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Optimal Laboratory Cultivation Conditions of *Limnospira maxima* for Large-Scale Production

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Simple Summary: This study addresses the challenge of harnessing *Limnospira maxima*, a cyanobacterium rich in proteins, carbohydrates, vitamins, and essential fatty acids, to enhance global food security. The problem lies in the lack of information regarding the optimal cultivation conditions, high production costs, and limited nitrogen sources. The objective is to identify the optimal cultivation conditions that maximize yields, minimize costs, and strengthen global food security. The results emphasize the importance of white and yellow light for the cultivation of *L. maxima*, as well as the use of KNO_3 as a cost-effective nitrogen source. It was observed that nitrogen deficiency increases dry biomass production but affects vital photosynthetic pigments such as chlorophyll and phycocyanin. *L. maxima*'s adaptability to various conditions, including nitrogen deficiency, is attributed to mechanisms involving phycobilisome degradation and glycogen storage. These findings offer new insights into cyanobacterial cultivation practices, which can significantly boost sustainable food production, global nutritional security, and promise a more sustainable future in both food production and environmental balance.

Abstract: Limited insights into optimal cultivation conditions, high production costs, and nitrogen source limitations have posed challenges for harnessing the potential of *Limnospira maxima*, a cyanobacterium rich in essential proteins, carbohydrates, vitamins, and fatty acids, to address global food security. This study seeks to overcome these obstacles by identifying optimal cultivation parameters that maximize yields, minimize costs, and bolster global food security efforts. Our research underscores the significance of white and yellow light spectra for optimizing *L. maxima* cultivation, and highlights KNO_3 as a cost-effective nitrogen source for large-scale production. While nitrogen deficiency enhances dry biomass production, it also affects vital photosynthetic pigments such as chlorophyll and phycocyanin. *L. maxima*'s adaptability to diverse environments, including nitrogen-deficient conditions, can be attributed to mechanisms involving phycobilisome degradation and glycogen storage. These findings offer novel insights into sustainable cultivation practices, paving the way for refined growth strategies that can significantly boost sustainable food production and global nutritional security. This research contributes to the advancement of

cyanobacterial cultivation, promising a more sustainable future in both food production and environmental equilibrium.

Keywords: nitrogen source; food security; cyanobacteria; *Arthrospira maxima*; phycobiliproteins; biomass production

1. Introduction

Cyanobacteria, previously misidentified as blue-green algae, is a group of gram-negative bacteria characterized by their proficiency in oxygenic photosynthesis and nitrogen fixation [1–3]. Among these, spirulina (*Limnospira maxima*), *synon. Arthrospira maxima* distinguishes as the most extensively cultivated cyanobacterium globally. Its cultivation contributes to over 30% of global microalgal biomass production [4], yielding approximately 20,000 metric tons annually across the world [5]. Notably, *L. maxima* possesses remarkable attributes, boasting a high protein content (60-70% of dry weight, DW), carbohydrates, and vitamin B12—nutrients rarely abundant in fruits, legumes, or vegetables [6,7]. Notably, it contains fourfold the amount of vitamin B_{12} compared to raw liver and a vitamin E quantity akin to that found in wheat grain [7,8]. The cyanobacterium also offers a rich profile of minerals, essential fatty acids, and pigments, including chlorophyll and carotenoids (surpassing carrot levels by 30 times). Additionally, *L. maxima's* valuable phycobiliproteins utilized in the food industry and potentially offering health benefits, further underscore its significance [8]. Remarkably, a mere kilogram of spirulina can provide nutritional value equivalent to 1,000 kilograms of diverse fruits and vegetables [9].

Securing food availability and access for all individuals remains a pivotal goal. Nonetheless, the persistence of global hunger poses a substantial challenge, particularly in developing nations [10]. This pressing concern is exacerbated by various factors, including population growth, economic disparities, and limited arable land. Addressing these challenges necessitates not only augmenting food production, but also exploring innovative solutions that can withstand climate fluctuations and resource constraints. Cultivating *L. maxima* emerges as a promising strategy owing to its exceptional nutritional content and unique adaptability to space- and resource-constrained environments [11]. By harnessing the potential of alternative food sources like *L. maxima*, we are a step forward in achieving sustainable food security and addressing global hunger.

Over the three past decades, *L. maxima* has experienced widespread trade and consumption across North and South America, Asia, Europe, and Africa. Its consumption has garnered acclaim from numerous governments, health agencies, and associations in over 60 countries [12]. The United Nations Food Conference has hailed *L. maxima* as a leading food for the future, and the World Health Organization (WHO) has hailed it as a paramount product for human health [13]. *L. maxima* has earned the title of "superfood," with even NASA recognizing its potential as sustenance for space travel due to its remarkable nutrient density [14]. The biomass of *L. maxima* is commonly consumed through nutritional supplements as dried powder, flakes, or capsules [15].

Nitrogen stands as a vital nutritional prerequisite, pivotal in biomass and pigment production while offering cost-efficient benefits for large-scale cultivation. Mousavi, *et al.* [16] examined the influence of a modified Zarrouk medium [17] supplemented with six nitrogen sources ($NaNO_3$, KNO_3, NH_4NO_3, NH_4SO_4, NH_4Cl, and urea) on biomass and phycocyanin production in *A. platensis*. They observed that growth media supplemented with $NaNO_3$ and KNO_3 resulted in the highest biomass production (~1.18 g L^{-1}) after an 18-day cultivation period. Recent studies have underscored the critical role of light in enhancing pigment production, notably phycobiliproteins, in cyanobacteria [18–20]. These studies elucidated how cyanobacteria adeptly adjust the concentration and composition of their photosynthetic pigments to optimize light absorption in response to irradiation and the light spectrum [21]. Notably, the application of red LEDs in *Arthrospira sp*. was driven by their coverage of the pigment absorption spectrum (620-645 nm), resulting in heightened energy utilization and greater biomass production [18]. Similarly, *Spirulina sp*. cultivated under yellow LEDs

in mixotrophic conditions exhibited phycocyanin content 5 to 6 times higher (380 g kg^{-1} DW) than the control (an autotrophic culture system employing a fluorescent lamp), which yielded 70 g kg^{-1} DW [22]. Additionally, research by Park and Dinh [19] delved into the effects of monochromatic LED lighting on growth, pigments, and photosynthesis in *L. maxima*. They found that red LEDs and white, fluorescent light yielded the highest growth and chlorophyll a (Chl a) concentration, while blue LEDs produced the highest phycobiliprotein levels (3.20 g kg^{-1} phycocyanin, 0.19 g kg^{-1} allophycocyanin, and 0.97 g kg^{-1} phycoerythrin). Building upon our central hypothesis, which posits $NaNO_3$ as the optimal nitrogen source for *L. maxima* cultivation and suggests that white and yellow LEDs would yield similar outcomes, our primary objective was to comprehensively evaluate various light spectra and nitrogen sources in *L. maxima* cultivation for large-scale implementation.

2. Materials and Methods

2.1. Cyanobacteria Strain and Production Medium

The strain *Limnospira maxima* was generously provided by AGROIMSA – Mexico as part of the reciprocal cooperation agreement MVZ – 002-2015. Registered under OR195505.1 in GenBank [25], this strain has been acclimatized to laboratory conditions and cultured using a modified Zarrouk medium [17]. The research was conducted at the Aquaculture Health and Water Quality Laboratory of the University of Cordoba (Montería, Colombia; 8°47'37" N; 75°51'51" W, 15 m a.s.l.). Culture containers of 4-liter capacity, containing 3 L of medium, were employed. The composition comprised 20% of the modified Zarrouk culture medium [17] infused with a nitrogen source ($NaNO_3$, KNO_3, or control – without nitrogen, WN), 20% inoculum of *L. maxima* (~1.2 g/L), and 60% deionized water supplemented with 1 g L^{-1} sea salt. To achieve the appropriate pH levels, NaOH, KOH, or $Ca(OH)_2$ were utilized for the $NaNO_3$, KNO_3, and WN treatments, respectively. Continuous aeration was maintained in all experimental units, supplied by an aquarium pump (Aquarium pump, PUMPOWER®, model PR-3710, Germany).

The study adopted a completely randomized design with a 4 x 3 factorial arrangement, incorporating 4 irradiation sources (white, red, blue, and yellow), 3 nitrogen sources ($NaNO_3$, KNO_3, and WN), and 5 repetitions (Supplementary Figure S1). Further details on the lamps and their emitted photosynthetically active radiation (PAR) are presented in Table 1. Within each plastic container, a 50 x 50 mm photovoltaic cell, regulated by a C and C++ Arduino Uno R3 Microcontroller A000066 (Arduino Inc., Belmont, CA, USA; accessed on 13 June 2023), was utilized to monitor the PAR received by each culture medium at the cyanobacterial level. Measurements were recorded at two-hour intervals. The cultures were maintained under controlled conditions of temperature (24 ± 3°C) and a photoperiod of 12 hours light / 12 hours darkness, for a total of 27 days.

Table 1. Characteristics of Light Sources and Measured Photosynthetic Active Radiation (PAR).

Light source	Model	Trademark	Color	Power	PAR (µmol m^{-2} s^{-1})	
					theoretical	measured[1]
LED T5	M22	Mercury, Kwangchow, China	Write - W	18W	82.8	79.14 ± 1.94
LED T5	M29	Mercury, Kwangchow, China	Red – R	18W	82.8	77.51 ± 2.23
LED T5	M21	Mercury, Kwangchow, China	Blue – B	18W	82.8	78.10 ± 1.81
LED T5	M24	Mercury, Kwangchow, China	Yellow – Y	18W	82.8	79.31 ± 1.85

[1] all value denotes mean (±SE), n = 15 p_{value} = 0.162 (ns).

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2.2. Optical Density and Growth Rate – Biomass

The evaluation of growth rate relied on measuring the optical density (OD) at 680 nm using a microplate reader (ThermoScientific™ Multiskan™ GO, Missouri City, TX, USA). The measurement process consisted of taking 1.5 mL of the culture medium for optical density measurement and 5 mL for dry weight determination. For dry weight assessment, the culture medium was vacuum-filtered using 47 mm diameter 0.5 µm Whatman® membrane filters (part number WHA7585004, Sigma-Aldrich, Darmstadt, Germany) that were pre-dried in an oven (70°C for 24h) and weighed on an analytical balance (Sartorius Analytical Balance mod. ENTRIS224-1S, Bradford, MA, USA) with a precision of 0.1 mg. The filtered biomass was subsequently washed with distilled water to remove excess salts, dried in an oven at 70°C for 24 hours, subjected to a desiccator for 1 hour, and re-weighed. The dry weight was determined by calculating the difference between the initial and final weights of the filter with the biomass, divided by the volume of the filtered sample, and expressed in g L^{-1}. Both optical density (OD) and dry weight (DW) were measured at intervals of 3 days, spanning up to the 27th day of the experiment.

2.3. Biochemical Analysis

2.3.1. Chlorophyll and Total Carotenoids

The determination of chlorophyll content was conducted following the method outlined by Wellburn [26], with adaptations to facilitate measurement using a microplate reader. Every 3 days, a 10 mL aliquot of the culture was extracted and rapidly frozen at -65ºC for a minimum of 18 hours. After thawing the tubes containing 10 mL of culture at 25ºC, they were centrifuged at 25ºC, 5,000 x g for 10 minutes. The supernatant was then discarded, and the pellet was used for chlorophyll extraction. To each 2 mL polypropylene tube (Sigma-Aldrich, Darmstadt, Germany, part number Z760951), pre-prepared with 0.1% poly(vinylpolypyrrolidone) (Sigma-Aldrich, part number 77627), 2 mL of 99% methanol (Sigma-Aldrich, part number 179337) was added. The tubes were mixed for 30 seconds, enveloped in aluminum foil, and placed in a thermo-shaker (Multitherm, Benchmark Scientific, Sayreville, NJ, USA) for incubation at 80ºC and 500 rpm for 5 minutes. Afterward, the tubes were centrifuged at 4ºC for 5 minutes at 15,000 x g. The supernatants were transferred to new microtubes for chlorophyll measurement, while the pellets were promptly frozen at -65ºC to measure soluble proteins. For chlorophyll analysis, 200 µL of the extracted solution was dispensed into a glass microplate and read using a microplate reader (ThermoScientific™ Multiskan™ GO, Missouri City, TX, USA). Absorbance was recorded at 652.4 nm, 665.2 nm, 470 nm, and 720 nm (as a negative control). The concentrations of chlorophyll "a" (Chla), chlorophyll "b" (Chlb), and total carotenoids (Car) were estimated using the equations proposed by Lichtenthaler and Buschmann [27].

2.3.2. Phycobiliprotein Measurement

Phycobiliproteins were quantified following the method proposed by Bennett and Bogorad [28], with adaptations for the current research. Samples of 10 mL of the *L. maxima* cell suspension were extracted and vacuum-filtered as previously described. The filtered biomass was re-suspended in 2 mL of a 100 mM pH 7.4 phosphate buffer (Sigma-Aldrich, part number P5244) and briefly mixed for 5 seconds. The resuspended samples were then shielded from light, frozen at -40ºC for 24 hours, and maintained at 4°C until the appearance of the blue pigment (~24 hours). Once pigment extraction was complete, the samples were kept chilled to prevent temperature-induced degradation, followed by centrifugation at 15,000 x g. The resulting supernatant was used to quantitatively assess all phycobiliproteins spectrophotometrically, as per the equations outlined by Bennett and Bogorad [28].

2.3.3. Soluble Proteins Measurement

For the determination of soluble proteins, the pellet retained from the chlorophyll analysis serves as the starting point. To extract soluble proteins, the pellet undergoes a series of washes using 70% Ethanol (Sigma-Aldrich, part number 493511), with each washing step performed 3 to 4 times. During

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each wash cycle, the pellet is thoroughly mixed and subsequently centrifuged at 4°C, at 13,000 x g for 10 minutes. After discarding the supernatant, the pellet remains for subsequent protein measurements.

Following this, the pellet is treated with 400 µL of 0.1M NaOH. The mixture is thoroughly mixed and then incubated at 95°C for 1 hour at 800 rpm. Subsequently, the tubes are centrifuged at 4°C and 13,000 x g for 5 minutes. After establishing a standard curve using Bovine Serum Albumin (BSA) as per the manufacturer's instructions, the samples are prepared accordingly. The Protein Assay Dye Reagent Concentrate (Bio-Rad, Hercules, California, USA, part number #5000006) was utilized in accordance with the manufacturer's guidelines for protein quantification.

2.4. Experimental Design and Statistical Analysis

The experiments were conducted using a completely randomized design, comprising four different light spectra (white, red, blue, and yellow), three nitrogen sources ($NaNO_3$, KNO_3, and a control without nitrogen) (Supplementary Figure S1), and were analyzed in 10 separate instances. All the analyzed features underwent replication with five repetitions.

All data were subjected to two-way repeated measures ANOVA, with time as the dependent variable. This analysis was performed using SigmaPlot for Windows v. 14.0 (Systat Software, Inc., San Jose, CA, USA). Furthermore, to elucidate the relationships between the ana-lyzed features, principal component analysis (PCA) was conducted through multivariate analysis. The PCA was performed using Minitab 18.1 (Minitab, Inc., Chicago, IL, USA).

3. Results

3.1. Photosynthetically Active Radiation Measurement

As shown in table 1, all light regimes exhibited consistent photosynthetically active radiation (PAR) levels across all treatments, with a PAR ranging from 77.51 ± 2.23 µmol photons m^{-2} s^{-1} under red LEDs to 79.31 ± 1.85 µmol photons m^{-2} s^{-1} under yellow LEDs. No significant differences in PAR were observed among the different media (p = 0.162). This uniformity ensures that all experimental units received equivalent PAR levels, differing only in terms of wavelength.

3.2. Optical Density (OD) and Growth Rate (DW)

The optical density (OD) exhibited a range from 0.19 ± 0.01 in the R-WN L. maxima cell suspension at day 0 to 1.31 ± 0.02 in the W-KNO_3 *L. maxima* cell suspension at day 27 (Figure 1). Meanwhile, the growth rate spanned from 0.23 ± 0.01 g L^{-1} in the Y-KNO^3 *L. maxima* at day 0 to 1.76 ± 0.06 g L^{-1} in the W-WN *L. maxima* at day 18 (Figure 2). Upon comparing only the 27-day *L. maxima* cell suspensions, the optimal outcomes were achieved under both white and yellow LEDs. When evaluating nitrogen sources, it was observed that both KNO_3 and $NaNO_3$ resulted in similar performance, with no significant differences between them (Figure 1).

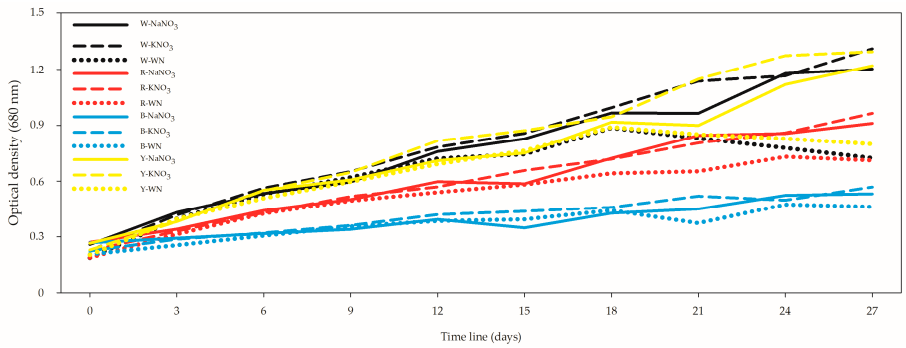

Figure 1. Effect of light spectra and nitrogen source on optical density of *Limnospira maxima* cultivated under white, red, blue, and yellow light spectra, supplemented with $NaNO_3$, KNO_3, and a control (WN). The values presented in the figure indicate the means (± standard error) of optical density.

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Specifically, the 27-day W-WN condition demonstrated a 40% and 45% decrease in OD when compared with W-$NaNO_3$ and W-KNO_3, respectively. Similarly, for the 27-day Y-WN condition, the OD showed a reduction of 34.4% and 38% when compared with Y-$NaNO_3$ and Y-KNO_3, respectively.

The growth rate trends like those observed for the optical density (OD), highlighting the superiority of white and yellow LEDs. Notably, over the 27-day period, the W-WN condition demonstrated a significant 12.6% increase in dry weight (DW) in comparison to W-$NaNO_3$ (Figure 2). Remarkably, this improvement was not significantly different when compared to the KNO_3 condition. Similarly, the Y-WN condition exhibited a noteworthy non-significant growth of 10.8% compared to Y-$NaNO_3$. These results underscore the influential role of both light spectrum and nitrogen source on the growth dynamics *of Limnospira maxima*. The OD and dry weight (DW) exhibited moderate correlation at both the 12- and 27-day time points, revealing their interdependence as growth indicators. However, an intriguing observation emerged when all datasets were aggregated as input data: the correlation between these two variables intensified significantly ($r = 0.798$; $p = 5.82 \times 10^{-41}$) (Supplementary data file). This suggests that the relationship between OD and DW is multifaceted, influenced not only by the growth period but also by various intrinsic and extrinsic factors. This enhanced correlation highlights the complexity of the interplay between growth kinetics and biomass accumulation in *L. maxima* under varying conditions.

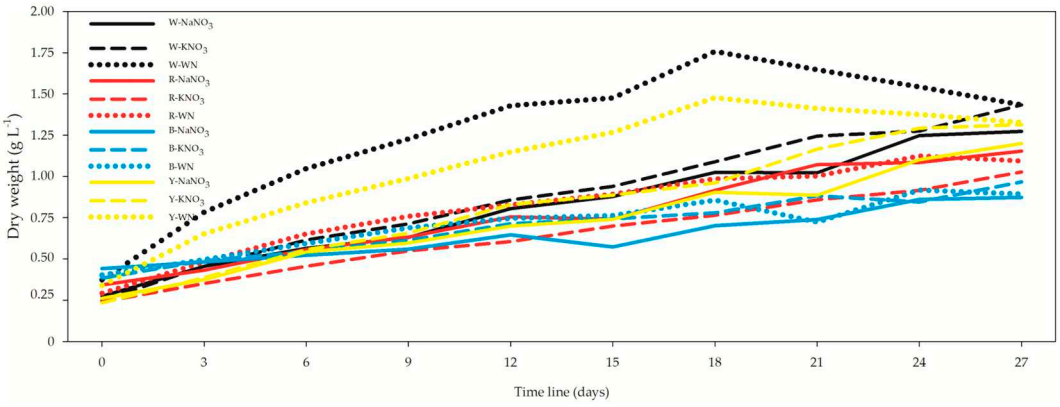

Figure 2. Effect of light spectra and nitrogen source on dry weight of *Limnospira maxima* cultivated under white, red, blue, and yellow light spectra, supplemented with $NaNO_3$, KNO_3, and a control (WN). The values presented in the figure denote the means (± standard deviation) of dry weight.

3.3. Chlorophyll (Chla and Chlb) and Total Carotenoids (Car)

Chlorophyll "a" concentration showed variation, ranging from 2.99 ± 0.11 g kg^{-1} DW at day 0 in the R-KNO_3 *L. maxima* cell suspension (Figure 3), to 3.09 ± 0.11 g kg^{-1} DW in a W-WN *L. maxima* cell suspension at day 18. Specifically considering the 27-day *L. maxima* cell suspension, W-WN exhibited the most favorable outcomes, followed by W-KNO_3, Y-$NaNO_3$, Y-WN, and R-$NaNO_3$. Interestingly, blue-LEDs and all other Red-LED nitrogen sources consistently displayed lower values throughout the study.

Regarding chlorophyll "b" a slightly distinct pattern emerged compared to Chla. Chlb varied from 9.86 ± 0.06 g kg^{-1} DW in a W-KNO_3 *L. maxima* cell suspension at day 0 (Figure 4), to 82.01 ± 0.06 g kg^{-1} DW in a W-$NaNO_3$ *L. maxima* cell suspension at day 27. Focusing solely on the 27-day *L. maxima* cell suspensions, it becomes apparent that W-$NaNO_3$ was effective in synthesizing 44.3% and 41.2% more Chl b com-pared to W-KNO_3 or W-WN, respectively. A similar behavior was observed under yellow lights, where Y-KNO3 demonstrated 2.3% and 67.8% higher Chl b synthesis when compared to Y-$NaNO_3$ and Y-WN (Figure 3).

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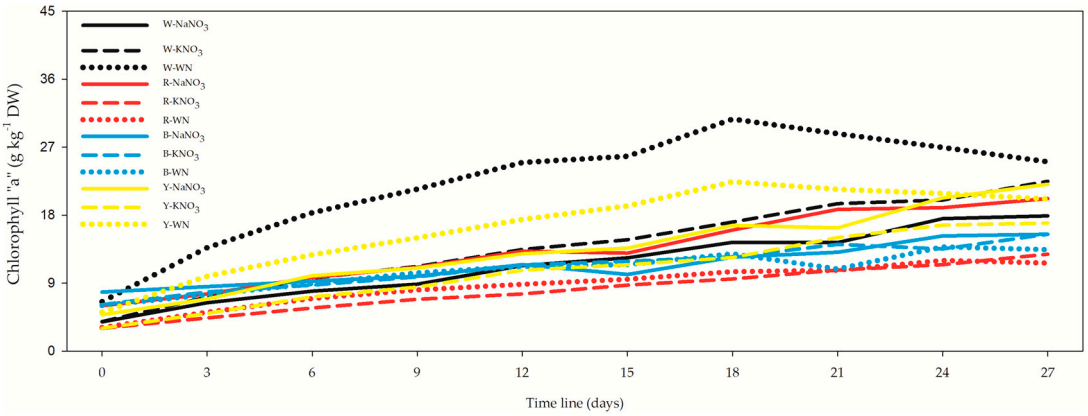

Figure 3. Influence of light spectra and nitrogen source on chlorophyll "a" concentration in *Limnospira maxima* cultivated under different conditions. This figure illustrates the impact of varying light spectra and nitrogen sources on the chlorophyll "a" concentration in *Limnospira maxima* cultures. The cultures were supplemented with $NaNO_3$, KNO_3, or maintained as controls (WN) under white, red, blue, and yellow light spectra.

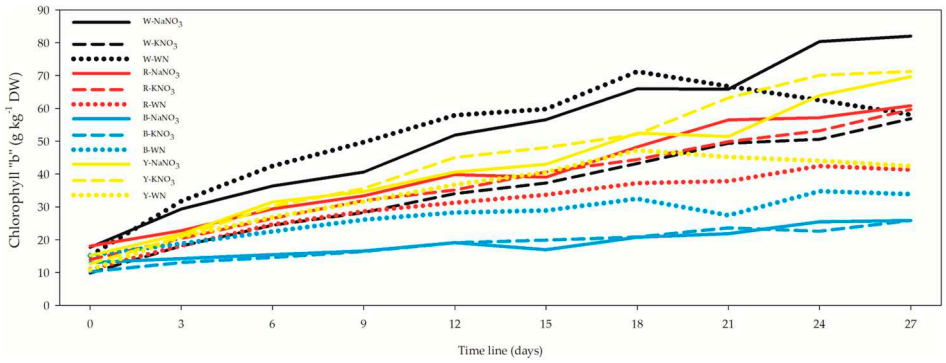

Figure 4. Effect of light spectra and nitrogen source on chlorophyll "b" concentration of *Limnospira maxima* grown in white, red, blue, and yellow light spectra, supplemented with $NaNO_3$, KNO_3, plus control (WN).

Total carotenoids exhibited a notably distinct pattern compared to the previous results. The variation in carotenoid concentration spanned from 0.20 ± 0.01 g kg^{-1} DW in the Y-$NaNO_3$ *L. maxima* cell suspension at day 0 (Figure 5) to 4.22 ± 0.101 g kg^{-1} DW in a W-$NaNO_3$ *L. maxima* cell suspension at day 27. Specifically focusing on the 27-day cell suspensions, the order of highest Carotenoid concentration was observed as follows: W-$NaNO_3$, B-WN, W-KNO_3, and Y-WN.

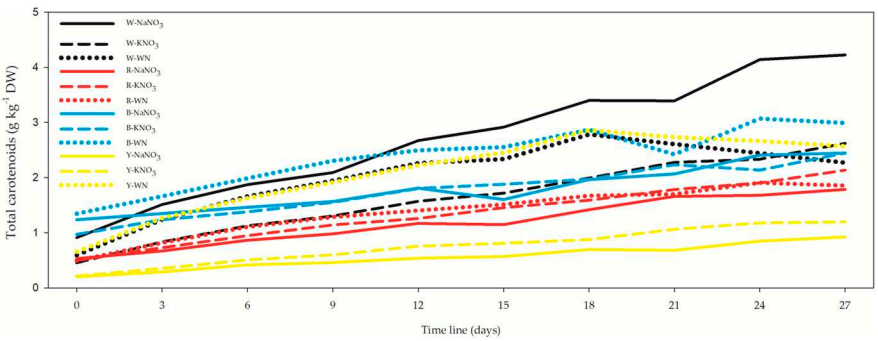

Figure 5. Effect of light spectra and nitrogen source on total carotenoids concentration of *Limnospira maxima* grown in white, red, blue, and yellow light spectra, supplemented with $NaNO_3$, KNO_3, plus control (WN).

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The correlation between Chlorophyll "a" (Chla) and Optical Density (OD) exhibited varying strengths over different time intervals. At 3-days, the correlation was non-significant (r = 0.312), and it remained weak at both 12-days (r = 0.312) and 27-days (r = 0.312). However, when considering the entire dataset with mean values, a more apparent correlation emerged (r = 0.669). Notably, the correlation between Chla and wet weight was consistently positive, with a moderately high strength at 27-days (r = 0.708), high at 3-days (r = 0.881) and 12-days (r = 0.878), and even stronger when all data were aggregated (r = 0.921).

Conversely, the correlation between Chlorophyll "b" (Chlb) and OD displayed differing trends. At 3-days, it showed weak correlation (r = 0.633), becoming moderate at 12-days (r = 0.795) and 27-days (r = 0.760). When considering the combined data, the correlation between Chlb and OD remained strong (r = 0.878). The correlation between Chlb and Dry Weight (DW) followed a similar pattern to Chlb x OD.

The relationship between total carotenoids (Car) and OD or DW showed distinct pattern. For Car x OD at 3- and 12-days, the correlation was negative, while Car x DW displayed a positive correlation during these time frames. At 27 days, the correlation between Car x OD or Car x DW was not statistically significant. However, when aggregating all data, the correlation for Car x OD was weak (r = -0.252), and Car x DW exhibited a moderate correlation (r = 0.587). For additional details, refer to the Supplementary Data File.

3.4. Phycocyanin, Allophycocyanin, Phycoerythrin, and Soluble Proteins

The concentration of phycocyanin exhibited a range from 52.02 ± 1.64 g kg^{-1} DW in the Y-WN *L. maxima* cell suspension at the outset (Figure 6) to 384.11 ± 9.52 g kg-1 DW in the W-KNO_3 *L. maxima* cell suspension at the 27-day mark. At the conclusion of the 27-day cultivation period, the sequence of phycocyanin concentration was as follows: W-KNO_3, W-$NaNO_3$, Y-$NaNO_3$, R-$NaNO_3$, Y-KNO_3. Notably, the W-WN treatment at day 27 demonstrated a reduction of 44.8% and 46% compared to W-$NaNO_3$ and W-KNO_3, respectively. Similarly, under red light at day 27, the R-WN treatment showed decreases of 26.9% (R-NaNO3) and 9% (R-KNO3). Conversely, the reduction seen in B-WN under blue light at day 27 was non-significant, with values dropping by only 0.6% (B-$NaNO_3$) and 1.7% (B-KNO_3). The Y-WN treatment under yellow light at day 27 displayed a decline to 38% and 34.8% of the values observed in Y-$NaNO_3$ and Y-KNO_3, respectively.

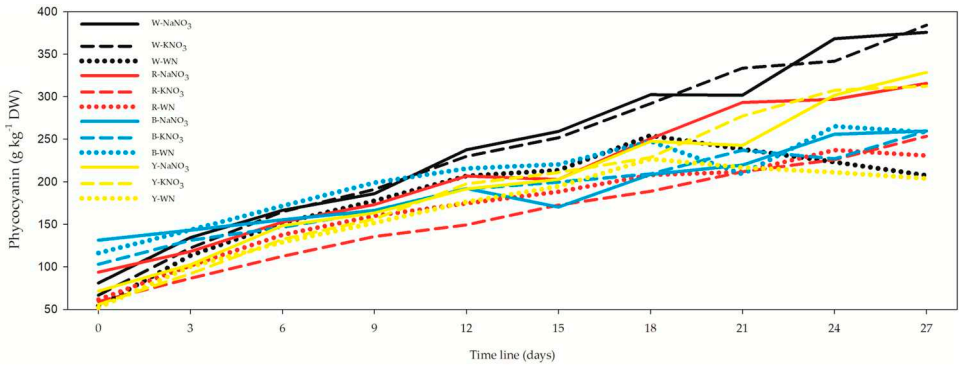

Figure 6. Impact of Light Spectra and Nitrogen Source on Phycocyanin Concentration in *Limnospira maxima* Cultivation. This figure showcases the influence of different light spectra and nitrogen sources on the concentration of phycocyanin in *Limnospira maxima* cultures. The cultures were supplemented with $NaNO_3$, KNO_3, or maintained as controls (WN) under white, red, blue, and yellow light spectra.

Regarding the correlation between phycocyanin and OD, it was weak and negative (r = -0.267), weak and positive (r = 0.252), moderate and positive (r = 0.663), and strong and negative (r = -0.825) for the 3-day, 12-day, 27-day datasets, and their combined data, respectively (Supplementary data file).

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Allophycocyanin demonstrated a range from 23.13 ± 1.64 g kg-1 DW in the W-KNO_3 *L. maxima* cell suspension at the commencement (Figure 7) to R-$NaNO_3$ *L. maxima* cell suspension at the 27-day interval. Focusing solely on the 27-day *L. maxima* cell suspension treatments, it becomes evident that R-$NaNO_3$ was the most productive treatment for allophycocyanin. Notably, the R-$NaNO_3$ medium exhibited increments of 24.4%, 28.5%, 34.8%, and 43.3% compared to B-WN, W-$NaNO_3$, Y-KNO_3, and R-KNO_3, respectively.

As for the correlation between allophycocyanin and OD, it displayed a moderately negative correlation (r = -0.633), indicating an inverse relationship.

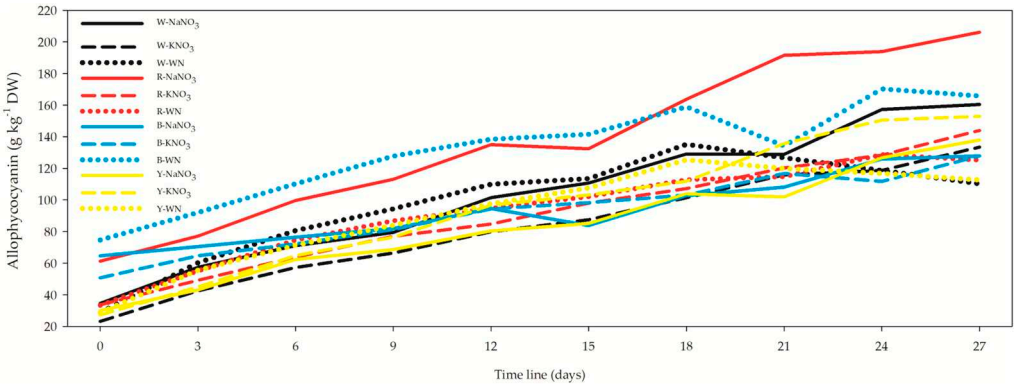

Figure 7. Effect of light spectra and nitrogen source on allophycocyanin concentration of *Limnospira maxima* grown in white, red, blue, and yellow light spectra, supplemented with $NaNO_3$, KNO_3, plus control (WN).

The correlation between allophycocyanin and OD was moderately negative (r = -0.321) and non-significant (p = 0.834) for 3-, 12-, and 27-days. However, the correlation between allophycocyanin and OD became moderately strong (r = -0.709) when all data were combined. Phycoerythrin, as the third phycobiliprotein in descending order, exhibited a range from 9.81 ± 0.14 g kg^{-1} DW for W-KNO_3 *L. maxima* cell suspension at 0 days (Figure 8) to 86.68 ± 2.20 g kg^{-1} DW for R-$NaNO_3$ *L. maxima* cell suspension at 27 days. Similarly, to allophycocyanin, phycoerythrin showed higher concentrations under the red LEDs. In the *L. maxima* cell suspension at 27 days, R-$NaNO_3$ provided 7.5%, 13.1%, and 40% higher phycoerythrin concentrations compared to W-$NaNO_3$, Y-KNO_3, and B-$NaNO_3$, respectively. The correlation between phycoerythrin and OD was moderate and negative (r = -0.539), negative and weak (r = -0.186), and positive and non-significant (p = 0.164) for the 3-, 12-, and 27-day *L. maxima* cell suspensions. However, a strong and negative correlation (r = -0.654) between phycoerythrin and OD was observed when all datasets were combined.

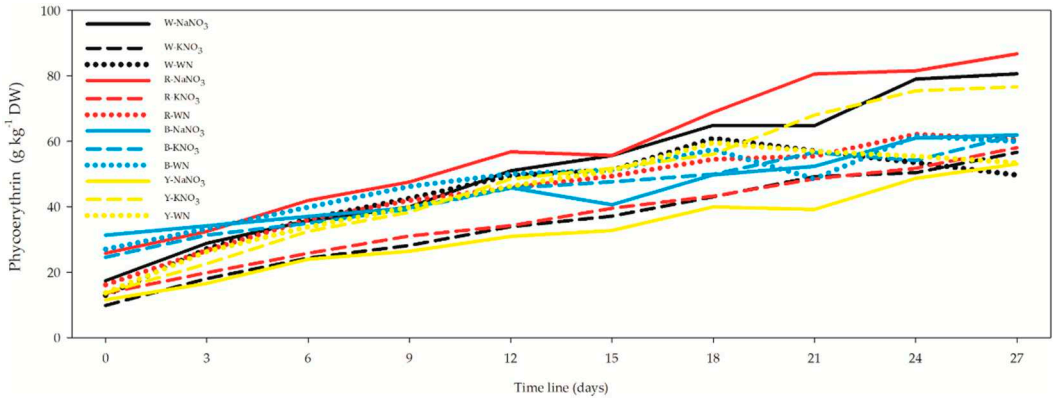

Figure 8. Effect of light spectra and nitrogen source on phycoerytrin concentration of *Limnospira maxima* grown in white, red, blue, and yellow light spectra, supplemented with $NaNO_3$, KNO_3, plus control (WN).

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Moreover, the results for soluble proteins displayed a completely different pattern compared to other proteins described above (Figure 9). For an *L. maxima* cell suspension at 27 days, a higher value was obtained for Y-WN (152.86 ± 2.88 g kg^{-1} DW), which was 7.5%, 24.6%, 40.1%, and 41.5% higher than Y-KNO_3, W-$NaNO_3$, W-WN, and Y-$NaNO_3$, respectively. The correlation between protein concentration and OD was moderate in the 12- (r = 0.515) and 27-day *L. maxima* cell suspension (r = 0.466), and strong in the 3-day suspension (r = 0.939), with r = 0.752 when all datasets were combined.

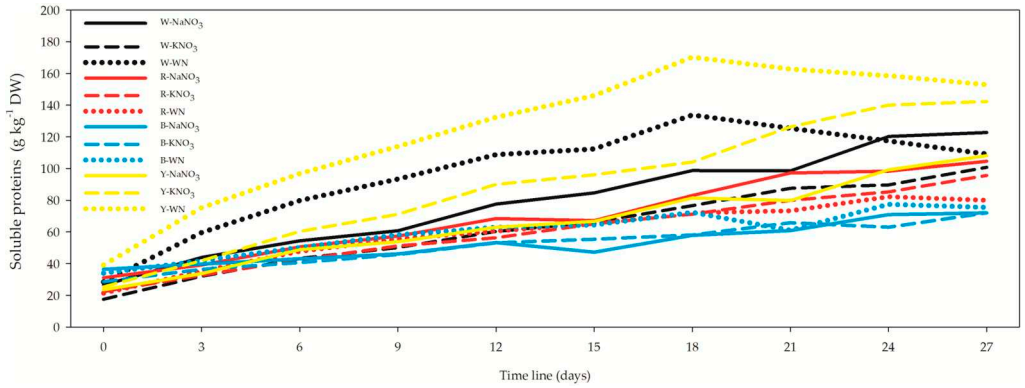

Figure 9. Effect of light spectra and nitrogen source on soluble proteins concentration of *Limnospira maxima* grown in white, red, blue, and yellow light spectra, supplemented with $NaNO_3$, KNO_3, plus control (WN).

3.5. Principal Component Analysis (PCA)

The principal component analysis (PCA) reveals the formation of data clusters into 5 distinct groups encompassing all treatments. The first group amalgamates four treatments with shared characteristics (W-$NaNO_3$, W-WN, Y-KNO_3, and Y-WN), while the second group aligns W-KNO_3 and Y-$NaNO_3$. The third group aggregates R-KNO_3 and R-WN, the fourth group aligns B-KNO_3 and B-WN, and the fifth group merges R-$NaNO_3$ and B-$NaNO_3$ (Figure 10). Figure 9B indicates that the vectors corresponding to OD, Chlb, and DW significantly con-tribute to the formation of the first group. Conversely, the presence of allophycocyanin and phycoerythrin chiefly influences the formation of the fifth group. Notably, groups 2, 3, and 4 lack distinct defining characteristics. These three groups exhibit substantial interconnectedness, with their dendrogram reflecting a lack of pronounced similarities among all indices above a threshold of 59.2 (data not shown).

The Pearson correlation analysis reveals strong correlations ($r \geq 0.850$) among all factors. Notably, optical density (OD) exhibits a negative correlation with total carotenoids, phycocyanin, allophycocyanin, and phycoerythrin (Supplementary data file). Therefore, it is reasonable to anticipate that changes in one characteristic would correspond to changes in others. This anticipation aligns with the actual observations, leading us to determine that the treatment involving yellow or white light supplemented with KNO_3 is the most favorable. It is suggested that even if the outcomes deviate from initial expectations, this treatment remains the optimal choice (further elaborated in the discussion section).

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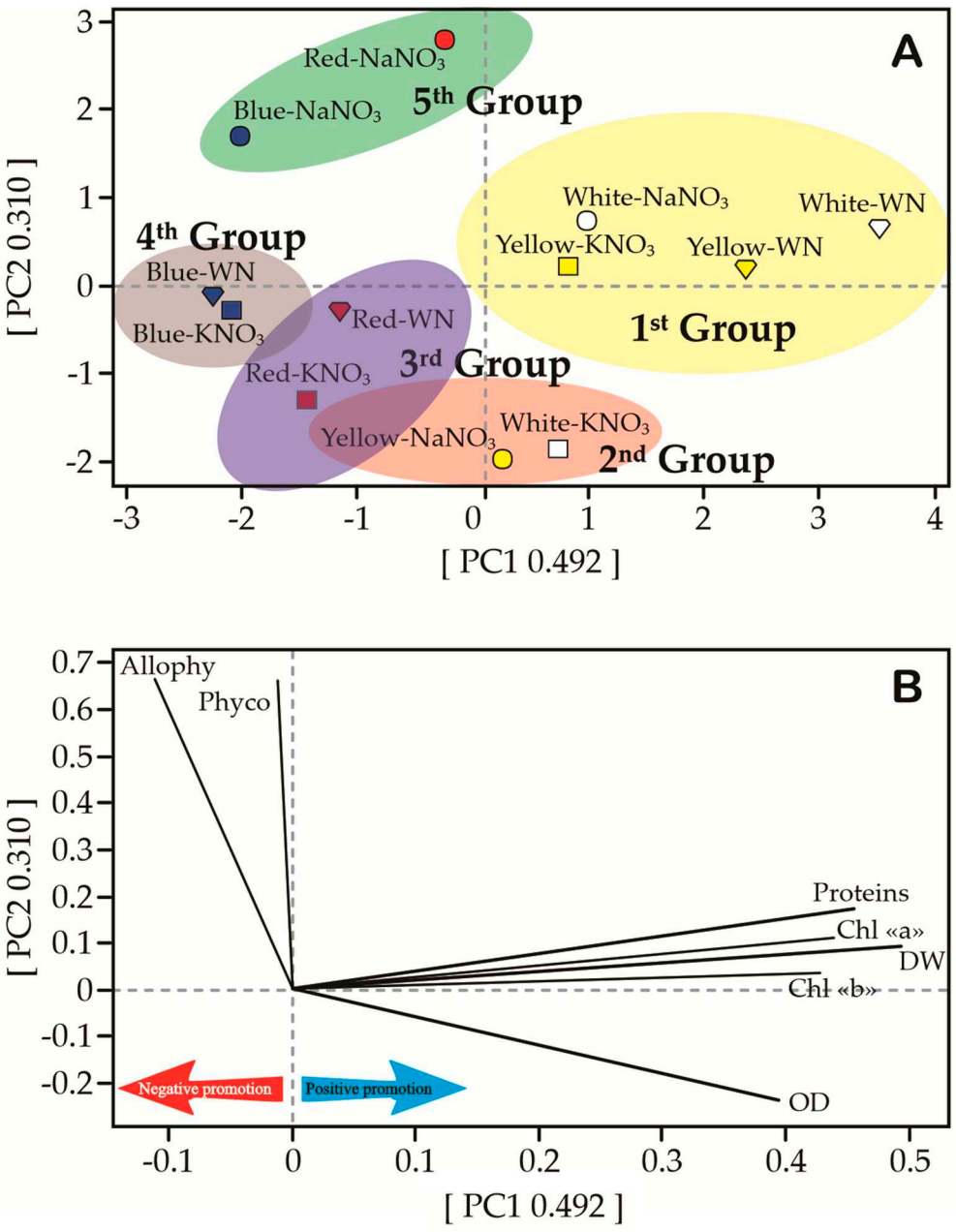

Figure 10. Multivariate Analysis of Light and Nitrogen Source Effects on Various Features of *Limnospira maxima* Cultivated in Different Light Spectra (White, Red, Blue, and Yellow), Supplemented with $NaNO_3$, KNO_3, and Control (WN). **A** The figure depicts a five-group clustering of treatments based on their similarities. B. The diagram illustrates the strength of each feature's influence on *L. maxima* growth, as observed through the increase in optical density (OD) and dry weight (DW). All measurements were taken after 27 days of *L. maxima* growth. Allophy refers to allophycocyanin; Phyco represents phycoerythrin; Proteins indicates soluble proteins; Chl "a" signifies chlorophyll a; Chl "b" represents chlorophyll b.

4. Discussion

The cyanobacteria *L. maxima* has evolved unique physiological and morphological characteristics that allow them to respond to changes in environmental parameters, such as, nutrients and light [29]. In our study, we contribute novel findings that shed light on the intricate interactions between light spectra, nitrogen sources and various physiological features of *L. maxima*. Our results reveals that when comparing *L. maxima* cell suspension at 27 days, white and yellow LEDS promotes the most favorable OD results. Moreover, the choice of nitrogen source, -either KNO_3 and $NaNO_3$ –

yielded growth outcomes with-out significant differences. The observation of higher growth (higher OD) and increased chlorophyll content under white light corroborates findings by Madhyastha and Vatsala [30], on pigment production in *Spirulina fussiformis*, where various conditions were explored. Similarly, our results are align with Ortiz-Moreno, *et al*. [31] who studied *Nostoc ellipsosporum*'s growth in relation to light wavelengths and concluded that white light followed by yellow light resulted in optimal cell growth.

In contrast, blue light showed a marked inhibitory effect on the growth of *N. ellipsosporum*. Khatoon, *et al.* [32], determined the effect of different light sources and media (wastewater and Bold's Basal Medium – BBM), on the growth and production of phyco-biliproteins of *Pseudanabaena mucicola*, describing that white light had a significantly high-er growth rate in terms of OD compared to blue light and natural light. The highest dry biomass was also produced when *P. mucicola* was grown under a white light source with no significant difference between BBM and wastewater media.

Contrary to our results, some studies have reported red light as promoting the highest growth, while blue light exhibited inhibitory effects [18,19,33–35]. The disparity in out-comes might be attributed to variations in light sources, cyanobacterial species (*A. platensis*, *L. maxima*, *A. fusiformis*), culture medium composition, pH levels, aeration rates, and other factors [33]. Our results also differ significantly from the Bahman, *et al.* [36] who describes a higher growth rate of *Arthrospira platensis* under blue light.

It should be noted that nitrogen plays a pivotal role in biomass and pigment production in *L. maxima*. Additionally, nitrogen usage holds economic significance in large-scale cultivation [16,37]. Our study demonstrates that at 27 days, both KNO_3 and $NaNO_3$ yield similar growth, as indicated by optical density measurements. Notably, KNO_3, being more cost-effective, produces comparable cell growth to $NaNO_3$, which is comparatively more expensive. These findings are in line with Mousavi, *et al*. [16], who studied the effect of various nitrogen sources on phycocyanin production in *A. platensis* and found that $NaNO_3$ and KNO_3-supplemented media resulted in the highest biomass production.

The increase in dry weight (DW) production under white and yellow lights, followed by a subsequent decrease, was observed in our study. This behavior was reported by Pelagatti, *et al.* [38] in their research of the effects of yellow and blue light on biochemical characteristics of *Limnospira fusiformis*. Notably, exposure to yellow light led to higher bi-omass production compared to blue light. Similar findings were documented by DeMooij, *et al*. [39], who explored *Chlamydomonas reinhardtii's* productivity concerning bio-mass-specific light absorption rates. They found that yellow light yielded the highest productivity per area, followed by white light, indicating its efficiency in biomass production.

Regarding nitrogen functionality, Möllers, *et al*. [40] noted that nitrogen limitation in *Synechococcus* sp. enhanced the carbon-nitrogen ratio and glycogen content, thus improving its viability as a biomass feedstock. Similarly, Cuellar-Bermudez, *et al.* [41] found that nitrogen-poor conditions prompted Arthrospira to accumulate carbohydrates while reducing protein content. This adaptation to nitrogen starvation results in metabolic shifts, amino acid balance, and carbon metabolism reconfiguration [42]. Our findings align with these insights, as we observed similar responses in *L. maxima* under varying nitrogen conditions.

For the functionality of nitrogen, Möllers, *et al*. [40] found that the nitrogen limitation on *Synechococcus* sp. was used to increase the carbon-nitrogen ratio and glycogen content to improve its utility as a biomass feedstock. In corroboration, Cuellar-Bermudez, *et. al*. [41], found that when *Arthrospira* is grown under nitrogen-poor conditions, it reduces the protein content and accumulates carbohydrates (up to 57-77% of dry weight), generally glycogen [43]. Similar results are shared by Saxena, *et al.* [44] in *A. platensis*. Acclimatization to N starvation is probably the best example of complexity in molecular responses. The biological response varies from metabolic changes to cell differentiation. During N starvation, the central metabolism is often reset, including energy production and energy con-version, amino acid metabolism, carbohydrate transport, and other types of metabolism. In fact, two simultaneous phenomena have been described: (i) the optimization and use of intracellular nitrogenous components (*e.g.* cyanophycin and phycobilisomes) and (ii) the

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redesign of the carbon metabolism to deal with excess carbon [42]. Also, N deficiencies induce phycobilisome degradation that is distinguished by a color change of the culture from blue-green to a paler shade [45]. In addition, some studies have shown that when limited by extracellular nitrogen, cyanophycin granules are degraded to meet the demands of metabolic nitrogen [46]. In addition, a proteomic analysis has shown that an abundance of cyanophykinase is greater under N-stressed conditions, providing a large amount of arginine and aspartate that are more likely to balance against an intracellular N deficiency [47].

Chlorophyll "a" concentrations in our study displayed a pattern similar to DW. Higher concentrations were noted on the 18th day under white and yellow light in the ab-sence of nitrogen. Comparable observations were made by Madhyastha and Vatsala [30], Milia, *et. al.* [34], and others, demonstrating a preference for white and orange lights in promoting chlorophyll "a" levels.

Chlorophyll "b" concentration variations can be attributed to *L. maxima's* surface po-sitioning, which influences its exposure to different light wavelengths. The prominence of chlorophyll "b" indicates its complementarity with chlorophyll "a" in light absorption and photosynthesis [48]. Our study suggests that the high concentration of *L. maxima* led to an almost light barrier-like situation, prompting a more significant role for chlorophyll "b."

Phycocyanin levels in our study peaked under white light, followed by yellow light with NaNO3 or KNO3 supplementation. Our findings highlight the potential of KNO_3 as a cost-effective alternative to $NaNO_3$ in promoting significant phycocyanin production. Interestingly, phycocyanin production responses to light color are varied in the literature [42,45,49,50], emphasizing the complexity of these interactions.

The best results for white light on phycocyanin production were also reported in the study by Ojit, *et al.* [51] who describe a higher concentration of this pigment after 15 days of culture in the cyanobacterium *Anabaena circinalis*. In contrast, they found the least amount of phycocyanin under blue light. Similarly, Park and Dinh [19], described a high-er production of phycocyanin in *L. maxima* in cultures exposed to blue light. Likewise, Milia, *et. al*. [34] reported greater production of this pigment under blue light.

Some scholars have shown that blue light positively influences phycocyanin production [42,45,49,50]. In contrast, white light covers a broader range of wavelengths and does not specifically increase phycocyanin production. Furthermore, the phycocyanin to chlorophyll a ratio and the allophycocyanin to chlorophyll a ratio was higher under blue light than under orange or white light for all strains, indicating that phycobilin content in-creased relative to Chl a when cells were exposed to blue light. Blue light stimulates *L. maxima* to produce more phycobilins to compensate for the lack of energy related to the limited range of radiation (400–475 nm), resulting in increased protein production [34]. In our study, blue light was overwhelmed by white, yellow, and sometimes by red light.

In a wide range of literature reviews, *L. maxima* exhibits a remarkable ability to with-stand nitrogen starvation and enabling it to thrive in various environments. Despite facing nutrient limitations, *L. maxima* displays exceptional adaptability and continues its growth. Regarding the absence of nitrogen, the cultures exposed to white and yellow light in the absence of nitrogen, showed a decrease in the concentration of phycocyanin from the 18th day, while the treatments exposed to red light began to decrease after the 24th day. Joseph, *et. al*. [52] describes that during prolonged nitrogen deprivation, the cyanobacterium *Synechocystis* sp. PCC 6803 stores glycogen and degrades nitrogen-rich phycobilisomes, resulting in a loss of the pigment phycocyanin, a condition known as photo-blanching or chlorosis. Phycobilisome proteins constitute about 50% of the total protein in the cyanobacterial cell under optimal growth conditions and therefore can provide massive amounts of nutrients along with a degradation in response to N starvation [27]. In the absence of N, protein synthesis is limited, and the photosynthetic energy and carbon are diverted from the synthesis of protein to carbohydrate production [41]. However, *L. maxi-ma* actively degrades its proteins and recycles the nitrogen associated with amino acids. This indicates that *L. maxima* could degrade its phycobilisomes to supply nitrogen for other metabolic processes. The degradation of the phycobilisome could be in accordance with the observed reduction in phycocyanin content.

5. Conclusions

In this study, we delved into the intricate interplay between environmental factors, particularly light and nitrogen availability, and the physiological responses of *Limnospira maxima*. The presented data underscores the significance of white and yellow light in promoting substantial growth and chlorophyll content, aligning with previous studies in the field. A comparison of nitrogen sources reveals that both KNO_3 and $NaNO_3$ exhibit similar effects on cell growth, with KNO_3 emerging as the more cost-effective option for large-scale cultivation. Our investigation into pigment production highlights the prominence of white light in enhancing phycocyanin levels, a critical pigment with implications for both nutritional and industrial applications. However, the nuanced influence of light color on phycocyanin content requires further exploration, given divergent results in the literature.

The remarkable adaptability of *L. maxima* to varying nitrogen conditions serves as a testament to its resilience and intricate metabolic responses. The discerned patterns of pigment degradation in the absence of nitrogen shed light on the organism's dynamic nature, where phycobilisomes are strategically harnessed as repositories of both nitrogen and energy. These insights deepen our comprehension of *L. maxima's* adept survival strategies and open new avenues for probing its potential role in nutrient recycling and adaptive metabolic responses.

In conclusion, our study uncovers the intricate relationships between light, nitrogen, and physiological responses in *Limnospira maxima.* These findings have implications for sustainable food production and resource management, particularly in the face of climate change and resource limitations. By unraveling the adaptive strategies of cyanobacteria like *L. maxima*, we contribute to the broader discourse on sustainability and offer insights into potential solutions for addressing global food security challenges. The study of cyanobacteria's metabolic flexibility and responses provides a promising avenue for revolutionizing food production and resource utilization in the pursuit of a more sustainable future.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, Y.Y.P.-R., M.F.P., L.A.R.-P., A.V.I. and D.S.H-C.; method-ology, Y.Y.P.-R. and M.F.P.; software, M.F.P. and K.A.P.; validation, Y.Y.P.-R. and M.F.P.; formal analysis, Y.Y.P.-R. and M.F.P.; investigation, Y.Y.P.-R., M.F.P., M.G.B., J.D.C-O., L.A.R.-P., K.A.P., D.V.A., A.P-G and A.R.A.-G.; resources, A.J.-O., D.S.H.-C., A.V.I., J.D.D.J.-N. and L.A.R.-P.; data curation, M.F.P.; writing—original draft preparation, Y.Y.P.-R. and M.F.P.; writing—review and editing, Y.Y.P.-R., M.F.P. and L.A.R.-P.; visualization, M.F.P., Y.Y.P.-R. and L.A.R.-P.; supervision, A.J.-O., D.S.H.-C., A.V.I. and J.d.D.J-N.; project administration, A.V.I.; funding acquisition, A.J.-O., D.S.H.-C., A.V.I. and J.d.D.J-N. All authors have read and agreed to the published version of the manuscript.

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References

1. Ciferri, O. Spirulina, the edible microorganism. *Microbiol Rev* **1983**, *47*, 551-578, doi:10.1128/mr.47.4.551-578.1983.
2. Pineda-Rodríguez, Y.Y.; Vegliante, D.; Ariza, A.; Pico, A.; Herazo, D.; Castro, G.R.; Vallejo, A.; Jarma-Orozco, A.; Jaraba-Navas, J.D. Effect of two culture media (Jourdan and Zarrouk) on the production of chlorophyll and phycobiliproteins of *Arthrospira maxima*. In Proceedings of the Asociacion Colombiana de Ciencias Biologicas, Riohacha, Colombia, 2021; pp. 136-139.

3. Singh, J.S.; Kumar, A.; Rai, A.N.; Singh, D.P. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Front Microbiol* **2016**, *7*, 529, doi:10.3389/fmicb.2016.00529.
4. Costa, J.A.V.; Freitas, B.C.B.; Rosa, G.M.; Moraes, L.; Morais, M.G.; Mitchell, B.G. Operational and economic aspects of spirulina-based biorefinery. *Bioresour Technol* **2019**, *292*, 121946, doi:10.1016/j.biortech.2019.121946.
5. Wang, J.; Cheng, W.; Liu, W.; Wang, H.; Zhang, D.; Qiao, Z.; Jin, G.; Liu, T. Field study on attached cultivation of *Arthrospira* (Spirulina) with carbon dioxide as carbon source. *Bioresour Technol* **2019**, *283*, 270-276, doi:10.1016/j.biortech.2019.03.099.
6. Ravindran, B.; Gupta, S.; Cho, W.M.; Kim, J.; Lee, S.; Jeong, K.H.; Lee, D.; Choi, H.C. Microalgae potential and multiple roles - current progress and future prospects - an overview. *Sustainability* **2016**, *8*, 1215, doi:10.3390/su8121215.
7. Yin, C.; Daoust, K.; Young, A.; Tebbs, E.J.; Harper, D.M. Tackling community undernutrition at lake Bogoria, Kenya: the potential of spirulina (*Arthrospira fusiformis*) as a food supplement. *Afr J Food Agric Nutr Dev* **2017**, *17*, 11603-11615, doi:10.18697/ajfand.77.15440.
8. Soni, R.A.; Sudhakar, K.; Rana, R.S. Spirulina – from growth to nutritional product: a review. *Trends Food Sci Technol* **2017**, *69*, 157-171, doi:10.1016/j.tifs.2017.09.010.
9. Chittora, D.; Meena, M.; Barupal, T.; Swapnil, P.; Sharma, K. Cyanobacteria as a source of biofertilizers for sustainable agriculture. *Biochem Biophys Rep* **2020**, *22*, 100737, doi:10.1016/j.bbrep.2020.100737.
10. Azadi, H.; Ghazali, S.; Ghorbani, M.; Tan, R.; Witlox, F. Contribution of small-scale farmers to global food security: a meta-analysis. *J Sci Food Agric* **2023**, *103*, 2715-2726, doi:10.1002/jsfa.12207.
11. Gala, J.L.; Cole, N.R.; Eggett, D.L.; Johnson, S.M. Growth comparison of *Arthrospira platensis* in different vessels: standard cylinder vs. enhanced surface area at low light. *Appl Phycol* **2023**, *4*, 1-14, doi:10.1080/26388081.2022.2125829.
12. García-López, D.A.; Olguín, E.J. Potential strategies and opportunities for the development of *Arthrospira maxima* (Spirulina) processes: A review. *Rev Lat Biotec Amb y Algal* **2020**, *11*, 1-14, doi: Available in http://www.solabiaa.org/ojs3/index.php/RELBAA/article/view/87.
13. Dolganyuk, V.; Sukhikh, S.; Kalashnikova, O.; Ivanova, S.; Kashirskikh, E.; Prosekov, A.; Michaud, P.; Babich, O. Food proteins: potential resources. *Sustainability* **2023**, *15*, 5863, doi:10.3390/su15075863.
14. Kulshreshtha, A.; Zacaria, J.A.; Jarouliya, U.; Bhadauriya, P.; Prasad, G.; Bisen, P.S. Spirulina in health care management. *Curr Pharm Biotechnol* **2008**, *9*, 400-405, doi:10.2174/138920108785915111.
15. Lafarga, T.; Mayre, E.; Echeverria, G.; Viñas, I.; Villaró, S.; Acién-Fernández, F.G.; Castellari, M.; Aguiló-Aguayo, I. Potential of the microalgae *Nannochloropsis* and *Tetraselmis* for being used as innovative ingredients in baked goods. *LTW Food Sci Technol* **2019**, *115*, 108439, doi:10.1016/j.lwt.2019.108439.
16. Mousavi, M.; Mehrzad, J.; Najafi, M.F.; Zhiani, R.; Shamsian, S.A.A. Nitrate and ammonia: two key nitrogen sources for biomass and phycocyanin production by *Arthrospira* (spirulina) *platensis*. *J Appl Phycol* **2022**, *34*, 2271-2281, doi:10.1007/s10811-021-02664-0.
17. Rajasekaran, C.; Ajeesh, C.P.M.; Balaji, S.; Shalini, M.; Siva, R.; Das, R.; Fulzele, D.P.; Kalaivani, T. Effect of modified Zarrouk's medium on growth of different *Spirulina* strains. *Agric Technol Biol Sci* **2016**, *13*, 67-75.
18. Prates, D.F.; Radmann, E.M.; Duarte, J.H.; Morais, M.G.; Costa, J.A.V. Spirulina cultivated under different light emitting diodes: enhanced cell growth and phycocyanin production. *Bioresour Technol* **2018**, *256*, 38-43, doi:10.1016/j.biortech.2018.01.122.
19. Park, J.; Dinh, T.B. Contrasting effects of monochromatic LED lighting on growth, pigments and photosynthesis in the commercially important cyanobacterium *Arthrospira maxima*. *Bioresour Technol* **2019**, *291*, 121846, doi:10.1016/j.biortech.2019.121846.
20. Garcia, A.K.; McShea, H.; Kolaczkowski, B.; Kacar, B. Reconstructing the evolutionary history of nitrogenases: evidence for ancestral molybdenum-cofactor utilization. *Geobiology* **2020**, *18*, 394-411, doi:10.1111/gbi.12381.
21. Morosinotto, T.; Poletto, L.; La Rocca, N. Oxygenic photosynthetic responses of cyanobacteria exposed under an M-Dwarf starlight simulator: implications for exoplanet's habitability. *Front Plant Sci* **2023**, *14*, 1070359, doi:10.3389/fpls.2023.1070359.
22. Bachchhav, M.B.; Kulkarni, M.V.; Ingale, A.G. Enhanced phycocyanin production from *Spirulina platensis* using light emitting diode. *J Inst Eng India Ser E* **2017**, *98*, 41-45., doi:10.1007/s40034-016-0090-8.
23. Gardner, N.L. The marine algae of the Pacific coast of North America. *University of California publications in botany* **1937**, *8*, 1-3, doi:10.5962/bhl.title.5719.

24. Nowicka-Krawczyk, P.; Mühlsteinová, R.; Hauer, T. Detailed characterization of the *Arthrospira* type species separating commercially grown taxa into the new genus *Limnospira* (Cyanobacteria). *Sci Rep* **2019**, *9*, 694, doi:10.1038/s41598-018-36831-0.
25. Pineda-Rodriguez, Y.Y.; Pompelli, M.F.; Jarma-Orozco, A.; Rodríguez, N.V.; Rodriguez-Paez, L.A. A new and profitable protocol to DNA extraction in *Limnospira maxima*. *Methods and Protocols* **2023**, *6*, 62, doi:10.3390/mps6040062.
26. Wellburn, A.R. The spectral determination of chlorophyll *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiol* **1994**, *144*, 307-313.
27. Lichtenthaler, H.K.; Buschmann, C. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. *Curr Protoc Food Anal Chem* **2001**, *F*, 1-8, doi:10.1002/0471142913.faf0403s01.
28. Bennett, A.; Bogorad, L. Complementary chromatic adaptation in a filamentous blue-green alga. *J Cell Biol* **1973**, *58*, 419-435, doi:10.1083/jcb.58.2.419.
29. Tandeau de Marsac, N.; Houmard, J. Adaptation of cyanobacteria to environmental stimuli: new steps toward molecular mechanisms. *FEMS Microbiol Rev* **1993**, *10*, 119-190, doi:10.1111/j.1574-6968.1993.tb05866.x.
30. Madhyastha, H.K.; Vatsala, T.M. Pigment production in *Spirulina fussiformis* in different photophysical conditions. *Biomed Eng* **2007**, *24*, 301-305, doi:10.1016/j.bioeng.2007.04.001.
31. Ortiz-Moreno, M.L.; Cárdenas-Poblador, J.; Agredo, J.; Solarte-Murillo, L.V. Modeling the effects of light wavelength on the growth of *Nostoc ellipsosporum*. *Univ Sci* **2020**, *25*, 113-148, doi:10.11144/Javeriana.SC25-1.mte.
32. Khatoon, H.; Kok Leong, L.; Abdu Rahman, N.; Mian, S.; Begum, H.; Banerjee, S.; Endut, A. Effects of different light source and media on growth and production of phycobiliprotein from freshwater cyanobacteria. *Bioresour Technol* **2018**, *249*, 652-658, doi:10.1016/j.biortech.2017.10.052.
33. Jung, C.H.G.; Waldeck, P.; Sykora, S.; Braune, S.; Petrick, I.; Küpper, J.-H.; Jung, F. Influence of different light-emitting diode colors on growth and phycobiliprotein generation of *Arthrospira platensis*. *Life* **2022**, *12*, 895, doi:10.3390/life12060895.
34. Milia, M.; Corrias, F.; Addis, P.; Chini Zitelli, G.; Cicchi, B.; Torzillo, G.; Andreotti, V.; Angioni, A. Influence of different light sources on the biochemical composition of *Arthrospira* spp. grown in model systems. *Foods* **2022**, *11*, 399, doi:10.3390/foods11030399.
35. Tayebati, H.; Pajoum Shariati, F.; Soltani, N.; Sepasi Tehrani, H. Effect of various light spectra on amino acids and pigment production of *Arthrospira platensis* using flat-plate photobioreactor. *Prep Biochem Biotechnol* **2021**, *21*, 1-12, doi:10.1080/10826068.2021.1941102.
36. Bahman, M.; Aghanoori, M.; Jalili, H.; Bozorg, A.; Danaee, S.; Bidhendi, M.E.; Amrane, A. Effect of light intensity and wavelength on nitrogen and phosphate removal from municipal wastewater by microalgae under semi-batch cultivation. *Environ Technol* **2022**, *43*, 1352-1358, doi:10.1080/09593330.2020.1829087.
37. Mirhosseini, N.; Davarnejad, R.; Hallajisani, A.; Cano-Europa, E.; Tavakoli, O.; Blas-Valdivia, V. Cultivations of *Arthrospira maxima* (Spirulina) using ammonium sulfate and sodium nitrate as an alternative nitrogen sources. *Iran J Fish Sci* **2021**, *20*, 475-489, doi:10.22092/ijfs.2021.351071.0.
38. Pelagatti, M.; Mori, G.; Falsini, S.; Ballini, R.; Lazzara, L.; Papini, A. Blue and yellow light induce changes in biochemical composition and ultrastructure of *Limnospira fusiformis* (Cyanoprokaryota). *Microorganisms* **2023**, *11*, 1236, doi:10.3390/microorganisms11051236.
39. De Mooij, T.; De Vries, G.; Latsos, C.; Wijffels, R.H.; Janssen, M. Impact of light color on photobioreactor productivity. *Algal Res* **2016**, *15*, 32-42, doi:10.1016/j.algal.2016.01.015.
40. Möllers, K.B.; Cannella, D.; Jørgensen, H.; Frigaard, N.-U. Cyanobacterial biomass as carbohydrate and nutrient feedstock for bioethanol production by yeast fermentation. *Biotechnol Biofuels* **2014**, *7*, 64, doi:10.1186/1754-6834-7-64.
41. Cuellar-Bermudez, S.P.; Struyf, T.; Versluys, M.; Van Den Ende, W.; Wattiez, R.; Muylaert, K. Quantification of extracellular and biomass carbohydrates by *Arthrospira* under nitrogen starvation at lab-scale. *Algal Res* **2022**, *68*, 102907, doi:10.1016/j.algal.2022.102907.
42. Deschoenmaeker, F.; Facchini, R.; Cabrera Pino, J.C.; Bayon-Vicente, G.; Sachdeva, N.; Flammang, P.; Wattiez, R. Nitrogen depletion in *Arthrospira* sp. PCC 8005, an ultrastructural point of view. *J Struct Biol* **2016**, *196*, 385-393, doi:10.1016/j.jsb.2016.08.007.
43. Liu, Q.; Yao, C.; Sun, Y.; Chen, W.; Tan, H.; Cao, X.; Xue, S.; Yin, H. Production and structural characterization of a new type of polysaccharide from nitrogen-limited *Arthrospira platensis* cultivated in

outdoor industrial-scale open raceway ponds. *Biotechnol Biofuels* **2019**, *12*, 131, doi:10.1186/s13068-019-1470-3.

44. Saxena, R.; Rodríguez-Jasso, R.M.; Chávez-Gonzalez, M.L.; Aguilar, C.N.; Quijano, G.; Ruiz, H.A. Strategy development for microalgae *Spirulina platensis* biomass cultivation in a bubble photobioreactor to promote high carbohydrate content. *Fermentation* **2022**, *8*, 374, doi:10.3390/fermentation8080374.
45. Sendersky, E.; Kozer, N.; Levi, M.; Moizik, M.; Garini, Y.; Shav-Tal, Y.; Schwarz, R. The proteolysis adaptor, NblA, is essential for degradation of the core pigment of the cyanobacterial light-harvesting complex. *Plant J* **2015**, *83*, 845-852, doi:10.1111/tpj.12931.
46. Deschoenmaeker, F.; Facchini, R.; Leroy, B.; Badri, H.; Zhang, C.-C.; Wattiez, R. Proteomic and cellular views of *Arthrospira* sp. PCC 8005 adaptation to nitrogen depletion. *Microbiology* **2014**, *160*, 1224-1236, doi:10.1099/mic.0.074641-0.
47. Wegener, K.M.; Singh, A.K.; Jacobs, J.M.; Elvitigala, T.; Welsh, E.A.; Keren, N.; Gritsenko, M.A.; Ghosh, B.K.; Camp, D.G.; Smith, R.D.; et al. Global proteomics reveal an atypical strategy for carbon/nitrogen assimilation by a cyanobacterium under diverse environmental perturbations. *Mol Cell Proteomics* **2010**, *9*, 2678-2689, doi:10.1074/mcp.M110.0001.
48. Xu, H.; Vavilin, D.; Vermaas, W. Chlorophyll b can serve as the major pigment in functional photosystem II complexes of cyanobacteria. *PNAS* **2001**, *98*, 14168-14173, doi:10.1073/pnas.251530298.
49. Zittelli, G.C.; Mugnai, G.; Milia, M., .; Cicchi, B.; Benavides, A.M.S.; Angioni, A.; Addis, P.; Torzillo, B. Effects of blue, orange and white lights on growth, chlorophyll fluorescence, and phycocyanin production of *Arthrospira platensis* cultures. *Algal Res* **2022**, *61*, 102583, doi:10.1016/j.algal.2021.102583.
50. Lee, S.H.; Lee, J.E.; Kim, Y.; lEE, S.-Y. The production of high purity phycocyanin by *Spirulina platensis* using light-emitting diodes based two-stage cultivation. *Appl Biochem Biotechnol* **2016**, *178*, 382-395, doi:10.1007/s12010-015-1879-5.
51. Ojit, S.K.; Indrama, T.; Gunapati, O.; Avijeet, S.O.; Subhalaxmi, S.A.; Silvia, C.; Indira, D.W.; Romi, K.; Minerva, S.; Thadoi, D.A.; et al. The response of phycobiliproteins to light qualities in *Anabaena circinalis*. *J Appl Biol Biotech* **2015**, *3*, 1-6, doi:10.7324/JABB.2015.3301.
52. Joseph, A.; Aikawa, S.; Sasaki, K.; Matsuda, F.; Hasunuma, T.; Kondo, A. Increased biomass production and glycogen accumulation in ApcE gene deleted *Synechocystis* sp. PCC 6803. *AMB Expr* **2014**, *4*, 17, doi:10.1186/s13568-014-0017-z.