ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 10, pp. 13930-13938, October, 2016

International Journal of Recent Scientific Research

Research Article

PROSPECTIVE STUDY ON NEW MICROALGAE "*TETRASELMIS SP.*, *PICOCHLORUM SP.*, AND *CHAETOCEROS SP*"ISOLATED FROM COASTAL WATERS OF TUNISIA AS PROMISING ANIMAL FOOD: GROWTH AND BIOCHEMICAL COMPOSITION

EL AbedMehdi*[1,2]., Zouiten Dora[1] and Ahmed NoureddineHelal[2]

[1]Institut National des Sciences et Technologies de la Mer, BP 56 Route de khénis 5000 Monastir
[2]Institut Supérieur de Biotechnologie Monastir, Avenue TaherHadded (B.P 74) 5000 Monastir

ARTICLE INFO

Article History:

Received 10th July, 2016
Received in revised form 14th August, 2016
Accepted 08th September, 2016
Published online 28th October, 2016

Key Words:

Micoalgae, fatty acid, sterol, diatoms, Prasinophyceae, Chlorophycae

ABSTRACT

In the present work we focused on three species of microalgaeisolated from Tunisian coast in which bivalves reproduced naturally: *Tetraselmis sp. (Tetra)*, *Picochlorum sp. (Pico)* and *Chaetoceros sp. (Chaeto)*. Class, family and genus were identified and their potential utilization in aquaculture examined. Growth and biochemical composition have been accordingly studied. Thus, the three isolated microalgae exhibited adequate growth as well as protein and lipid contents for potential hatchery uses. Relative protein content, ranged from 50 to 53%, 39 to 45% and 42 to 45% respectively for *Chateoceros sp., Tetraselmis sp.* and *Picochlorum sp.* in exponential and stationary phases. Lipids content reached 15 to 18%, 12% and 15 to 20% respectively for *Chateoceros sp., Tetraselmis sp.* and *Picochlorum sp.* Relative fatty acid composition of *Chateoceros sp.* was characterized by the presence of EPA (20:5n-3) with 10 to 19% and low amount of DHA (22:6n-3) with 1 to 2%. Low EPA content was also recorded in *Tetraselmis sp* (5%); whereas fatty acid profile of *Picochlorum sp* was dominated by linolenic and linoleic acids. Sterol analysis showed that *Picochlorum sp* was composed of94% of stigmasterol; whereas the major components was campeserol (71-73%) and 24-methylene (25-27%) in *Tetraselmis sp.* More sterol diversity was found in *Chaetoceros sp.* in its exponential growth phase with 24-methylene (51%), isofucosterol (15%) and cholesterol (15%). Molecular identification was carried out on *Picochlorum sp*; the 18S rRNA gene phylogeny revealed that such strain was a sequence for *Picochlorum*.

INTRODUCTION

The large presence of microalgae in aquatic environment (bioavailability), their high growth and nutritional value make them an interesting topic for several current scientific research fields. Indeed, these features and especially their biochemical characteristics (lipids, proteins, pigments, enzymes and bioactive substances) have potential application in human and animal nutrition, energy production and treatment of anthropogenic waste (Muller-Feuga 1997; El Abed *et al*. 2008; Sialve and Steyer 2013; Sung-Suk *et al*. 2015).

In aquaculture, microalgae play a fundamental role as primary production source. Feeding of farmed aquatic organisms is based on algal cultures as an indirect food source to fish larvae through zooplankton (such as enriched rotifers, Artemia, and copepods), or for direct consumption for mollusks and crustaceans (Lavens and Sorgeloos 1996; Minh Thi *et al*. 2015). In shellfish hatchery, phytoplankton represents nowadays the only suitable feed for bivalve because substitution diet led to low mollusks' development (Laing *et al*. 1990; Robert and Trintignac 1997a; Langdon and Onal 1999), larval production remains strictly dependent of the nutritional quality and quantity of microalgae produced in hatchery. Research on feeding bivalves is ultimately focused on two areas: identification and selection of species of high nutritional value (Volkman *et al*. 1989; Ponis *et al*. 2006, Costard *et al*. 2012) and improving of culture technology essentially by development of photobioreactors system (Carvalho *et al*. 2006, Loubiere *et al*. 2009).

Many factors contribute to the efficiency of feed for farmed aquatic organism but for phytoplankton itsnutritional value is of crucial importance as a first step of food chain. Proximate composition and microalgae growth rely, on the one hand, on species and strains and, on the other hand, on culture conditions and growth phases (Costard *et al*. 2012). Qualitatively, microalgae are generally characterised by a high proportion of polyunsaturated fatty acids (PUFA) that are of major importance in aquatic organism development (Lavens and Sorgeloos 1996). For bivalves, relationship between biochemical composition, ratio of microalgae and nutritional

*Corresponding author: **EL AbedMehdi***
Institut National des Sciences et Technologies de la Mer, BP 56 Route de khénis 5000 Monastir

value was mainly studied (Epifanio 1979; Browen*et al*. 1997; Loor*et al*. 2016). Lipid contents and especially of highly unsaturated fatty acids (HUFAs) were reported as crucial factors in survival and growth of farmed bivalves (Langdon and Waldock, 1981; Marty *et al* 1992; Rico Villa *et al* 2006, Da Costa *et al* 2015). However, criteria such as size, digestibility and ease of cultivation are also important to define the nutritional value of phytoplancton (Muller-Feuga *et al*. 2003).

In order to contribute to the Tunisian national effort for the development of bivalve cultures and to initiate creation of local strains collection, three microalgae species were isolated from Tunisia coast where edible molluscs are naturally present, as potential food for shellfish production. Two green microalgae: *Tetraselmis sp (Tetra), Picochlorum sp (Pico)* and one diatom *Chaetoceros sp(Chaeto)* were thus retained. These species were selected according to two prerequisite criteria: presence in bloom in natural bivalve reproduction areas and fitted size for larvae.

To evaluate their nutritional value and suitability for aquaculture, the three microalgae were characterized at two different stages, in terms of cell density, dry weight, proteins, carbohydrates, lipids, fatty acid and sterol contents. The second step that will be exposed in a forthcoming paper will be its use as single microalgae or in combination as food for clams and oysters larvae on which its effects on growth, survival, competence and metamorphosis will be exposed.

METHODS

Enrichment cultures, isolation and purification of microalgal strains

Seawater samples (n= 3) were collected from tunisian coasts (South Mediterranean, table 1). Sample of 500 ml from each location was filtered through 30 µm pore size membrane and transferred into a flask containing Conway medium (2ml/L). Cultures were incubated with shaking at 25 C, provided by continuous illumination intensity (2000 lux) supplied by cool-white fluorescence tubes. Every 3 days, flasks were visually examined and the algal growth was confirmed microscopically. Enriched cultures were used for the isolation of microalgae in pure cultures. Microalgae concentration and quality were estimated using a "cellule of Malassez". Serial 10-fold dilutions from each enriched culture were prepared in sterilized sea water containing Conway medium (1ml /L). From the serial-dilution tube intended to contain about 1 cell ml 1, aliquots of 1ml served to inoculate new tubes in same conditions as described above. Otherwise, 2 ml was taken from the serial dilution tube, and spread in agar plates prepared with the growth medium "Conway". When the algal cell colonies are visible, a sample of each colony was removed and suspended in sterile seawater containing Conway medium. Microscopic analysis was regularly undertaken to verify the purity of the culture.

Culture conditions and biomass measurements of microalgae

Isolated microalgae (Table 1) were examined in triplicate cultures. They were previously grown under defined conditions in 2lbatch culture in sterilised seawater at 34PSU salinity, enriched with Conway medium, silicate being added to the diatom strain. Temperature and light intensity, were respectively 22.6°C ± 0.5 and 205µE m^2 s^{-1}, the whole cultures were aerated with CO_2-air mixture (3% of the total injected) to support growth and stabilize the pH (7.1± 0.4). Cell concentration was daily recorded on a Beckman Multisizer III Coulter and growth rates (µ) calculated from biomass increase per unit of time during logarithmic phase (Pirt 1975) as µ=[ln (N1 N0) /(t1 t0)] , where N1= measurement at time 1 (t1), N0 = measurement at time 0 (t0). Aliquots were harvested from each culture during exponential and stationary phase's growth for dry weight, protein, lipid, fatty acid and sterol analysis.

Biochemical analysis

For dry weight 50 ml of microalgae culture were filtered through pre-combusted (450 °C; 24 h), pre-weighed, 47-mm filters. Samples were then rinsed with 30 ml of 0.5 M Ammonium Formate to remove residual salts. These filters were finally dried overnight (80 °C) then reweighted to determine dry weight (DW). For total lipid and protein determinations, 25 to 50 ml of culture, depending on algae concentration, were filtered through 47-mm filters (Whatman GF/C). Protein was analysed according to Bradford method (Bradford 1976). For lipid extraction , the sample was put in 6 ml of Folch reagent (Folch *et al*. 1957) (Chloroform / Methanol 2: 1) and extracted for 10 minutes in an ultrasonic tub and stored at -20 °C for later analysis. The separation of neutral and polar lipids was made on a micro column of silica (SI100). A sample aliquot was evaporated and taken three times 500 µmol of chloroform / methanol (98: 2) and filed in column. In each sample, 2.3 µg of C23: 0 as internal standard fatty acids and 2.3µg cholestane as internal standard sterols were added. For fatty acids, the transesterification was carried by BF3 at 100 °C, the methyl esters of fatty acids then dissolved in hexane (Marty 1976). To identify sterols in the neutral lipids, we made a basic transesterification by a methoxide Sodium (MeONa). In polar lipids, it is necessary to make an acid transmethylation in sulfuric acid at 50 ° C.

Molecular identification

Isolated microalgae strains were sent to Ifremer Laboratory of Environment Physiology and Biotechnology of Algae in liquid form and maintained in enriched seawater for molecular identification. They were used for molecular analysis performed on 10 ml sample. DNA extraction was carried out by the phenol chloroform method. Microalgal 18S rRNA gene fragments were amplified by polymerase chain reaction using the (18 S dir: 5'- AACCTGGTTGATCCTGCCAGT -3, 18S rev: 5'- TCCTTCTGCAGGI rCACCTAC-3'). Polymerase chain reaction was performed in a total volume of 25 µL reaction mixture containing approximately 50 ng of genomic DNA (diluted 100×), 5 µL 10× Dream Taq buffer (MBI Fermentas, St. Leon-Rot, Germany), 500 µmol L 1 of each deoxynucleoside triphosphate, 0.2 µmol L 1 of each primer (EukA and EukB) and 2.5 U of Dream Taq DNA polymerase (Fermentas). Thermal cycling consisted of an initial denaturation of 3 min at 94 C, followed by 30 cycles of 45 s at 94 C, 1min at 60 C, 3min at 72 C, and a final extension of 5 min at 72 C. The 18S rDNA was cloned in the pGEMT Easy Vector System (Promega, Madison, USA). The sequencing of PCR fragments obtained was done by CATC society (GATC

Biotech SARL- Mulhouse, France). To determine the first phylogenetic affiliation, each sequence was compared with sequences available in databases using the Basic Local Alignment Search Tool (BLAST) from the National Centre for Biotechnology Information (NCBI).

Statistical analysis

Correlation between the biochemical components of microalgae (total lipids, proteins, carbohydrates, total saturated, monounsaturated, polyunsaturated fatty acids and sterol) and growth phase of culture were analyzed by one-way ANOVA and significant differences between treatments were determined by the Tukey test (Zar 1999) using Stat View 5.0 software.

RESULTS

Microalgae culture and biomass growth

All cultures were performed under similar conditions (light, temperature, aeration). The evolution of the three microalgae cell concentrations during 10 days of culture is reported in Figure 1.

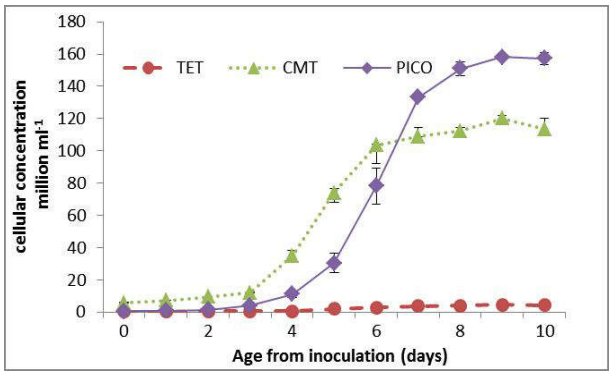

Figure 1 Mean growth of *Picochlorum sp. (Pico), Chaetoceros sp. (Chaeto) and Tetraselmis sp. (Tetra)* cultured in 2L batch culture; mean ±SD (n=3).

Table1 Class, genus, and origin of isolated microalgae

Class	Genus	Species	Origin
Chlorophyceae	*Picochlorum*	N.I.	Khnis lagoon(Monastir, Tunisia : 35°44'40.3"N 10°49'29.8"E)
Diatom	*Chaetoceros*	N.I.	OuedMaltine (Golfe de Gabes, Tunisia 34°24'30"N 10°21'15"E)
Prasinophyceae	*Tetraselmis*	N.I.	Khnislagoon (Monastir, Tunisia : 35°44'40.3"N 10°49'29.8"E)

For *Chaetoceros sp,* the exponential phase lasted 4 days (from days 3 to 7), while for *Tetraselmis sp* and *Picochlorum sp*it occurred from days 4 to 8. Maximum concentrations occurred on day 9 with 158.15 for *Picochlorum sp,* 119.98 for *Chaetoceros sp* and 4.67 for *Tetraselmis sp* 10^6 cells ml^{-1} (Table 2). The cellular concentrations of the threemicroalgae showed marked variations between species. Specific growth rates µ, estimated from days 2 to 9, were equal to 0.20 for *Tetraselmis sp*, 0.67 for *Chaetoceros sp* and 0.72 for *Picochlorum sp.* With 6.39 to 6.58 µg 10^{-6} C, *Chaetoceros sp*dry weight remained constant between both phases of cultures (Table 2). In contrast, values fluctuated from 8.41 to 3.75 µg 10^{-6} C for *Picochlorum sp* respectively in exponential and stationary phases whereas opposite trends occurred with *Tetraselmis sp* with values ranging from 196.56 to 214.97 µg 10^{-6} C in both phases (Table 2).

Table 2 Mean size and weight characteristics of the three isolated microalgae, cultured in 2L batch culture; mean ±SD (n=3).

Algal species	*Chaetoceros sp.*	*Tetraselmis sp.*	*Picochlorum sp.*
Size (µm)	2.83±0,01	8.74±0.02	2.48±0,03
Cell volume ((µm^3)	12.80±0,14	369.0±4.24	9.39±0,43
Growth rate (divisions day^{-1}*)	0.67 ± 0.05	0.20± 0.01	0.72 ± 0.04
Maximum cells concentration (Cell 10^6ml^{-1})	119.98 ±1.87	4.67 ±0.38	158.15 ±0.21
Dry weight (µg C*10-^{6})	Exp 6.39±0.66	196.56±14.09	8.41±0.29
	Sta 6,58±0,54	214.97±22.67	3.75±0,29
Cell mass (mg DW ml^{-1}**)	Exp 0.82 ± 0.03	0.8 ± 0.07	0.63 ± 0.02
	Sta 1.05 ± 0.02	1.14 ± 0.02	0.97 0.01

* From days 2 and 9.
** After 5 days in exponential phase (Exp) and 8 days in stationary phase (Sta).

Evolution of size

Both green species showed gradual increase in equivalent cell size from exponential to stationary phases with however an homogeneous cells distribution, with dominance of 2-3 µm cell size for *Picochlorum sp* (Fig. 2a) and 8-10 µm for *Tetraselmis sp* (Fig. 2b). In contrast, for *Chaetoceros sp* cell size remained constant over time with the most observed frequency of 2.5-3 µm (Fig. 2c).

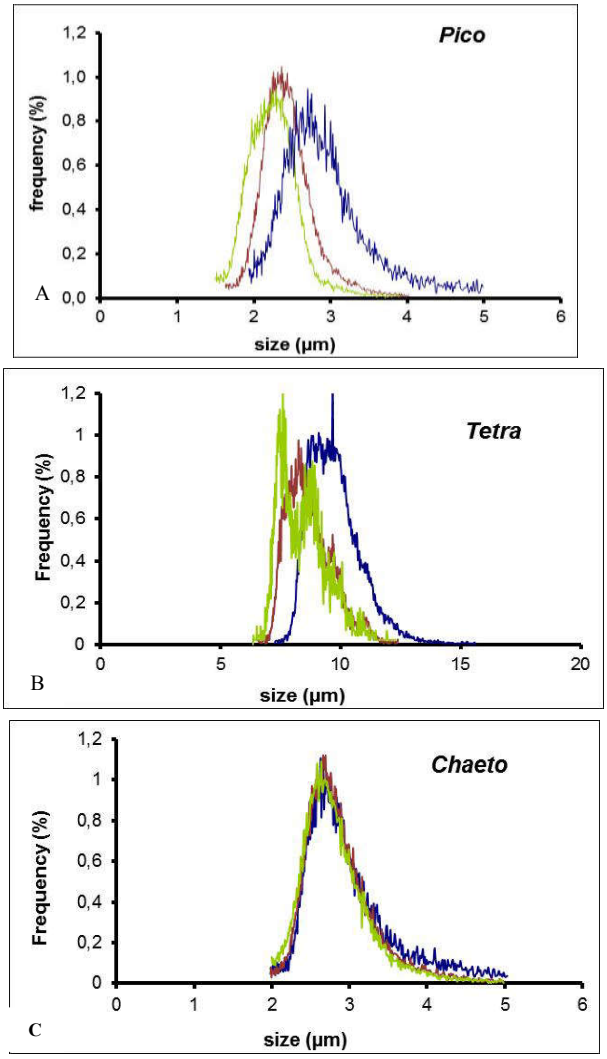

Figure 2 Evolution of cell size (equivalent cell size diameter) with time for *Picochlorum* sp. (Pico) "A", *Tetraselmis* sp. (Tetra) "B", and *Chaetoceros* sp.(Chaeto) "C", in 2 L batch culture on day 4 (blue) ; day 6 (red) and day 10 (green).

Proximate composition

Protein, carbohydrate and lipid were determined as relative contents in both growth phases. Protein levels remained constant between the two culture phases, with values ranging from ≈ 50 to 54, ≈ 39 to 45 and ≈ 42 to 46% of dry weight respectively for *Chaetoceros sp, Tetraselmis sp* and *Picochlorum sp* (Table 3). Similar trends were observed in lipid contents for *Tetraselmis sp* with values ranging from ≈ 11 to 12% of dry weight and *Chaetoceros sp* (15 to 18% of dry weight) except for *Picochlorum sp* exhibiting significant decrease in stationary phase (≈ 15 vs 20% of dry weight in exponential phase). For carbohydrate, values increased significantly from exponential to stationary phases for all strains, from ≈ 1 to 8% of dry weight for *Chaetoceros sp,*≈ 2 to 5% of dry weight for *Tetraselmis sp*and ≈ 2 to 8% of dry weight for *Picochlorum sp.*

Table 3 Mean relative biochemical composition of three isolated microalgae, cultured in 2 L batch culture in exponential (Exp) and stationary (Sta) phases; mean ±SD (n=3). The different letter (a/b) indicate a statistical difference between phase for each microalgae.

		Exp	Sta
Lipids (%)	*Chaetoceros sp.*	15.21±0.11(a)	18.52±0.64(a)
	Tetraselmis sp.	12.19±0.81(a)	11.72±0.22(a)
	Picochlorum sp.	20.13±0.21(a)	15.34±0.51(b)
Carbohydrates (%)	*Chaetoceros sp.*	1.14±0.05(a)	7.69±0.01(b)
	Tetraselmis sp.	2.09±0.03(a)	4.81±0.03(b)
	Picochlorum sp.	1.83±0.01(a)	8.11±0.06(b)
Proteins (%)	*Chaetoceros sp.*	53.58±2.08(a)	49.95±2.46(a)
	Tetraselmis sp.	44.77±0.73(a)	39.42±0.51(a)
	Picochlorum sp.	45.87±2.49(a)	41.80±0.60(a)

Fatty acids

Polyunsaturated fatty acid (PUFA) contents represent the major fraction of total fatty acid in exponential phase. However, a significant decrease of PUFA was observed with an increase of SFA (Saturated fatty acid) in stationary phase for the three isolated microalgae. MUFA content tended to increase significantly in stationary phase in *Tetraselmis sp* with no statically variation in *Chaetoceros sp* and *Picochlorum sp* (Table 4).

Data analysis, showed that the most important fraction of fatty acids in *Chaetoceros sp* were C14:0 (range 17-19% of totally fatty acid between growth phase), C16:0 (11-22%), C16:1n7 (23-27%), and EPA (10-19%). In *Tetraselmis sp,* the major fatty acids were C16:0 (22-26%), C18:1n9 (9-23%), C16:4n3 (8-15%), C18:3n3 (8-14%), C18:2n-6 (6-7%) C18:4n3 (4-9%), and EPA C20:5n3 (4-5%). High percentage of PUFA dominated by C16-C18 fraction was found in *Picochlorum Sp* (54-66 %). This microalga consists mainly of C16:0 (22-26%), C16:3n-3 (8-13%), C18:2n6 (15-24%), C18:3n3 (18-30%) and a marked deficiency of C20-C22 range PUFAs (Table 4).

Sterols

Sterol composition of *Picochlorum sp* consists mainly of stigmasterol (94% of total sterol in both growth phases: Table 5). For *Tetraselmis sp,* the major sterol was campesterol (range 71-73% between growth phases) and 24-methylene (25-27%). No significant changes in major sterols occurred between culture growth phases in green microalgae (Table 5); whereas the diatoms *Chaetoceros sp* exhibited more variations with 24-methylene (11 to 51%), isofucosterol (15 to 24%), cholesterol (14 to 20%), bistosterol (1 to 10%), fucosterol (8 to 18%). These sterols showed significant changes between phases with a decrease in 24-methylene from exponential to stationary phases and values increases for the others sterols (Table 5).

Molecular identification

Sequencing of Picochlorum sp 18S rRNA gene was performed (Fig. 3) because there are few studies on biochemical characteristics of this species and accordingly other approach was necessary.

AACCTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGT
ATAAACTGCTTTATACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTATTTGATGG
TACCTACTTACTCGGATACCCGTAGTAATTCTAGAGCTAATACGTGCGTACATCCCGACTTCTG
GAAGGGACGTATTTATTAGATAAAAGGCCGACCGGGCTTGCCCGACTCGCGGTGACTCATGAT
AACTTCACGAATCGCATGGCCTCGCGCCGGCGATGTTTCATTCAAATTTCTGCCCTATCAACTTT
TGATGGTAGGATAGAGGCCTACCATGGTGGTAACGGGTGACGGAGAATTAGGGTTCGATTCCG
GAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCA
ATCCTGACACAGGGAGGTAGTGACAATAAATAACAATACCGGGCCTTTGGTCTGGTAATTGGA
ATGAGTACAACCTAAACACCTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCG
CGGTAATTCCAGCTCCAATAGCGTATATTTAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATT
TCGGGTGGGGCCTGCCGGTCCGCCGTTTCGGTGTGCACTGGCCGGGCCCACCTTGTTGCCGGGG
ACGAGCTCCTGGGCTTTATTGTCCGGGACTCGGAGTCGGCGAGGTTACTTTGAGTAAATTAGAG
TGTTCAAAGCAGGCCACCGCTCTGAATACATTAGCATGGAATAACACGATAGGACTCTGGCCT
ATCTTGTTGGTCTGTAGGACCGGAGTAATGATTAAGAGGGACAGTCGGGGGCATTCGTATTTCA
TTGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAGGA
TGTTTTCATTAATCAAGAACGAAAGTTGGGGGCTCGAAGACGATTAGATACCGTCCTAGTCTCA
ACCATAAACGATGCCGACTAGGGATCGGCGGGTGTTTTTTTGATGACCCCGCCGGCACCTTATG
AGAAATCAAAGTTTTTGGGTTCCGGGGGGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTG
ACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAAACTTAC
CAGGTCCAGACATAGTGAGGATTGACAGATTGAGAGCTCTTTCTTGATTCtATGGGTGGTGGTG
CATGGCCGTTCTTAGTTGGTGGGTTGCCTTGTCAGGTTGATTCCGGTAACGAACGAGACCTCAG
CCTGCTAACTAGTCACGCGTGCTCCGGCACGCGGCGGACTTCTTAGAGGGACTATTGGCGACTA
GCCAATGGAAGCATGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCG
CGCTACACTGATGCATTCAACGAGCCTATCCTTGGCCGACAGGTCCGGGTAATCTTTGAATCTG
CATCGTGACGGGGATAGATTATTGCAATTATTAATCTTCAACGAGGAATGCCTAGTAGGCGCA
AGTCATCAGCTTGCGTCGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTCCTACCGATT
GCGTGTGCTGGTGAAATGTTCGGATTGGCGGCTTTGGGCGGTTTCCGCCCTCGGCTGTCGAAAA
GTTCATTAAACCCTCCCACCTAGAGGAAGGAGAAGTCGTAACAAGGTTTCCGTAGGTGAACCT
GCAGAAGGATACACTGATGCATTCAGCGAGTCGTCTCCCTTGACCGAGAGG7CCGGGTAATCTT
GTGAACTTGCATCGTGATGGGGATAGATTATTGCAACTATTAATCTTCAACGAGGAATTCCTAG
TAAGCGTGTGTCATCAGCGCACGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTC
CTACCGATTGAATGATCCGGTGAGGCCCCCGGACTGCtGCGCCGCCGCTGGTTCTCCAGCGCGG
CGTCGCGGGAAGCTGTCCGAACCTTATC

Figure 3 Sequence of of 18S rRNA gene for *Picochlorum* sp. strain.

The length of 18S rRNA gene sequence of Picochlorum sp was 2,064 bp.When compared to NCBI BLAST (Zheng *et al*.2000; Aleksander *et al*. 2008) the sequences exhibiting ≥ 95% of similarity were retained. The 18S rRNA gene phylogeny constructed by Neighbor joining (max sequence difference 0.3) indicated that this strain was Picochlorum (Fig. 4).

DISCUSSION

Microalgae play a key role in aquaculture hatchery; they are an important direct or indirect feed source for early developmental stages of many farmed finfish, shellfish and invertebrate species (Shields and Lupatsch 2012).

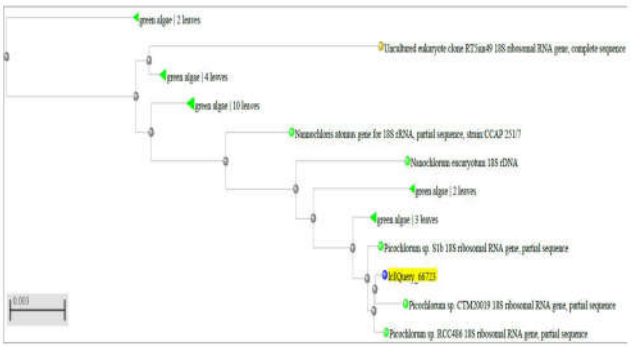

Figure 4 Phylogenetic tree of 18S rRNA of *Picochlorum sp*. (lcl Query_102991)

Table 4 Mean relative fatty acids composition of three isolated microalgae, cultured in 2 L batch culture in exponential (Exp) and stationary (Sta) phases; mean ±SD (n=3). The different letter (a/b) indicates a statistical difference.

	Chaetoceros sp.				*Picochlorum sp.*				*Tetraselmis sp.*			
	Exp		Sta		Exp		Sta		Exp		Sta	
14:0"	17.03	±0.28	19.90	±0.20	1.37	±0.14	0.61	±0.14	1.34	±0.06	0.76	±0.13
16:0"	11.47	±0.45	22.07	±1.08	24.61	±1.24	30.44	±0.53	22.22	±0.74	26.37	±1.30
18:0"	0.43	±0.02	0.42	±0.04	1.10	±0.20	1.17	±0.15	0.37	±0.01	0.59	±0.10
20:0"	0.05	±0.01	0.08	±0.01	0.04	±0.01	0.04	±0.01	0.01	±0.01	0.04	±0.02
22:0"	0.10	±0.02	0.09	±0.01	0.01	±0.01	0.02	±0.01				
24:0"	0.08	±0.01	0.13	±0.03	0.09	±0.02	0.05	±0.07	0.04	±0.01	0.05	±0.001
∑ SFA	**29.15**	**±0.80** (a)	**42.69**	**±1.38**(b)	**27.22**	**±1.44**(a)	**32.31**	**±0.91**(c)	**23.97**	**±0.84**(a)	**27.81**	**±1.56**(e)
16:1(n-9)"	0.32	±0.14	0.19	±0.14	1.10	±0.27	0.91	±0.12	1.39	±0.10	4.06	±0.24
16:1(n-7)"	23.82	±0.42	27.89	±0.83	0.36	±0.08	0.23	±0.01	0.34	±0.03	0.36	±0.03
16:1(n-13)"			1.05	±0.12	0.00	±0.01	0.00	±0.01	0.98	±0.001	0.32	±0.10
18:1(n-9)"	0.77	±0.01	0.84	±0.09	3.36	±0.10	7.37	±0.65	9.40	±1.07	23.28	±0.81
18:1(n-7)"	2.09	±0.01	0.89	±0.39	0.73	±0.74	0.11	±0.15	1.42	±1.72	2.93	±0.13
20:1(n-9)"	0.06	±0.01	0.02	±0.001	0.12	±0.02	0.15	±0.01	0.88	±0.17	1.78	±0.10
∑ MUFA	**27.06**	**±0.58**(c)	**30.88**	**±1.58**(c)	**5.66**	**±1.23**(b)	**8.76**	**±0.94**(b)	**14.40**	**±3.11**(a)	**32.72**	**±1.42**(e)
16:2(n-7)"	3.90	±0.24	0.98	±0.27	4.25	±0.01	3.17	±1.07				
16:2(n-6)"	0.02	±0.02	0.03	±0.01	1.94	±0.01	0.00	±0.01	0.17	±0.19	0.26	±0.14
16:2(n-4)"	3.85	±0.05	2.36	±0.05	0.02	±0.20	0.01	±0.01				
16:3(n-6)"									1.78	±0.02		
16:3(n-4)"	7.39	±0.05	3.70	±0.06	0.00	±0.01	0.00	±0.01	0.01	±0.01		
16:3(n-3)"					13.23	±0.15	8.34	±0.16				
16:4(n-3)"	0.27	±0.04	0.06	±0.07	0.52	±0.43	0.00	±0.01	15.08	±0.51	8.34	±0.62
16:4(n-1)"	1.17	±0.01	0.78	±0.01	0.08	±0.01	0.06	±0.01	0.03	±0.01		
18:2(n-6)"	0.50	±0.06	1.29	±0.15	15.60	±0.93	24.32	±0.27	7.52	±0.11	6.81	±0.19
18:2(n-4)"	0.02	±0.01	0.01	±0.01	0.00	±0.01	0.00	±0.01	0.02	±0.001		
18:3(n-6)"	0.33	±0.01	0.41	±0.02	0.00	±0.01	0.00	±0.01	0.36	±0.01	0.49	±0.03
18:3(n-3)"	0.10	±0.02	0.23	±0.02	30.05	±0.29	18.33	±1.14	14.75	±0.07	8.51	±0.66
18:4(n-3)"	1.92	±0.04	0.53	±0.01	0.14	±0.20	0.00	±0.01	9.83	±0.04	4.31	±0.08
18:5(n-3)"	0.02	±0.01	0.01	±0.01	0.05	±0.06	0.00	±0.01	2.88	±0.08	0.78	±0.04
20:4(n-6)"	0.25	±0.01	0.95	±0.08	0.00	±0.01	0.01	±0.01	0.47	±0.04	0.52	±0.04
20:4(n-3)"									0.70	±0.08	0.37	±0.001
20:5(n-3)"	19.06	±0.27	10.71	±0.42	0.11	±0.14	0.00	±0.01	5.33	±0.11	4.81	±0.52
22:5(n-6)"	0.05	±0.01	0.18	±0.01	0.02	±0.20	0.00	±0.01	0.01	±0.01	0.04	±0.01
22:5(n-3)"	0.08	±0.01	0.06	±0.01	0.00	±0.01	0.00	±0.01	0.05	±0.01	0.01	±0.01
22:6(n-3)"	1.64	±0.07	1.41	±0.16	0.09	±0.01	0.07	±0.01	0.05	±0.01	0.01	±0.01
∑ PUFA	**40.58**	**±0.95**(a)	**23.69**	**±1.39**(b)	**66.06**	**±2.30**(c)	**54.30**	**±2.70** (a)	**59.01**	**±1.68**(d)	**35.26**	**±2.39**(a)

Table 5 Sterol composition of three isolated microalgae, cultured in 2 L batch culture in exponential (Exp) and stationary (Sta) phases; mean ±SD (n=3);The different letter (a/b) indicate a statistical difference between phase for each microalgae.

	Picochlorum sp.				*Tetraselmis sp.*				*Chaetoceros sp.*			
	Exp		**Sta**		**Exp**		**Sta**		**Exp**		**Sta**	
Cholesterol	2.12	±0.39(a)	1.10	±0.11(a)	1.82	±0.31(a)	1.54	±0.29(a)	14.92	±0.39(b)	20.50	±0.14(f)
Desmosterol									2.37	±0.04		
Campesterol					71.05	±4.00(d)	73.18	±0.57(d)	6.20	±0.16(e)	13.40	±0.20(e)
24-Methylene					27.13	±3.68(c)	25.28	±0.86(c)	51.15	±0.22(a)	11.36	±1.25(b)
Stigmasterol	93.98	±0.55(a)	94.55	±0.17(a)							2.39	±0.09
Bisto									1.63	±0.2(a)	10.11	±1.42(b)
Fucosterol									8.59	±0.1(a)	18.10	±0.51(b)
Isofucosterol									15.15	±0.04(a)	24.14	±0.66(b)

The choice of microalgae in aquaculture hatchery is based on practical considerations of strain availability, ease of culture, growth rate and nutritional composition (Shields and Lupatsch 2012). In our study, the cellular concentrations of the threemicroalgaeexhibitednoticeable variations between species. The highest densities were associated with twolocal strains*Picochlorum sp* and *Chaetoceros sp*, these variations being probably related to their small sizes as pointed out by Phatarpekar *et al*. (2000). The growth rate µ (division per day) ranged from 0.2 and 0.72 and could be considered as satisfying. Thus, Renaud *et al*. (1999) and Martínez-Fernández *et al*. (2006) indicated that these rates varied from 0.4 to 0.7 for Diatoms and from 0.2-0.5 for Prasinophyceae.

Dry weights, corresponding to the exponential and stationary growth phases, were measured for each strain, to determine their relative biochemical content. With regard to dry weight, microalgae yield seemed to be appropriate with respect to commonly used strains in aquaculture with range from 8 to 292 pg cell^{-1} in literature (Lavens and Sorgeloos 1996; Ponis *et al*. 2006).

Determination of cell size is important for identify suitable species for larval nutrition in shellfish hatchery. Thus, difficulties in some French oyster hatcheries due to the use of inappropriate size microalgae for larval rearing in early stages have been reported (Robert and Trintignac 1997 b). In the present study, the three microalgae cell size ranged from 2.5 to 13 µm and is accordingly in agreement with the data reported

in the literature for filter feeders (1 to 15 µm : Riisgard *et al*. 1980; Baldwin and Newell 1991; Fritz *et al*. 1984; Priyadarshani *et al*. 2012).

The relative biochemical composition of the three microalgae varied between 41 to 53% for proteins, 12-20% for lipids and 1-2% for carbohydrates. Generally, microalgae used in aquaculture, contain over 25% proteins, 10 to 30% lipids and 5 to 30% carbohydrates (Lavens and Sorgeloos 1996; Renaud *et al*. 1999; Volkman and Brown 2006). However, for biochemical composition, literature comparison makes sense only in identical culture condition. The changes in carbohydrate contents might be explained by a limitation of nitrate during the stationary culture phase, which leads to an increase of sugars to the detriment of proteins (Harison *et al*. 1990; Brown *et al*. 1993).

The nature and profile of fatty acids in microalgae have been intensively described in the literature under many conditions (Renaud *et al*. 1999; Pernet *et al*. 2003; Lang *et al*. 2011). The correlation between microalgae PUFA composition and nutritional values has been established (Robert *et al*. 2001; Ponis *et al*. 2008; Sung-Suk *et al*. 2015). Gudes and Malcata (2012) indicated that the fatty acid contents of microalgae exhibit systematic differences according to taxonomic group. In this work, result showed that the most important fraction of fatty acids in *Chaetoceros sp* were C14:0, C16:0 and C16:1n7. This microalga also included a moderate amount of DHA. This composition is typically characteristic of diatoms. Indeed, Volkman *et al*. (1989), indicate that in most diatoms these four components represent up to 60% of total fatty acids with low proportions of C18 and C22. For *Tetraselmis sp*, the major fatty acids were essentially C16:0, C18:1n9, C16:4n3 and EPA C20:5n3 (4-5%). These results are closed to those previously reported (Zhukova and Aizdaicher 1995; Pratoomyot *et al*. 2005). These authors agree that Prasinophyceae contain C16:0, C18:1n9 and C18:3n3 as main fatty acids. Moreover, they exhibit moderate amount of EPA, C20:5n3. High percentage of PUFA dominated by C16-C18 fraction was found in *Picochlorum sp*. This microalga consists mainly of C16:0, C16:3n-3, C18:2n6, C18:3n3 and a marked deficiency of C20-C22 range PUFAs. The nutritional value of this microalgae is thus arguable: with a lack of EPA and DHA, known as crucial in aquaculture feed (Thompson *et al*. 1996; Glencross 2009; Da Costa *et al*. 2015) and high proportion of two essential fatty acids, C18:3n3 and Cl8:2n6, it would be very informative to test their larval feeding effect in monospecific and mixture algal diets. The composition of fatty acids in *Picochlorum sp* are similar as that described for most green microalgae; dominated by C16 and C18 PUFA (Pratoomyot *et al*. 2005; Widianingsih *et al*. 2013; Sung-Suk *et al*. 2015). However C18:3n-3 was more abundant in *Picochlorum sp* comparatively to other green algal species reported in the literature.

Many authors highlighted the importance of sterol composition of microalgae for nutrition of bivalve (Teshima *et al*. 1974; Wikforsand Ohno1996; Soudant *et al*. 1996). Sterol composition of *Picochlorum sp* mainly consists in stigmasterol whereas for *Tetraselmis sp*, the major sterols were campesterol and 24-methylene. In contrast, *Chaetoceros sp* showed a more diversify profile with 24-methylene, isofucosterol, cholesterol, bistosterol and fucosterol. Generally microalgae have wide variety of sterol structures and synthetic pathways (Villanueva *et al*. 2014). Sterol profiles can be characteristic of a particular class, family, genus, or even species of microalgae, and so are often used for chemotaxonomic and phylogenetic comparisons (Volkman 1986; Ponomarenko *et al*. 2004). However, changes in sterol composition may also result from fluctuations in environmental and growing conditions (Rampen *et al*. 2009). In the present study, qualitative sterol profile of *Chaetoceros sp* is consistent with that reported by Rampen *et al*. (2010) in batch cultures growing conditions. Similarly *Tetraselmis sp*sterol composition is in accordance with the data of Fabregas *et al*. (1997). Few studies have been devoted to *Picochlorum* species. However, its high stigmasterol content has to be pointed out because such characteristic raises question. Indeed, in the one hand, such content was not found in any of the 14 microalgae commonly used in aquaculture (Robert *et al*. 2004) and in the other hand, this compound is considered as a molecule of therapeutic interest (Gabay*et al*. 2010; Ghosh *et al*. 2011) and its potential role in aquaculture should be accordingly support by further research.

CONCLUSION

In this study we evaluated the nutritional value of three isolated microalgae in Tunisia for its possible use in aquaculture. Selection was performed according to tow criteria: bioavailability of these species on the natural reproductive sites of bivalves and their suitable size for larval rearing. Biochemical composition allowed us to correlate two species profiles among the three isolated microalgae: a diatom *Chaetoceros sp* and Prasinophyceae *Tetraselmis sp*. Biochemical composition and both microalgae growth revealed their potential use in bivalve hatchery. The relatively small size of *Chaetoceros sp* is a great potential interest, particularly for the rearing of early larval stages for which the size of preys is highly limiting. For the third microalgae molecular identification was required for its classification in *Picochlorum sp*. This microalga exhibited contrasting information on an aquaculture point of view: relatively small (+), C20 deficiency (-) and high content of stigmasterol. It would be interesting to investigate its nutritious value in aquaculture and its potential for human health.

Acknowledgments

The first author is thankful toDr R. ROBERT, searcher in IFREMER for hospitality and assistance for the realization of this work.

References

Aleksander M, George C, Yan R, Thomas LM, Richa A, Alejandro AS (2008) "Database Indexing for Production Mega BLAST Searches". Bioinformatics 24:1757-1764

Baldwin BS, Newell R (1991) Omnivorous feeding by planktotrophic larvae of the Eastern oyster Crassostreavirginica. Marine Ecology. Progress Series 78: 285-301

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram. Anal. Biochem 72: 248– 254

Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture. Aquaculture 151: 315-331

Brown MR, Garland CD, Jeffrey SW, Jameson ID, Leroi JM (1993) the gross and amino acid compositions of batch and semi-continuous cultures of *Isochrysis sp.* (clone T.ISO), Pavlova lutheri and Nannochloropsisoculata. *J ApplPhycol* 5: 285-296.

Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: A review of enclosed system designs and performances. Biotechnol. Prog 22:1490-1506

Costard GS, Machado RR, Barbarino E, Martino RC, Lourenço SO (2012) Chemical composition of five marine microalgae that occur on the Brazilian coast. Int. *J. Fish. Aquac* 4 (9): 191-201

Da Costa GF, Robert R, Quere C, Wikfors GH, Soudant P (2015) Essential Fatty Acid Assimilation and Synthesis in Larvae of the Bivalve *Crassostreagigas*. Lipids 50(5): 503-511

El Abed MM, Marzouk B, Medhioub MN, Helal AN, Medhioub A (2008) Microalgae: A Potential Source of Polyunsaturated Fatty Acids. Nutr Health 19: 221-226

Epifanio CE (1979) Growth in bivalve mollusc: nutritional effect of two or more species of algae in diets fed to the American oyster *CRASSOSTREA VIRGINICA* (GMELIN) and the hard clam *MERCENARIA MERCENARIA*(L.). Aquaculture 18: l-12

Fabregas J, Aran J, Morales ED, Lamela T, Otero A (1997) Modification of Sterol concentration in marine microalgae. Phytochemistry 46(7):1189-1191

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem* 266: 497-509.

Fritz LW, Lutz RA, Foote MA, Van Dover CL, Ewart JW (1984) Selective Feeding and grazing rates of oyster (*Crassostreamrgillica*) larvae on natural phytoplankton assemblages. Estuaries, 7(413): 513-518.

Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, Wolf C, Jacques C, Berenbaum F (2010) Stigmasterol: a phytosterol with potential anti-osteoarthritic properties, Osteoarthr Cartilage 18:106-116

Ghosh T, Maity TK, Singh J (2011) Evaluation of antitumor activity of stigmasterol, a constituent isolated from *Bacopamonnieri* (Linn) aerial parts against Ehrlich ascites carcinoma in mice. Orient Pharm Exp Med 11:41–49

Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture 764 species. Reviews in Aquaculture 1:71–124

Guedes AC, Malcata FX (2012) Nutritional Value and Uses of Microalgae in Aquaculture. Aquaculture, Dr. Zainal Muchlisin (Ed.).Doi: 10.5772/30576

Harrison PJ, Thompson PA, Calderwood GS (1990) Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *J ApplPhycol* 2: 45–56

Laing I, Child AR, Janke A (1990) Nutritional value of dried algal diets for larvae of Manila clam (*Tapes philippinarum*). *J. Mar. Biol. Assoc*. U. K. 70: 1 – 12

Lang I, Ladislav H, Thomas F, Ivo F (2011) Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology 11:124

Langdon C, Onal E (1999) Replacement of living microalgae with spray-dried diets for the marine mussel *Mytilusgalloprovincialis*. Aquaculture 180: 283– 294.

Langdon CJ, Waldock MJ (1981) the effect of algal and artificial diets on the growth and fatty acid composition of *Crassostreagigas*. *J. Mar. Biol. Assoc*. U. K. 61: 431-448.

Lavens P, Sorgeloos P (1996) Manual on the production and use of live food for aquaculture FAO Fisheries Technical Paper

Loor A, Daniel O, César L, Stanislaus S (2016) Early life cycle and effects of microalgal diets on larval development of the spiny rock-scallop, *Spondyluslimbatus* (Sower by II, 1847). Aquaculture 450: 328–334

Loubiere K, Olivo E, Bougaran G, Pruvost J, Robert R, Legrand J (2009) A New Photobioreactor for Continuous Microalgal Production in Hatcheries Based on External-Loop Airlift and Swirling Flow. Biotechnol Bioeng 102(1), 132-147.

Martínez-Fernández E, Acosta-Salmón H, Southgate PC (2006) The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctadamargaritifera*, L.) larvae. Aquaculture 257 (1–4) :491-503

Marty Y (1976) Méthode de dosage des acides gras libres dissous dans l'eau de mer. Application à l'étude de leurs variations dans les eaux de la rade de Brest. Thèse de spécialité, Université de Bretagne Occidentale

Marty Y, Delaunay F, Moal J, Samain JF (1992) Changes in the fatty acid composition of *Pecten maximus* (L.) during larval development. *J Exp Mar Biolecol1* 63: 221–234

Minh TTV, Claire Do, Rayner TA, Thoisen C, Laurentius SN, Hansen BW (2015) Optimization

of photosynthesis, growth, and biochemical composition of the microalga *Rhodomonassalina*—an established diet for live feed copepods in aquaculture. *J Appl Phycol*. DOI 10.1007/s10811-015-0722-2 accepted: 24 September 2015

Muller-Feuga (1997) Microalgues marines «Les enjeux de la recherche», IFREMER

Muller-Feuga A, Robert R, Cahu C, Robin J, Divanach P (2003) Uses of microalgae in aquaculture. In: Støttrup, J.G., McEvoy, L. A. (Eds.), Live Feeds in Marine Aquaculture. Blackwell, Oxford, pp. 253–299

Pernet F, Tremblay R, Dermers E, Roussy M (2003) Variation of lipid class and fatty acid composition of *Chaetoceros mulleri* and *Isochrysis sp.* Grown in semicontinuous system. Aquaculture 211: 393-406

Phatarpekar P.V, Sreepada RA, Pednekar C, Achuthankutty CT (2000) Acomparative study on growth performance and biochemical composition of mixed culture of *Isochrysisgalbana* and *Chaetoceros calcitran*s with monocultures. Aquaculture 181:141-155

Ponis E, Probert I, Véron B, Mathieu M, Robert R (2006) New microalgae for the Pacific oyster *Crassostreagigas* larvae, Aquaculture 253(1-4): 618-627

Ponis E, Parisi G, Chini ZG, Lavista F, Robert R, Tredici MR (2008) *Pavlova lutheri*: Production, preservation and use as food for *Crassostreagigas* larvae. Aquaculture 282: 97–103

Ponomarenko LP, Stonik IV, Aizdaicher NA, OrlovaTYu, Popovskaya GI, Pomazkina GV, Stonik VA (2004) Sterols of marine microalgae *Pyramimonascf.cordata* (Prasinophyta), *Attheyaussurensis sp. nov*. (Bacillariophyta) and a spring diatom bloom from Lake Baikal. Comp BiochemPhysiol Part B 138: 65–70

Pratoomyot J, Srivilas P, Noiraksar T (2005) Fatty acids composition of 10 microalgal species. Songklanakarin *J. Sci. Technol*, 27(6): 1179-1187

Priyadarshani I, Sahu D, Rath B (2012). Algae in Aquaculture. *International Journal of Health Sciences & Research* 2(1):108-113

Rampen SW, Abbas BA, Schouten S, Damste JS (2010) A comprehensive study of sterols in marine diatoms (Bacillariophyta): Implications for their use as tracers for diatom productivity. Limnol. Oceanogr 55(1):91–105

Rampen SW, Schouten S, Schefuß E, SinninghеDamsté JS (2009) Impact of temperature on long chain diol and mid-chain hydroxyl methyl alkanoate composition in Probosciadiatoms: results from culture and field studies. Org Geochem 40: 1124–1131

Renaud MS, Thinh L, Parry DL (1999) the gross composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. Aquaculture, 170: 147-159.

Rico Villa B, Le Coz JR, Mingant C, Robert R (2006) Influence of phytoplankton diet mixtures on microalgae consumption, larval development and settlement of the Pacific oyster *Crassostreagigas* (Thunberg). Aquaculture256 (1-4):377-388

Riisgard HU, Randlov A, Kristensen PS (1980) Rates of water processing, oxygen comsumption and efficiency of particle retention in veligers and young postmetamorphic*M. edulis*. Ophelia 19: 37-47

Robert R, Trintignac P (1997a). Substitutes for live microalgae in mariculture: a review. Aquatic Living Ressource 10: 315–327

Robert R, Trintignac P (1997b) Microalgues et nutrition larvaire en écloserie de mollusques. Haliotis, 26:1-13.

Robert R, Chrétiennot-Dinet MJ, Kaas R, Martin-Jézéquel V, Moal J, Le Coz JR., Nicolas JL, Bernard E, Connan JP, Le Dean L, Gourrierec GLB, Quéré C (2004) Amélioration des productions phytoplanctoniques en écloserie de mollusques: caractérisation des microalgues fourrage. IFREMER, RI DRV/RA-2004-05

Robert R, Parisi G, Rodolfi L, Poli BM, Tredici MR (2001) Use of fresh and preserved *Tetraselmis suecica* for feeding *Crassostreagigas* larvae. Aquaculture 192:333–346

Shields, Lupatsch (2012).Algae for Aquaculture and Animal Feeds. Schwerpunkt: Challenges and Perspectives of Microalgae, TATuP - Journal by ITAS on Technology Assessment, Nr. 1, 21. Jahrgang, S. 23-37

Sialve B, Steyer JP (2013) Les microalgues, promesses et défis. Innovations Agronomiques 26: 25-39

Soudant P, Marty Y, Moal J, Robert R, Quéré C, Le Coz JR, Samain JF (1996) Effect of food fatty acid and sterol quality on *Pecten maximus* gonad composition and reproduction process. Aquaculture 143:361-378

Sung-Suk S, So JK, Jinik H, MP, Taek-Kyun L, Eui-Joon K, Sukchan L (2015) Fatty acid methyl ester profiles and nutritive values of 20 marine microalgae in Korea. *Asian Pacific Journal of Tropical Medicine*, 191-196

Teshima S, Kanazawa A (1974) Biosynthesis of sterols in abalone *Haliotisgurneri* and *mussel Mytilus edulis*. Comp. Biochem. Physiol. 43(B):555–561.

Thompson PA, Guo M, Harrison PJ (1996) Nutritional value of diets that vary in fatty acid composition for larval Pacific oyster (*Crassostreagigas*). Aquaculture 143:379-391.

Villanueva L, Irene WCR, Schouten S, Sinninghe DJS (2014) Genetic biomarkers of the sterol-biosynthetic pathway in microalgae. Environ Microbiol Rep 6(1):35–44

Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J Exp Mur BioiEcol* 128: 219-240

Volkman JK, Brown MR (2006) Nutritional value of microalgae and applications. Algal cultures, analogues of blooms and applications. 1:407-457

Volkman JK (1986) A review of sterol markers for marine and terrigenous organic matter. Org Geochem 9:83–99

WidianingsihRH, HadiE, Jane M (2013) Fatty acid composition of marine microalgae in Indonesia. Journal of tropical biology and conservation 10: 75-82, 2013

Wikfors GH, Ohno M (2001). Impact of algal research in aquaculture. *J Phycol* 37: 968-974

Young-Sang K, Xi-Feng L, Kyong-Hwa K, BoMi R, Se KK (2014) Stigmasterol isolated from marine microalgae *Naviculaincerta* induces apoptosis in human hepatoma HepG2 cells. BMB Rep 47(8): 433-438

Zar, JH (1999) Biostatistical analysis. 4ème edt, Prentice Hall International Ed., London

Zheng Z, Scott S, Lukas W, Webb M (2000) A greedy algorithm for aligning DNA sequences. *J ComputBiol* 7(1-2):203-14

Zhukova NV, Aizaicher NA (1995) Fatty acid composition of 15 species of marine microalgae. Phytochemistry 39(2):351-356

****

How to cite this article:

EL AbedMehdi *et al*. 2016, Prospective Study on New Microalgae "Tetraselmis sp., Picochlorum Sp., and Chaetoceros sp" Isolated from Coastal Waters of Tunisia As Promising Animal Food: Growth And Biochemical Composition. *Int J Recent Sci Res.* 7(10), pp. 13930-13938.