouni K, Nasri A, Diwani N, Grati I, Belhaj D, et al. Optimization, isolation, characterization and hepatoprotective effect of a novel pigment-protein complex (phycocyanin) producing microalga: Phormidium versicolor NCC-466 using response surface methodology. International Journal of Biological Macromolecules. 2019;**137**:647-656

[19] Boukhris S, Athmouni K, Hamza-Mnif I, Siala-Elleuch R, Ayadi H, Nasri M, et al. The potential of a brown microalga cultivated in high salt medium for the production of high-value compounds. BioMed Research Internationa. 2017;**2017**:1-10

[20] Belhaj D, Frikha D, Athmouni K, Jerbi B, Ahmed MB, Bouallagui Z, et al. Box-Behnken design for extraction optimization of crude polysaccharides from Tunisian Phormidium versicolor cyanobacteria (NCC 466): Partial characterization, in vitro antioxidant and antimicrobial activities. International Journal of Biological Macromolecules. 2017;**105**:1501-1510

[21] Fogg GE, Westlake D. The importance of extracellular products of algae in freshwater: With 3 figures and 2 tables in the text. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen. 1953;**12**(1):219-232

[22] Santini A, Tenore GC, Novellino E. Nutraceuticals: A paradigm of proactive medicine. European Journal of Pharmaceutical Sciences. 2017;**96**:53-61

[23] Harun R, Singh M, Forde GM, Danquah MK. Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable and Sustainable Energy Reviews. 2010;**14**(3):1037-1047

[24] Marfaing H, Lerat Y. Les algues ont-elles une place en nutrition? Phytothérapie. 2007;**5**(1):2-5

[25] Zeid A, Sigamani S. Pharmaceutically valuable bioactive compounds of algae. 2016;**9**(6):43-47

[26] Shariati M, Hadi MR. Microalgal biotechnology and bioenergy in Dunaliella. In: Progress in Molecular and Environmental Bioengineering-from Analysis and Modeling to Technology Applications. IntechOpen; 2011

[27] Li H-B, Cheng K-W, Wong C-C, Fan K-W, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry. 2007;**102**(3):771-776

[28] Suali E, Sarbatly R. Conversion of microalgae to biofuel. Renewable and Sustainable Energy Reviews. 2012;**16**(6):4316-4342

[29] Kobbi-Rebai R, Annabi-Trabelsi N, Khemakhem H, Ayadi H, Aleya L.

Impacts of restoration of an uncontrolled phosphogypsum dumpsite on the seasonal distribution of abiotic variables, phytoplankton, copepods, and ciliates in a man-made solar saltern. Environmental Monitoring and Assessment. 2013;**185**(3):2139-2155

[30] Elloumi J, Guermazi W, Ayadi H, Bouaïn A, Aleya L. Detection of water and sediments pollution of an arid saltern (Sfax, Tunisia) by coupling the distribution of microorganisms with hydrocarbons. Water Air and Soil Pollution. 2008;**187**(1):157-171

[31] Guermazi W, Masmoudi S, Boukhris S, Ayadi H, Morant-Manceau A. Under low irradiation, the light regime modifies growth and metabolite production in various species of microalgae. Journal of Applied Phycology. 2014;**26**(6):2283-2293

[32] Stanier R, Kunisawa R, Mandel M, Cohen-Bazire G. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriological Reviews. 1971;**35**(2):171-205

[33] Guillard RR. Culture of phytoplankton for feeding marine invertebrates. In: Culture of Marine Invertebrate Animals. Boston, MA: Springer; 1975. pp. 29-60

[34] Speziale BJ, Schreiner SP, Giammatteo PA, Schindler JE. Comparison of N, N-dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll. Canadian Journal of Fisheries and Aquatic Sciences. 1984;**41**(10):1519-1522

[35] Jeffrey ST, Humphrey G. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemie und Physiologie der Pflanzen. 1975;**167**(2):191-194

[36] Chamovitz D, Sandmann G, Hirschberg J. Molecular and biochemical characterization of herbicide-resistant mutants of cyanobacteria reveals that phytoene desaturation is a rate-limiting step in carotenoid biosynthesis. Journal of Biological Chemistry. 1993;**268**(23):17348-17353

[37] Salguero A, de la Morena B, Vigara J, Vega JM, Vilchez C, León R. Carotenoids as protective response against oxidative damage in Dunaliella bardawil. Biomolecular Engineering. 2003;**20**(4-6):249-253

[38] Silveira ST, Burkert JDM, Costa JAV, Burkert CAV, Kalil SJ. Optimization of phycocyanin extraction from Spirulina platensis using factorial design. Bioresource Technology. 2007;**98**(8):1629-1634

[39] Horwitz W, Albert R. The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. Journal of AOAC International. 2006;**89**(4):1095-1109

[40] Classics Lowry O, Rosebrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry. 1951;**193**(1):265-275

[41] Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 1956;**28**(3):350-356

[42] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965;**16**(3):144-158

[43] Cunniff P, Washington D. Official methods of analysis of aoac international. Journal of AOAC International. 1997;**80**(6):127A

[44] Lepage G, Roy CC. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. Journal of Lipid Research. 1984;**25**(12):1391-1396

[45] Ho SH, Huang SW, Chen CY, Hasunuma T, Kondo A, Chang JS. Characterization and optimization of carbohydrate production from an indigenous microalga *Chlorella vulgaris* FSP-E. Bioresour Technol. 2013;**135**:157-165

[46] Nguyen-Deroche TLN, Caruso A, Le TT, Bui TV, Schoefs B, Tremblin G, et al. Zinc affects differently growth, photosynthesis, antioxidant enzyme activities and phytochelatin synthase expression of four marine diatoms. The Scientific World Journal. 2012;**2012**:1-15

[47] Elloumi J, Carrias JF, Ayadi H, Sime-Ngando T, Bouaïn A, Communities structure of the planktonic halophiles in the solar saltern of Sfax. Tunisia, Estuarine: Coastal and Shelf Science; 2009;**81**(1):19-26

[48] Corrêa PS, Morais Júnior WG, Martins AA, Caetano NS, Mata TM. Microalgae biomolecules: Extraction, separation and purification methods. PRO. 2021;**9**(1):10

[49] Widowati I, Zainuri M, Kusumaningrum HP, Susilowati R, Hardivillier Y, Leignel V, et al. Antioxidant activity of three microalgae Dunaliella salina, Tetraselmis chuii and Isochrysis galbana clone Tahiti. In: IOP Conference Series: Earth and Environmental Science. Vancouver, BC, Canada: IOP Publishing; 2017. p. 012067

[50] Pouvreau J-B, Taran FDR, Rosa P, Pin S, Fleurence Jl, Pondaven P. Antioxidant and free radical scavenging properties of marennine, a blue-green polyphenolic pigment from the diatom Haslea ostrearia (Gaillon/Bory) Simonsen responsible for the natural greening of cultured oysters. Journal of Agricultural and Food Chemistry. 2008;**56**(15):6278-6286

[51] Chtourou H, Dahmen I, Jebali A, Karray F, Hassairi I, Abdelkafi S, et al. Characterization of Amphora sp., a newly isolated diatom wild strain, potentially usable for biodiesel production. Bioprocess and Biosystems Engineering. 2015;**38**(7):1381-1392

[52] Singh NK, Parmar A, Madamwar D. Optimization of medium components for increased production of C-phycocyanin from Phormidium ceylanicum and its purification by single step process. Bioresource Technology. 2009;**100**(4):1663-1669

[53] Khatoon H, Banerjee S, Yusoff F, Shariff M. Evaluation of indigenous marine periphytic Amphora, Navicula and Cymbella grown on substrate as feed supplement in Penaeus monodon postlarval hatchery system. Aquaculture Nutrition. 2009;**15**(2):186-193

[54] Lee S-H, Karawita R, Affan A, Lee J-B, Lee K-W, Lee B-J, et al. Potential of benthic diatoms Achnanthes longipes, Amphora coffeaeformisand Navicula sp.(Bacillariophyceae) as antioxidant sources. Algae. 2009;**24**(1):47-55

[55] Brown MR. The amino-acid and sugar composition of 16 species of microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology. 1991;**145**(1):79-99

[56] Liska AJ, Shevchenko A, Pick U, Katz A. Enhanced photosynthesis and redox energy production contribute to salinity tolerance in Dunaliella as revealed by homology-based proteomics. Plant Physiology. 2004;**136**(1):2806-2817

[57] Boulay C, Abasova L, Six C, Vass I, Kirilovsky D. Occurrence and function of the orange carotenoid protein in photoprotective mechanisms in various cyanobacteria. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 2008;**1777**(10):1344-1354

[58] Gao C, Zhai Y, Ding Y, Wu Q. Application of sweet sorghum for biodiesel production by heterotrophic microalga Chlorella protothecoides. Applied Energy. 2010;**87**(3):756-761

[59] Desbois AP, Walton M, Smith VJ. Differential antibacterial activities of fusiform and oval morphotypes of Phaeodactylum tricornutum (Bacillariophyceae). Journal of the Marine Biological Association of the United Kingdom. 2010;**90**(4):769-774

[60] Iglesia I, Huybrechts I, Gonzalez-Gross M, Mouratidou T, Santabárbara J, Chajès V, et al. Folate and vitamin B12 concentrations are associated with plasma DHA and EPA fatty acids in European adolescents: The healthy lifestyle in Europe by nutrition in adolescence (HELENA) study. British Journal of Nutrition. 2017;**117**(1):124-133

[61] Zheng W, Wang X, Cao W, Yang B, Mu Y, Dong Y, et al. E-configuration structures of EPA and DHA derived from Euphausia superba and their significant inhibitive effects on growth of human cancer cell lines in vitro. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2017;**117**:47-53

[62] Apt KE, Behrens PW. Commercial developments in microalgal biotechnology. Journal of Phycology. 1999;**35**(2):215-226

[63] Ward OP, Singh A. Omega-3/6 fatty acids: Alternative sources of production. Process Biochemistry. 2005;**40**(12):3627-3652

[64] Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: Effects on risk factors, molecular pathways, and clinical events. Journal of the American College of Cardiology. 2011;**58**(20):2047-2067

[65] Bourre J-M. Dietary omega-3 fatty acids for women. Biomedicine & Pharmacotherapy. 2007;**61**(2-3):105-112