Turk. J. Fish.& Aquat. Sci. 22(10), TRJFAS20844
https://doi.org/10.4194/TRJFAS20844

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

The Effects of Probiotic-Prebiotic on the Biomass and Protein Content of *Spirulina platensis* in Different Temperatures and Illuminations

Şafak Seyhaneyıldız Can[1,*], Elif Seyhaneyıldız[2], Mustafa Dörücü[2]

[1] Dokuz Eylül University, Institute of Marine Sciences and Technology, Izmir, Turkey (38000).
[2] Munzur University, Fisheries Faculty, Aquaculture Department, Tunceli, Turkey.

How to cite

Seyhaneyıldız Can, Ş., Seyhaneyıldız, E., Dörücü, M. (2022). The Effects of Probiotic-Prebiotic on the Biomass and Protein Content of *Spirulina platensis* in Different Temperatures and Illuminations. *Turkish Journal of Fisheries and Aquatic Sciences*, *22(10)*, *TRJFAS20844.*

Article History
Received 01 November 2021
Accepted 20 May 2022
First Online 30 May 2022

Corresponding Author
Tel.: +905446727922
E-mail: seyhaneyildiz@gmail.com

Keywords
Spirulina platensis
Probiotic-Prebiotic
Biomass
Protein

Abstract

The blue-green algae *Spirulina platensis* Geitler 1925 has been used by people since ancient times due to its high protein content. This study was conducted to determine the effect of a Probiotic-Prebiotic (P-P) product, which contains *Bifidobacterium infantis*, *Bifidobacterium thermophilum, Leuconostoc mesenteroides* as probiotic bacteria, and beta-glucan and oligosaccharide as a prebiotic mixture on *S. platensis*. In the study, four groups were one of which was a control. In this study, we investigated whether the P-P product contributed to the biomass development and protein production of *S. platensis* under different temperatures and lighting conditions. In terms of biomass, the P-P product gave different responses at different temperatures and light intensities, but its results were positive in all groups in terms of protein ratio. The most positive effect on biomass and protein content was in the group where 0.1 ml/L P-P product was added, at 40°C and 3200 lux with 68.71% protein content and an average OD of 0.727.

Introduction

Probiotics are bacteria and yeasts that are beneficial for human health, especially in relation to the digestive system. The digestive system naturally contains a wide variety of bacteria, some of which are harmful, and some that are benefical. Probiotics are bacteria that are beneficial to the body, and these bacteria contribute to resistance to many diseases. In particular, studies support their protective properties against cancer (Davani-Davari et al., 2019; Ram et al., 2020; Sharma et al., 2020).

Prebiotics are non-digestible compounds that provide nutrients for beneficial bacteria in the intestinal flora and help them reproduce, enabling inactive bacteria to become active and to multiply. Prebiotics are usually found in plants; when they are consumed they are not digested immediately and manage to reach the intestine undigested. They are then used as an energy source in the intestine to support the growth of beneficial bacteria (Gibson & Roberfroid, 1995; Gibson et al., 2017). While probiotics can help regulate the digestive system and support the immune system, without the nutritive effect of prebiotics, their effect is limited. Studies have proven that appropriate combinations of probiotic and prebiotic products provides a more protective effect than either acting alone (Bengmark, 2005).

Spirulina is a multicellular, filamentous blue-green algae used as a protein and vitamin supplement in the health food industry. The quality and high protein content of *Spirulina* (up to 70%) is much higher than the widely known vegetable protein sources as dry beans (35%), peanuts (25%), or grains (8%-10%). Another feature of *Spirulina* is that - unlike eukaryotic green microalgae such as *Chlorella*, *Ankistrodesmus*,

Selenastrum, *Scenedesmus* - it can be easily digested due to the absence of cellulose in the cell walls. Approximately 18 h after ingestion, 77%-95% of the protein is digested (Habib, 2008). Found mainly in alkaline lakes, *Spirulina* has been used as food since ancient times, especially by people living in areas close to those lakes (Holman & Malau-Aduli, 2013). Today, *Spirulina* is commercially produced and actively used worldwide (Muhling et al., 2005).

Clinical studies have shown that *Spirulina* has a supportive effect on the treatment of many diseases. *Spirulina* capsules are effective in lowering the lipid level in the blood and improving the immunological function that is weakened due to the reduction of leukocytes after radiotherapy and chemotherapy (Liu & Zhang, 2002).

Studies undertaken so far on *Spirulina* have studied its antioxidant properties, protein content, lipid content, and influence on cancer cells. Studies on prebiotic-probioticinteractions generally have focused on the effects of these products on human and animal health. Unlike other studies, in this study, the contribution of probiotic-prebiotic products on the development and nutritional content of *Spirulina platensis* was investigated. Thus, the content of Spirulina, which is of critical importance for human and animal health, has been made more effective by using prebiotic probiotics even at low temperatures, and preparations have been made for future antioxidant and cancer studies.

Material and Method

Microorganism and Culture Medium

S. platensis obtained from the Ben-Gurion University of the Negev was used in this study. Zarrouk's medium (Zarrouk, 1966) used for culture was autoclaved before the prebiotic-probiotic product was added. A probiotic-prebiotic (P-P) product containing *Bifidobacterium infantis*, *Bifidobacterium thermophilum*, and *Leuconostoc mesenteroides* as a probiotic mixture, and a beta-glucan and oligosaccharide mixture as prebiotics was added to the autoclaved nutrient medium after cooling as 0.01, 0.05, 0.1 mL/L. Zarrouk's medium without P-P products were used as the control groups. Apart from the experimental groups, 0.01, 0.05, 0.1 mL/L P-P were added to the Zarrouk's medium separately to determine the effect of bacteria in the P-P product on optical density, and algae were not cultivated in these groups. These groups were cultured for 20 days as in the experimental groups. Measurements made during the 20-day trial were too small to affect the experiment, so they were excluded from the calculations.

Experimental Design

The effect of the P-P product on *S. platensis* was tested under four environmental conditions at different temperatures and lighting. These were 1600 lux illumination at 20°C, 3200 lux illumination at 20°C, 1600 lux illumination at 40°C, and 3200 lux illumination at 40°C. Cool White illumination was applied under a 12 h light, 12 h dark cycle. *S. platensis* was added to the prepared media at a ratio of 1/10. The biomass of algae was measured at 680 nm using SP-3000 Nano (UV/Vis Spectrophotometer, OPTIMA Tokyo, JAPAN) at the same time each day for 20 days.

After sufficient biomass was obtained, the algae were harvested by centrifugation and dried at room temperature. After the dried algae were pulverized, protein analysis was performed by the Kjeldahl method to determine the effect of P-P on the protein content of the algae.

Protein Analysis with the Kjeldahl Method

To be used in crude protein analysis, 0.32 g of dried algae was weighed, and Two of these weighed samples were taken into the tubes of the Kjeldahl device as blanks. One catalyst tablet (1.5 g K_2SO_4 and 7.5 mg Selenium mixture) was placed into each tube with 6 ml of sulfuric acid (H_2SO_4) and 1 ml of hydrogen peroxide (H_2O_2). The samples were burned in the incinerator at 420°C for about 3 h, until the tubes turned a green-yellow color, then the samples were cooled to room temperature. For the distillation process, 40 ml of 40% NaOH, 20 ml of 4% boric acid, and 20 ml of purified water were also added. The distillation occurred at a minimum temperature of at least 100°C. Two drops of indicator (methyl red) were dropped into an empty Erlenmeyer and placed in the distillate capture part of the Kjeldahl instrument for distillation. The process continued until approximately 150 ml of liquid accumulated in the Erlenmeyer (10 minutes). The samples were then titrated with 0.1 N HCl to calculate the percentage protein (Feldsine et al., 2002). The protein amount was made according to the following formula (Kjeldahl, 1883).

$$\text{Protein\% (dw)= Nitrogen\% x 6.25} = \frac{(V1-V0)\text{x N x } 0.014}{\text{m}} \text{ x 100=...g/100 g}$$

V_1 : Volume of 0.1 N HCl used in the sample titrations

V_0 : Volume of 0.1 N HCl used in the blank titrations

m : Sample weight (g)

N : Normality of the standard acid (eq/l)

dw : Dry weight

Statistical Analysis

The optical density and the extracted protein contents from the four groups were compared using a one-way ANOVA and Duncan's multiple comparison method. The level of significant difference was at $P<0.05$.

Results

***S. platensis* Culture at 20°C and 1600/3200 Lux Light Intensities**

The opitical density (OD) of the culture performed at 20°C and 1600 lux light intensity was measured to be 0.736, 0.993, 0.881, in a nutrient medium enriched with 0.01 mL/L P-P, 0.05 mL/L P-P, and 0.1 P-P mL/L, respectively (Figure 1). At the same temperature at 3200 lux light intensity, OD was as 0.947, 1.291, 1.099 in nutrient media enriched with 0.01 mL/L P-P, 0.05 mL/L P-P, and 0.1 P-P mL/L, respectively (Figure 2).

When the growth chart of *S. platensis* was examined, no differences were observed at either 1600 or 3200 lux illumination until day 7. However, from the day 7, the growth was faster than the control groups at both light intensities. This rapid growth occurred at both light intensities, but OD was higher at 3200 lux light intensity (Figures 1, 2).

***S. platensis* Culture at 40°C and 1600/3200 Lux Light Intensities**

In the culture study conducted at 40°C, there were no significant differences between the groups until day 7 at 1600 lux light intensity or 3200 lux light intensity. (Figures 3, 4).

The Protein Content of *S. platensis* Cultured at 20/40°C Temperatures and 1600/3200 Lux Light Intensities

When the experiments were examined, it was seen that all cultures made at 3200 lux illumination and 40°C were the most efficient in terms of protein content compared to other culture conditions. However, it was observed that the most efficient group in the culture in the study conducted at 3200 lux and 40°C was the group with 0.1 mL/L P-P added (Figure 5).

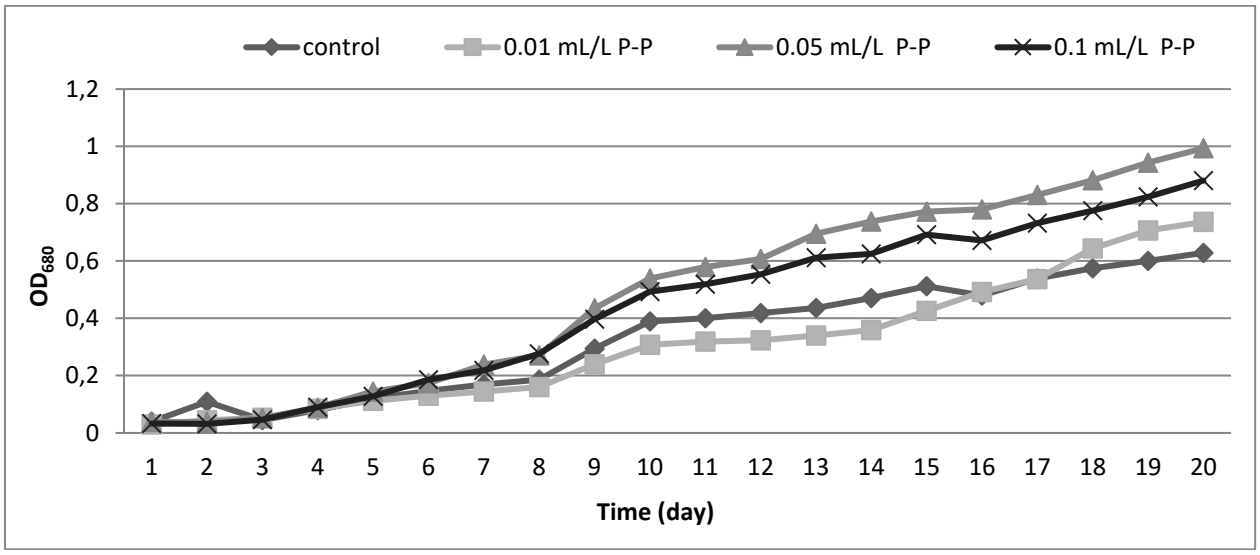

Figure 1. The optical density of *S. platensis* cultured at 20 °C and 1600 lux.

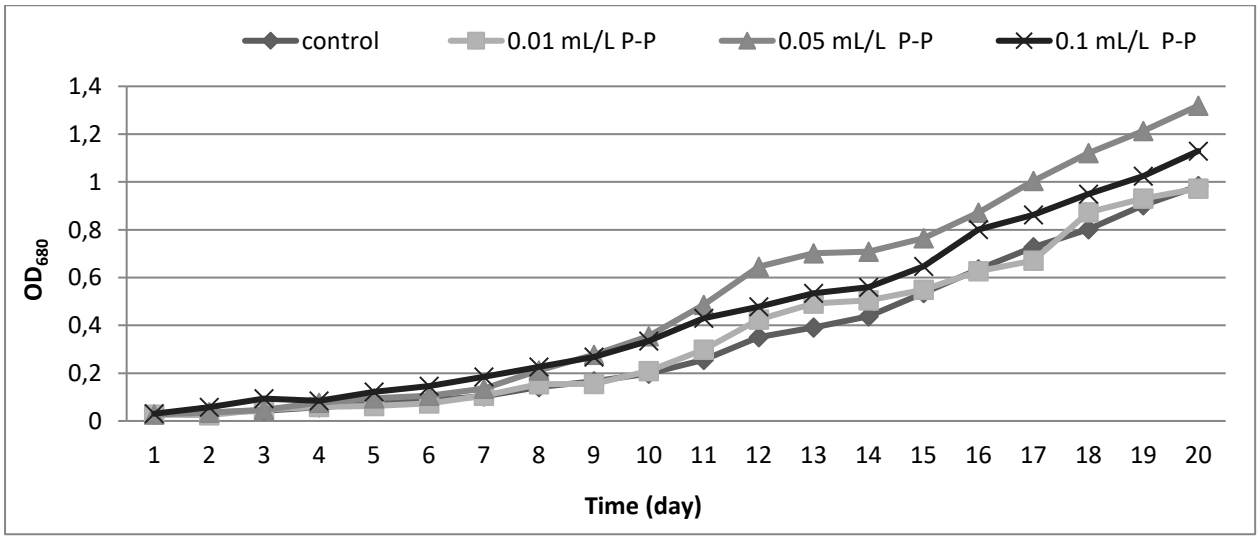

Figure 2. The optical density of *S. platensis* cultured at 20 °C and 3200 lux.

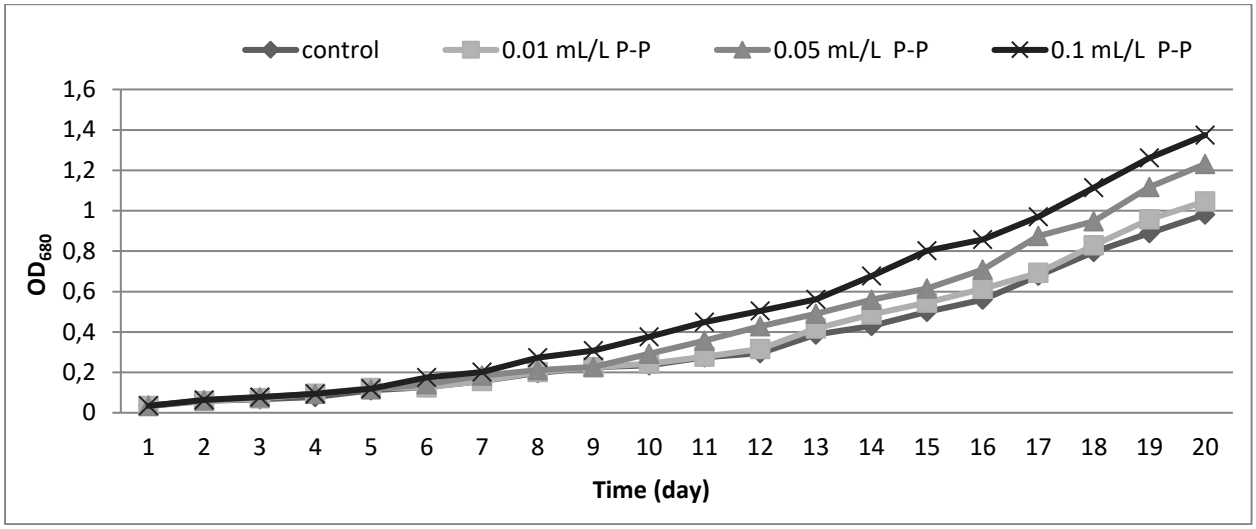

Figure 3. The optical density of *S. platensis* cultured at 40°C and 1600 lux.

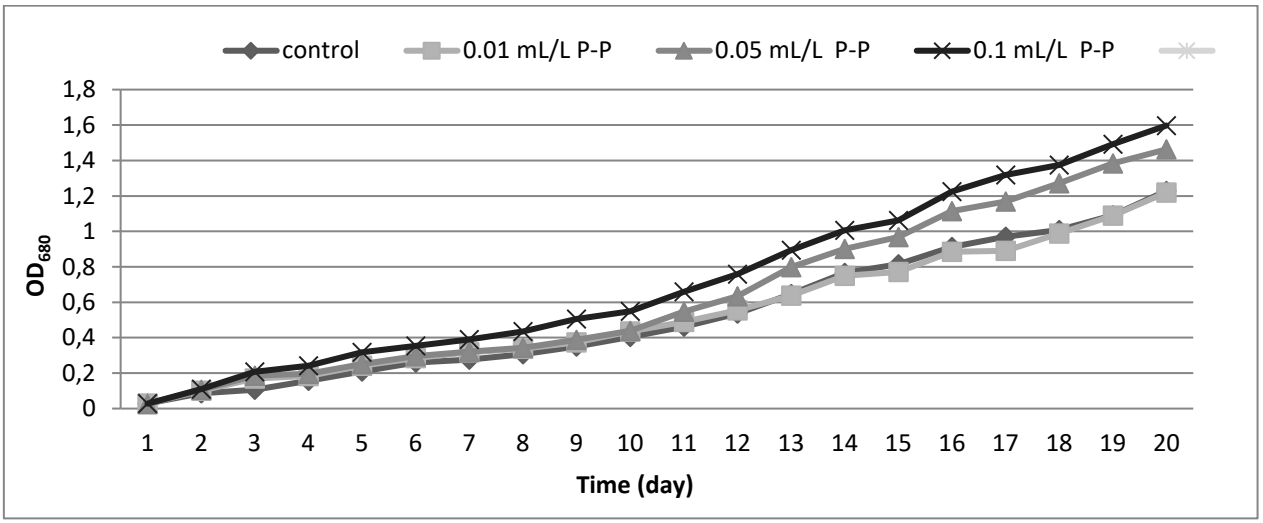

Figure 4. The optical density of *S. platensis* cultured at 40°C and 3200 lux.

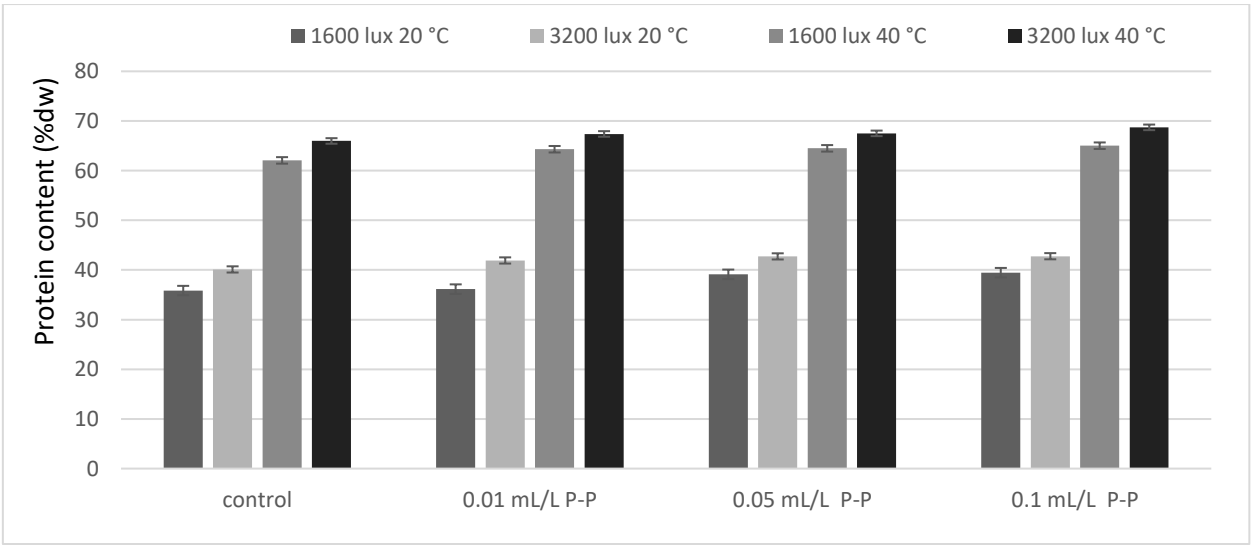

Figure 5. The total protein amount of *S. platensis* cultured at 20/40 °C and 1600/3200 lux

Statistical data on the OD and protein content of biomass obtained from *S. platensis* culture with 20/40ºC and 1600/3200 lux light intensities are given in Table 1. Considering culture grown at 20°C and 1600 Lux, the highest protein content was observed in the 0.1 mL/L P-P group with 39.45%. In the culture grown at 20°C and 3200 Lux, the highest protein content was found again in the 0.1 mL/L P-P group with 42.76%. In the culture at 40°C at 1600 lux and 3200 lux, the highest protein was observed in the 0.1 mL/L P-P group, 65.02%, and 68.71%respectively. When all experimental groups were examined, it was found that the 0.1 mL/L P-P group was the ideal group for *S. platensis*.

Discussion

For centuries, people have harvested *Spirulina* in Lake Chad in Africa and Lake Texcoco in Mexico due to its high nutritional content (Tomaselli, 1997). *Spirulina* an important algal species with a high nutritional value and nutraceutical properties relating to its, chemical composition, provitamins, minerals, proteins, polyunsaturated fatty acids, and antioxidant activity (Miranda et al., 1998; Belay et al., 1993). Therefore, both biomass efficiency and biomass content of *Spirulina* are important. The biomass yield and biomass content of *Spirulina* depends on the nutrient availability of the culture medium and environmental factors such as pH, light, and temperature. Zarrouk's medium is considered the standard medium for growing *Spirulina*. In most previous studies, Zarrouk's medium or environmental conditions were altered, and the effects of these changes on algal biomass, protein, lipid, or other cell contents were investigated. In this study, the contribution of P-P added to the Zarrouk's medium on the optical density and protein content of Spirulina cultured in different temperature and lighting conditions was investigated (Tomaselli et al., 1988; Colla et al., 2007; Yucetepe et al., 2021). According to the data obtained from the experiments at 20°C, it was determined that the best OD measured at 680 nm at both light intensities was in the nutrient medium in which 0.05 ml/L P-P product adds. It was determined that the best optical density of the culture made at 20**°**C and 1600 lux was 0.4922±0.3284, and the optical density of the culture made at 3200 lux was 0.5100±0.4256. Additionally, in the study at 20**°**C, it was determined that the algae culture in the nutrient media added P-P product showed better growth than the control group. These results show us that the bacteria in the product are effective even at low temperatures.

At high temperatures, the biomass development of Spirulina is already higher than at low temperatures. This result has been proven in many previous studies (Torzillo et al., 1984; Tomaselli, 1997; Seyhaneyildiz Can et al., 2017). Furthermore, Koru and Cirik (2002) determined that the biochemical structure of *S. platensis* did not deteriorate at temperatures up to 40°C and that phycobiliproteins increased with the increase in temperature. Therefore, in this study, above 40**°**C was not studied. In the study conducted at 40°C, at 1600 lux, the highest biomass increase was found as 0.5146±0.4151 in the group with 0.1 mL/L P-P added. In the study conducted at 3200 lux at 40°C, the highest biomass increase was found to be 0.7270±0.4812 in the group with 0.1 mL/L P-P added. Compared to previous studies, it is seen that a higher optical density was obtained in this study (Hadiyanto et al., 2014; Michael et al., 2019). These results show that the nutrient mediums enriched with P-P are more efficient concerning algal biomass. In previous studies, it has been determined that the protein content of algae, like biomass, is affected by seasons and changing environmental factors (Tomaselli et al., 1988; Colla et al., 2007; Adalıoğlu and Çalışkan, 2020; Yücetepe et al., 2021). Although S. platensis is an alga that grows more efficiently in tropical climates, in this study, it was cultured at both 20°C and 40°C to investigate the effect of P-P product on algin protein content at low and high temperatures. Additionally, the optimum growth temperatures, of *L. acidophilus* and *Bifidobacterium spp.* are 35–38°C and 37–40°C, respectively (Lee et al.,1999). Therefore, in yogurt production, it is recommended that the incubation temperature is usually between 37 and 40°C to accelerate the development of probiotic bacteria and to keep the viability of the cultures at the highest level. (Lee et al., 1999). Despite all these results, in the study at 20°C, it was determined that the algae culture in the nutrient media added P-P product showed better growth than the control group. These results show us that the bacteria in the product are effective even at low temperatures. When the protein content of the culture made at 20°C and 1600 lux was examined, it was observed that the best result with 39.4533±0.0777 in the 0.1 mL/L P-P added group. At the same temperature, in the 3200 lux, the highest protein amount was 42.7633±0.5859 in the 0.1 mL/L P-P group. The present study showed that compared to other experiments performed at low temperatures (Uslu et al., 2009), the protein content was higher, and P-P has a positive effect on algal protein even at low temperatures.

The study at 40 degrees yielded more effective results in terms of protein as in biomass compared to 20 degrees. In the examination conducted at 40°C, 1600 lux, the highest protein content was in the group with 0.1 ml/L P-P added with 65%. In the study conducted at the same temperature at 3200 lux, the highest protein content was 68.7% in the group with 0.1 mL/L P-P added.

Torzillo et al. (1984) state that protein content decreased above 40ºC in their studies. However, the P-P product given to the medium in this study had a positive effect on the protein of *S. platensis*, and the algal protein was increased compared to the control group at both low and high temperatures. When the studies are examined, there is no example of probiotic-prebiotic application to algae. However, some

polysaccharides obtained from algae are used as probiotics. For example, it has been reported that the polysaccharides called laminarin and fucoidan, obtained from brown algae such as *Ascophyllum nodosum* and *Laminaria sp*., increase the probiotic feature in the microflora and immune system of piglets. They also determined that the extracts obtained from *Laminaria hyperborea* positively affected the growth of lactic acid bacteria and *Bifidobacterium* species in the large intestine. (Reilly et al., 2008, O'Doherty et al., 2010, Smith et al., 2011). Many similar studies have also been conducted on the use of algae as P-P. As is known, algae have many uses as health, foods, and in cosmetics. *Spirulina* has been used as a protein-rich species for a long time mainly. However, today it has become one of the species that attracts attention in the field of health not only with its protein content but also with its antioxidant properties. For this reason, enriching *Spirulin*''s ingredients, especially its nutritional content, will make the algae more efficient. Both the biomass and protein content of algae was successfully increased by using P-P in this study. The fact that there is no previous study to determine the contribution of P-P products applied to algae to algae development and protein content is valuable in terms of the originality of this study. Additionally, it provides a starting point for further studies on the effect of P-P on the antioxidant properties of algae, which are planned the future.

Conclusion

Spirulina platensis is protein-rich species. Especially today, where the population is increasing rapidly, there is a problem of finding quality food. Considering that the world population will increase, even more, it is an undeniable fact that there will be a shortage of agricultural land to be planted. In such a case, it is essential to carry out studies that can obtain more efficiency from algae, the aliment of the future. In this paper, we showed that P-P has a significant effect on the biomass and protein production of *S. platensis*, depending on temperature and lighting. *Spirulina* production is not preferred in cold regions since the amount of biomass and protein decreases at low temperatures. In this study, even at 20°C using P-P, we achieved a sufficient percentage of biomass and protein 0.4390±0.2861 (OD_{680}) and 39.4533±0.0777 (%dw), respectively. The biomass and protein of the culture at 40°C were much higher (OD680: 0.7270±0.4812 and protein: 68.7133±0.3050 % dw). In this study, it was determined that algae culture made with the addition of P-P was more efficient in terms of both biomass and protein.

Ethical Statement

This article does not contain any studies with animals performed by any author.

Table 1. Statistical data on OD and protein ratio of *S. platensis* at 20 °C / °40 C and 1600/3200 lux.

Groups	20°C				40°C			
	1600 lux		3200 lux		1600 lux		3200 lux	
	OD_{680}	Protein (% dw)	OD_{680}	Protein (% dw)	OD_{680}	Protein (% dw)	OD_{680}	Protein (% dw)
Control	0.3317^{b}±0.2022	35.8433^{b}±0.1193	0.3474^{b}±0.3090	40.1067^{c}±0.8392	0.3543^{b}±0.2862	62.0600^{d}±0.0888	0.5309^{b}±0.3657	65.9733^{c}±0.0351
0.01 mL/L P-P	0.3095^{b}±0.2195	36.1433^{b}±0.2873	0.3630^{b}±0.3166	41.9200^{b}±0.1709	0.3756^{b}±0.3061	64.3100^{c}±0.1114	0.5375^{b}±0.3477	67.3867^{b}±0.2665
0.05 mL/L P-P	0.4922^{a}±0.3284	39.1200^{a}±0.7709	0.5100^{a}±0.4256	42.7300^{a}±0.1732	0.4383^{ab}±0.3636	64.4900^{b}±0.1114	0.6406^{ab}±0.4492	67.5033^{b}±0.0153
0.1 mL/L P-P	0.4390^{a}±0.2861	39.4533^{a}±0.0777	0.4492^{ab}±0.3451	42.7633^{a}±0.5859	0.5146^{a}±0.4151	65.0233^{a}±0.0611	0.7270^{a}±0.4812	68.7133^{a}±0.3050

Funding Information

This work was funded by Munzur University Scientific Research Projects Coordination Unit (MUNIBAP) (Grant Number YLMUB017-02).

Author Contribution

All authors contributed to the study conception and design. Authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

This study is of the project entitled Investigation of Protein Content of *Spirulina platensis* Enriched with Probiotic-Prebiotic supplementation in Zarouk's Medium in Different Environmental Conditions. We would like to thank Munzur University.

References

Adalıoğlu S, Çalıskan G (2020). Effect of Nutrient, Light Intensity and Temperature on the Growth Rates and Metabolism of a Stress-Resistant Bacillariophyta–*Entomoneis sp*.-in Izmir Bay (Aegean Sea). Mediterranean Marine Science *21*(1), 1-10. https://doi.org/10.12681/mms.19439

Belay, A., Ota, Y., Miyakawa, K., & Shimamatsu, H. (1993). Current knowledge on potential health benefits of *Spirulina*. Journal of Applied Phycology5(2), 235-241. https://doi.org/10.1007/bf00004024.

Bengmark, S. (2005). Bioecologic control of the gastrointestinal tract: the role of flora and supplemented probiotics and synbiotics. Gastroenterology Clinics of North America34(3), 413-436. https://doi.org/10.1016/j.gtc.2005.05.002.

Colla, L.M., Reinehr, C.O., Reichert, C., & Costa, J.A.V. (2007). Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioresource Technology 98(7), 1489-1493. https://doi.org/10.1016/j.biortech.2005.09.030.

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S.J., Berenjian A. & Ghasemi, Y. (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods*. 8(3), 92. https://doi.org/10.3390/foods8030092.

Feldsine, P., Abeyta, C., & Andrews, W.H. (2002). AOAC International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. Journal of AOAC International 85(5), 1187-1200. https://doi.org/10.1093/jaoac/85.5.1187

Gibson, G.R., & Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. The Journal of Nutrition 125(6), 1401-1412. https://doi.org/10.1093/jn/125.6.1401.

Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K., & Reid, G. (2017). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology and Hepatology14(8), 491. https://doi.org/10.1038/nrgastro.2017.75.

Habib, M.A.B. (2008). Review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals and fish. Food and agriculture organization of the united nations. http://www.fao.org/3/i0424e/i0424e00.htm.

Holman, B.W.B., & Malau-Aduli, A.E.O. (2013). *Spirulina* as a livestock supplement and animal feed. Journal of Animal Physiology and Animal Nutrition (Berl). 97(4), 615-623. https://doi.org/10.1111/j.1439-0396.2012.01328.x.

Kjeldahl, J.G.C.T. (1883). Neue methode zur bestimmung des stickstoffs in organischen körpern. Zeitschrift für Physikalische Chemie (NF). *22*(1), 366-382. https://doi.org/10.1007/bf01338151.

Koru, E., & Cirik, S. (2002). Biochemical composition of *Spirulina* biomass in open-air system. *Proceedings of the 10th IEEE International Conference on Network Protocols, Trabzon.* 97-100. November 12-15 2002.

Lee, Y.K., Nomoto, K., Salminen, S., & Gorbach, S.L. (1999). Handbook of probiotics. John Wiley and Sons. https://doi.org/10.1002/9780470432624.

Liu, X.M., & Zhang, H.Q. (2002). Effect of spirulina platensis polysaccharide on hematopoietic recovery and related cytokines in mice with transplanted tumor treated by chemotherapy. Chinese Journal of Integrative Medicine 8(2), 130-133. https://doi.org/10.1007/BF02934440.

Michael, A., Kyewalyanga, M.S., & Lugomela, C.V. (2019). Biomass and nutritive value of *Spirulina* (*Arthrospira fusiformis*) cultivated in a cost-effective medium. Annals of Microbiology 69(13), 1387-1395. https://doi.org/10.1007/s13213-019-01520-4.

Miranda, M.S., Cintra, R.G., Barros, S.D.M., & Mancini-Filho, J. (1998). Antioxidant activity of the microalga *Spirulina maxima*. Braz. J. Med. Biol. Res. 31(8), 1075-1079. https://doi.org/10.1590/s0100-879x1998000800007.

Mühling, M., Belay, A., & Whitton, B.A. (2005). Variation in fatty acid composition of Arthrospira (Spirulina) strains. Journal of Applied Phycology*17*(2), 137-146. https://doi.org/10.1007/s10811-005-7213-9.

O'Doherty, J.V., Dillon, S., Figat, S., Callan, J.J., & Sweeney, T. (2010). The effects of lactose inclusion and seaweed extract derived from Laminaria spp. on performance, digestibility of diet components and microbial populations in newly weaned pigs. Animal Feed Science and Technology157(3-4), 173-180. https://doi.org/10.1016/j.anifeedsci.2010.03.004.

Ram, G., Sharma, V.R., Sheikh, I., Sankhyan, A., Aggarwal, D., & Sharma, A.K. (2020). Anti-cancer potential of natural products: recent trends, scope and relevance. Letters in Applied NanoBioScience. 9(1), 902-907. https://doi.org/10.33263/lianbs91.902907.

Reilly, P., O'doherty, J.V., Pierce, K.M., Callan, J.J., O'sullivan, J.T., & Sweeney, T. (2008). The effects of seaweed extract inclusion on gut morphology, selected intestinal microbiota, nutrient digestibility, volatile fatty acid concentrations and the immune status of the weaned pig. Animal, 2(10), 1465-1473.

https://doi.org/10.1017/s1751731108002711.

Seyhaneyildiz Can, S., Koru, E., & Cirik, S. (2017). Effect of temperature and nitrogen concentration on the growth and lipid content of *Spirulina platensis* and biodiesel production. Aquaculture International25(4), 1485-1493. https://doi.org/10.1007/s10499-017-0121-6.

Sharma, V., Panwar, A., & Sharma, A.K. (2020). Molecular dynamic simulation study on chromones and flavonoids for the in silico designing of a potential ligand inhibiting mTOR pathway in breast cancer. Current Pharmacology Reports 6, 373–379. https://doi.org/10.1007/s40495-020-00246-1.

Smith, A.G., O'doherty, J.V., Reilly, P., Ryan, M.T., Bahar, B., & Sweeney, T. (2011). The effects of laminarin derived from Laminaria digitata on measurements of gut health: selected bacterial populations, intestinal fermentation, mucin gene expression and cytokine gene expression in the pig. British Journal of Nutrition*105*(5), 669-677. https://doi.org/10.1017/s0007114510004277.

Tomaselli, L. (1997). Morphology, ultrastructure and taxonomy of Arthrospira (Spirulina) maxima and *Arthrospira* (*Spirulina*) *platensis*. *Spirulina platensis (Arthrospira):* physiology, cell-biology and biotechnology. A. Vonshak (Ed), Taylor and Francis Group, London. pp1-16. https://doi.org/10.1201/9781482272970-9.

Tomaselli, L., Giovannetti, L., Sacchi, A., & Bocci, F. (1988). Effects of temperature on growth and biochemical composition in *Spirulina platensis* strain M2. In: Algal Biotechnology, T. Stadler, J. Mellion, M.C. Verdus, Y. Karamanos, H. Morvan, D. Christiaen (Eds.), Elsevier Applied Science, London. pp 19-34. https://doi.org/10.1201/9781482272970-9.

Torzillo, G., Giovannetti, L., Bocci, F., & Materassi, R. (1984). Effect of oxygen concentration on the protein content of *Spirulina* biomass. Biotechnology and Bioengineering. 26(9):1134-5. https://doi.org/10.1002/bit.260260920.

Uslu, L. H., Oya, I. Ş. I. K., Sayin, S., Durmaz, Y., Göksan, T., & Gökpınar, Ş. (2009). The Effect of Temperature on Protein and Amino Acid Composition of *Spirulina platensis*. Ege Journal of Fisheries and Aquatic Sciences. *26*(2), 139-142. http://www.egejfas.org/tr/download/article-file/57436 (21.01.2022).

Yücetepe, A., Yavuz-Düzgün, M., Şensu, E., Bildik, F., Demircan, E., & Özçelik, B. (2021). The impact of pH and biopolymer ratio on the complex coacervation of Spirulina platensis protein concentrate with chitosan. Journal of Food Science and Technology. 58(4), 1274-1285.

Zarrouk, C. (1966). Contribution a l'etude d'une Cyanophycee. Influence de Divers Facteurs Physiques et Chimiques sur la croissance et la photosynthese de Spirulina mixima. [Contribution to the study of a Cyanophycee. Influence of Various Physical and Chemical Factors on the Growth and Photosynthesis of Spirulina mixima]*Thesis. University of Paris, France.* (in French).