Effect of nitrogen and phosphorus amendment on the yield of a Chlorella sp. strain isolated off the Lebanese coast.

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Abstract— *A strain of microalgae was isolated from phytoplankton samples collected from the sea coast of Amsheet, North Lebanon. Molecular diagnosis based on ribosomal RNA genes showed it to be most closely related to Chlorella sp. (GenBank accession KC188335.1) with over 90 % nucleotide identity. It was then evaluated whether N and P amendments of seawater fertilized with Guillard's f/2 medium would improve algal growth and production. Addition of nitrogen (30 ppm) and/or phosphorus (2 ppm) to microalgae grown under laboratory conditions in 3L bioreactors resulted in improved biomass yield (mg dry matter/ L) by approximately 48%, and increased protein yield by approximately 56%, from 19.5% to 30.6% of DM content. Total protein yield/L of culture medium was therefore increased by approximately 83%. Total lipid content and carotenoid levels of the microalgal culture were not affected by the N+P amendement, whereas chlorophyll content was almost doubled. When lower levels of N+P supplementations, 10 and 20 ppm N, were tried, the biomass yield was also improved. The experiment was repeated in 20 L bioreactors in a plastic greenhouse, under normal environmental conditions, with an average temperature of 28°C and a maximum temperature of 36°C. At these relatively high temperatures, the growth rate was slowed down, but N supplementations at 10 and 20 ppm resulted in improved dry matter yield by 25 and 45% respectively, and protein content by 17 and 35%, respectively. Knowledge of the optimal culturing conditions of this local Chlorella strain is essential for its efficient production and is expected to serve future environmental and biotechnological purposes.*

Keywords— Microalgae, nutrient amendment, nitrogen, phosphorus, dry weight, yield, protein yield, chlorophyll.

I. INTRODUCTION

Microalgae are prokaryotic or eukaryotic microorganisms characterized by their efficient photosynthetic activity, capacity to survive in different environments and high rate of reproduction (Mata, 2010). The most common habitat of many microalgae is open waters, thus constituting the phytoplankton. The large biodiversity of microalgae manifested in many marine studies (Norton *et al.*, 1996; Cappo *et al*., 2003; Guiry, 2012), coupled with their ability to accumulate high quantities of biomass within a relatively short time, has attracted the attention of researchers since several decades as potential source of food, feed and feedstock for renewable bioenergy (Sheehan *et al*., 1998; Chisti, 2007; Chisti, 2008).

Given their simple structures and photosynthetic ability, microalgae are capable of rapidly generating important primary and secondary metabolites such as lipids, proteins, carbohydrates and antioxidants, from which high value products including food and feed supplements, industrial chemicals, para-pharmaceutical, pharmaceuticals (Borowitzka, 2013) and biofertilizers (Rani *et al.*, 2008).

Microalgae are often used as food and feed due to their nutritional value. Many species are known to have double the protein content (up to 60%) of the traditional protein supplements like meat or eggs, and contain essential amino acids that are responsible of the major metabolic processes such as energy and enzyme production, high amounts of simple and complex carbohydrates which provide the body with a source of additional energy, an extensive fatty acid profile, including Omega 3 and Omega 6, as well as an abundance of vitamins, minerals, and trace elements; hence, they are being cultivated to be used as food or food supplements (Kay & Barton, 1991). For instance, *Spirulina (Arthrospira)* is often used commercially as a nutritional supplement to treat for malnutrition because it has high protein content and other important nutrients (Habib *et al.*, 2008). Microalgae are also used as animal feed or feed supplements. Improvement in growth rates, carcass quality and coloration, increase in survival rates, reduction in the requirement for medication and higher immunity are the main benefits associated with the use of feed containing microalgae biomass (Belay *et al*., 1996). However, the high cost of most of these algae may limit their commercial uses to few applications.

Microalgae are exposed to a variety of environmental factors and nutrient availability that influence the growth rate and

cellular composition in both natural and engineered systems. Hence, to develop a suitable high productivity bio-algal system, understanding synergistic interactions between multiple nutritional factors and environmental variables is essential (Radzun *et al.*, 2015).

Microalgae require inorganic nutrients, light, and favorable temperatures to grow; the primary inorganic nutrients are nitrogen and phosphorus (Fogg, 1973; Bold and Wynne, 1978). In laboratory studies, Guillard's F/2 medium and Walne medium are the two enriched media extensively used for the growth of most marine microalgae except for cyanobacteria (Lavens and Sorgeloos, 1996; Bartley *et al.*, 2013; Roleda *et al.*, 2013). These media contain a mixture of macro- and micro-mineral elements and some vitamins required for microalgae growth. However, for large-scale production, agricultural grade fertilizers such as urea, ammonium sulfate and calcium superphosphate may be used as cost effective alternatives for the macronutrients (Lavens and Sorgeloos, 1996). Microalgae growth, lipid and protein content or composition can be altered by the composition of culturing media. The form and concentration of nitrogen in the medium has been found to affect growth significantly. When both ammonium and nitrate are available in a culture, ammonium is often preferred over nitrate as a nitrogen source since ammonium does not need to be reduced prior to amino acid synthesis (Grobbelaar, 2004). However, ammonium concentrations greater than 25μM are reported to be toxic to phytoplankton (Grobbelaar, 2004). Nitrogen starvation has been extensively studied as a means of increasing total lipid production for using microalgae biomass as feedstock for biodiesel production (Huang *et al.*, 2012). However, relatively fewer studies were conducted on the effect of nitrogen supplementation on the production of primary or secondary metabolites, and considerable variations in the response of various isolates were reported (Ratha *et al.*, 2013; Aremu *et al.*, 2016).

Temperature is a major influencing factor for optimal growth of microalgae as temperature can go below or above the optimal for each species. Microalgae can tolerate a temperature ranging from 18-24°C depending on the species and culture media. However, some microalgae can survive beyond that range. Temperatures lower than 16°C will slow down growth, while those higher than 35°C become lethal to many species. The effect temperature exerts on biochemical reactions and how it affects the biochemical composition of algae makes temperature one of the most important environmental factors (Hu, 2004; Wei *et al.*, 2015).

The aim of this study was to select, identify and culture a local strain of microalgae promising for potential biotechnological exploitation in the MENA (Middle East and North Africa) region. The isolate was cultured under optimal laboratory and greenhouse conditions experimenting on the effect of two major nutrient requirements (nitrogen and phosphorus) on the total biomass production, and on its protein, lipids and chlorophyll content. The isolated strain was identified as a taxon of the green alga *Chlorella*, a genus already known for its commercial by-products.

II. MATERIALS AND METHODS

2.1 Isolation and Molecular characterization

During a survey conducted in 2013-2014 (Abdelkader et al., 2014; Saleh et al., 2014), a green microalgae taxon of *Chlorella* was isolated by streaking technique from a seawater sample collected in Amsheet, northern coast of Lebanon. For molecular characterization, the DNA extracts were amplified by PCR using several primers targeting different genes or gene loci, i.e. the Internal Transcribed Spacer (ITS) region (White *et al*., 1990) and the Large Subunit (LSU) of ribosomal RNAs (Harper and Saunders, 2001), and the elongation factor EF-Tu (Tuf) gene (Fama *et al*., 2002). The amplicons obtained were sequenced and the sequences were deposited in the Genbank and were subjected to BLASTN analysis (NCBI).

2.2 Effect of N and P supplementation

Three trials were conducted, trials 1 and 2 under laboratory conditions and trial 3 under greenhouse conditions.

2.2.1 Trial 1

The local *Chlorella* isolate was grown in 3L flat bioreactors. Seawater (salinity 41 g/L; pH 8.3) was fertilized with Guillards f/2 medium (Guillard & Ryther, 1962; Guillard, 1975) in addition to different concentrations of ammonium chloride and sodium phosphate tribasic. In one trial, the nitrogen and phosphorus levels were adjusted to reach the same levels, 40 ppm and 3 ppm respectively, present in effluents of a Lebanese wastewater treatment plant (Bashour 2015, personal communication). Five treatments were included: f/2, f/2+N, f/2+P, f/2+N+P and f/2 +N2, where F/2 refers to the Guillard f/2 medium; N and P, 40 and 3 ppm respectively and N2, 70 ppm). Each treatment was replicated three times. The cultures were incubated in a growth chamber at room temperature (23-25°C), with an illumination for 16 hr light at 450 lux and 8 hours of darkness. The cultures were aerated using air pumps with an airstone in the center of each bioreactor. Under the experimental conditions, the pH reached 8.5-8.6 and remained stable at that level. Samples were collected every day and their optical

density was measured at 560 nm. For dry matter determination, samples were collected starting from day 5. At the end of the experiment the total lipid, protein, carotenoid and chlorophyll contents were determined.

2.2.2 Trial 2

The same experimental procedures were followed as described in the previous trial, except that the N and P supplementation were done simultaneously at a ratio of 7N:1 P; this ratio was selected based on literature review (Vuorio *et al.*, 2005). Four treatments were included: Guillards f/2 medium without supplementation or with addition of three levels of N and P: 10 ppm N + 1.4 ppm P, 20 ppm N + 2.8 ppm P, and 30 ppm N + 4.3 ppm P. Each treatment was replicated three times.

2.2.3 Trial 3

This trial was conducted in a single span plastic greenhouse, during the month of June under natural light and temperature conditions. The volumes of culture media were upscaled in 20L flat bioreactors. Three treatments were evaluated: f/2 medium, f/2 + 10 ppm N + 1.4 ppm P and f/2 + 20 ppm N + 2.8 ppm P. Each treatment was replicated twice.

2.3 Dry matter determination

Microalgae were harvested at late exponential / early stationary phase stage by centrifugation (5000 rpm for 10 min). The harvested cells were placed at -20°C for some time and then freeze-dried overnight (LABCONCO lyophilizer, USA). The lyophilized material was weighed for determination of dry matter content.

2.4 Determination of total lipid content

Total lipid content was determined gravimetrically using the Folch method (Folch *et al.* 1956). A solvent mixture of dichloromethane: methanol (2:1) was added to the freeze dried algal cells. Tubes were placed in an ultrasonic bath for one hour (at 40ºC) then vortexed well. Homogenates were then filtered into new screw capped tubes and 8 ml of water were added for 20 ml of solvent mixture. After vortexing for few seconds, the mixtures were centrifuged for 10 minutes at 4000 rpm to allow phase separation. The upper phase was discarded while the lower phase representing the lipids was recovered. To the lower phase, 3 g of magnesium sulfate were added for moisture absorption. The mixture was then filtered into a new tube and the solvent was allowed to evaporate under normal atmospheric pressure for 24 hrs.

Dry weights of lipids were measured and lipid contents (% in biomass) were determined using the following formula (1):

Lipid content = (weight of lipids in g/ dry weight of sample in g) x 100

2.5 Protein Analysis

The Kjeldahl method (Owusu-Apenten, 2002) was followed: A sample of 0.25 g of dried microalgae was weighed on a nitrogen free filter paper and digested using concentrated sulfuric acid. Then, NaOH was added to the digested sample to allow the separation of nitrogen from the mixture and obtain ammonia that was trapped in 50 ml boric acid. To quantify the amount of ammonia trapped, titration with sulfuric acid was done until a color change was observed. A blank and a reference were used as a control. The volume of acid needed in the titration was used to calculate the crude protein percentage.

Since not all of the nitrogen in microalgae is associated with protein, the nitrogen-to-protein conversion factor used for microalgae was 5.95 for Kjeldahl method (Lourenço *et al*., 2004) instead of the 6.25 conventional conversion factor. The following formula was sued (2)

Protein% = (ml H2SO4 - blank) × 5.95 × 14 × normality H_2SO_4 × 0.1 / sample weight

2.6 Determination of Chlorophyll and carotenoid contents

To 20 mg of dried algae, 5 ml of distilled water were added followed by vortexing. The mixture was then subjected to disruption by freezing and defreezing for three times using liquid nitrogen and then freeze dried overnight. 100% methanol was added to the culture tubes and then vortexed to ensure pigment extraction. Extracts were then filtered over a 0.2 µm cellulose acetate filter (SIGMA) and 3 ml of the extracted pigments were used to measure the absorbance at fixed wavelengths specific for the solvent.

Quantification of pigment concentration was determined based on spectrophotometric readings of methanol algal extracts at 3 or 4 specific wavelengths depending on pigment to be quantified. Then empirical correlations, reported in the literature, were used to calculate the concentration of chlorophylls and carotenoids (Porra, 1989 & Lichtenthaler, 1983).

Calculations of pigment concentrations were obtained based on the following formulas:

Porra *et al.* (1989) (3)

$$\mu g \text{ Chlorophyll/ ml medium} = (16.29A_{665}-8.54A_{652})\ v/\ (l*V)$$

Lichtenthaler (1983) (4)

$$\mu g \text{ Total Carotenoids/ ml medium} = (1000A_{470} - 44.76A_{666})/221$$

*where *A* is the absorbance (nm), *v* means the volume of solvent used (mL), *l* is the spectrophotometric cell length (1cm) and *V* is the sample volume (mL).

2.7 Statistical analysis

Statistical analysis was conducted using SPSS 23 Software. One way ANOVA was performed to analyze protein, lipid and dry weight. Variation among means was investigated using the Tukey's HSD test (P< 0.1).

III. RESULTS

3.1 Identification of the microalgae isolate

The best PCR amplification products were observed with primers targeting the ribosomal LSU region. The amplicons were purified and sequenced using forward and reverse primers, and the sequences were deposited in the GenBank under accession number KX709906.

BLASTN analysis of the LSU sequences showed that the Lebanese isolate is most closely related: i) to another Lebanese *Chlorella* strain isolated in our lab (KF021312.1) with 92% nucleotide identity and 94% querry coverage, ii) to three isolates of *Chlorella vulgaris* (GenBank accession numbers KC188335.1, JX401407.1, JX401408.1) with 90% nucleotide identity and 85% - 79% querry coverage, and iii) to *C. variabilis* (GenBank accession number JX401412.1) with 90% nucleotide identity and 79% querry coverage.

3.2 Trial 1

3.2.1 Growth kinetics and biomass production

The optical density readings at 560 nm showed that the faster growth occurred in the Guillard f/2 supplemented with N and/or with N+P with a growth rate µ= 1.04 and 1.10 respectively, as compared to f/2 alone or f/2+P with a µ= 0.94 and 0.80, respectively (Fig. 1). Nitrogen supplementation improved the rate of growth while phosphorus alone did not increase it. However, a combined N+P supplementation gave the highest growth rate.

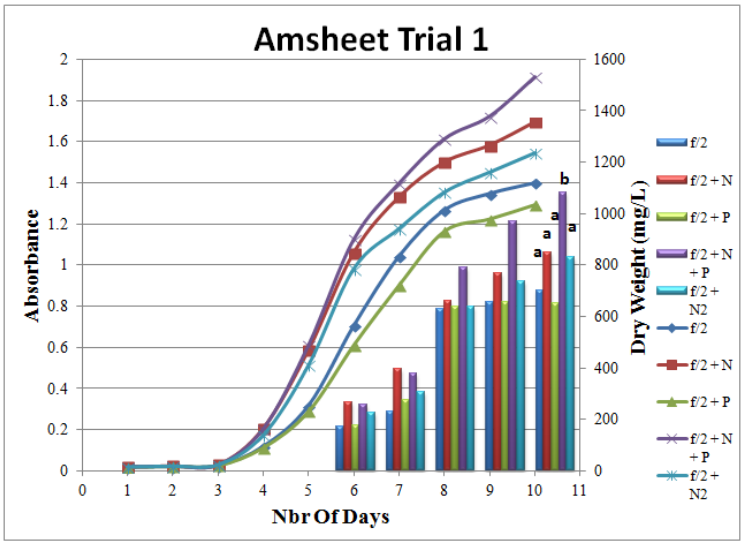

FIG.1 GROWTH KINETICS AND DRY MATTER PRODUCTION OF THE AMSHEET *CHLORELLA* ISOLATE GROWN IN GUILLARD f/2 MEDIUM WITH OR WITHOUT N AND P AMENDMENTS UNDER LABORATORY CONDITIONS. N and N2 = NITROGEN SUPPLEMENTATION AT 30 AND 60 ppm, RESPECTIVELY; AND P= PHOSPHORUS SUPPLEMENTATION AT 2 ppm. COLUMNS WITH DIFFERENT LETTERS ARE STATISTICALLY DIFFERENT USING TUKEY'S HSD TEST (P< 0.1).

Biomass yield (mg dry weight/ Liter) of the different treatments were recorded from day 6 until the end of the experiment (Fig. 1). After 10 days of growth the highest biomass production was obtained with the f/2+N+P followed by the f/2+N treatment with a dry matter content of 1080 and 850 mg/L respectively, which were significantly higher than in the basic medium (700 mg/L). Phosphorus amendment alone did not have a positive effect on dry matter yield, while in combination with nitrogen, it allowed to get a better yield. Doubling the N amendment to 60 ppm did not improve biomass yield as compared to 30 ppm.

3.2.2 Lipid content

The effect of N and P amendment of the f/2 nutrient medium on lipid content of the Amsheet *Chlorella* isolate was evaluated (Fig. 2). Total lipid content ranged between 13.35% in the reference treatment to 15.3% for the treatment supplemented with nitrogen (Fig. 2). This difference was considered statistically significant only between the f/2 and f/2 + N with an increase of 16% over the control treatment. However, no statistical differences were observed when all treatments containing N supplementation were compared with all treatments without N supplementation.

3.2.3 Protein content

The protein content, as measured by the Kjeldahl method, ranged between 19.5 and 19.8 % in the two treatments without nitrogen supplementation (f/2 or f/2 +P) and ranged between 27.14% (f/2 +N) to 33.35 % (f/2+2N) with nitrogen supplementation. A statistically significant increase in protein content, of an average of around 50% over the control (19.5 to 33.35%), was observed upon nitrogen supplementation (Fig. 2).

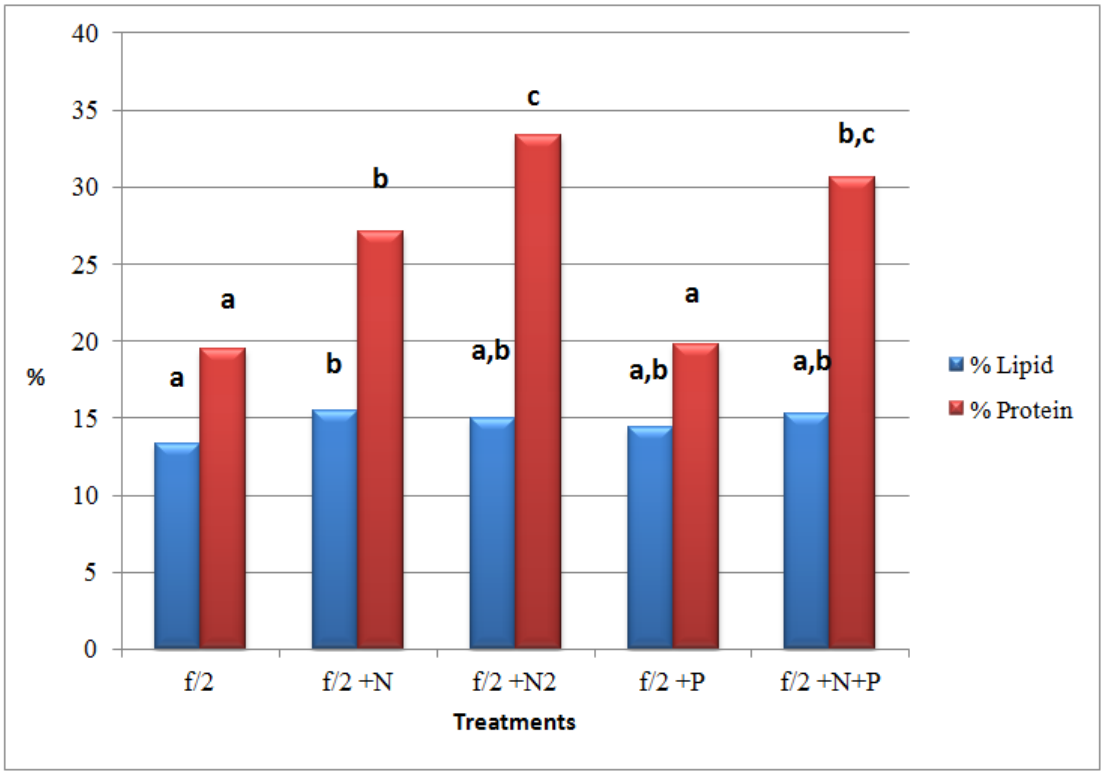

FIG. 2 PERCENT LIPID AND PROTEIN CONTENT FOR THE AMSHEET *CHLORELLA* ISOLATE GROWN IN DIFFERENT MEDIA UNDER LABORATORY CONTROLLED CONDITIONS. F/2 = GUILLARD F/2 WITHOUT SUPPLEMENTATION; N AND N2 = NITROGEN SUPPLEMENTATION AT 30 AND 60 PPM, RESPECTIVELY; AND P= PHOSPHORUS SUPPLEMENTATION AT 2 PPM. COLUMNS OF THE SAME COLOR WITH DIFFERENT LETTERS ARE STATISTICALLY DIFFERENT USING TUKEY'S HSD TEST (P< 0. 05).

3.2.4 Pigment content

The effect of N and P amendment on chlorophyll and carotenoid content were determined using methanol extraction.

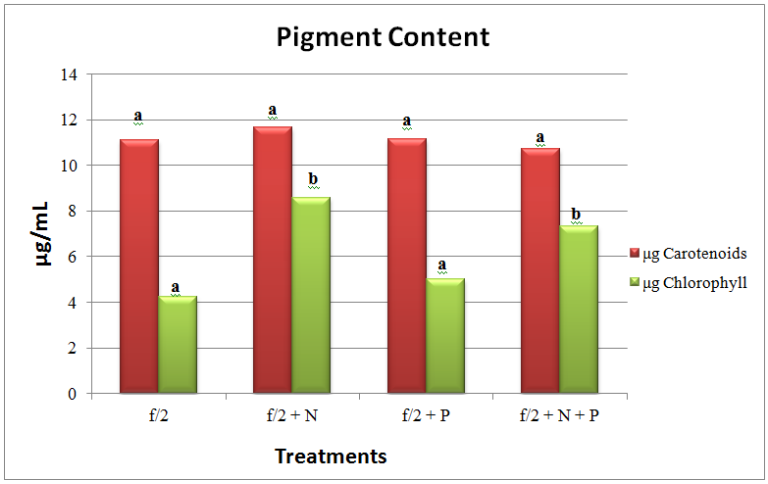

FIG. 3 EFFECT OF N AND P SUPPLEMENTATION ON CHLOROPHYLL A AND CAROTENOIDS CONTENT (µG/L) USING METHANOL EXTRACTION AND LICHTENTHALER (1983) & PORRA (1989) EQUATIONS FOR CALCULATIONS. COLUMNS OF THE SAME COLOR WITH DIFFERENT LETTERS ARE STATISTICALLY DIFFERENT USING TUKEY'S HSD TEST (P< 0.1)

Nitrogen supplementation of f/2 medium resulted in a significant increase in chlorophyll content, with the highest concentration recorded in the nitrogen supplementation alone 8.57µg/mL which was roughly double of that determined in the basic f/2 medium, 4.25µg/mL. Phosphorus supplementation increased slightly the chlorophyll content but this increase was not statistically significant from the control (Fig. 3).

The carotenoid content ranged between 10.71 and 11.67µg/mL in all treatments, and does not seem to have been affected by the N or P supplementation.

3.3 Trial 2

The second trial was conducted a month after finishing the first trial and included three levels of N. The optical density readings showed that all three levels of N + P supplementation improved the growth rate over the control, the non-supplemented f/2 medium (Fig. 4). However, the difference between the three N levels was not as evident especially in the early growth stages, till 7 days post inoculation.

Biomass production expressed in mg dry matter/L seemed to have reached peak production 8-9 days post inoculation (Fig. 4). At that time the maximum yield in the f/2 medium ranged between 305 and 363 mg/L, while it reached between 682 and 820 mg/L for the N+P supplementation (Fig. 4).

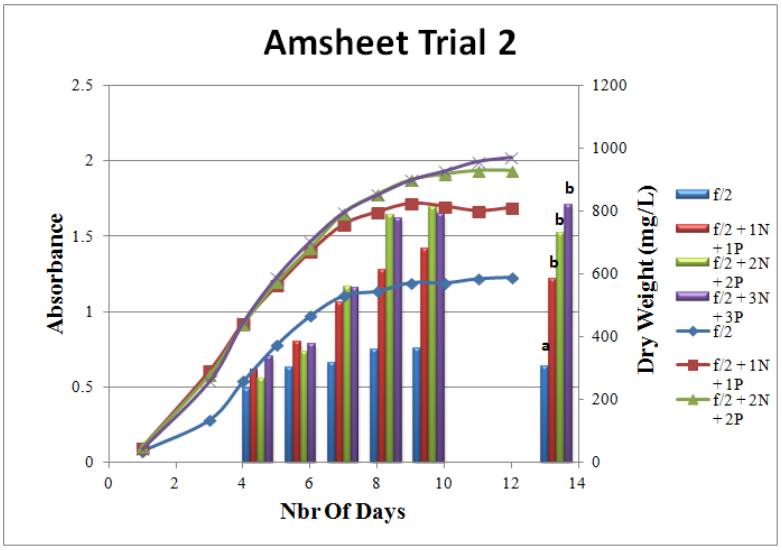

FIG. 4 GROWTH KINETICS AND DRY MATTER PRODUCTION OF THE AMSHEET CHLORELLA ISOLATE GROWN IN GUILLARD F/2 MEDIUM WITH OR WITHOUT N + P AMENDMENT UNDER LABORATORY CONTROLLED CONDITIONS. F/2= GUILLARD F/2 WITHOUT SUPPLEMENTATION; 1N, 2N & 3N = NITROGEN SUPPLEMENTATION AT 10, 20 AND 30 PPM, RESPECTIVELY; AND P= PHOSPHORUS SUPPLEMENTATION AT A RATIO OF N:P=7.

3.4 Trial 3

When the experiment was up-scaled to 20 L bioreactors and repeated under normal lighting and temperature conditions in a plastic greenhouse, the temperature fluctuated between 25 and 36°C with an average daily temperature of 28°C and average minimum of 26°C, while the maximum daily temperature varied between 33°C and 36°C. The growth rate (μ) in the greenhouse experiment was slower than in the lab conditions (in greenhouse μ of control was 0.356 as compared to 0.656 in the laboratory). This delayed growth was also reflected in a reduced yield, whereby the best yield obtained in the greenhouse was about 420 mg/L after 18 days of growth as compared to over 815 mg/L after 9 days under laboratory conditions.

The greenhouse trials using 20 L bioreactors confirmed the previous results. The supplementation with 10 ppm N resulted in a 25 % increase in DM and 17% increase in protein (Figs. 5 and 6). This increase in protein content was statistically significant from the control. On the other hand, the 20 ppm resulted in a 45% increase in DM content and a statistically significant increase in protein content ($P < 0.01$) that reached 35% percent over that of the control.

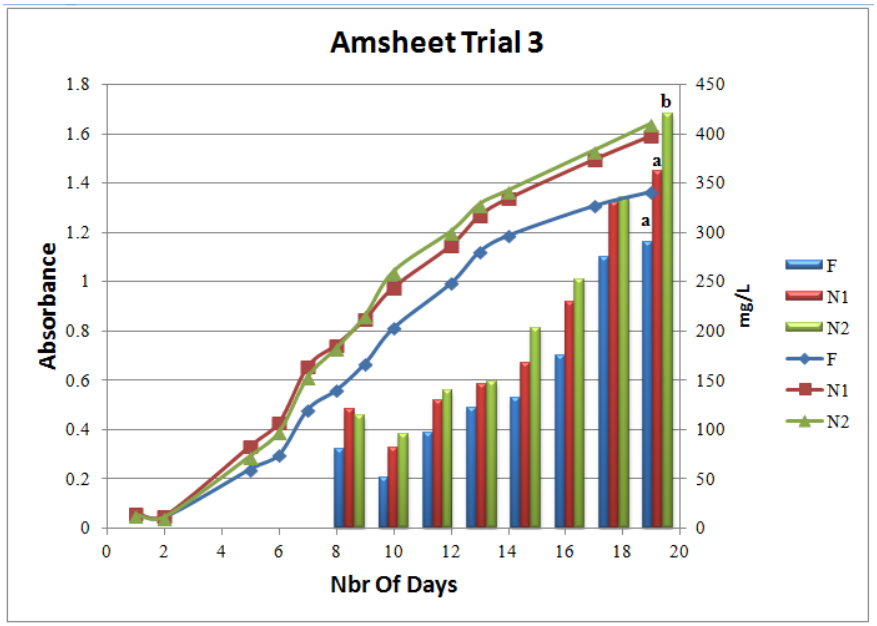

FIG. 5 GROWTH KINETICS AND DRY MATTER PRODUCTION OF THE AMSHEET CHLORELLA ISOLATE GROWN IN GUILLARD F/2MEDIUM WITH OR WITHOUT N + P AMENDMENT UNDER GREENHOUSE NATURAL CONDITIONS. F= GUILLARD F/2 WITHOUT SUPPLEMENTATION; N1 AND N2 = NITROGEN SUPPLEMENTATION AT 10 AND 20 PPM, RESPECTIVELY; AND P= PHOSPHORUS SUPPLEMENTATION AT AN N/P RATIO OF 7:1.

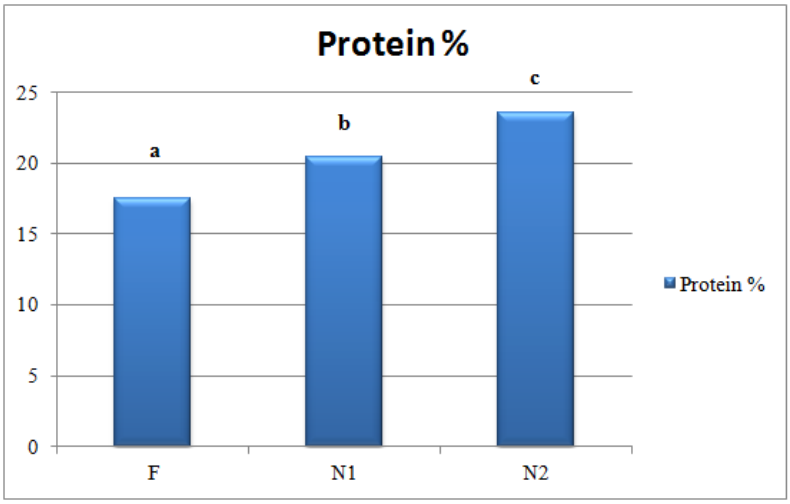

FIG. 6 PERCENT PROTEIN CONTENT IN THE AMSHEET CHLORELLA SP. GROWN IN F/2 GUILLARD MEDIUM WITH OR WITHOUT N + P AMENDMENT UNDER GREENHOUSE NATURAL CONDITIONS. F= GUILLARD F/2 WITHOUT SUPPLEMENTATION; N1 & N2 = NITROGEN SUPPLEMENTATION AT 10 AND 20 PPM, RESPECTIVELY; AND P= PHOSPHORUS SUPPLEMENTATION AT AN N/P RATIO OF 7:1. COLUMNS WITH DIFFERENT LETTERS ARE STATISTICALLY DIFFERENT USING TUKEY'S HSD TEST (P< 0.1).

IV. DISCUSSION

"Climate is changing, Food and agriculture must too". This is the theme for 2016 World Food Day (WFD), a day of action against hunger, organized yearly by the Food and Agriculture Organization of the United Nations (FAO). Effectively, microalgae have a highly efficient photosynthetic ability (Miao & Wu, 2006) and were considered as potential protein food and feed sources since the early fifties (Becker, 2007). They were also initially examined as a potential replacement fuel source for fossil fuels in the 1970s (Barclay *et al*., 1987) due to their high oil content and rapid biomass production. This latter interest rose considerably following the upsurge of fuel costs; therefore, new efficient production methods were developed. With the current decline in fuel costs, the economic production of microalgae will have to address all the potential uses of microalgae products and byproducts.

Molecular characterization of the green algae species isolated from Amsheet, Lebanon indicated a taxon of *Chlorella* based on the LSU region. Better primers may be designed for other DNA regions including the ITS and Tuf genes. However, these genomic regions may not be sufficient to allow an accurate identification to the species level. Therefore, other DNA barcode markers (Hadi *et al*., 2016) should be tried and the results will be presented in a separate publication.

Optimization of culturing conditions is important for efficient microalgae production. It has been documented that nitrogen was the most important nutrient affecting the biomass yield of several microalgae genera (Piorreck *et al*., 1984; Griffiths & Harrison, 2009; Huang *et. al*., 2012; Aremu *et al*., 2016). Guillard's medium is one of the most commonly used media for culturing marine microalgae (Guillard *et al.,* 1962; Lavens & Sorgeloos, 1996; Bartley *et al.,* 2013; Roleda *et al.,* 2013). In this study nitrogen supplementation of Guillard's f/2 medium in the range of 10-30 ppm lead to a significant increase in biomass production. The optimal N concentration for this *Chlorella* isolate ranged between 2.1 and 5 mM, this was close to 5-10 mM reported for *Scendesmus* (Arumugam *et al*., 2013). Under laboratory conditions, about 1g dry matter/L was harvested within 9-10 days. Nitrogen supplementations also lead to a significant increase (over 50%) in protein yield, with an increase from 18-19% to about 33%, a level similar to that present in red meat. Similar results were reported earlier (Piorreck *et al*., 1984). Some of the other microalgae strains isolated in Lebanon, yielded about 50% protein, but their growth rate was slower and took about 20 days to reach the early stationary phase (data not shown), rendering *Chlorella* more economically promising. The cyanobacterium *Spirulina* (*Arthrospira)* and the green alga *Chlorella* are the two most important microalgae species commercially produced and used in human/animal nutrition (Spolaore *et al*., 2006; Priyadarshani & Rath 2012). It would be highly interesting to further evaluate the local isolate and study the amino acid profile, especially its content in some essential amino acids, like lysine and methionine, which are normally limited in most plant foods, as well as its content in other nutrients including omega fatty acids, minerals and vitamins. In this study supplementation with N also lead to a significant increase in chlorophyll, which is rich in antioxidants, vital minerals such as magnesium, iron, potassium, calcium and essential fatty acids (Pangestutia & Kim, 2011). Increased levels of chlorophyll may have contributed to the significant increase in biomass yield through an increase in photosynthetic activity. However, supplementation with N did not lead to a significant change in total content of carotenoids which are highly valued for their health benefits, especially in decreasing the risk of certain cancers and eye diseases and in protecting the skin from environmental toxins (van Rooyen *et al*., 2008; Pangestutia & Kim, 2011). Similarly, nitrogen supplementation did not lead to a significant increase in total lipid production. While in this study the effect of nitrogen starvation was not evaluated, several other studies showed that nitrogen starvation leads to a significant increase in total lipid production, a highly valuable characteristic when growing microalgae for biofuel production (Hu, 2004; Huang *et. al*., 2013).

Another important factor to be evaluated is the source of nitrogen. Other studies showed that potassium nitrate was the best source for biomass production of the green microalgae *Botryococcus braunii* (Dayananda *et al*., 2006) and *Scenedesmus* (Arumugam *et al*., 2013). The most plausible explanation for the better performance of KNO_3 is that N and K are two important nutrients for algal growth; however, it was suggested that KNO_3 may be replaced by urea as an economical source for large scale production (Arumugam *et al*., 2013). On the other hand, phosphorus supplementation alone did not have a significant effect on biomass yield nor on protein content, but its addition to the N supplemented culture media helped improving biomass production.

Temperatures above 30°C were reported to be detrimental for the production of several microalgae species (Li, 1980; Ras *et al*., 2013). Since the present isolate continued growth and production under maximum temperatures ranging between 33 and 36°C, the Amsheet *Chlorella* isolate may be considered as a moderately heat tolerant isolate.

Climate change in the Mediterranean region will be accompanied with an increase in average temperature and a corresponding increase in drought. In search for adapting for climate change, bio-diversification of food and feed resources is essential and microalgae may represent one important element in the diversification process, since microalgae represent a rich source of proteins, carbohydrates, lipids including omega 3 FAs, fibers, and enzymes, in addition to many vitamins and minerals like vitamin A, C, B1, B2, B6, niacin, potassium, iodine, iron, calcium and magnesium (Kay & Barton, 1991; Habib *et al.*, 2008). Many microalgae are currently used for food supplements or as animal feed and feed supplements (Spolaore *et al.*, 2006; Priyadarshani & Rath, 2012). Microalgae are a diversified group of microorganisms and locally adapted strains will play a role in adaptation to climate change and sustainable food and feed production. Furthermore, nitrogen and phosphorus are known as the two major nutrient requirements and are abundant in waste water. Microalgae growth and harvesting may serve a dual purpose, reducing environmental pollution as well as providing feed or food supplements and the byproducts may be used for energy production. Another major concern in the Middle East and North Africa (MENA) region is the shortage of fresh water resources. The identification of locally adapted marine microalgae species may play a key role in the search for improving water use efficiency and finding alternative saline aquaculture systems.

V. CONCLUSION

The green alga *Chlorella* sp. is a widely used nutritional supplement and an excellent vegan source of protein. To cope with climate change and diminishing fresh water resources, research should be conducted in order to search for locally adapted microalgae strains and to optimize growth conditions to improve the yield quantity and quality per unit area. This study showed that amendment of the culture media with N and P improved dry matter, protein and chlorophyll yield of a heat tolerant *Chlorella* strain isolated locally improving the economics of potential production.

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