

Effects of Nitrogen and Sulfur Deprivation on β -Carotene and Fatty Acid content of *Dunaliella salina*

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Abstract

Dunaliella salina, a green unicellular chlorophycean microalga, is famous as a robust producer of carotenoid, fatty acids, and also biomass. Biomass, lipid, and β -carotene levels in *D. salina* increases during nutrient deprivation conditions. In this study, the effects of nitrogen and sulfur starvation, on β -carotene, lipid, and biomass production and composition in a naturally isolated *D. salina* strain was studied. Johnson culture medium was exploited for subculturing and growth of the studied strain. Direct cell counting method and also dry cell weight measurement were used for monitoring the cell growth. β -carotene production was measured using spectrophotometry method. The experiments were performed in 22 days with two different growth stages composed of 8 days of nutrient rich and 14 days of nitrogen and sulfur deprived media. The studied microalgal strain in nutrient condition showed a higher biomass production and cell growth rate in comparison with the starvation condition. Moreover, a significant increase in cellular β -carotene and lipid contents was observed under nitrogen and sulfur limitation. The studied microalgal strain contained some important fatty acids with food, feed, and biodiesel potentials. The obtained results suggested the applicability of macroelements deprivation strategy to elevate the carotenoids and lipid accumulation in *D. salina* with the minimum biomass reduction.

Keywords: β -carotene, *Dunaliella salina*, Lipid profile, Nitrogen, Pharmaceutical Biotechnology, Sulfur.

1. Introduction

Currently, carotenoids have achieved different applications in various pharmaceutical, food, and cosmetic industries as feed and food supplement, antioxidant, coloring agent, and also radical scavenger (1, 2). *Dunaliella salina* is a halophile green chlorophycean microalga which is extensively studied as a robust candidate for the overproduction of various bioactive compounds including carotenoids, glycerol, fatty acids, and

some vitamins. The carotenoids, fatty acids and also biomass contents of *D. salina* is shown to be elevated during macroelements deprivation (3). Glucose, nitrogen and sulfur starvation will lead to β -carotene and fatty acids aggregation in *D. salina*. This phenomenon is mainly observed during the late exponential phase of growth. Therefore, it could be suggested that the macro- and micro-element contents of the culture medium define the ruling conditions for microalgal growing, fatty acids and carotenoids accumulation, and productivity (4). Some studies have proven the significant influences of N limitation on *D. salina* growth rate,

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carotenoid production, cell physiology, and also lipid production pathways (5, 6). Besides, S starvation is accompanied by higher lipid and β -carotene levels in *D. salina* cell (7). Additionally, nutrient starvation causes a significant reduction in growth and photosynthesis rates (8). Nevertheless, the synergistic influences of concurrent nitrogen and sulfur deprivation on biomass composition, cell growth rates, lipid and β -carotene production in *D. salina* is still unidentified. Besides, the exact mechanisms involved in carotenoid biosynthesis are poorly determined.

Here, we investigate the influences of nitrogen and sulfur starvation on the biomass, lipid, and carotenoid production in an unstudied strain of *D. salina*. Moreover, its lipid content and composition was also determined to assess its potential for application as the human food or animal feed.

2. Materials and methods

2.1. Strain, culture media, and cultivation mode

The studied *D. salina* strain was obtained from Maharlu Salt Lake, south of Iran, during a screening program. The isolated strain was identified using morphologic and molecular-based methods. To preserve and cultivate the studied microalgal strain, Johnson culture medium was utilized. Cell growth rates were observed for 22 days. In the initial 8 days of the cultivation procedure, the *D. salina* cells were cultivated in nitrogen (1 g L^{-1}) and sulfur (0.5 g L^{-1}) rich Johnson medium to obtain the ultimate achievable growth levels. After 8 days of cultivation in the nutrient rich medium, the second stage of the study was commenced in which the microalgal cells were transferred to the nutrient starved culture medium (100 mL). The microalgal cells were filtered using a membranous filter apparatus with 1 mm pore size. It was then washed two times using the normal saline solution. The second phase of the study was continued for 14 extra days of experiment. In this stage, the freshly prepared Johnson culture medium (100 mL) with normal nitrogen and sulfur concentrations as 1 g L^{-1} and 0.5 g L^{-1} , respectively; and starved conditions including nitrogen starved (0 g L^{-1}) medium with normal sulfur concentration (0.5 g L^{-1}), and sulfur starved (0 g L^{-1}) medium with normal nitrogen concentration (1 g L^{-1}), were added to each

medium. Each study was performed in triplicate with 500 mL Erlenmeyer flasks. The cultivation experiments were performed at the light intensity of $60 \text{ mol m}^{-2} \text{ s}^{-1}$, the agitation rate of 160 rpm and the controlled cultivation temperature of $25 \text{ }^\circ\text{C}$.

2.2. Cell growth determination

Direct cell counting method was performed daily using Neubauer haemocytometer and a light microscope with 40 X magnification. Besides, dried cell weight technique was used for cell growth rate determination in each mode of cultivation every day during 22 days of experiment.

2.3. Molecular identification of the strain

For molecular identification of the isolated microalgal strain, 18S rRNA gene was amplified using PCR method. DNA extraction was performed using heat shock ($95 \text{ }^\circ\text{C}$ for 2 min) method. The microalgal culture medium (1 mL) was centrifuged for 3 min at 13000 g at $25 \text{ }^\circ\text{C}$. The microalgal pellet was resuspended in PBS buffer (0.5 mL) and then the mixture was shaken slowly. The employed primers for 18S rRNA amplification were 5'-GTCAGAGGTGAAATTCTTGGATTTA-3' (the forward primer) and 5'-AGGGCAGGGACGTAATCAACG-3' (the reverse primer). The total volume of the reaction was set to $25 \text{ }\mu\text{L}$ using $10 \text{ }\mu\text{g }\mu\text{L}^{-1}$ genomic DNA, 0.5 U Taq polymerase, $200 \text{ }\mu\text{M}$ dNTPs, 50 mM MgCl_2 , 0.5 pM from each primer and also $2.5 \text{ }\mu\text{L}$ PCR buffer 10x. The amplified sequence was electrophoresed using agarose gel system (1% w/v) with tris borate EDTA (TBE) electrophoresis buffer system. The amplified ribosomal gene were then purified. The sequencing procedure was performed by CinnaGen Company, Tehran, Iran.

2.4. Phylogenetic analysis of the isolated strain

The obtained sequence from 18S rRNA gene amplification of the studied *D. salina* strain was aligned and compared with nine related *Dunaliella* strains from the NCBI database through BLAST analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). MAFFT multiple sequence alignment software (version 7) was employed for multiple sequence alignment analysis (9). MEGA software (version 7) was employed for phylogenetic studies

(10) using the Maximum Likelihood method. For identification of the conserved domains through the ten studied *D. salina* strains, the CLC sequence viewer software (Qiagen, Aarhus, Denmark) version 8.0, was employed.

2.5. Analytical methods

The chloroform and methanol (1:2) solvent extraction system was used to extract the lipid fraction and the gravimetric method was used to assess the total lipid content in each study. The results of lipid determination assay were obtained and reported as % w/w in comparison with the ultimate biomass obtained from each experiment after 22 days of investigation. The fatty acid profile of the studied *D. salina* strain was also determined using methanol and sulfuric acid method. The β -carotene production was measured using spectrophotometry (450 nm) method using a previously reported protocol (11). The carbohydrate compartment of the studied *D. salina* microalga was measured using phenol (5%)-sulfuric acid (150 μ L) method in 96-well microplate at 490 nm (12). A glucose solution (serially diluted) with predetermined concentration was used to plot the requested standard curve for total carbohydrate determination. Moreover, the Bradford method was employed in triplicate to determine the total proteins in each experiment.

2.6. Statistical analysis

For statistical analysis of the observed results in each experiment, the IBM SPSS software (version 23.0) provided by Armonk, NY: IBM Corp. and also the GraphPad prism version 7.00 provided by GraphPad Software, La Jolla California, USA were employed. In ANOVA analysis, the statistical difference of 5% was regarded as significant.

3. Results

3.1. Molecular identification and phylogenetic analysis

A band of about 950 bp, which represents the partial sequence of 18S rRNA, was detected in agarose gel electrophoresis. The amplified sequence of 18S rRNA from the isolated *Dunaliella* strain was compared with the available ribosomal

genes in the NCBI database using BLAST analysis. The results showed 98-100% similarity with the previously reported *D. salina* strains. After obtaining the results of multiple sequence alignments between the amplified sequence and nine related *D. salina* strains, the similarity between the studied sequences and also the conserved domains were detected and presented in a color scale (Figure 1). The green residues were regarded as the least conserved domains and the red residues were the most conserved ones. Besides, the name of each sequence is shown at the start of every row. Additionally, the position of each residue is shown through the presented numbers located above the alignment columns. Moreover, the consensus sequence is represented under each of the ten investigated *D. salina* ribosomal genes. A turquoise-colored plot with percent scale, indicates the conservation level for each studied domain.

The phylogenetic tree of the ten studied *D. salina* strains was constructed using the Maximum Likelihood method (Figure 2). To express the evolutionary history of the ten studied microalgal strains, the bootstrap analysis with 500 replicates was performed. For drawing the phylogenetic tree, the branches with 50% or lower values of bootstrap replicates were shown as collapsed. The amount of replicate trees for clustering each strain or microalgal taxa is indicated just close to each branch. The Neighbor-Joining and BioNJ algorithms were used for drawing the phylogenetic tree between the ten selected microalgal strains. The accession number for each studied sequence is shown in the parenthesis. The MEGA7 software, (10) was exploited for drawing the tree.

3.2. Effects of nitrogen and sulfur starvation on the cell growth

The isolated *D. salina* strain was grown in two distinct cultivation phases. On the other word, at the first growth phase, a nutrient (nitrogen and sulfur) rich Johnson culture medium was added to the harvested cells for 8 days. For the second growth phase, with 14 days of duration, the nitrogen starved and also the sulfur starved culture media were supplemented. The growing pattern of the studied *D. salina* microalga was determined on a regular basis. The cell sampling process

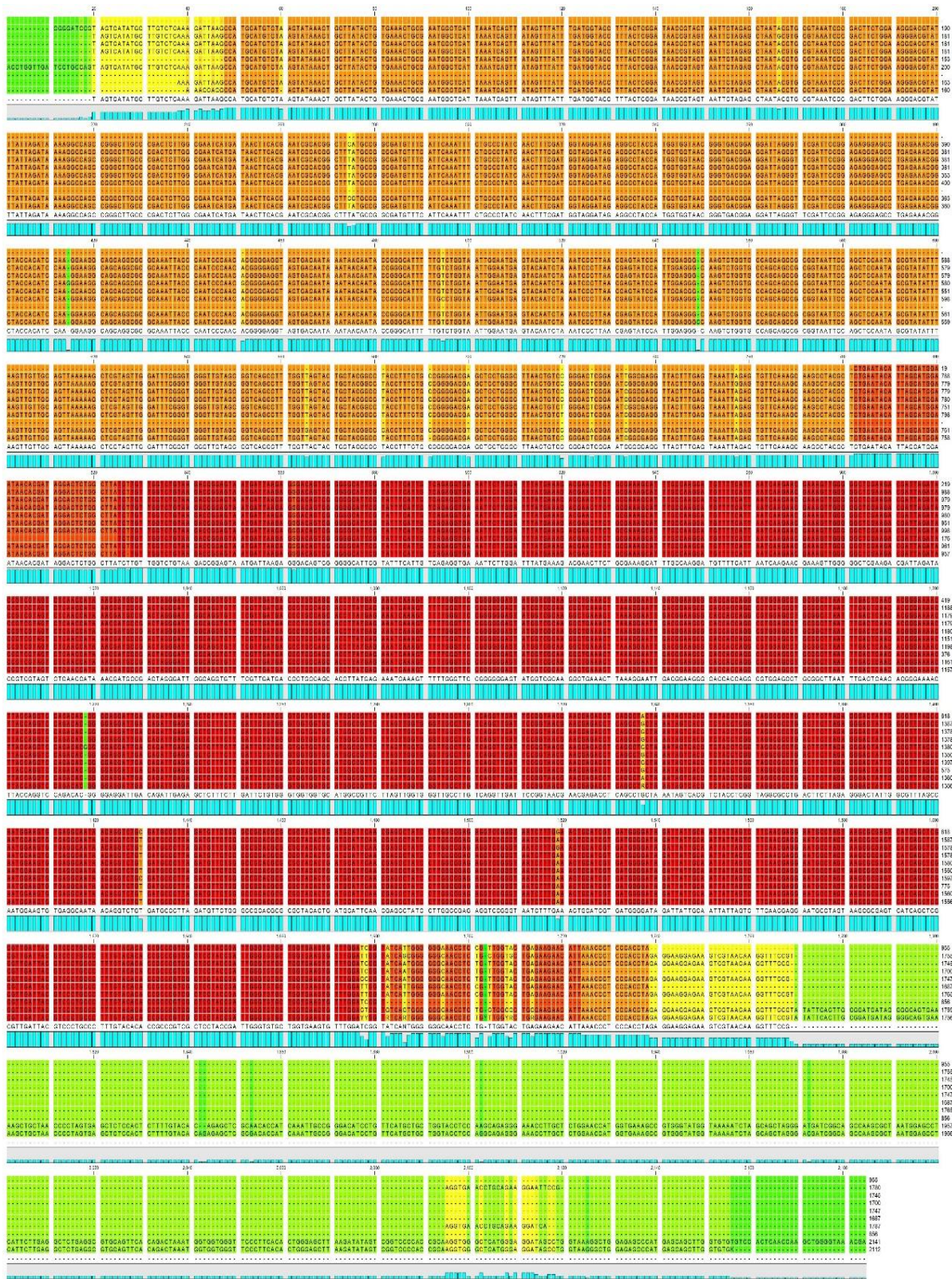


Figure 1. The tabular format of the multiple sequence alignment from ten studied *D. salina* strains. The conservation levels for each domain is shown on a color scale with green residues representing the least conserved and red residues showing the most conserved ones. Moreover, a turquoise colored plot in percent scale, represents the conservation level for each studied domain.

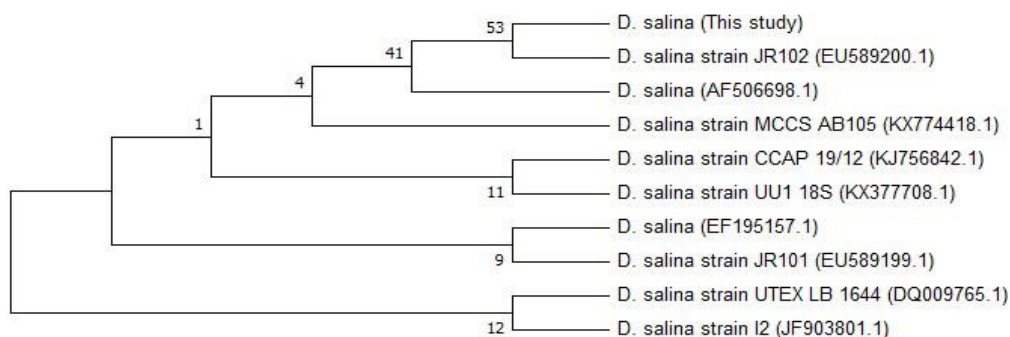


Figure 2. Molecular phylogenetic analysis of the ten studied *D. salina* strains using MEGA 7. The evolutionary history was inferred by using the Maximum Likelihood method. The bootstrap consensus tree inferred from 500 replicates.

was performed every day for direct cell counting and dried cell weight method. The results of cell growth measurements are presented in Figure 3. It was indicated that in the initial first two days of the experiment, the studied microalgal cells displayed the lag phase of growth. Continuing their growth, the *D. salina* cells reached the exponential stage of growth from the second to the eighth day of the study. The microalgal cells, then achieved the stationary stage of growth. The obtained results from cell numbering study revealed that the microalgal cell number of *D. salina* strain was 0.118×10^6

cells mL^{-1} at the beginning of the study (Figure 3a). It was in quite agreement with the results of cell dried weight values (0.089 g L^{-1}) (Figure 3b). In the second stage of cultivation experiment, *D. salina* cell numbers was increased to the maximum level of 2.275×10^6 cell mL^{-1} in 22nd day (D_{max}) of the experiment at the basic (nutrient rich) culture medium. It was equal to 0.832 g L^{-1} at the 22nd day of experiment in dried cell weight analysis. Nevertheless, in the nitrogen starved culture medium, a maximum cell number and dried cell weight of 0.942×10^6 cells mL^{-1} and 0.579 g L^{-1} was found

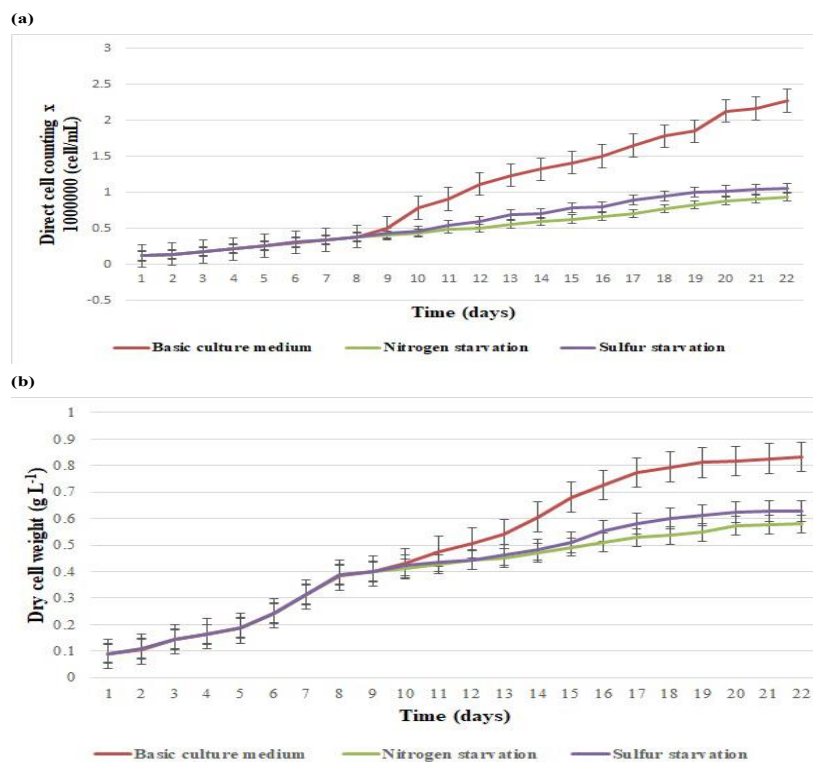


Figure 3. Growth trend of the studied *D. salina* strain in basic and nutrient deficient culture media during 22 days of study using direct cell counting method (a) and dried cell weight method (b).

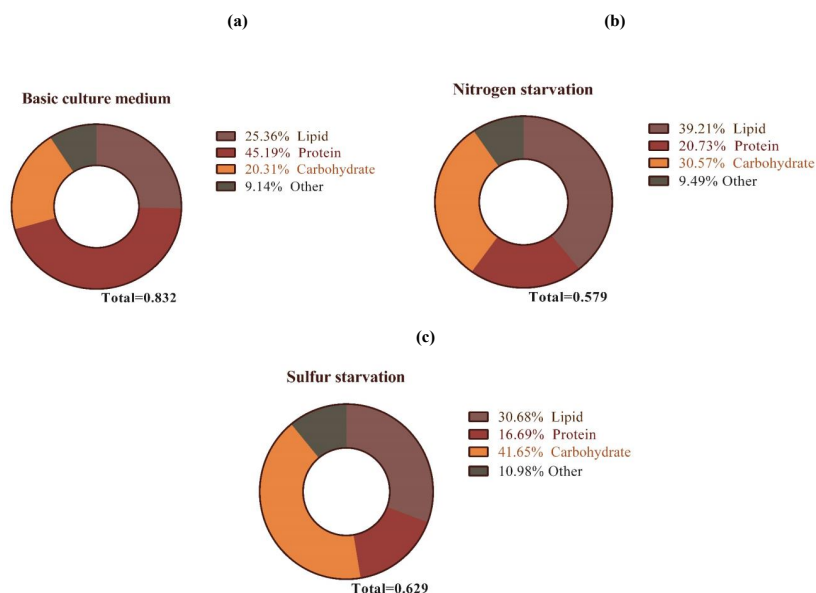


Figure 4. Total biomass production and composition in basic culture medium (a), nitrogen starved (b) and sulfur starved media (c).

in the 22nd day of study, respectively. In the sulfur starved medium experiment, the maximum cell number of 1.059×10^6 cells mL⁻¹ in 22nd day of experiment was comprehended, whilst a final cell dried weight of 0.629 g L⁻¹ was observed.

On the other word, the total achieved cell number and dried cell weight from the nitrogen starved media were decreased up to 41.41% and 69.59%, respectively in comparison with the basic cultivation medium containing the normal concentrations of each macro- and micronutrients. In the sulfur starved culture medium, there were a 46.55% and 75.60% decrease in the total cell number and dried cell weights, respectively in comparison with the nutrient rich medium. Besides, the ultimate cell number and dried cell weights in nitrogen starved medium depicted 88.95% and 92.05% decrease in comparison with the sulfur starved media.

3.3. Effects of nitrogen and sulfur starvation on biomass production and composition

To observe the biomass production pattern in each of these three distinct culture media, the dried cell weight measurement method was performed. After the first step of growth, at the end of the 8th day of experiment, the final cell dry weight found to be 0.401 g L⁻¹. At the second step of experiment, which was continued for another 14 days, the maximum amounts of 0.832 g L⁻¹, 0.579

g L⁻¹, and 0.629 g L⁻¹ were observed in nutrient rich Johnson medium, nitrogen starved, and sulfur starved media, respectively (Figure 3b).

Moreover, the final lipid content of *D. salina* was found to be 0.211 g L⁻¹ (25.36% in the total obtained biomass) in nutrient rich medium, 0.227 g L⁻¹ (39.21%) in nitrogen starved medium, and 0.193 g L⁻¹ (30.68%) in sulfur starved medium. On the other words, after 22 days of microalgal growth, the obtained lipid amount was increased in nitrogen and sulfur starvation experiments.

Nitrogen starvation showed a higher increase (127.80%) in the lipid concentration in comparison with the sulfur starvation condition. Moreover, the presented data in Figure 4 revealed the critical roles of nitrogen and sulfur concentrations on biomass, lipid, protein and carbohydrate production in *D. salina*. The total concentrations of protein and carbohydrates in the ultimate biomass were also determined. It was revealed that the final concentration of the proteins and carbohydrates in the microalgal cells grown in nutrient rich medium were 0.376 g g⁻¹ (45.19%) and 0.169 g g⁻¹ (20.31%), respectively (Figure 4a). Moreover, a cumulative amount of 0.076 g g⁻¹ comprising the 9.14% of the final biomass (0.832 g L⁻¹) was considered as impurities, nucleic acids, and probable errors. Results of the same studies

in nitrogen starved culture medium indicated that the final protein and carbohydrate concentration at the end of nitrogen starvation analysis was 0.120 g g^{-1} (20.73%) and 0.177 g g^{-1} (30.57%), respectively. The quantity of the remained ingredients and compounds were found to be 0.055 g g^{-1} , which is regarded to be 9.49% of the final attained biomass (0.874 g L^{-1}) (Figure 4b). Moreover, in sulfur starvation condition, the final amounts of proteins and carbohydrates were found to be 0.105 g g^{-1} (16.69%) and 0.262 g g^{-1} (41.65%), respectively. Notably, 0.069 g g^{-1} (10.98%) of the remaining materials were considered as nucleic acids and impurities, whilst the ultimate amount of microalgal biomass was detected as 0.579 g L^{-1} (Figure 4c). The maximum amounts of protein and carbohydrate production was occurring in nutrient rich medium.

3.4. β -carotene content of *D. salina*

The final concentration of the β -carotene achieved from three distinct experiments including nutrient rich, nitrogen starved, and sulfur starved culture media was measured after 22 days of study. Figure 5 presents the mean values of β -carotene concentration with error bars attained from the studied strain in each experiment. It is observed that, the total concentration of β -carotene was in-

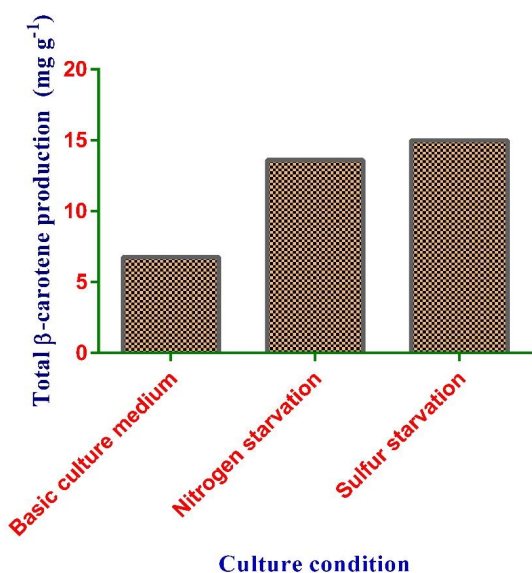


Figure 5. β -carotene concentration (mg g^{-1}) obtained from *D. salina* after 22 days of cultivation in nutrient rich and starved media.

creased from 6.753 mg g^{-1} in, the initial Johnson culture medium as the nutrient rich medium, to 13.616 mg g^{-1} in the nitrogen deprived medium and 14.994 mg g^{-1} in the sulfur deprived medium. Based on this, it could be suggested that the macroelement deprivation approach led to an increase in carotenoid level in the studied microalgal strain to 201.63% in the starved medium and 222.03% in the sulfur starved medium in comparison with its primary levels in basic Johnson medium with physiologic concentrations of nitrogen and sulfur. The final β -carotene production levels in sulfur starved medium was 110.12% higher than its obtained levels in the nitrogen starved medium.

3.5. Fatty acid profile of the studied strain

Commencing the stationary stage of growth, the ultimate amounts of lipids from the studied *D. salina* strain were firstly esterified and determined using GC/MS apparatus. The esterification procedure leads to fatty acids (FAs) and fatty acid methyl esters (FAMES). The identification procedure for each FA/FAME was done by comparing each mass spectrum with the reported values in the Wiley database. Findings of this investigation were categorized and presented as FAs and FAMES profile. The physicochemical properties of each studied FA/FAME are presented in Table 1. Various categories of FAs/FAMES from 3-24 carbon atoms were identified in the *D. salina* strain. The shortest identified FA was 2-propenoic acid (acrylic acid), whilst the largest detected fatty acid was tetracosanoic acid (lignoceric acid) with 24 carbon atoms. Findings of FA/FAME determination indicated some monounsaturated fatty acids (MUFAs) such as 2-propenoic acid, 2-pentenoic acid, 2-heptenoic acid, 3-octenoic acid, 9-hexadecenoic acid, and 6-octadecenoic acid in the extracted lipids. The major polyunsaturated fatty acid (PUFA) with two double bonds was found to be 9,12-octadecadienoic acid (linoleic acid). Besides, a PUFA with three double bonds were identified as 11,14,17-eicosatrienoic acid which is considered as an (n-3, 6, 9) omega-3 fatty acid (Table 1).

4. Discussion

Macro- and micronutrient concentrations in the culture medium determines the nutritional

Table 1. The identified fatty acids (FAs) and fatty acid methyl esters (FAMES) from the studied *D. salina* strain

Systematic name	Common name	Formula	No. of carbon atoms	No. of double bond(s)	Position of double bond(s)	Family
2-Propenoic acid	Acrylic acid	C ₃ H ₄ O ₂	3	1	2	Mono unsaturated FA
Butanoic acid	Butyric acid	C ₄ H ₈ O ₂	4	0		Saturated FA
Pentanoic acid	Valeric acid	C ₅ H ₁₀ O ₂	5	0		Saturated FA
2-pentenoic acid		C ₅ H ₈ O ₂	5	1	2	Mono unsaturated FA
Hexanoic acid	Caproic acid	C ₆ H ₁₂ O ₂	6	0		Saturated FA
Heptanedioic acid	Pimelic acid	C ₇ H ₁₂ O ₄	7	0		Saturated (dioic) FA
2-Heptenoic acid		C ₇ H ₁₂ O ₂	7	1	2	Mono unsaturated FA
Octanoic acid	Caprylic acid	C ₈ H ₁₆ O ₂	8	0		Saturated FA
3-Octenoic acid		C ₈ H ₁₄ O ₂	8	1	3	Mono unsaturated FA
Decanoic acid	Capric acid	C ₁₀ H ₂₀ O ₂	10	0		Saturated FA
Undecanoic acid	Hendecanoic acid	C ₁₁ H ₂₂ O ₂	11	0		Saturated FA
Methyl decanoate	Methyl caprylate	C ₁₁ H ₂₂ O ₂	11	0		Branched saturated FAME
Dodecanoic acid	Lauric acid	C ₁₂ H ₂₄ O ₂	12	0		Saturated FA
Tridecanoic acid		C ₁₃ H ₂₆ O ₂	13	0		Saturated FA
Tetradecanoic acid	Myristic acid	C ₁₄ H ₂₈ O ₂	14	0		Saturated FA
Pentadecanoic acid	Palmitic acid	C ₁₅ H ₃₀ O ₂	15	0		Saturated FA
Methyl tetradecanoate	Palmitoleic acid	C ₁₅ H ₃₀ O ₂	15	0		Branched saturated FAME
Hexadecanoic acid	Margarinic acid	C ₁₆ H ₃₂ O ₂	16	0		Saturated FA
9-Hexadecenoic acid	Methyl palmitate	C ₁₆ H ₃₂ O ₂	16	1	9	Mono unsaturated (ω7) FA
Heptadecanoic acid	Stearic acid	C ₁₇ H ₃₄ O ₂	17	0		Saturated FA
Methyl hexadecanoate	Petroselinic acid	C ₁₇ H ₃₄ O ₂	17	0		Branched saturated FA
Octadecanoic acid	Linoleic acid	C ₁₈ H ₃₆ O ₂	18	0		Saturated FA
6-Octadecenoic acid		C ₁₈ H ₃₄ O ₂	18	1	6	Mono unsaturated FA
9,12-Octadecadienoic acid		C ₁₈ H ₃₂ O ₂	18	2	9, 12	Poly unsaturated FA
Nonadecanoic acid		C ₁₉ H ₃₈ O ₂	19	0		Saturated FA
11,14,17-Eicosatrienoic acid	Eicosatrienoic acid or ETE	C ₂₀ H ₃₄ O ₂	20	3	11, 14, 17	Poly unsaturated (ω3) FA
Heneicosanoic acid		C ₂₁ H ₄₂ O ₂	21	0		Saturated FA
Tricosanoic acid		C ₂₃ H ₄₆ O ₂	23	0		Saturated FA
Tetracosanoic acid	Lignoceric acid	C ₂₄ H ₄₈ O ₂	24	0		Saturated FA

and chemical circumstances for *D. salina* growth and metabolism. Nitrogen, and sulfur deprivation strategy is supposed to have a complex crosstalk in molecular, metabolic, and physiological levels which leads to both transcriptional and posttranscriptional regulation levels (8). Besides, it has been shown that *D. salina*, aggregates β-carotene and lipids in the nutrient starved media (13). On

the other hand, optimizing a suitable ratio of nitrogen and phosphorus concentration could be of importance to reach the best conditions for growth, biomass, lipid and β-carotene production.

As shown in the Figure 3, at the second phase of the experiment, after addition of the nitrogen and sulfur starved regimen, *D. salina* growth pattern presented a typical sigmoidal trend in the

three different observed cultivation media. However, the nutrient rich medium (the basic culture medium), containing normal physiologic amounts of nitrogen and sulfur presented a higher slope compared with the other two nutrient starved media. In the second phase of *D. salina* cultivation, the cell growing trend in the nitrogen and sulfur deficient media was slightly reduced. It might be attributed to the nitrogen and sulfur deprivation strategy. As presented in Figure 3, nitrogen starved medium brought more decrease (88.95%) in *D. salina* cell numbers in comparison with the sulfur starved culture media. The observed decrease in *D. salina* growth in nitrogen and sulfur starved culture media might be attributed to the unsuitable growth conditions. The experienced decrease in the growth in the investigated *D. salina* strain was in quite agreement with the results of previous studies (13, 14).

The investigated *D. salina* strain presented a higher biomass production and cell growth rate in comparison with the nitrogen and sulfur starvation conditions. *D. salina* microalga is known to be a promising microalgal candidate for biomass production as the single cell protein (SCP). Regarding the biomass production results of this study, the possibility of utilizing this microalgal strain for SCP production is warranted.

Essential element starvation is assumed to recruit the lipid aggregation in different microalgal strains (15). In this study, it was also revealed that the nitrogen and sulfur starved regimen could increase the lipid biosynthetic pathways in *D. salina* cells. These results might be utilized to elevate the biomass production levels or promote carotenoids accumulation in *D. salina*. The produced lipids could be investigated for food or feed applications and also for green energy production. The observed changes in *D. salina* biomass ingredients revealed the variations of microalgal cell metabolism and physiology during the different experiments with various concentrations of nitrogen and sulfur in the culture medium. Based upon the biomass composition data, the investigated *D. salina* strain could be considered as a robust candidate for biomass, lipid and carotenoid production. Nutrient starvation approach could be utilized to attain higher carotenoid and lipid concentrations.

Regarding the increased levels of β -carotene in nitrogen and sulfur starved regimen, it could be concluded that nitrogen and/or sulfur starvation approach could be considered as a process engineering approach to maximize the β -carotene production process. Besides, it could be comprehended that the available concentrations of nitrogen and sulfur elements in the culture medium for growing the *D. salina* cells are in inverse relation with the β -carotene producing metabolic networks. This phenomenon is quite in agreement with other studies reporting more β -carotene levels in nitrogen, sulfur, iron and manganese starvation conditions (5, 13). Nevertheless, it should be considered that increasing the β -carotene levels in *D. salina* strains after nutrient starvation approach is a species-specific procedure with a general repetitive pattern (16).

The fatty acid profiling results specified that the studied strain was not producing more precious PUFAs such as DHA or EPA, as two major omega-3 compounds. On the other hand, considering the significant growth trend, biomass, protein, and lipid productivity, and an acceptable fatty acid profile, the investigated microalgal isolate could be regarded as a promising candidate for large scale purposes. Besides, the identified FAs/FA-MEs might be useful as metabolic selection markers in lipid profiling study for taxonomic purposes and also for the identification of new strains.

The current study, reveals the influences of nitrogen and sulfur limitation on the cell growth, biomass production and composition, and also more importantly the β -carotene production levels in an unstudied *D. salina* strain. The highest β -carotene production level was observed as 14.994 mg g⁻¹ in sulfur deficient culture medium. Nitrogen and sulfur starvation conditions is assumed to hamper the microalgal cell division and also reduce the rate of many biomass biosynthetic pathways, hence it could be quite rational that the cell division rate is decreased during nitrogen and sulfur limitation.

5. Conclusion

Briefly, the possibility of nutrient starvation strategy to increase the β -carotene and lipid production was confirmed. Nitrogen and sulfur

were defined as the crucial nutritional elements involved in β -carotene biosynthesis, *D. salina* growth, and lipid production. Considering the significant growth trend, the amounts of β -carotene production, and higher lipid contents, the isolated *D. salina* strain might be regarded as a potential candidate for industrial purposes. Moreover, the nutrient starvation strategy, as a bioprocess engineering-based method, might be considered for optimization and scale up studies.

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Conflict of Interest

None declared.

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