PIGMENTARY POTENTIAL OF VARIOUS INLAND WATER MICROALGAE AND THEIR APPLICATIONS

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ملخص

الطَّاقة الصبغيّة لعدد من طحالب المياه الدَّاخلية واستخداماتها : هناك طلب متزايد في الوقت الحاضر على المنتجات الطبيعية المتأتية من النباتات الدقيقة مثل الأصباغ الطبيعية، الملوّنات، مضادات الأكسدة و الفيتامينات من أجل الاستخدام التجاري سواء في مجال الصناعات الغذائية أو في مجالات حيويّة أخرى كقطاع المستحضرات الصيدلانية، والمغذيات، ومستحضرات التجميل. في هذا الإطار قمنا بإجراء بحث تحليلي دقيق على إحدى عشرة (11) سلالة من النباتات الدقيقة التي تمّ عزلها من مياه داخلية تابعة لعدّة مسطحات مائية مختلفة بالبلاد التونسية و ذلك بغرض تحديد و انتقاء الأنواع و السلالات التي تتميّز بقدرتها على إنتاج و تخزين مستويات مهمّة من الأصباغ الطبيعية مثل الفيكوبيليبروتينات و الكاروتينات و اليخضور باعتبارها جزيئات ثمينة. عشرة من هذه السلالات موضوع البحث تنتمي إلى مجموعة الزّراقم وواحدة تنتمي إلى الأشنيات الخضراء. نتائج هذه الدراسة بيّنت أن نوع الزّراقم سيلندروسبارموبسيس راسيبورسكي (*Cylindrospermopsis raciborskii*) (Cyl-NB-05)، يمكن اعتباره خزّان طبيعي غني جدا بالفيكوسيانين (Phycocyanin) بمقدار يصل إلى 2.87 مغ/مل و ذلك بمعدّل يفوق بعشرة أضعاف كاملة بقية أنواع الفيكوبيليبروتينات الأخرى مجتمعة (الألوفيكوسيانين و الفيكوئريترين) و كذلك نحو ضعفي مقدار الفيكوسيانين مقارنة ببقية السلالات العشرة الأخرى مجتمعة. هذه النتيجة هي ذات أهميّة خاصّة بالنسبة للقطاعات الطبية و الصيدلانية باعتبار الخصائص الوقائية و العلاجية المفيدة جدا للصحة التي تعزى إلى هذه الصنف من الفيكوبيليبروتينات بما في ذلك تأثيره المضاد للسرطان و للالتهابات ،و الحامي للأعصاب والكبد كما هو مبيّن في العديد من الدراسات. من جهة أخرى، بيّنت نتائج هذه الدراسة أيضا أنّ الأشنيّة الخضراء وحيدة الخليّة، ديناليلّا غ م (*Dunaliella sp.*) كانت السلالة الأعلى إنتاجا لأربعة من بين أصناف الأصباغ الطبيعية التي تمّ تحليلها في إطار هذه الدراسة و هم الألوفيكوسيانين، الكاروتينات فضلا عن اليخضور صنف 'أ' و 'ب'. و بالتّالي، فإنّ هذه السلالة تمثّل مصدرا ممتازا لهذه الأصناف من الأصباغ الطبيعية القيّمة للغاية باعتبار دورها في الوقاية من العديد من الأمراض المزمنة و الأمراض التنكسيّة خاصّة أمراض القلب والأوعية الدموية والسكري وترقق العظام بالإضافة إلى العديد من أنواع السرطان (الرئة والبروستات والقولون والثدي والجهاز الهضمي وعنق الرحم والمبيض والبنكرياس) كما ثبت في العديد من الدراسات الوبائية.

الكلمات المفاتيح : نباتات الدقيقة، فيكوبيليبروتينات، كاروتينات، يخضور، مياه داخلية.

RÉSUMÉ

Potentiel pigmentaire de diverses micro-algues isolées à partir des eaux continentales et leurs applications: De nos jours, il existe une demande croissante pour les produits naturels issus des microalgues comme les pigments, les colorants, les antioxydants et les vitamines en vue d'un usage commercial en industrie alimentaire ainsi que dans les secteurs pharmaceutique, nutraceutique et cosmétique. Dans ce contexte, des investigations ont été menées sur 11 souches de microalgues isolées à partir de différents habitats aquatiques afin d'identifier et de sélectionner les souches présentant des niveaux intéressants de production et d'accumulation des pigments naturels (phycobiliprotéines, caroténoïdes, chlorophylles) en tant que molécules à haute valeur ajoutée. Parmi ces souches, dix appartiennent au phylum des cyanobactéries (Oscillatoriales, Nostocales et Chroococcales) et une à la classe des Chlorophyceae (Chlamydomonadales). Les résultats ont montré que la cyanobactérie *Cylindrospermopsis raciborskii* se révélait être un réservoir naturel très riche en C-phycocyanine (PC) avec une teneur atteignant 2,87 mg/ml, soit dix fois plus élevée que celles de l'allophycocyanine et de la phycoérythrine de la même souche. Elle présente également une teneur deux fois plus élevée que celles des 10 autres souches investiguées. Ce résultat présente un intérêt particulier pour les domaines thérapeutiques et pharmaceutiques. En effet ce groupe de phycobiliprotéines présente des propriétés extrêmement bénéfiques pour la santé y compris leurs effets anticancéreux, anti-inflammatoires, neuroprotecteurs et hépatoprotecteurs tels que mis en évidence dans de nombreuses études. D'autre part, les résultats ont également indiqué que la microalgue verte unicellulaire, *Dunaliella sp.*, était la souche la plus productrice de quatre parmi l'ensemble des pigments analysés dans la présente étude (allophycocyanine, caroténoïdes et les deux formes de chlorophylle a et b). Cette souche représente une excellente source de ces pigments très précieux vu leur rôle dans la prévention de nombreuses maladies chroniques et dégénératives telles que les maladies cardiovasculaires, le diabète, l'ostéoporose ainsi que plusieurs types de cancer (poumon, prostate, côlon, sein, col de l'utérus, ovaire, pancréas) comme ça était prouvé dans plusieurs études épidémiologiques.

Mots clés : microalgues; phycobiliprotéines; caroténoïdes; chlorophylles; eaux continentales.

ABSTRACT

Nowadays there is an increasing demand for microalgal natural products, such as pigments, colorants, antioxidants and vitamins, for commercial use in the food and feed industry as well as in the pharmaceutical, nutraceutical and cosmetic sectors. Thereby, in order to select candidate strains with interesting accumulation levels of pigments (phycobiliproteins, carotenoids, chlorophylls) as valuable molecules, investigations were carried out and focused on 11 inland water microalgal strains isolated from different aquatic habitats. Ten strains belonged to cyanobacteria (Oscillatoriales, Nostocales and Chroococcales) and one to Chlorophyceae (Chlamydomonadales). Results showed that the cyanobacterium *Cylindrospermopsis raciborskii*, was found to be a very rich natural reservoir of C-phycocyanin (PC) with an amount reaching to 2.87 mg/ml. This level is ten-folds higher than the other two phycobiliprotein groups combined (Allophycocyanin and Phycoerythrin) and about two-folds higher compared with the PC amount in all the 10other strains together. This result is of particular interest for the therapeutic and pharmacological fields given the extremely health beneficial properties attributed to this phycobiliproteins group including anticancer, anti-inflammatory, neuroprotective and hepatoprotective effects as highlighted in many studies. On the other hand, results also indicated that the unicellular green microalgae, *Dunaliella sp*., was the top producer strain of four among the pigments analyzed in the present study (Allophycocyanin, total carotenoids as well as the two forms of chlorophyll a and b). This strain represents, thus, an excellent source of these very valuable pigments regarding their role in the prevention of numerous chronic and degenerative diseases such as cardiovascular diseases, diabetes, osteoporosis and several types of cancer (Lung, prostate, colon, breast, gastrointestinal, cervical, ovarian and pancreatic cancers) as proved in several epidemiological studies.

Keywords: microalgae; phycobiliproteins; carotenoids; chlorophylls; inland waters.

INTRODUCTION

Microalgae are photosynthetic microscopic phototrophs that exhibit a huge genetic diversity; they may appear as individual cells, complex, three-dimensional colonies or extended filaments. While unicellular types exist as single cells, suspended or benthic, or aggregates, filamentous types may be thin or thick, single trichome or bundles either with or without a sheath (Abed et al., 2009; Bellinger and Sigee, 2010). Some species of a particular group, cyanophyceae, have evolved specialized cells, such as heterocysts for atmospheric nitrogen fixation and akinetes for survival in stressed conditions (Gupta et al., 2013). They are ubiquitously distributed throughout the biosphere given their exceptional ecological plasticity and can be found in almost every conceivable habitat on earth ranging from freshwater to extreme salinity, and can proliferate in moist, black earth, ice, thermal springs, ferruginous waters, acidic peat bogs, carbonate waters and even desert sands (Couté and Bernard, 2001). Their diverse morphological, physiological and biochemical properties allow them to spectacularly adapt to a wide spectrum of environmental stresses such as heat, cold, drought, salinity, photo-oxidation, anaerobiosis, osmotic pressure and UV exposure (Samarakoon and Jeon, 2012). In addition, they are known to be capable of performing different modes of metabolism with the possibility to switch from one mode to another (Stal, 1995). For example, all microalgae carry out oxygenic photosynthesis but some cyanobacterial species can switch to the typical bacterial anoxygenic photosynthesis using sulfide as electron donor (Cohen et al., 1986). They are also known to carry out fermentation during the dark and under anoxic conditions (Stal and Moezelaar, 1997).

During the last decades, there has been a high interest in the cultivation of microalgae as a sustainable natural source of high value, naturally synthesized products that can be valued in food and pharmaceutical industry (Lamers et al., 2010). Aside from being identified as a rich source of biologically active compounds with antiviral, antibacterial and anticancer activities, microalgae are also used in aquaculture, wastewater treatment, food, fertilizers, production of secondary metabolites including exo-polysaccharides, vitamins, toxins, enzymes and depsipeptides (Mimouni et al., 2012). In addition, microalgae are considered as some of the most promising stakeholders in blue biotechnology: besides their notable metabolic versatility, they utilize commonly available material for growth and require only low cost inorganic N- and P-sources, sequester CO2 as base nutrient, and rely on sunlight to fulfill energy requirements (Amaro et al., 2011; Jalal et al., 2013). Besides, they are among the fastest growing autotrophs on earth with spectacularly high productivity levels and can be produced in controlled conditions with a low cost due to their ability to grow in a wide variety of environments favors to an exceptional biochemical production (Moreau et al., 2006).

Phycobiliproteins and carotenoids are among the most valuable accessory pigments that can be extracted from microalgae given their extremely health beneficial effects. They are largely considered as potent functional ingredients with very interesting applications in various fields such as agro-food,

aquaculture, biotechnology, medicine and pharmacy (Lamers et al., 2010; Jalal et al., 2013).

Phycobiliproteinsare the principal photoreceptor for photosynthesis in cyanobacteria, red algae, and cryptomonads (Glazer, 1994). These water soluble fluorescent proteins are used as accessory or antenna pigments for photosynthetic light collection which absorb energy in portions of the visible spectrum (450–650 nm). They are classified into three main groups depending on the inherent color and absorbance properties: C-phycocyanin (dark cobalt blue, λmax = 610 − 620 nm), allophycocyanin (brighter aqua blue, λmax = 650 − 655 nm) and phycoerythrin (bright pink, λmax = 540 − 570 nm) (Eriksen, 2008). In many algae, phycobiliproteins are arranged in subcellular structures called phycobilisomes, which allow the pigments to be arranged geometrically in a manner which helps to optimize the capture of light and transfer of energy. This latest feature appears to be of a great practical usefulness since it enables them to use a large portion of the solar spectrum and to grow even at low light intensities(Pangestuti and kim, 2011).

Carotenoids are linear C40 polyene backbone with conjugated double bonds that function as light energy harvesters. These essential structural components of the photosynthetic antenna are considered as accessory pigments since they augment the light-harvesting properties of algae by passing on light excitation to chlorophyll (Larkum and Kuhl, 2005). They are largely considered as essential components of the human diet as they are precursors for vitamin A biosynthesis and have antioxidant functions that inactivate reactive oxygen species (ROS) formed by exposure to light and air (Fraser and Bramley, 2004; Murthy et al., 2005). More than 600 naturally occurring carotenoids, widely distributed in plants, animals, and micro-organisms, have been identified and they can be classified into two types: carotenes, which are unsaturated hydrocarbons; and xanthophylls, which present one or more functional groups containing oxygen (Fraser and Bramley, 2004).

As for chlorophylls, they are a group of greenish lipid-soluble pigments which contain a porphyrin ring and found in all photosynthetic organisms from prokaryotic unicellular algae to the higher plants. These photosensitive light harvesting pigments with special electronic properties occur in microalgae mainly in four kinds; the first and most important is chlorophyll a which is an essential compound for photosynthesis absorbs most energy from wavelength of violet blue and orange-red light. The other chlorophyll types are chlorophylls b c and d. The later type was the last to be discovered and was found in red algae (Rhodophyta) over 60 years ago. It was considered to be accessory chlorophyll but now better known as light-harvesting chlorophyll and it can be formed artifactually from chlorophyll a (Jackson, 1976 ; Larkum and Kuhl, 2005).

Currently, the increasing demand for functional foods and nutraceuticals had also triggered a growing interest in the use of microalgae as naturally occurring sources of high value added products, particularly phycobiliproteins and carotenoids, in the food industry globally, which automatically had made them sky-scraping in demand in global market of human health, nutrition and aquaculture (Jalal et al., 2013; Yang et al., 2013). Whoever, its broad industrial application still requires studies to isolate and characterize more candidate strains from various terrestrial and marine environments in order to select the ones with interesting accumulation levels of these valuable molecules along with exceptional growth performance to ensure maximum production yields of the desired products and meet the constantly unceasing market demand (Abed et al., 2009).

Therefore, owing the importance of phycobiliproteins and carotenoids, the present work was conducted to investigate11 microalgal candidate strains, from different classes and orders, sampled and isolated from several Tunisian inland waters, in terms of production and accumulation of these accessory pigments as well as to determine their growth parameters expressed as specific growth rates and generation times. This study aimed to analyze their pigmentary potential and identify the candidate taxa with outstanding profiles in order to be selected for possible consideration as interesting natural sources of these high value added molecules.

MATERIALS AND METHODS

Algal culture, media and cultivation conditions

The microalgal strains investigated in the present work were collected from eight different Tunisian inland waters (river, lagoon, dam reservoir, spring water) located in northern, central and southern of the country (table I). After being sampled and taken to the laboratory, the strains were isolated by capillary isolation on liquid medium and using an inverted microscope (Leica microsystems, Wetzlar, Germany). For each strain, a single individual was isolated (a single cell, a colony or a filament) in 300 µl of the appropriate culture medium and the growing was carried out by scaling-up culture volumes gradually from the initial isolation to the final batch. All strains were cultivated in 2 L volume laboratory flasks using monospecific batch culture system under sterile conditions. Growing was carried outat a temperature of 25 ± 1 °C in a thermostatically controlled room and using BG11 (Rippka et al., 1979) or CONWAY (Blancheton, 1985) mediums depending on the origin of each strain (table I). Culture mediums were sterilized by autoclaving at 120 °C for 20 min before use. All isolates were grown in a 16:8 h light: dark

Table I. Microalgal strains isolated from Tunisian inland waters (river, lagoon, dam reservoir, spring water) with location and culture mediums.

Strain	Class/Order	Sampling site	Culture medium	Sampled and isolated in
Dunaliella sp. Duna-GR	Chlorophyceae/ Chlamydomonadales	El Grine Sabkha Lat (33.385); Long (10.323) S: 56 PSU	CONWAY	Present work
Chroococcus sp. Chroo-CH	Cyanobacteria /Chroococcales	Chanchou river Lat (33.540); Long (9.432) S: 4.2 PSU	BG11	Present work
Arthrospira platensis Arthro-KB	Cyanobacteria /Oscillatoriales	Korba lagoon Lat (36.613); Long (10.884) S: 16 PSU	BG11	Present work
Leptolyngbya sp1. Osci-NB-01		Nabhena reservoir Lat (36.061); Long (9.865) S: 0.5 PSU	BG11	Fathalli et al. (2011b)
Leptolyngbya sp2. Lepto-CH		Chanchou river Lat (33.540); Long (9.432) S: 4.2 PSU	BG11	Present work
Limnotrix sp. Osci-BM-01		Bir Mcherga reservoir Lat (36.509); Long (10.010) S: 1.6 PSU	BG11	Fathalli et al. (2011b)
Planktothrix agardhii Plank-SS-01		Sidi Saad reservoir Lat (35.381); Long (9.689) S: 1.9 PSU	BG11	Fathalli et al. (2011b)
Spirulina sp. Spir-ML		Maltine river Lat (34.245); Long (10.194) S: 45 PSU	CONWAY	Present work
Lyngbya sp. Lyng-ML		Maltine river Lat (34.245); Long (10.194) S: 45 PSU	CONWAY	Present work
Anabaenopsis circularis Pseud-01	Cyanobacteria /Nostocales	Siliana spring water Lat (35.911); Long (9.335) S: 0.2 PSU	BG11	Present work
Cylindrospermopsis raciborskii Cyl-NB-05		Nabhena reservoir Lat (36.061); Long (9.865) S: 0.5 PSU	BG11	Fathalli et al. (2011b)

S : Salinity; Lat : Latitude; Long : Longitude.

regime with illumination from cool white fluorescent tubes at a light intensity of approximately 10 µmol m^{-2} s^{-1}. The cells were harvested in the exponential growth phase only by centrifugation (3000 rpm; Thermo IEC CL31R Multispeed centrifuge; Massachusetts - USA) and then lyophilized using a Christ Alpha 2-4 LD Plus freeze-dryer (Harz, Germany) after a period of cultivation of 18 to 25 days.

Morphological analysis using microscopy

Morphologies and sizes of the microalgae strains were observed through a LeicaDM LS2 microscope (Leica microsystems, Wetzlar, Germany) using a specialized digital camera (HDCE - 50B, Olympus Corporation, Tokyo, Japan) monitored by an AxioVision softeware, version 4.8.

Molecular identification

Depending of the nature of the strain , total genomic DNA was extracted using either a DNeasy® Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) for prokaryotic strains following the protocol for Gram-negative bacteria as recommended by the manufacturer or the Salting Out technique according to the procedure of Aljanabiand Martinez (1997) for the eukaryotic unicellular green microalgae (*Dunaliella sp.*, table II). The DNA was subsequently kept frozen at -20 °C until use. DNA extracts were Checked by electrophoresis in a 1% agarose geland then quantified with a Thermo Fisher spectrometer (Heaios Y, Thermo Scientific; Massachusetts - USA) to be subsequently diluted to 200 ng/µl solution.

Polymerase chain reaction (PCR) amplification was performed with a DNA Thermal Cycler model 2720 (Applied Biosystems/ California, USA) by using 100 µl of the PCR reaction mixture. For prokaryotic strains, the nuclear-encoded 16S rRNA gene segments were amplified with PCR primers 27 Fw and 1494 Rev (Fathalli et al., 2011a; Fathalli et al., 2011b) under conditions as described in Fathalli et al. (2011b). For Duna-GR, PCR primers 18SCF and 18SDR were used for the PCR amplification of 18S rDNA gene segmentsunder conditions applied by Kato et al. (1997).

All PCR amplifications were performed in 20 µl aliquots containing 10 pmol of each forward and reverse primers (Invitrogen, Carlsbad, California), 1x Reaction Buffer (Invitrogen, Carlsbad, California), 250 µM of each deoxynucleosides triphosphate (OMEGA bio-tec TQAC136), 2.5 mM $MgCl_2$ (Invitrogen, Carlsbad, California), 0,5 U of Taq DNA polymerase (Invitrogen, Carlsbad, California) and 5 to 10 ng of genomic DNA template. All PCR amplicons were analyzed by electrophoresis in 1 %agarose gel (Invitrogen, Carlsbad, California) run in 1× TBE buffer, stained with ethidium bromide and photographed under UV trans-illumination.

A total of 100 µl of PCR of each amplified product was purified using a PureLink® PCR Purification Kit (Invitrogen, Carlsbad, California) following the protocol supplied by the manufacturer before being sent for direct sequencing. Nucleotide sequences were obtained and submitted to the (Basic Local Alignment Search Tool) BLAST database (http://www.ncbi.nlm.nih.gov/BLAST) for identification.

All sequences were subsequently submitted to the GenBank database (accession numbers are given in Table II).

Growth assessment

For all strains, the growth assessment was carried outby monitoring the chlorophyll a content following a spectrophotometric method every other day according to the protocol of Chen et al. (2011). 5 ml of fresh microalgal culture was centrifuged at 5000 rpm for 10 min (Thermo IEC CL31R Multispeed centrifuge; Massachusetts - USA), and washed twice with PBS buffer (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4). The cell pellets were then mixed with 5 ml of pure methanol and centrifuged at 5000 rpm for 10 min after being heated in a 70 °C water bath for 10 min. Once the supernatant was separated from the cell debris, its absorbance (AB) was determined at wavelengths of 665 and 750 nm against pure methanol as a blind. Chlorophyll a amount was then calculated by the equation given by Oncel and Sukan (2008), where chlorophyll a in (mg/l) equals [13.9 x (AB_{665} - AB_{750})]. The growth parameters were then calculated for each strain: the specific growth rate (µ) as the increase in cell density per time unit using the following equation (Kang et al., 2011):

$$\mu\left(\frac{1}{day}\right) = \frac{\ln\left(\frac{X_1}{X_0}\right)}{\frac{t_1}{t_0}}$$

where X_0 and X_1 are cell density at the beginning (t_0) and the end (t_1) of a selected time interval between inoculation and maximum cell density, respectively. The second growth parameter, Generation time (G), is defined as the time interval required for the cells to divide and was calculated as: $G = \ln(2)/\mu$. All the measurements were performed in triplicate to ensure repeatability.

Phycobiliproteins determination

Content of phycobiliproteins (phycocyanin: PC, allophycocyanin: APC and phycoerythrin: PE) in the microalgal strain was determined during their exponential growth rate and using the spectrophotometric method of Bennett and Bogorod (1973). 10 ml of fresh cultures were centrifuged at 3000 rpm for 5 minutes. The collected cell mass was then washed with buffer solution 1 M Tris−Cl (pH 8.1) and one volume of cell mass was subsequently resuspended in five times of the volume of the same buffer. In order to extract pigments, it was necessary to make splitting the cell wall of microalgal strains. For that, continuous freezing at - 20 ° C and thawing at +4 ° C, and sonication (10 minutes with cycles of 30 seconds) were applied to all samples which allowed the destruction of the cell wall of the strains. After that, the cell fragments were separated by

Table II. Molecular identification of the microalgal strains isolated from different Tunisian inland waters with % of similarity according to BLAST database and accession numbers of 16S rRNA, rpoC1 or 18S rDNA sequences.

Strain	Total genomic DNA extraction method	Primers used for PCR amplification	Taxon according to BLAST database	% of similarity	Accession numbers		
					16S rRNA	rpoC1	18S rDNA
Osci-NB-01	DNeasy® Plant Mini Kit	27 Fw and 1494 Rev	*Leptolyngbya sp.*	99 %	MG762090	-	-
Osci-BM-01			*Limnotrix sp.*	100 %	MG762091	-	-
Plank-SS-01			*Planktothrix agardhii*	100 %	MG762092	-	-
Pseud-01			*Anabaenopsis circularis*	99 %	MG098078	-	-
Arthro-KB			*Arthrospira platensis*	100 %	MG098079	-	-
Lepto-CH			*Leptolyngbya sp.*	99 %	MG098077	-	-
Spir-ML			*Spirulina sp.*	99 %	MG518486	-	-
Lyng-ML			in progress	in progress	in progress	-	-
Chroo-CH			in progress	in progress	in progress	-	-
Cyl-NB-05		CYL 2 and CYL 4	*Cylindrospermopsis raciborskii*	96 %	-	HQ389355	-
Duna-GR	Salting Out	18SCF and 18SDR	in progress	in progress	-	-	in progress

centrifugation at 12000 rpm for 10 minutes and the supernatant was taken for the spectrophotometric estimation of phycobiliprotein contents by measuring absorbance at wavelengths of 620, 652 and 562 nm. Absorbance measurements were performed on the UV – visible spectrophotometer (Heaios Y, Thermo Scientific; Massachusetts - USA) and the amount of PC, APC and PE in the sample was calculated using the following formula (Bennett and Bogorad, 1973; Horváth et al., 2013):

$$PC\ [mg/ml] = (A_{620}-0.474xA_{652})/5.34$$
$$APC\ [mg/ml] = (A_{652}-0.208xA_{620})/5.09$$
$$PE\ [mg/ml] = (A_{562}-2.41xPC-0.849xAPC)/9.62$$

Each sample was analyzed in triplicate and buffer was used as a blank.

Chlorophylls a and b and total carotenoids contents measurement

Total carotenoids as well as the two chlorophyll forms a and b were extracted from each microalgal strain's biomass according to the adapted method of Dere et al. (1998). A known amount of freeze-dried algal sample were manually ground with pestle and mortar in 96% methanol and centrifuged at 2500 rpm for 10 min at room temperature. The supernatant was separated and the absorbances were spectrophotometrically measured at wavelengths of 470, 653 and 666 nm on ThermoFisher Scientific - Heaios Y, spectrophotometer (Massachusetts - USA). The experiments were repeated three times to ensure repeatability.

The amounts of Chlorophyll a (Ch a), Chlorophyll b (Ch b) and Total carotenoids (Car) were calculated according to the following formulas (Dere et al., 1998):

$$Chl\ a\ (\mu g/gdw) = 15.65\ A_{666} - 7.340\ A_{653}$$
$$Chl\ b\ (\mu g/gdw) = 27.05\ A_{653} - 11.21\ A_{666}$$
$$Car\ (\mu g/gdw) = (1000\ A_{470} - 2.860\ Chl\ a - 129.2\ Chl\ b)/245$$

Statistics

All experiments were done in triplicates and the results are expressed as the mean ± SD of three independent measurements.

RESULTS

Growth parameters

Table III displays the growth parameters of all investigated strains expressed as specific growth rate and generation time. The former ranged from 0.06 day^{-1} to 0.43 day^{-1}while the later extended between 1.6 and 10.5 days. From this table, it can be observed that the highest rate was equitably shared between *Lyngbyasp.* (Lyng-ML) and *Leptolyngbya sp2.* (Lepto-CH) strains (0.43 day^{-1} each). However, *Leptolyngbyasp1.* (Osci-NB-01) held the lowest growth rate among all investigated strains by only 0.06 day^{-1}.

Table III. Growth parameters: specific growth rate and generation time of the studied cyanobacterial strains. Values are means of three replicates ± standard deviation.

Strain	Specific growth rate (day^{-1})	Generation time(day)
Lyng-ML	0.43±0.06	1.6±0.2
Lepto-CH	0.43±0.09	1.6±0.4
Osci-BM-01	0.30±0.06	2.3±0.4
Spir-ML	0.26±0.05	2.7±0.6
Chroo-CH	0.20±0.01	3.4±0.3
Duna-GR	0.18±0.006	3.8±0.1
Cyl-NB-05	0.14±0.003	4.7±0.1
Plank-SS-01	0.14±0.03	5.08±1.3
Arthro-KB	0.11±0.01	6.1±0.8
Pseud-01	0.07±0.02	9.1±2.5
Osci-NB-01	0.06±0.009	10.5±1.3

Phycobiliproteins content

Quantitative evaluation of C-phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) content was performed in all investigated microalgal strains, the results are displayed in fig. 1.

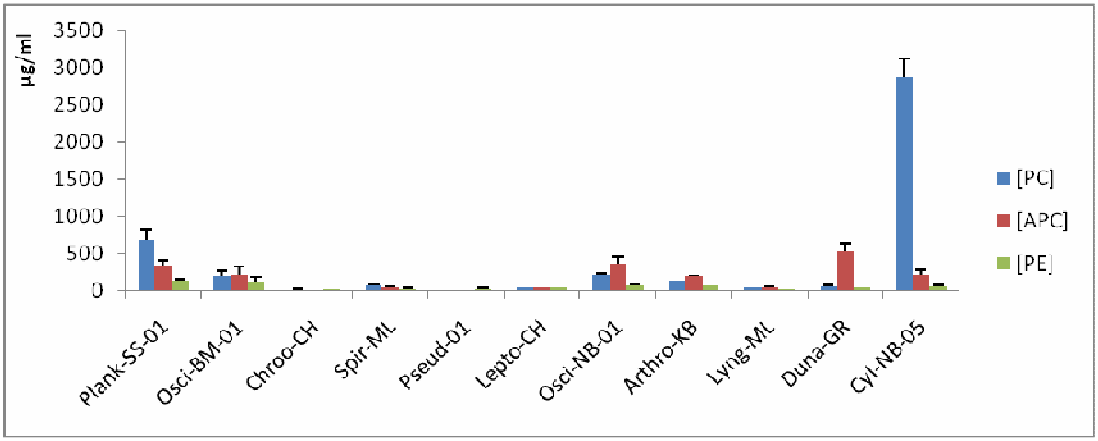

Fig. 1: Phycobiliproteins concentration of microalgal strains isolated from different aquatic habitats expressed in µg per ml of fresh culture. Bars indicate the standard deviation of three replicates (PC = C-phycocyanin; APC = Allophycocyanin; PE = Phycoerythrin).

Among all the essayed isolates, the Nostocale cyanobacterium Cyl-NB-05contained maximum total phycobiliprotein content (3150 µg/ml of fresh culture) while the minimum amount was found in both Chroo-CH and Pseud-01(44.08 and 44.80 µg/ml respectively). For the top phycobiliprotein producer strain, PC level occurs at more than ten-folds higher than the other two constituents (2.87mg/ml of PC against 0.27 mg/ml for APC and PE combined) and about two-folds higher compared with the PC amount in all the other 10 strains. The other tested strains had smaller amounts of PC which were in the range betwen11 (Pseud-01) and 671µg/ml (Plank-SS-01).

In terms of APC production, the maximum yield was found in the *Dunaliella sp.* strain (0.51mg/ml).It is also observed that this green alga represents the only strain where APC content largely exceeds PC level (almost ten-folds). This top APC producer strain is closely followed by the Oscillatoriales cyanobacteria strains Osci-NB-01and Plank-SS-01by 0.36 and 0.32 mg/ml respectively.

In all investigated strains, PE was present at lower quantities comparing with the other two constituents except for Chroo-CH and Pseud-01when it was the major phycobiliprotein produced.

Chlorophylls and carotenoids content

The chlorophyll a content in the eleven microalgae strains whose pigment levels were studied was

determined the highest in the Chlorophyceae strain, Duna-GR(fig. 2). Although chlorophyll a levels in the other isolates were very close to each other (except for Chroo-CH and Spir-ML who presented a particularly low chlorophyll a levels), it was found a little higher in Arhtro-KB in comparison with the other taxa (fig. 2). Regarding the chlorophyll b content, the maximum yield was also found in the same Chlorophyceae strain (Duna-GR) although it remains remarkably low in comparison with the chlorophyll a content (3-folds lower) as was the case for all the other strains (between 3 and 17-folds lower than chlorophyll a level). The only exception is the Oscillatoriale cyanobacterium, Spir-ML, in which the chlorophyll b level exceeds that of chlorophyll a (fig. 2).

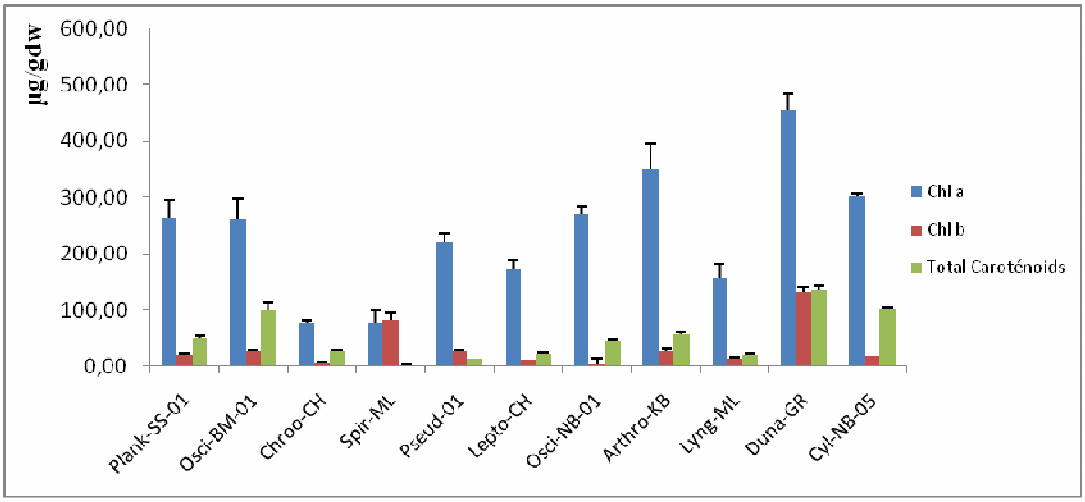

Fig. 2: Chlorophylls a and b and total carotenoid content of microalgal strains isolated from different aquatic habitats expressed in µg per g of dry weight. Bars indicate the standard deviation of three replicates (Chl a = Chlorophyll a; Chl b = Chlorophyll b).

In terms of carotenoids concentrations, the results showed that the highest content among all the investigated isolates was also characterized in the Chlorophyceae strain, Duna-GR (134µg/gdw), which was closely followed by the ostocale and the Oscillatoriale cyanobacteria, Cyl-NB-05 and Osci-BM-01 respectively (about 100 µg/gdw for both strains). Meanwhile, the lowest content was found in Pseud-01 (13µg/gdw) and Spir-ML where the carotenoids amount was almost undetectable (0.6 µg/gdw) (fig. 2).

DISCUSSION

Growth potential

The growth monitoring of the investigated microalgal strains in the present work showed that one of the two isolates of the *Leptolyngbya* genius (Lepto-CH) was the most fast growing among all essayed strains with a specific growth rate reaching 0,43 day^{-1}, while the other strain of the same genius (Osci-NB-01) possessed the lowest growth rate by only 0.06 day^{-1}. Many other studies assessed the growth features of some cyanobacterial strains cultivated under similar laboratory conditions compared with this work (temperature, photoperiod, light intensity) and recorded particularly low biomass productivity related to this genius. One of these studies is the work carried out by Da Rós et al. (2013) in which the authors compared the growth potential of many isolates belonging to four different cyanobacterial orders (Synechococcales, Chroococcales, Nostocales and Oscillatoriales) and found that *Leptolyngbya sp.* had the lowest growth rate compared with the representatives of the other orders. In another study, Abazari et al. (2013) assessed the growth potential of two different strains of *Leptolyngbya sp.* isolated from the fresh waters in East Azerbaijan province of Iran and grown in BG11 culture medium. The authors also reported a similarly week growth potential of both their *Leptolyngbya sp.*strains with a slightly higher specific growth rates, compared to the present study (0.13 day^{-1}in their study). Furthermore, the authors demonstrated that the growth parameters of this species are highly sensitive to some cultured conditions and that it was possible to markedly improve the growth performance by modifying the concentration of one nutrient each time. For instance they were able to obtain a considerable increase in the specific growth rates (up to twice) by increasing the concentrations of some medium components such as $NaNO_3$, K_2HPO_4 or Ferric ammonium citrate. By the same token, other studies confirmed the great sensitivity of biomass productivity and growth rates towards the culturing conditions. One of the best examples is the study conducted by Lürling et al. (2012) in which they assessed the growth potential of eight cyanobacterial isolates and found that their

mean growth rates can be increased from 0.42 day^{-1} to 0.92 day^{-1} by applying the mean optimum growth temperatures (29.2°C) instead of 20°C. According to the same work, chlorophytes show the same behavior as cyanobacteria although the gain amplitude was less pronounced (from 0.62 day^{-1} to 0.92 day^{-1}).

Phycobiliproteins

According to our results, the *Cylindrospermopsis* strain Cylind-NB occupies an outstanding position among all investigated strains by being by far the most productive of total phycobiliproteins with a total amount exceeding 3000 µg/ml. More interestingly, it can be observed that PC level in this Nostocale cyanobacterium occurs at more than ten-folds higher than the other two phycobiliprotein groups taken together (2873µg/ml of PC against 277 µg/ml for APC and PE combined). Compared to the results obtained by other authors, the *Cylindrospermopsis* strain essayed in the present study seems to be an extraordinarily very rich source of PC. For example, Horváth et al. (2013) who evaluated the PC content in a *Cylindrospermopsis raciborskii* strain isolated from surface water samples of Lake Balaton (Hungary) using the same extraction method (repeated cycles of freezing and thawing combined with sonication) found a PC amount lower than our strain (about 5 µg/ml). Aside from the strain effect, which may be the main factor explaining the huge difference observed between the two isolates, other factors may also have intervened especially the high light intensity applied in Horváthet al. (2013) (40 µmol m^{-2} s^{-1}) compared to the present work (27µmol m^{-2} s^{-1}). In fact, the negative effect of applied light intensities of more than 25 µmol m^{-2} s^{-1}on the PC production by cyanobacterial isolates was highlighted in several studies such as the one carried out by Takano et al. (1995). The authors demonstrated that a light intensity of 25 µmol m^{-2} s^{-1}at the flask surface provided by fluorescent lamps was associated to maxim PC content in the cell of the marine cyanobacterium, *Synechococcus sp.* and that this PC content in the cell decreased when the light intensity was increased from 25 µmol m^{-2} s^{-1}. Similarly, De Oliveira et al. (2014) reported that the level of incident light is indeed one of the factors that most influences the cyanobacteria metabolism and that lower light intensities were more advantageous in terms of the yield of total phycobiliproteins produced by two isolates of edaphic algae from the genus *Nostoc*. The authors suggested that when the light availability increased, the content of these pigments decreased as a strategy for prevention of photo-oxidative damage caused by the production of free radicals.

With such a huge and exceptional PC productivity, the *Cylindrospermopsis* isolate investigated in the present study may indeed be considered as a super-rich natural source of this highly valuable phycobiliprotein group which had been proved to possess numerous potent bioactivities with extremely health beneficial effects. Among the most interesting therapeutic and nutritional functions attributed to this pigment and especially sought for food and pharmaceutical industry is the antioxidant and radical scavenging activities. In fact, the role of PC extracted from different cyanobacterial isolates as an effective and non-toxic substance with potent antioxidant activity in vivo and in vitro has been well documented (Romay et al., 1998; Liu et al., 2000; Samarakoon and Jeon, 2012). According to many authors, several synthetic commercial antioxidants such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) have been used widely to retard the oxidation and peroxidation processes (Kim et al., 2001; Je et al., 2005). However, the use of these synthetic antioxidants is often associated with potential health hazards (Park et al, 2001; Safer and Al-Nughamish, 1999) making the search for natural antioxidants as safe alternatives in food and pharmaceutical industry even more appealing and urgent. Furthermore, it had been shown that antioxidants protective action may be valued on many levels, firstly and most importantly they have a positive effect on human health as they can protect the human body against damage by ROS (Reactive Oxygen Species), which attack macromolecules such as membrane lipids, proteins and DNA, leading to many health disorders such as cancer, diabetes mellitus, aging and neurodegenerative diseases (Ngo et al, 2010). Besides, they can prevent food deterioration caused by lipid oxidation and the formation of undesirable secondary lipid peroxidation products by ROS such as superoxide anion, hydroxyl radicals and H_2O_2 which are known to be responsible for the decrease in the nutritional value of lipid foods affecting their safety and appearance (Pangestuti et kim, 2011).

In addition to the previous beneficial effects, some studies also reported the effectiveness of PC as a proliferation inhibitor of cancer cells in vivo and in vitro. For example, Liu et al. (2000) studied the effect of PC from the Oscillatoriale cyanobacterium, *Spirulina platensis*, on the growth of human chronic myelogenous leukemia-blastcrisis K562 cells and had found that this phycocyanobilin group significantly inhibited the growth of K562 cells in a dose-dependent manner. The authors specified that K562 cells were blocked to progress through S-phase and arrested at G1 phase suggesting that PC may be able to inhibit the growth of K562 cells by pathways other than apoptosis, and that changed expression pattern of the c-myc protein may be involved in such inhibition. The anti-cancer activity of PC was also reported by Morcos (1988) who evaluated the resultant

cytotoxicity from activation of PC by measuring the viability of mouse myeloma cells in culture after incubation with PC (0.25 mg/ml) and irradiated by 300 J/cm^2 at 514 nm. The author recorded that PC enhanced the laser cytotoxic effect for cancer laser therapy after tumor cells showed 15% viability within 72 hours of the treatment, compared to 69% for control cells exposed to laser only.

Moreover, many other authors attributed another very interesting therapeutic property to the PC that may open huge possibilities in the medical field especially the prevention of tissue damages caused by inflammatory changes. For instance, in what they reken to be the first study ever to demonstrate the anti-inflammatory effect of PC, Romay et al. (1998) used the peroxide-induced inflammatory response model in vivo in order to test the PC ability to inhibit the inflammatory response induced by glucose oxidase (GO). They had found that PC significantly reduced the edema produced by GO in the mouse paw. The authors suggested that this anti-inflammatory effect must be due, at least in part, to the scavenging of hydroxyl radicals, taking into account the fact that much of the damage induced by H_2O_2 in vivo is due to its conversion to highly reactive oxidants, mainly hydroxyl radicals. Another evidence of the PC positive effect on the immune system and particularly the defense mechanisms against infectious diseasesis the case of the study carried out by Nemoto-Kawamura et al (2004). Trying to prove the influence of this phycocyanobilin ingestion on the secretary IgA antibody response and the allergic IgE antibody response in mice, the authors found that PC from *Spirulina platensis* enhances biological defense activity against infectious diseases through sustaining functions of the mucosal immune system and reduces allergic inflammation by the suppression of antigen-specific IgE antibody. They concluded that *Spirulina* products containing PC are not only useful dietary supplements, but also strengthen the defense mechanisms against infectious diseases, food allergies and other inflammatory diseases.

Otherwise, there are some other appreciable therapeutic and pharmacological applications associated with microalgal PC that are far to be less interesting than the ones previously described. For instance, its hepatoprotective effect highlighted by Vadiraja et al.(1998) who indicated that a single dose of PC (200 mg/kg) from *Spirulina platensis* administrated to rats one hour prior to treatment by different hepatotoxins (R-(+)-pulegone and CCl_4) significantly reduced the hepatotoxicity caused by these chemicals. They suggested that, in the presence of PC, responses to both hepatotoxins are significantly reduced possibly due to its capacity to inhibit some of the cytochrome P450 mediated reactions involved in the formation of reactive metabolites. On the other hand, the neuroprotective role of PC was examined in vivo by Rimbau et al. (1999) on the neurobehavioral and neuronal damage in rats induced by kainic acid (KA) who put in evidence that the incidence of neurobehavioral changes was significantly lower in animals receiving PC. Also, the neuroprotective effect of PC was highlighted in a study carried out by Rimbau et al. (2001) in which the protective role of PC against cell death caused by 24 h potassium and serum (K/S) withdrawal in rat cerebellar granule cell (CGC) cultures was demonstrated. These findings from the two later studies are of particular interest as they open very promising perspectives about the utilization of this pigment as a useful natural and non toxic drug to treat oxidative stress-induced neuronal injury in neurodegenerative diseases, such as Alzheimer's and Parkinson's.

Regarding the APC production, our results showed that the *Dunaliella* strain (Duna-GR) had the highest yield with an amount of 0.51mg/ml. Besides, it can be observed that this Chlorophycae Chlamydomonadale was the only strain where APC content largely exceeds (by almost ten-folds) the PC levels.

This remarkably high APC productivity may select this green alga strain as an excellent candidate to be valued in some strategic sectors and fast growing markets such as nutraceuticals and therapeutic industry. In fact, numerous studies highlighted the ability of APC to act like potent antioxidant and antiviral agent. This later activity was particularly reported by Shih et al. (2003) in a study that was aimed to prove the inhibitory effect of enterovirus 71-Induced apoptosis by APC isolated from *Spirulina platensis*. As an important mechanism in disease pathogenesis (Lai et al., 2002), apoptosis can be triggered in host cells by some viruses at the late stages of infection to spread viral progeny (Everett and Mcfadden, 2001). The authors registered that APC was able to delay viral RNA synthesis in the infected cells and to abate the apoptotic process in enterovirus 71-infected rhabdomyosarcoma cells with evidence of characteristic DNA fragmentation, decreasing membrane damage and declining cell sub-G1 phase. Moreover, it was concluded that APC would have a rather protective than curative action since the antiviral activity was more efficient in cultures treated with this phycocyanobilin fraction before viral infection compared with that in the cultures treated after infection.

Taking into account these interesting findings along with our results, we suggest that the top ACP producing strain characterized in the present work (Duna-GR) can be valued for its potential for development as an anti-enterovirus 71 agents especially in the current context in which the enterovirus 71 infections are causing significant morbidity and mortality in children in a lack of any

effective treatment yet (Shih et al., 2003). Besides, this may give a new light of hope in the fight against virus-induced apoptosis in nonrenewable cells, such as those in the central nervous system, where most neurons are postmitotic and therefore cannot be replaced resulting in an irreversible pathology.

Aside from these therapeutic and pharmaceutical functionalities of APC, it was established that this phycobiliprotein fraction, whether alone or as PE-APC complexe, has several more biotechnological applications especially in highly sophisticated biotechnological domains such as immunofluorescence experiments (Tjioeet al., 2001) and fluorescence resonance energy transfer (FRET) (Batard et al., 2002). In fact, as immunofluorescence experiments become more complex, the demand for new dyes with different properties increases and fluorescent dyes with large Stoke's shifts that are highly bright and have low background binding to cells are especially desirable. According to Tjioe et al. (2001), PE-APC complexes showed promising fluorescence spectral properties like excellent excitation at 488 nm and greatest emission at the APC emission maximum with efficiency of transfer of energy from PE to APC of about 90%. The authors concluded that PE-APC can be considered an excellent substitute for Cy5PE having similar brightness, slightly greater compensation requirements with PE and lower non specific background binding.

Chlorophylls

The chlorophylls a and b quantification in the different microalgal isolates conducted in the present study showed that the Chlorophycae, Duna-GR, possessed the most interesting profile with the highest levels of both pigments among all essayed strains. The powerful antioxidant activity of these chlorophyll groups from micro and macroalgae has been demonstrated in several studies (Endo et al., 1985; Higashi-Okai et al., 2001) presenting thus a promising potential as an accessible and safe alternative to synthetic antioxidants. Moreover, Higashi-Okai et al. (2001) reported a significant suppression capacity of chlorophylls and other pigments including some carotenoids and pheophytins against hydroperoxide generation in a dose-dependent manner. The authors found that chlorophyll a exhibited the most pronounced antioxidant activity and that the ranks of suppressive activity against hydroperoxide generation were chlorophyll a > lutein > pheophytin a > chlorophyll b > beta-carotene > pheophytin b. Similarly, chlorophyll a showed the strongest antioxidant activity compared with three other chlorophyll derivatives (chlorophylls b, pheophytin a and b) and was found to possess the greatest ability to retard the oxidative deterioration of triglycerides in rapeseed and soybean oils at 30 °C (Endo et al., 1985). Furthermore, in another study, Hsu et al. (2013) explored the mechanisms through which the a and b forms of chlorophyll reduce oxidation and measured their ability to prevent H_2O_2 DNA damage and to chelate Fe(II) in a chemical assay. They reported that both chlorophylls a and b showed significant dose-dependent activity in the assays and that these chlorophyll forms can prevent oxidative DNA damage and lipid peroxidation both by reducing reactive oxygen species, such as DPPH, and by chelation of metal ions, such as Fe(II), which can form reactive oxygen species. These later findings confirm that chlorophyll a and b are important health promoting dietary factors which can protect the body through multiple chemical mechanisms. Besides, they can be very useful and valuable compounds for the food and feeding industry due to their ability to prevent food deterioration caused by lipid oxidation and the formation of undesirable secondary lipid peroxidation products by ROS such as superoxide anion, hydroxyl radicals and H_2O_2.

Carotenoids

The total carotenoid measurements carried out in the present study showed that the green microalgae, Duna-GR, was the top carotenoids producer among all assayed strains with an interesting yield reaching 134 µg/gdw (fig. 2). It was followed by the Nostocale strain, cylindro-NB, and the Oscillatoriales train, Limno, by about 100 µg/gdw each (fig. 2).

This high carotenoid productivity seems to be of particular interest for the medical and pharmacological fields due to the numerous health beneficial effects associated with carotenoids as highlighted in many studies. In fact, several epidemiological studies have shown that carotenoid-enriched diet has been found to diminish the risk of suffering from degenerative diseases such as Alzheimer and Parkinson (Guerin et al., 2003; Murthy et al., 2005), cardiovascular diseases such as coronary heart disease, acute myocardial infarction and arteriosclerosis (Kristenson et al., 1997; Klipstein-Grobush et al., 2000) and even cancer (Moreau et al., 2006; Emtyazjoo et al., 2012). Carotenoids activity against this later disease seems to be of particular importance given the high efficiency of this pigment group against a wide range of cancer types such as lung cancer (Wright et al.,2003), prostate cancer (DePrimoet al.,2001) and colorectal cancer (Slattery et al., 2000). Besides, there is now growing evidence in support of the protective role of some carotenoids in other cancer categories including breast, gastrointestinal, cervical, ovarian and pancreatic cancers (Nahum et al., 2001; Fraser and Bramley, 2004; Rao and Rao, 2007).

In addition to the previous valuable functions, carotenoids were also proved to possess potent anti-

inflammatory properties (Yang et al., 2013). The beneficial effect of carotenoids has also been shown in patients with a particular skin inflammatory pathology called psoriasis. Lima and Kimball (2010) found low levels of carotenoids in the skin correlate well with psoriasis prevalence. Taken together, these findings should give the carotenoid-rich microalgal strains a great potential to be exploited in the treatment of inflammation-associated diseases.

Moreover, It had been published that a high intake of carotenoids prevents the development of disorders caused by Helicobacter pylori (Molnár et al., 2010), a Gram negative bacteria genus that colonizes the gastric mucosa of at least half of the human on the planet (Kusters et al., 2006). This provides additional evidence that, in recent years, carotenoids are also starting to be considered as important protective molecules in gastric disorders.

Furthermore, results from epidemiological studies indicate that carotenoid concentration in plasma seems to be directly associated with the mortality rate in the elderly (Akbaraly et al., 2009). This negative correlation between total plasma carotenoid levels and mortality risk in the elderly may suggest that total plasma carotenoid levels could be a health indicator in elderly populations.

It is interesting to note that most of the previously discussed interesting therapeutic functions attributed to carotenoids are actually considered as a direct consequence of their powerful antioxidant activity. In fact, many authors suggested that the antioxidant properties of carotenoids are the main mechanism by which they afford their beneficial effects. For example, Vílchez et al., (2011) reported that, in humans, the most relevant biological functions of carotenoids and their effects on a long list of degenerative, chronic and other diseases are actually linked to their antioxidant properties, which are in turn directly dependent on their molecular structures. Indeed, it had been well established that this group of pigments have antioxidant properties by virtue of their highly unsaturated nature, which enable them to lend themselves to oxidation instead of other molecules. The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties and their ability to trap free radicals, which mainly depends on the number of conjugated double bonds of the molecule and the carotenoid end groups or the nature of substituents in carotenoids containing cyclic end groups (Britton, 1995). By the same token, the benefits of some carotenoids to human health have been shown based on the positive impacts of the antioxidant bioactivity of carotenoids, especially their scavenging action on ROS, in inmuno-response modulation, in signaling transduction between cells and in anti-inflammatory response mechanisms (Le Marchandet al., 1993; Biesalski, 2001; Kim et al., 2009). However, recent studies have also demonstrated that carotenoids may mediate their effects via other mechanisms such as gap junction communication, cell growth regulation, modulating gene expression, immune response and as modulators of Phase I and II drug metabolizing enzymes (Paiva and Russell, 1999; Bertram, 1999). Meanwhile, specific carotenoids such as α- and β-carotein and β-cryptoxanthin have the added advantage of being able to be converted to Vitamin A which is well recognized as a factor of great importance for child health and survival. It is particularly essential for the vision cycle (Bendich and Olson, 1989) and the immune response (Hughes et al., 1997) and its deficiency causes disturbances in vision and various related lung, trachea and oral cavity pathologies (World Health Organization, 1998).

CONCLUSION

Recently, the marked trend and consumers growing interest in natural and healthy products have forced researches and industry to develop novel products with functional ingredients. Microalgal pigments, especially phycobiliproteins and carotenoids, are among the most valuable accessory pigments given their powerful bioactivities with extremely health beneficial properties. The results of the present study showed that some local microalgal strains isolated from Tunisian inland waters had an exceptional potential for pigments production (phycobiliproteins, carotenoids and chlorophylls a and b). In fact, the *Cylindrospermopsis raciborskii* strain, Cyl-NB-05, occupied an outstanding position among all strains investigated in the present work and strains from other studies by its extraordinary and unique C-phycocyanin production and accumulation capacity almost two times higher than that of all the other essayed strains. This feature, may give this Nostocale cyanobacterium a very interesting potential to be valued in the therapeutic and medical sectors due to the extremely health beneficial effects attributed to this phycobiliproteins group including anticancer, anti-inflammatory and hepatoprotective effects. Furthermore, the Chlorophycae Chlamydomonadale, Duna-GR, appeared to be an interesting natural source of allophycocyanin, total carotenoids and both chlorophyll forms a and b having the highest production levels of these pigments among all the tested isolates. With such a rich and interesting pigments profile, this strain should be the subject of great attention in terms of potential pharmaceutical and medicinal applications given the highly valuable and beneficial properties of these pigments for human health especially their biological role in the prevention and/or treatment of human chronic diseases from degenerative and cardiovascular diseases to several types of cancers as demonstrated in several epidemiological studies.

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