http://www.pjbs.org



ISSN 1028-8880

Pakistan Journal of Biological Sciences



ISSN 1028-8880 DOI: 10.3923/pjbs.2020.55.62.



Research Article Application of Three Cyanobacteria in Foods and Feeds Biotechnology: Phosphorus Affects

^{1,2}Ahmed Issa, ^{1,3}Esmat Ali, ^{1,2}Refaat Abdel-Basset, ^{1,4}Sabry Hassan, ^{1,5}Mohamed Awad and ^{1,6}Abd El-Basst Ebied

¹Department of Biology, Faculty of Science, Taif University, P.O. Box 888, Taif, Saudi Arabia

²Department of Botany and Microbiology, Faculty of Science, Assiut University, 71516 Assiut, Egypt

³Department of Ornamental Plants (Floriculture), Faculty of Agriculture, Assiut University, Assiut, Egypt

⁴Department of Microbiology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt

⁵Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Assiut, Egypt

⁶Department of Zoology, Faculty of Science, South Valley University, Qena, Egypt

Abstract

Background and Objective: Cyanobacteria grown under abiotic stress affect on some metabolites that used as promising for foods and feeds biotechnology. Thus, the objective of the study was to evaluate the 3 local cyanobacteria isolates for production of foods and feeds under various concentration of phosphorus. **Material and Methods:** Cyanobacteria namely; *Anabaena* sp., *Merismopedia tenuissima* and *Spirulina platensis* were grown photoautotrophically in modified medium. The growth pattern in the medium containing various phosphorus concentrations were followed and harvested around 14 days. **Results:** A decrease in phosphorus concentrations were followed and harvested around 14 days. **Results:** A decrease in phosphorus concentrations by 50% led to an increase in chlorophyll-a of *M. tenuissima* and *S. platensis*. The application of high concentration of phosphorus (+100%) to the culture of *Anabaena* sp. led to an increase in dry weight and growth rate by 0.382 mg mL⁻¹ and 0.013 h⁻¹, respectively. The deficiency of phosphorus concentrations led to a decrease in carbohydrate contents of *Anabaena, Merismopedia* and *Spirulina* with compared to the control culture. In general, the total lipid contents of *Anabaena* sp. and *M. tenuissima* were stimulated by phosphorus deficiency. The phosphorus-free media and increase in phosphorus concentration by 100% resulted in an increase in protein fractions such as soluble, insoluble, globulins, prolamines, glutelins and total protein content of *Anabaena* sp. The application of high concentration of phosphorus (+100%) to the culture of *S. platensis* led to an increase in total lipid contents in comparison to control. The highest phycobiliprotein contents of *S. platensis* were recorded at 50% phosphorus deficiency. **Conclusion:** Cyanobacteria has a soft cell wall that makes it especially easy to digest and is additionally full of live active enzymes which further enhances metabolism and the efficient intake of nutrients.

Key words: Anabaena, Spirulina, Merismopedia, globulins, prolamines, glutelins

Citation: Ahmed Issa, Esmat Ali, Refaat Abdel-Basset, Sabry Hassan, Mohamed Awad and Abd El-Basst Ebied, 2020. Application of three cyanobacteria in foods and feeds biotechnology: Phosphorus affects. Pak. J. Biol. Sci., 23: 55-62.

Corresponding Author: Esmat Ali, Department of Biology, Faculty of Science, Taif University, P.O. Box 888, Taif, Saudi Arabia

Copyright: © 2020 Ahmed Issa *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Besides, phosphorus (P) has vital role in growth of cyanobacteria, there are many enzymes critical in major metabolic pathways and cell division cycle progression is activated by phosphorylation. The preferred form of phosphorus, dissolved inorganic phosphorus often falls below growth-limiting concentrations in many parts of the ocean, including oligotrophic oceans and some coastal waters^{1,2}. Phosphorus in surface waters is higher than in groundwater, where soils are become saturated with phosphorus after extended over fertilization³. Phosphorus (P) having a dominant role in controlling freshwater primary production and eutrophication, leading to tight regulation on P inputs from agricultural, urban and industrial sources^{3,4}. However, P reductions alone are not enough to stop eutrophication.

Cyanobacteria are found in freshwater lakes and oceans, as well as in deserts, hot and acidic springs and even in the arctic ice. The adaptability of cyanobacteria is based on their enormous diversity in species and strains and their ability to synthesis structurally and functionally diverse natural products. They are considered one of the potential organisms, which can be useful to humankind in various ways; an important source of vitamins, minerals, proteins, polyunsaturated fatty acids, antioxidants, etc. Since they utilize sunlight energy more efficiently, their potential for the production of valuable compounds or biomass is widely recognized and they can be used to enhance the nutritional value of food and feed⁵. In view of the cyanobacterial significance as a food source. Little of researches were conducted on the role of phosphorus in cyanobacteria metabolism. The current work aimed to screen the production of the carbohydrates, lipids, proteins and phycobilins from three native cyanobacteria isolated from soils, fresh and marine water in western Saudi Arabia under various phosphorus concentrations.

MATERIALS AND METHODS

Isolation and purification of cyanobacteria: This study was carried out at Taif University, Biology Department in summer season (June-October), 2018. Cyanobacteria namely, *Anabaena* sp., *Merismopedia tenuissima* and *Spirulina platensis* were isolated and purified from three regions in Saudi Arabia (Taif, Bisha and Jeddah) either from soil, freshwater and marine water and grown in BG 11-modified medium⁶ under continuously illuminated with a light intensity of 3500 Lux⁷.

Determination of growth and other metabolites in the cyanobacteria: The three tested cyanobacteria were grown in modified medium containing various phosphorus concentrations (control, 50% P (-), 75% P (-), 100% P (-) and 100% P (+)). The cultures were continuously illuminated with a light intensity of 48 µmol m⁻² sec⁻¹ at pH 7.8 and incubated at a temperature of 28-30 °C. The cultures were followed by daily measurements of chlorophyll-a according to Holden⁸. The growth rate (µ) and generation time (G) were calculated as chlorophyll-a content. The cultures were harvested in the late of exponential phase or beginning of the stationary phase (14 days after inoculation) according to the growth curve as shown later. The dry weight of the algal biomass was estimated after drying the pre-weighed filters at 105 °C for 24 h at the end of the study.

The net (P_N) and true (P_T) photosynthetic oxygen evolution as well as dark respiratory oxygen uptake (R_D) were monitored using a Clark type electrode computerized to an Oxygen Monitoring System (OMS, Hansatech instruments Inc.). Phycobiliproteins contents were determined according to the method described by Bennett and Bogorad⁹ For the determination of carbohydrate, (soluble, insoluble and totals), the anthrone sulfuric acid method were used¹⁰⁻¹². Protein contents (soluble, insoluble and protein fractions) were estimated in the tested cyanobacteria according to the method adapted by Lowry et al.13. Free amino acids were estimated according to the method adopted by Lee and Takahashi¹⁴. The total lipid contents in the three tested cyanobacteria grown under various phosphorus concentrations were estimated using the methods described by Drevon and Schmitt¹⁵.

Statistical analysis: ANOVA (one-way analysis of variance) were calculated for the means of treatments to assess the heterogeneity of samples around their means.

RESULTS AND DISCUSSION

Cyanobacteria are one of the oldest life forms; they have the ability to fix atmospheric carbon (CO₂) in to organic form through oxygen evolving photosynthetic process (photoautotrophs). Phosphorus is the most abundant element on earth crust and remains unavailable to the microorganisms^{16, 17}. Majority of cyanobacteria are mineralize organic phosphorus by producing alkaline phosphatases, thus they play an important role in cycling of carbon, nitrogen and phosphorus¹⁸.

Pak. J. Biol. Sci., 23 (1): 55-62, 2020



Fig. 1(a-c): Effect of different concentrations of phosphorus on growth curves, (a) *Anabaena*, (b) *Merismopedia* and (c) *Spirulina*

Growth parameters: *Anabaena, Merismopedia* and *Spirulina* growth curves were enhanced by phosphorus deficiency in comparison to control. The growth pattern in the medium treatment showed that around day 12, the exponential growth phase ended and there was very little production of new biomass between day 12 and 14 (Fig. 1). By this time, the three species produced very similar yields in this respect, change in the cellular N:P ratio influence on the cellular protein content¹⁹. The irradiance-dependent change in N:P ratios among cyanobacteria may be due to association of protein with the phycobilisome²⁰.

The data in Table 1 showed the effect of various phosphorus concentrations (control, 50% P (-), 75% P (-), 100% P (-) and 100% P (+)) on the chlorophyll-a, dry weight, growth rate and generation time of the studied cyanobacteria. The results cleared that, a decrease in phosphorus concentrations by 50% led to an increase in chlorophyll-a of *M. tenuissima* and *S. platensis*. The application of high concentration of phosphorus (+100%) to the culture of *Anabaena* sp. led to an increase in dry weight and growth rate by 0.382 mg mL⁻¹ and 0.013 h⁻¹, respectively. The maximum growth rate and the

	Anabaena	sp.				Merismope	dia tenuissir.	na			Spirulina plā	itensis			
^r reatments	Control	50% P (-)	75% P (-)	100% P (-)	100% P (+)	Control	50% P (-)	75% P (-)	100% P (-)	100% P (+)	Control	50% P (-)	75% P (-)	100% P (-)	100% P (+)
Chlorophyll-a ug mL ⁻¹)	5.64 ± 0.4^{a}	5.91±0.3ª	5.43±0.7ª	6.51±0.1 ^a	5.69±0.1ª	5.58±0.3 ^{ab}	6.41±0.2 ^b	6.1 ± 0.2^{ab}	4.24±1.1ª	6.36±0.0 ^b	3.49±0.7 ^{ab}	3.56±0.1 ^b	2.44±0.2 ^{ab}	2.33±0.1ª	3.02±0.0 ^{ab}
(mg mL ⁻¹)	0.308	0.331	0.37	0.131	0.382	0.474	0.528	0.527	0.49	0.607	0.65	0.637	0.61	0.643	0.641
$\lambda_{max}(h^{-1})$	0.012	0.013	0.01	0.011	0.013	0.019	0.015	0.015	0.02	0.02	0.013	0.017	0.013	0.00	0.016
(l1) 5	59.7	53.8	72.7	60.6	53.2	37.1	47	44	42.5	34.2	55.1	39.6	52.1	74.2	43.3
Photo-P _N	96	82	76	167	140	120.9	86	70	54	40	815	826	922	658	973
Resp. R_D	18	29	19	16	22	7.5	7	6	22	22	30	29	42	40	29
^o hoto/Resp.	5	2	4	10	9	16.2	12	8	2	1.8	27	27	21	16	32

minimal generation time (0.02 and $34.2 h^{-1}$, respectively) were observed by the treatment of *M. tenuissima* with 100% increase in phosphorus concentration.

P deficiency among phytoplankton species is inhibition of population growth, directly from the completion of the cell cycle. P limitation has been observed to cause lengthened G1 (growth stage before S phase) phase in *Synechococcus* and P starvation caused cell cycle arrest at any phase (including unrecoverable arrest at S) in *Prochlorococcus* spp. The decrease in chlorophyll content probably because both the cell density and the phosphorus limitation decreased as well²¹.

Photosynthesis and respirations: Table 1 showed that photosynthesis, net and true (P_N or P_T), of *Spirulina* was increased by decreasing phosphorus concentrations in the culture media to 50 and 25% that of the control. However, complete removal of phosphorus decreased the photosynthetic oxygen evolution. Doubling phosphorus content in the culture media enhanced this activity. Photosynthesis/ Respiration $(P_N/R_D \text{ and } P_T/R_D)$ was not noticeably affected by the content of phosphorus in the culture media; except at zero phosphorus where this ratio sharply lowered due to inhibited photosynthesis and the simultaneous rise in respiration. On the other wise photosynthesis, net and true (PN or PT), of Anabaena sp. was decreased by decreasing phosphorus concentrations in the culture media to 50 and 25% that of the control. However, complete removal of phosphorus markedly enhanced the photosynthetic oxygen evolution. Doubling phosphorus content in the culture media of Anabaena also enhanced this activity. Respiratory oxygen uptake has been enhanced at 50 or 100% phosphorus while has not been remarkably affected at 25 and 0% phosphorus. Photosynthesis/respiration (P_N/R_D and P_T/R_D not affected by the content of phosphorus in the culture media; except at zero phosphorus where this ratio sharply elevated due to enhanced photosynthesis and the simultaneous decline in respiration. The photosynthesis, net

and true $(P_N \text{ or } P_T)$ of *Merismopedia* was continuously decreased by decreasing phosphorus in the culture media relative to that of the control. Furthermore, doubling phosphorus content in the culture media further inhibited this activity. Respiratory oxygen uptake has been relatively enhanced by decreasing phosphorus concentration to 25%, while greatly enhanced up to 300% at both 0 and 100% phosphorus. Photosynthesis/respiration $(P_N/R_D \text{ and } P_T/R_D)$ gradually decreased as the content of phosphorus in the culture media decreased or even doubled; it reached about 10% the ratio of the control. Cyanobacteria are distinct from all other algae in that most of them possess two light harvesting systems (as opposed to one). Maintaining two light harvesting systems is costly in terms of protein and N requirements and manifests strongly in their cell biology. For example, the extra protein requirement means that cyanobacteria have a high tissue nitrogen: phosphorus (N:P) ratio and a high N requirement for growth. Despite this, light harvesting is necessary in photosynthetic organisms to (1) collect light energy from the sun and (2) convert it to chemical energy in the form of electrons and ATP that can be used to power carbon fixation with two photosystems²² (PSI and PSII). Inorganic phosphate regulates the partitioning of carbon and utilization of photo assimilates, furthermore, suppression in photosynthetic efficiency in photosystem (PS) II has been reported under P limitation in some phytoplankton species²³. However, different responses to P limitation have been reported on Ribulose 1, 5-bisphosphate carboxylase/ oxygenase (Rubisco), the key enzyme for CO₂ fixation in the Calvin-Benson-Bassham pathway. P limitation was found to decrease Rubisco abundance in the marine diatom Skeletonema costatum²⁴.

Carbohydrate metabolism: The culture of *Anabaena* and *S. platensis* treated with 100% decrease in phosphorus concentration resulted in an increase in soluble, insoluble and total carbohydrate contents (Table 2). Furthermore, the

Table 2: Effect of different concentrations of phosphorus on carbohydrate contents of cyanobacterial species (mg g^{-1} DW)

	<i>Anabaena</i> sp			Merismopedi	a tenuissima		Spirulina pla	tensis	
Treatments	SC	Ins. C	TC	SC	Ins. C	TC	SC	Ins. C	TC
Control	22.83±0.2ª	21.22±3.0ª	44.05±2.8ª	69.48±0.7°	30.14±2.1°	99.62±1.3℃	28.06±0.0ª	2.97±0.3 ^b	31.03±0.3ª
50% P (-)	18.36±0.5ª	21.78±3.9ª	40.14±3.3ª	90.75±4.6 ^d	5.08±0.1ª	95.83±4.5℃	27.72±0.3ª	1.39±0.0ª	29.11±04ª
75% P (-)	41.28±3.9 ^b	22.64±2.7ª	63.92±1.1 ^b	70.00±0.6°	$14.25 \pm 1.4^{\text{b}}$	84.26±2.0 ^b	34.39±2.9ª	1.13±0.1ª	35.53±3.1 ^{ab}
100% P (-)	69.66±10.9℃	44.94±2.5 ^b	114.60±8.4°	47.48±0.1ª	6.49±0.2ª	53.97±0.1ª	46.09±4.5 ^b	4.61±0.0℃	50.70±4.5°
100% P (+)	14.10±1.6ª	27.14±2.8ª	41.24±4.4ª	59.24±4.1 ^b	50.10±2.7 ^d	109.34±1.4 ^d	34.36±2.3ª	4.39±0.5℃	39.96±1.8 ^b

SC: Soluble carbohydrates, Ins. C: Insoluble carbohydrates, TC: Total carbohydrates, data are given as averages of 3 replicates±standard error, values followed by the different letters are significantly different at p<0.05

application of high concentration of phosphorus (+100%) to the culture of *M. tenuissima* and *S. platensis* led to significantly increase in insoluble (mainly glycogen) and total carbohydrate contents. On the other side, the deficiency of phosphorus concentrations led to a decrease in carbohydrate contents of *M. tenuissima* with compared to the control culture. In general, the total carbohydrate contents in the tested cyanobacteria were 11.46% from Anabaena at 100% P deficiency, 10.9% from Merismopedia at P 100% and 5.07% from Spirulina at 100% P deficiency. In this respect, al.²⁵ Mota et showed that the cyanobacterium Cyanothece sp. CCY 0110 is among the most efficient extracellular polysaccharides producers, releasing the majority of the carbohydrates into the culture medium. Phosphate variation did not show any significant effect on the carbohydrates of A. platensis, in which phosphorus was limited²⁶. The produced carbohydrates used as feedstock for biofuel generation, A drastic increase in the microalga biomass carbohydrate content under phosphorus starvation, that explained by ADP-glucose pyrophosphorylase (carbohydrates synthesis) is activated by the 3-phosphoglycerate^{27,28}.

Protein metabolism: Phosphorus-free media and increase in phosphorus concentration by 100% resulted in an increase in protein fractions such as soluble, insoluble, globulins, prolamines, glutelins and total protein content of *Anabaena* sp. Generally, a decrease and/or an increase in phosphorus concentrations led to decrease in globulins, prolamines, glutelins insoluble and total protein contents of *M. tenuissima* and *S. platensis* (Table 3). Cyanobacteria fix nitrogen assimilated which affected by P in the form of amino acids and proteins. Protein compounds increase soil fertility²⁹, absorbing heavy metals in wastewater³⁰ (metallothionein protein), kill mosquito eggs in water (larvicidal protein), used in health products³¹, (human superoxide dismutase) and new feed sources for all living³².

Amino acid metabolism: Increase in phosphorus concentrations by 100% resulted in an increase in free amino acid contents of *M. tenuissima* and *S. platensis*. The highest amount of free amino acid however, was observed by the treatment of *Anabeana* with 100% phosphorus deficiency (Table 4). Amino acid profiles of many algae studied to present are much higher than the recommended value. In this respect, *Spirulina, Anabaena* and *Nostoc* are consumed as human food in many countries in the form of powder,

able 3: Effe	ect of differen	t concentrat.	ions of phos	phorus on pr	otein conten	ts of cyanoba	cterial species	: (mg g ^{_1} DV	V)									
	Anabaena .	sp.					Merismop	edia tenuis.	sima				Spirulina pla	atensis				
reatments	SP	Glob.	Prol.	Glut.	Ins. P	ΤP	Ъ	Glob.	Prol.	Glut.	lns. p	L L	SP	Glob.	Prol.	Glut.	Ins. P	F
ontrol	87.5±0.7 ^a	50.4±0.8 ^b	80.4±0.8 ^b	37.9±0.0⊆	25.0±1.2 ^c	281.1 ± 2.1^{b}	68.9±2.1 ^{ab}	9.6±1.9 ^b	29.1±1.9 ^b	5.1±0.2℃	2.9±0.3 ^b	115.6±7.1⊆	131.9±2.6 ^a	69.1±1.8 ^d	95.6±1.8℃	68.0±2.5 ^a	53.1±0.7 ^d 4	±27.7±
(-) A %C	113.9±0.8 ^b	40.0 ± 0.5^{a}	68.0 ± 0.5^{a}	17.8 ± 1.2^{a}	12.7 ± 2.0^{a}	252.4 ± 1.5^{a}	72.3±3.1 ^b	6.0 ± 0.0^{ab}	23.5 ± 0.0^{a}	3.2±0.2 ^b	1.7 ± 0.6^{a}	106.6±2.7 ^{bc}	135.4 ± 5.8^{a}	34.6 ± 0.9^{ab}	62.1 ± 0.9^{a}	69.4±2.6 ^a	33.1±0.5 ^a 3	34.7±
5% P (-)	110.6土7.3 ^b	36.9±2.2ª	61.9±2.2 ^a	23.3 ± 2.5^{a}	18.1 ± 2.2^{a}	250.9 ± 1.8^{a}	65.2 ± 3.5^{ab}	6.9 ± 1.6^{ab}	24.4 ± 1.6^{ab}	2.2±0.1 ^a	2.4土0.1 ^{ab}	101.1 ± 0.1^{abc}	150.5±9.4 ^{ab}	37.4±0.8 ^{bc}	67.3±0.8 ^b	74.3±4.0 ^a	39.5±2.6 ^{bc} 3	£1.69
(-) A %0C	118.5 ± 0.8^{b}	79.2±0.4 ^d	86.2±0.4 ^b	45.9±0.8 ^d	33.6±2.2 ^d	363.4±3.1 ^d	57.7 ± 4.4^{a}	3.9土1.8ª	22.8 ± 1.8^{a}	2.4 ± 0.1^{ab}	2.6土0.1 ^{ab}	89.2±7.9ª	147.9±1.5 ^{ab}	32.8±0.1 ^a	59.9±0.1ª	67.0±0.1 ^a	35.9±0.4 ^{ab} 3	43.5±
(+) d %0C	105.5 ± 6.6^{b}	60.8±5.6 ^c	85.1 ± 5.6^{b}	30.9±3.1 ^b	$19.8 \pm 1.6^{\rm bc}$	302.1±3.1℃	60.3 ± 3.5^{a}	6.8土1.5 ^{ab}	21.2 ± 1.7^{a}	2.8 ± 0.5^{ab}	2.3±0.1 ^{ab}	93.4±0.9ªb	160.7±8.6 ^b	40.1±0.2℃	67.2±0.3 ^b	71.2±1.4ª	41.3±0.3 ^c 3	80.5±
P: Soluble	oroteins, Glob	h.: Globulins,	Prol.: Prolam	nines, Glut.: G	ilutelins, Ins. p	: Insoluble pr	oteins, TP: Tot	tal proteins,	data are give.	n as averad	es of 3 repl	icates±stand.	ard error, valu	les followed h	ov the differe	ent letters ar	e significantly	/ differ

0.7^d 9.7^a 17.7^{bc} 2.3^{ab} 10.2^c ent at

Pak. J. Biol. Sci., 23 (1): 55-62, 2020

Table 4: Effect of	different	concentrations of	phosphorus of	on free amino	acid and total lir	oid contents of c	vanobacterial si	oecies (ma a−1 l	DW)
			P						- /

	Anabaena sp.		Merismopedia ten	nuissima	Spirulina platensi	is
Treatments	Amino acids	Total lipids	Amino acids	Total lipids	Amino acids	Total lipids
Control	95.89±9.6ª	75.4±0.8 ^b	30.86±2.4 ^b	34.0±1.4 ^b	44.39±0.9 ^{ab}	158.4±1.4 ^{ab}
50% P (-)	45.46±3.3 ^{ab}	98.6±3.8 ^d	26.64±2.1 ^b	38.1±0.2°	55.03±4.1b ^c	175.3±19.7 ^ь
75% P (-)	55.57±7.1 ^b	84.3±1.9°	20.66±0.6ª	41.0±0.7°	37.26±2.8ª	151.4±1.3 ^{ab}
100% P (-)	138.76±10.5 ^d	85.6±1.9°	16.78±1.6ª	31.2±0.4 ^b	38.39±1.3ª	137.5±10.6ª
100% P (+)	26.30±5.3ª	22.0±0.7ª	38.65±2.1°	24.7±1.9ª	56.54±5.6°	183.2±5.3 ^b

Data are given as averages of 3 replicates±standard error, values followed by the different letters are significantly different at p<0.05

Table 5: Effect of different phosphorus concentrations on the phycobiliprotein contents of cyanobacterial species (mg g^{-1} DW)

	<i>Anabaena</i> s	p.			Merismopedi	ia tenuissima	1		Spirulina p	olatensis		
Treatments	PC	APC	PE	ТР	PC	APC	PE	TP	PC	APC	PE	ТР
Control	115.2±9.6ª	25.9±3.6ª	11.7±0.8ª	152.8±13.9ª	168.6±11.7°	37.2±3.2 ^b	5.4±2.3ª	211.3±6.3°	59.3±3.3 ^b	52.4±0.9 ^b	22.7±0.4°	134.3±4.6 ^b
50% P (-)	118.3 ± 4.0^{a}	25.4±9.7ª	14.0 ± 1.7^{a}	157.8±4.1ª	137.1±13.9 ^b	20.4 ± 1.1^{ab}	5.7 ± 0.8^{a}	163.2±3.0 ^b	80.3±4.9°	65.9±1.9°	28.2 ± 0.7^{d}	174.4±4.6°
75% P (-)	117.9 ± 7.4^{a}	38.7 ± 5.9^{ab}	12.0 ± 1.9^{a}	168.6±15.2ª	129.7±11.1 ^b	34.5 ± 5.6^{ab}	4.9±1.5ª	169.2±18.3 ^b	55.1 ± 1.2^{b}	49.9±3.4 ^b	$18.1 \pm 1.3^{\text{b}}$	123.2±8.9 ^b
100% P (-)	114.9±0.9ª	27.3±1.5ª	10.6 ± 1.9^{a}	152.8±0.5ª	110.4±4.9 ^{ab}	25.9 ± 0.4^{ab}	13.5±6.4ª	149.9±1.2 [♭]	38.3±3.3ª	34.3 ± 1.6^{a}	12.1±0.5ª	84.7 ± 5.4^{a}
100% P (+)	$165.0 \pm 2.4^{\text{b}}$	55.1 ± 3.2^{b}	15.9±1.5ª	236.0 ± 14.0^{b}	87.0±4.1ª	16.6 ± 1.4^{a}	4.1±1.5ª	107.8±4.5ª	$62.0{\pm}0.5^{\scriptscriptstyle b}$	$52.4\pm0.1^{ ext{b}}$	$18.5 \pm 1.3^{\text{b}}$	$132.9 \pm 0.6^{\text{b}}$
PC: Phycocy	anin, APC: All	ophycocyani	in, PE: Phyco	erythrin, TP: To	otal phycobilip	oteins, data a	are given as a	averages of 3	replicates±s	standard erro	or, values foll	owed by the

different letters are significantly different at p<0.05

flakes, tablets and capsules^{33,34}. It is used as a food supplement, feed fishes in aquacultures and in human diet.

Lipid metabolism: Cyanobacteria having high reproduction rates, high photosynthetic capacity and low nutritional requirements and do not compete for fertile and arable lands, that promising for the production of biofuels^{35,36}. The total lipid contents of *Anabaena* sp. and *M. tenuissima* were stimulated by phosphorus deficiency. The application of high concentration of phosphorus (+100%) to the culture of *S. platensis* led to an increase in total lipid contents in comparison to control (Table 4). Phosphorus deficiency and /or starvation for a short period supplemented with CO₂ increase the lipid contents in many Cyanobacteria and microalgae³⁷⁻⁴⁰.

Phycobiliprotein metabolism: Table 5 showed that the phycocyanin, allophycocyanin, phycoerythrin and total phycobiliprotein contents were stimulated by 50 and 75% reduction as well as 100% increase of phosphorus concentration in *Anabaena* sp. Generally, the decrease and/or increase in phosphorus concentrations significantly decreased the phycobiliprotein fractions in *M. tenuissima*. However, the highest phycobiliprotein contents of *S. platensis* were recorded at 50% phosphorus deficiency

In spite of most of cyanobacteria species containing phycocyanin, the small number used for commercial production in large scale especially in tropical and subtropical regions^{41,42}, which are used as coloring agents and antioxidants applications as food additives^{43,44}.

CONCLUSION

In conclusion, *Merismopedia* contains the highest carbohydrates about 20% as well as 11% total phycobiliproteins, *Spirulina* contains 43% proteins and 18 total lipids. In addition, *Anabaena* sp. contains about 13% amino acids based on dry weight, these metabolites efforts should be directed towards phosphorus concentrations in the medium. The produced carbohydrates used as feedstock for biofuel generation and used as a food supplement, feed fishes in aquacultures and in human diet and new feed sources for all living organisms. Phycocyanin used as coloring agents and antioxidants applications as food additives.

SIGNIFICANCE STATEMENT

This study discovers that, Phosphorus is the first or the second elemental factors affecting growth and metabolism of three cyanobacteria. *Merismopedia* contains the highest carbohydrates about 20% as well as 11% total phycobiliproteins. While, *Spirulina* contains 43% proteins and 18% total lipids. In addition, *Anabaena* sp. contains about 13% amino acids based on dry weight, in addition, some nutritional components including chlorophyll, carbohydrate (mainly, glycogen) proteins, amino acids, lipids and Phycocyanin. This study will help the researcher to use these species of cyanobacteria in foods and feeds technology.

ACKNOWLEDGMENTS

The results presented in this paper were obtained with financial support from the King Abdulaziz City for Science and Technology, Saudi Arabia (AT-35-139). We are grateful to the Agency of Taif University for graduate studies and scientific research.

REFERENCES

- 1. Karl, D.M., 2014. Microbially mediated transformations of phosphorus in the sea: New views of an old cycle. Annu. Rev. Mar. Sci., 6: 279-337.
- 2. Lin, S., R.W. Litaker and W. Sunda, 2015. Phosphorus physiological ecology and molecular mechanisms in marine phytoplankton. J. Phycol., 1: 10-36.
- 3. Xu, H., H.W. Paerl, B. Qin, G. Zhu and G. Gao, 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic lake Taihu, China. Limnol. Oceanogr., 55: 420-432.
- 4. Yoshiyama, K. and J.H. Sharp, 2006. Phytoplankton response to nutrient enrichment in an urbanized estuary: Apparent inhibition of primary production by overeutrophication. Limnol. Oceanogr., 51: 424-434.
- 5. Issa, A.A., M.H. Abd-Alla and T. Ohyama, 2014. Nitrogen Fixing Cyanobacteria: Future Prospect. In: Advanced in Biology and Ecology of Nitrogen Fixation, Ohyama, T. (Ed.)., IntechOpen Limited, London, ISBN: 978-953-51-1216-2, pp: 23-47.
- 6. Rippka, R. and M. Herdmann, 1993. Pasteur Culture Collection of Cyanobacterial Strains in Axenic Culture: Catalogue of Strains. Vol. 1, Institut Pasteur, France, pp: 103.
- Stanier, R.Y., R. Kunisawa, M. Mandel and G. Cohen-Bazire, 1971. Purification and properties of unicellular blue-green algae (order *Chroococcales*). Bacteriol. Rev., 35: 171-205.
- Holden, M., 1976. Chlorophylls. In: Chemistry and Biochemistry of Plant Pigments, 2nd Edn., Vol. 2, Goodwin, T.W. (Ed.)., Academic Press, London, pp: 1-37.
- 9. Bennett, A. and B. Bogorad, 1973. Complementary chromatic adaptation in a filamentous blue-green alga. J. Cell Biol., 58: 419-435.
- 10. Fales, F.W., 1951. The assimilation and degradation of carbohydrates by yeast cells. J. Biol. Chem., 193: 113-124.
- 11. Schlegel, H.G., 1956. Die ver wertung organisch ersauren duch *Chlorella* in licht. Planta, 117: 510-526.
- 12. Badour, S.S.A., 1959. Analytisch chemische unter suchung des kalimangels bei *Chlorella* in vergleich mit anderen manget zamstanden. Ph.D. Thesis, Goettingen.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
- 14. Lee, Y.P. and T. Takahashi, 1966. An improved colorimetric determination of amino acids with the use of ninhydrin. Anal. Biochem., 14: 71-77.

- Drevon, B. and J.M. Schmitt, 1964. La reaction sulfophospho vanillique dans l'ét.ude des lipids seriques. Bull. Trau. Soc. Pharm. Lyon., 8: 173-178.
- Singh, S.K., S.S. Singh, V.D. Pandey and A.K. Mishra, 2006. Factors modulating alkaline phosphatase activity in the diazotrophic rice-field cyanobacterium, *Anabaena oryzae*. World J. Microbiol. Biotechnol., 22: 927-935.
- 17. Singh, P., S.S. Singh, J. Elster and A.K. Mishra, 2013. Molecular phylogeny, population genetics and evolution of heterocystous cyanobacteria using *nif*H gene sequences. Protoplasma, 250: 751-764.
- Henson, B.J., S.M. Hesselbrock, L.E. Watson and S.R. Barnum, 2004. Molecular phylogeny of the heterocystous cyanobacteria (subsections IV and V) based on *nii*D. Int. J. Syst. Evol. Microbiol., 54: 493-497.
- 19. Geider, R.J. and J.L. Roche, 2002. Redfield revisited: Variability of C:N:P in marine microalgae and its biochemical basis. Eur. J. Phycol., 37: 1-17.
- 20. Leonardos, N. and R.J. Geider, 2004. Responses of elemental and biochemical composition of *Chaetoceros muelleri* to growth under varying light and nitrate: Phosphate supply ratios and their influence on critical N: P. Limnol. Oceanogr., 49: 2105-2114.
- Luque, I. and K. Forchammer, 2008. Nitrogen Assimilation and C/N Sensing. In: The Cyanobacteria Molecular Biology, Genomics and Evolution, Herrero, A. and E. Flores (Eds.)., Caister Academic Press, Norfolk, UK., ISBN: 978-1-904455-15-8, pp: 334-382.
- De Ruyter, Y.S. and P. Fromme, 2008. Molecular Structure of the Photosynthetic Apparatus. In: The Cyanobacteria Molecular Biology, Genomics and Evolution, Herrero, A. and E. Flores (Eds.)., Caister Academic Press, Norfolk, UK., pp: 217-269.
- 23. Fromme, P., J. Kern, B. Loll, J. Biesiadka and W. Saenger *et al.*, 2002. Functional implications on the mechanism of the function of photosystem II including water oxidation based on the structure of photosystem II. Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci., 357: 1337-1345.
- 24. Liu, X.J. and F. Chen, 2003. Cell differentiation and colony alteration of an edible terrestrial cyanobacterium *Nostoc flagelliforme*, in liquid suspension cultures. Folia Microbial., 48: 619-626.
- 25. Mota, R., R. Guimaraes, Z. Buttel, F. Rossi and G. Colica *et al.*, 2013. Production and characterization of extracellular carbohydrate polymer from *Cyanothece* sp. CCY 0110. Carbohydr. Polym., 92: 1408-1415.
- 26. Cade-Menun, B.J. and A. Paytan, 2010. Nutrient temperature and light stress alter phosphorus and carbon forms in culture-grown algae. Mar. Chem., 121: 27-36.
- 27. Markou, G., I. Chatzipavlidis and D. Georgakakis, 2012. Effects of phosphorus concentration and light intensity on the biomass composition of *Arthrospira* (*Spirulina*) *platensis*. World J. Microbiol. Biotechnol., 28: 2661-2670.

- Milovanović, I., A. Mišan, J. Simeunović, D. Kovač, D. Jambrec and A. Mandić, 2015. Determination of volatile organic compounds in selected strains of cyanobacteria. J. Chem., Vol. 2015. 10.1155/2015/969542.
- 29. Minocheherhomji, F.P. and A. Pradhan, 2016. Evaluation of total protein production by soil cyanobacteria in culture filtrate at various incubations periods. Int. J. Adv. Phar. Biol. Chem., 5: 346-349.
- Melis, A., 2009. Solar energy conversion efficiencies in photosynthesis: Minimizing the chlorophyll antennae to maximize efficiency. Plant Sci., 177: 272-280.
- Khasdan, V., E. Ben-Dov, R. Manasherob, S. Boussiba and A. Zaritsky, 2003. Mosquito larvicidal activity of transgenic *Anabaena* PCC 7120 expressing toxin genes from *Bacillus thuringiensis* subsp. *israelensis*. FEMS Microbiol. Lett., 227: 189-195.
- Akgül, R., B. Kızılkaya, F. Akgül and H. Erduğan, 2015. Amino acid composition and crude protein values of some Cyanobacteria from Çanakkale (Turkey). Pak. J. Pharm. Sci., 28: 1757-1761.
- 33. Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv., 25: 207-210.
- Diniz, G.S., E. Barbarino, J. Oiano-Neto, S. Pacheco and S.O. Lourenço, 2011. Gross chemical profile and calculation of nitrogen-to-protein conversion factors for five tropical seaweeds. Am. J. Plant Sci., 2: 287-296.
- 35. Taboada, C., R. Millan and I. Miguez, 2010. Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. J. Sci. Food Agric., 90: 445-449.
- Sarsekeyeva, F.K., B.K. Zayadan, A. Usserbaeva, V.S. Bedbenov, M.A. Sinetova and D.A. Los, 2015. Cyanofuels: Biofuels from cyanobacteria. Reality and perspectives. Photosynth. Res., 125: 329-340.

- Li, Y., M. Horsman, B. Wang, N. Wu and C.Q. Lan, 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Applied Microbiol. Biotechnol., 81: 629-636.
- Mayers, J.J., K.J. Flynn and R.J. Shields, 2014. Influence of the N: P supply ratio on biomass productivity and time-resolved changes in elemental and bulk biochemical composition of *Nannochloropsis* sp. Bioresour. Technol., 169: 588-595.
- Makareviciene, V., V. Skorupskaite and V. Andrulevicute, 2013. Biodiesel fuel from microalgae-promising alternative fuel for the future: A review. Rev. Environ. Sci. Biotechnol., 2: 119-130.
- Rodolfi, L., G.C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini and M.R. Tredici, 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol. Bioeng., 102: 100-112.
- 41. Sørensen, N., N. Daugbjerg and K. Richardson, 2013. Choice of pore size can introduce artefacts when filtering picoeukaryotes for molecular biodiversity studies. Microb. Ecol., 65: 964-968.
- 42. Eriksen, A., 2007. Gender, Christianity and Change in Vanuatu: An Analysis of Social Movements in North Ambrym. 1st Edn., CRC Press, UK., ISBN: 9780754672098, Pages: 216.
- 43. Pandey, V.C., J.S. Singh, D.P. Singh and R.P. Singh, 2014. Methanotrophs: Promising bacteria for environmental remediation. Int. J. Environ. Sci. Technol., 11: 241-250.
- Panjiar, N., S. Mishra, A.N. Yadav and P. Verma, 2018. Functional Foods from Cyanobacteria: An Emerging Source for Functional Food Products of Pharmaceutical Importance. In: Microbial Functional Foods and Nutraceuticals, First Edition, Gupta, V.K., H. Treichel, V.O. Shapaval, L.A. de Oliveira and M.G. Tuohy (Eds.)., Chapter 2. John Wiley & Sons Ltd., New York, ISBN: 9781119049012, pp: 21-37.