JOURNAL OF
EMERGING INVESTIGATORS

Article

Heterotrophic culture of *Spirulina platensis* improved its growth and the study of its nutritional effect

Luming Jin[1], Ningcheng Yu[1], Yuzhe Jiang[1], Yiming Li[1], Xiaoming Wang[1]
[1] Hefei Thomas School, Hefei, China

SUMMARY

With the development of human civilization, many people in the world still suffer from hunger due to wars, epidemics, and other causes such as not being able to afford a healthy diet. As a food, raw material with huge market potential, *Spirulina platensis* can be used as a new resource for food and has been in large-scale industrialized production worldwide. However, one of the biggest limitations to growing microalgae on an industrial scale is the high cost of the culturing medium. To reduce the culture cost, we hypothesized that *S. platensis* could grow better in a medium supplemented with glucose. In this study, we investigated the effects of Zarrouk's medium, Central Food Technological Research Institute (CFTRI) medium, and CFTRI with glucose on the growth rate and biomass of *S. platensis*. Our results showed that compared with the expensive Zarrouk's medium containing trace elements, adding a small amount of glucose to the low-cost CFTRI medium significantly increased *S. platensis*'s growth rate and biomass. Then, to further reduce breeding costs, we continued to explore the growth of *S. platensis* in a CFTRI medium supplemented with glucose under dark conditions. Our statistical data showed that the heterotrophic glucose consumption of *S. platensis* was affected by light, and the growth of *S. platensis* was limited in the absence of light. Finally, we used *Drosophila melanogaster* to verify the function of *S. platensis* and found that *S. platensis* supplementation increased longevity and reproductive ability in fruit flies.

INTRODUCTION

According to World Population Prospects 2022, the global population hit a milestone in human development by surpassing 8 billion (1, 2). By 2050, it is estimated that the world will have up to 10 billion people to feed (1, 2). Today, with the development of human civilization, many people still suffer from hunger and cannot afford a healthy diet due to wars, epidemics, and other causes. Taken together, these problems pose an unprecedented threat, as current agricultural methods and food distribution systems cannot cover current needs or increase nutritional demands in the future (3).

Currently, in addition to traditional farming methods, scientists have developed many new plant-based and laboratory-grade nutrient substitutes (4). In addition, single-celled organisms such as bacteria, yeast, or algae have also been shown to be excellent alternative sources of organic matter, such as protein and lipids (5). Around 1940, a French scientist discovered locals harvesting of *Spirulina platensis* around the shallow Lake Chad in Africa (6). The unusually healthy and long lifespan of the local population sparked further interest. Today, *S. platensis* is eaten by millions of people around the world who have discovered its many health benefits, in addition to its nutritional value (7). For example, *Spirulina maxima* supplementation has been reported to enhance the hypolipidemic effect of a systematic physical exercise program in men with excess body weight and dyslipidemia, a systemic chronic metabolic disease, and has been known to decrease cardiovascular disease risks factors (8, 9). Controlling and lowering blood lipids, known as the hypolipidemic effect, can reduce the risk of cardiovascular and cerebrovascular diseases. However, long-term use of lipid-lowering drugs can produce a variety of side effects, such as liver and kidney damage, the emergence of drug resistance and so on, while *Spirulina maxima* has been shown to be a good drug alternative (10).

S. platensis is an aquatic prokaryote of cyanobacteria, which has a simple structure and a long evolutionary history. It is a filamentous body composed of single or multicellular cells, loose or tightly twisted in a regular spiral shape like a clockwork (11). Numerous studies have shown that *S. platensis* is rich in protein, unsaturated fatty acids, beta-carotene, chlorophyll, vitamins, acidic heteropolysaccharides, minerals, etc. Its protein content is as high as 60%-70% of the total mass, which is two times more than soybeans, 3.5 times more than beef, and four times more than eggs. Additionally, *S. platensis* contains a complete range of essential amino acids, which makes it a good protein supplement (11). Studies have shown that *S. platensis* can reduce the toxic side effects of cancer radiotherapy and chemotherapy and limit nervous system, liver, and kidney damage, indicating *S. platensis* is a functional food to improve sub-health state (12-14). Also, Spirulina and its components show a good influence in antioxidant and anti-inflammatory action. Thus, Spirulina promotes a healthy environment for the skin's cells and structure, cooperating for the highlighted anti-aging, photoprotection, and wound-healing effects (15).

In recent years, the use of microalgae as an innovative platform for food, feed, and health products has attracted great interest. *S. platensis* is suitable for high-temperature and alkaline environments, with short growth cycles and high photosynthetic efficiency (16, 17). As a raw food material with excellent market potential, *S. platensis* can be developed and utilized as a new resource for food and has been produced in large-scale industrialization worldwide (18). Currently, *S. platensis* products are available in several pharmaceutical forms, such as tablets, liquid or capsules, and baking ingredients for pasta and snacks (19).

However, one of the limitations to cultivating microalgae on

https://doi.org/10.59720/23-116

an industrial scale is the high cost, mainly due to the mineral costs required to make suitable mediums for high biomass production. There are several standard culture mediums reported to culture microalgae. *S. platensis* is cultured mainly in Zarrouk or modified Zarrouk's medium. This media is expensive because it requires increased amounts of sodium bicarbonate ($NaHCO_3$), sodium nitrate ($NaNO_3$), and trace metals (20). Because of the high cost, Zarrouk's medium is not utilized in the mass production of *S. platensis*. In addition, CFTRI medium is also used for *S. platensis* culture, but the culture efficiency is low due to its nutrient deficiency. *S. platensis* contains 47% carbon, so the medium in which it is grown has to have a very high concentration of sodium bicarbonate or sodium carbonate to provide adequate carbon for growth (21).

Drosophila melanogaster is a common model organism used extensively for modern biological science research. They are easy to cultivate, have a short lifespan, and are well-studied in anatomy and genomes (22). Drosophila is increasingly recognized as a model organism in food and nutrition research (23). For example, supplementation with 9-cis-β-carotene in ageing *D. melanogaster* improved mitochondrial function in terms of ATP production and whole-body respiration and extended mean lifespan (24).

S. platensis is a cyanobacteria, and thus can absorb freely available solar light, which is converted into chemical energy during photosynthesis, and later used to convert CO_2 into carbohydrates. Moreover, many microalgae can also utilize organic substrates as carbon and energy sources (25). In this context, in order to reduce the breeding cost, we hypothesized that *S. platensis* could grow better in CFTRI medium supplemented with 1g/L glucose than in Zarrouk's medium, thus reducing the culture cost. We cultured *S. platensis* in three different mediums, Zarrouk's medium, CFTRI medium, and CFTRI with 1g/L glucose. The OD_{560} value and biomass indicated glucose benefited cell division and organic accumulation in *S. platensis*. Furthermore, we evaluated the effect of glucose on the growth of *S. platensis* in dark and light conditions. *S. platensis* grew more slowly in the dark than in the light, and the cells in the no-glucose medium barely divided. Finally, we used fruit flies as model organisms to examine the health function of *S. platensis*, and we determined that *S. platensis* could improve the reproductive capacity and prolong the lifespan of *D. melanogaster*.

RESULTS

Morphological observation of *S. platensis*

We cultured *S. platensis* for three days, to monitor the growth conditions and then viewed cells under the microscope to evaluate their morphology. Over time, the microalgae cells continued to divide, and the number of *S. platensis* increased. The slightly different colors of *S. platensis* with different densities were seen, with colors ranging from light to dark green (**Figure 1A**). We collected the cells in the logarithmic growth phase for observation. The *S. platensis* body was blue-green and were spiral-like filaments. The tip was obtuse or slightly tapered and the filaments were 250~400μm long (**Figure 1B**).

Growth kinetic measurement of *S. platensis*

To test whether glucose can accelerate the growth rate of *S. platensis* and improve the accumulation of organic matter,

Figure 1: Morphological observation of *S. platensis*. A) S. platensis in the flasks at different growth stages, the cells were cultured for 1 day, 5 days, 9 days, 14 days respectively (Flask 1, 2, 3, 4). **B)** Micrograph of *S. platensis*. The slide ruler and scale bar is equal to 100μm and 10μm respectively, and the magnification is 100x.

we inoculated *S. platensis* into Zarrouk's medium, CFTRI medium, or CFTRI medium with 1g/L glucose with the same initial optical density at 560nm wavelength (OD_{560}) value of 0.2. During the first two days of culture, there were no obvious differences in *S. platensis* density among the three groups with the OD560 value of 0.3, which indicated that the growth rate of *S. platensis* in the three cultures were similar at the beginning (**Figure 2A**). We added additional glucose after day 2 of culture to maintain the glucose concentration at 1g/L. The density of *S. platensis* in the CFTRI medium with glucose was much higher than in the other two groups. On day 12, the OD_{560} value in medium with glucose peaked at 1.4 and then leveled off, revealing that glucose could significantly increase the growth rate of *S. platensis*. In the whole culture process, the OD_{560} of *S. platensis* in Zarrouk's medium was always higher than that in CFTRI medium without glucose, indicating that Zarrouk's medium was more nutrient-rich and could promote the division and growth of cells.

In addition, we wanted to test whether *S. platensis* could grow in the dark with glucose to reduce the culture cost further. Two flasks of *S. platensis* were inoculated into CFTRI medium and CFTRI medium with 1g/L glucose, and cultured without light. The growth rate of the dark group was much slower compared with the *S. platensis* growing in light (**Figure 2B**). In the dark group without glucose, the OD560 remained almost unchanged, indicating that light is necessary for *S. platensis* growth. While cultured in the dark with glucose, *S. platensis* multiplied at first, but after the third day the OD_{560}

https://doi.org/10.59720/23-116

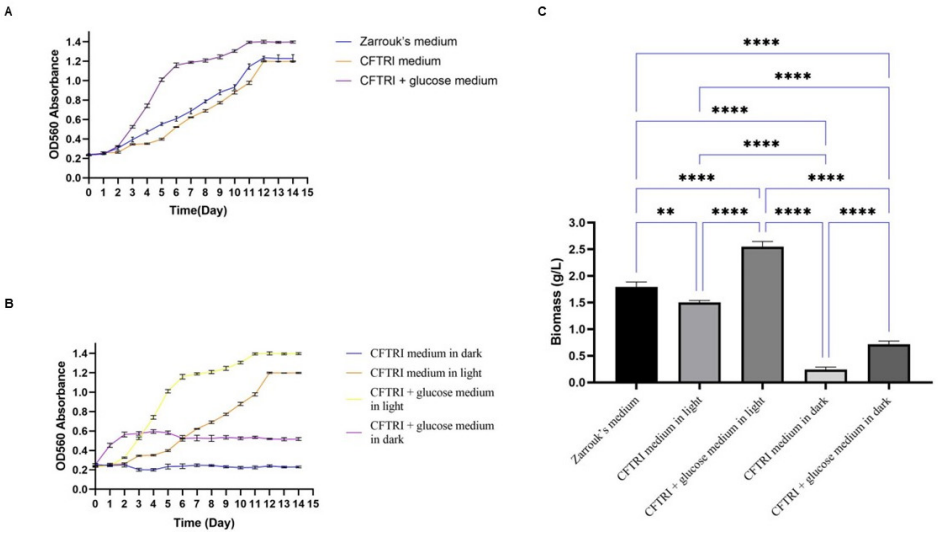

Figure 2. Growth of *S. platensis* under different culture conditions. A) Mean OD_{560} value of S. platensis measured daily from three different mediums. All samplings were performed in triplicate, error bars represent standard deviation. **B)** *S. platensis* were cultured in dark or light conditions in the CFTRI medium in the absence or presence of glucose (n=3). **C)** Mean biomass of *S. platensis* in different mediums (n=3). One-way ANOVA with Tukey HSD test, ** p<0.01, **** p<0.0001.

value remained almost the same, even though we added extra glucose as we had done for the experiment with light. These results indicate that *S. platensis* consumes glucose heterotrophically, and the growth was limited in the absence of light.

After 14 days of culture, we removed the *S. platensis* from five bottles of different mediums. We weighed the dry mass to measure its biomass (weight of organic matter or dry weight in a unit area at any given time). The dry weight of *S. platensis* obtained from CFTRI medium containing glucose under light conditions was the largest, and the biomass was 2.55g/L. While the dry weight of *S. platensis* cultured in CFTRI medium in the dark was the lowest with a biomass of 0.24g/L (**Figure 2C**). These results were consistent with the OD_{560} value measured previously - that is, *S. platensis* density is proportional to its biomass. The biomass of cultures in the five different mediums were statistically different from each other (p<0.01, one-way ANOVA with Tukey HSD), illustrating that glucose could increase the biomass of *S. platensis* under light.

Effects of *S. platensis* on the reproductive capacity and lifespan of *D. melanogaster*

We used fruit flies to test whether *S. platensis* could prolong the flies' lives and enhance their reproductive capacity. Flies that emerged within 10 hours were collected and separated into male and female groups. Flies were cultured in a standard fly growth medium as a control and experimental group with 6g/L *S. platensis* powder (**Figure 3A**). To determine the effects of *S. platensis* on fruit flies' reproduction, we cultured 15 female and 15 male fruit flies in a vial. Two weeks later, the average number of offspring was counted. Thirty-nine offspring were seen in the control group, compared with 57 in the experimental group ($p < 0.05$, **Figure 3B**).

For the fruit fly lifespan test, the number of male and female flies in the control group and the experimental group were counted every other day until all flies died, and the remaining flies were transferred to fresh vials (**Figure 4A**). The impact of the extract was greater in females than in males, but there was no increase in absolute lifespan, as animals in both experimental and control groups were dead by around day 40 (**Figure 4B-C**). Hence, the positive impact increased the probability of survival in middle-aged and late-middle-aged flies. Log-rank (Mantel-Cox) test indicated *S. platensis* had a positive impact on females but not on males when compared with the controls, elevating the median survival of females from 31 days to 38 days.

DISCUSSION

In our study, we investigated a modified CFTRI medium to reduce the breeding cost of *S. platensis*. First, *S. platensis* was cultured in three different mediums, Zarrouk's, CFTRI, and CFTRI with 1g/L glucose. The results showed that glucose could greatly increase the growth rate of *S. platensis*. To further reduce the cost of breeding, we looked at whether *S. platensis* could grow in the dark using glucose. Interestingly, the heterotrophic metabolism of *S. platensis* using glucose was affected by light. Further, we employed *D. melanogaster* as a model organism to determine the function of *S. platensis*. *S. platensis* when added in the medium could improve the reproductive ability of fruit flies and elevate the median survival of females. Our study provided a reference for the low-cost breeding of *S. platensis*. Also, the functional test using *D. melanogaster* provided a theoretical basis for further development of *S. platensis* as an edible health food.

S. platensis was recognized as "a fantastic food source of the future" by the International Association for Applied Microbiology in 1967 (26). In 1970, Lake Texcoco was the site of the world's first commercial production of *S. platensis* (23, 27). Several environmental factors, such as lighting and temperature, inoculation volume, stirring speed, and total micronutrient presence, can all impact the productivity of *S. platensis* (28). Based on the literature, we set the optimal value of light intensity, temperature, pH, etc., and changed different medium components to explore the influence of

https://doi.org/10.59720/23-116

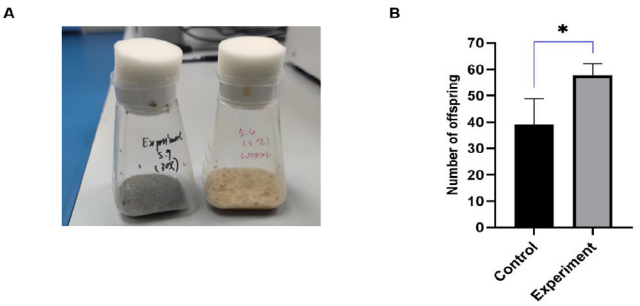

Figure 3. Effects of *S. platensis* on the reproductive capacity of D. melanogaster. A) Fruit flies were cultured with and without spirulina supplementation (left and right flasks, respectively). **B)** The mean ± SD number of offspring produced by mating male and female fruit flies (n=3). t-tests (and nonparametric tests), * $p < 0.05$.

different nutrients on the growth of *S. platensis* (29).

Like plant cells, microalgae can fix carbon dioxide through the Calvin cycle during photosynthesis. Thus, microalgal cells can capture light energy as an energy source and absorb CO_2 as the carbon source, which can be cultured autotrophically (30). In addition, the mixotrophic growth of algae can be achieved by adding organic matter to the medium, and both light and fixed carbon are used as energy sources. Thus, altering the nature of the carbon and energy sources makes it possible to illuminate the different underlying metabolic states of the cell. In this context, 1g/L glucose was added into the CFTRI medium to test whether *S. platensis* could grow on glucose heterotrophically. By measuring the optical density, we can clearly observe the growth phase of *S. platensis* (**Figure 2A**). *S. platensis* shows a lag period of 0-2 days, a logarithmic growth period of 3-11 days, and a stable period of 12-14 days. There were no obvious differences in the growth rate of *S. platensis* in the three mediums in the first two days, which may be because *S. platensis* cells are photosynthetic autotrophic and needed some time to adapt to the use of glucose after being transferred to the mixed nutrient condition. On the third day of culture, *S. platensis* in the logarithmic stage creates an increased demand for nutrients. Therefore, for the *S. platensis* cells in the CFTRI medium with glucose, the glucose supplement promoted the rapid growth of cells, suggesting that the presence of glucose, which contains six carbon atoms per molecule, may compensate for the insufficient supply of carbon sources in the medium. Interestingly, glucose increased the growth rate and biomass of *S. platensis* correlated with light (**Figure 2B-C**). Without light, *S. platensis* grew slowly in a medium containing glucose. Our results indicated that the mixotrophic cultures grew faster and achieved a higher biomass concentration than the photoautotrophic cultures.

Previous experiments showed that adding 20% washed marine macroalgae *Ulva lactuca* into 80% standard medium led to extended lifespans and stable body weights in flies (31). In this context, we found that *S. platensis* can improve the reproductive ability of *D. melanogaster* (**Figure 3B**). In addition, compared to the control group, the flies fed with *S. platensis* powder had a statistically similar maximum lifespan, whereas females showed remarkably increased median survival (**Figure 4B-C**). These results were consistent with the anti-aging and anti-oxidation functions of *S. platensis*. Further work should be performed, including a study of whether these functions are related to the concentration of *S. platensis*, such as adding different doses of *S. platensis* in the medium so as to provide a reference for the daily dose of *S. platensis* ingested by the human.

MATERIALS AND METHODS

S. platensis (Cat# FACHB-314) were provided by Freshwater Algae Culture Collection at the Institute of Hydrobiology, National Aquatic Biological Resource Center. Before the exposure experiment, the microalgae were closely cultivated in the flask (1 L for culture vessel and 0.5 L for culture volume) fulling the Zarrouk's medium (Guide Chem, Cat# ZY6AMP272) to activate the microalgae cells and gradually expand the cultivation.

D. melanogaster were generous gifts from the Liu lab at Hefei University of Technology and they were cultured using Medium for D. melanogaster (Qingdao Haibo Biology, Cat# HB8590) at 25°C.

Morphological observation of *S. platensis*

20 µL of *S. platensis* (Freshwater Algae Culture Collection at the Institute of Hydrobiology, National Aquatic Biological Resource Center, Cat# FACHB-314) was taken at the logarithmic growth stage, and a drop of sterile water was added to the slide. A ring of *S. platensis* suspension was lifted from the algal liquid through an aseptic operation and evenly dispersed in the sterile water. A clean, sterile cover glass was gently placed on the algal liquid to ensure it was free of bubbles, and the morphology was observed under a light microscope.

Growth kinetic measurement of *S. platensis*

All the experiments were conducted in batches using a 1000 mL Erlenmeyer flask with a working volume of 400 mL. The pH culture medium was adjusted before the sterilization at 9.5 for all the flasks. The stock culture was placed on a shaking platform at 140 rpm at 25°C with a photoperiod at 12 h per day and a light intensity of 3000 Lux for 14 days. The optical density of algae in each column was recorded at 560 nm wavelength, representing the algae concentration in the fluid. The initial OD_{560} value was about 0.2 for each flask with Zarrouk's medium, CFTRI medium, and CFTRI with 1g/L glucose, respectively. Additional glucose was added to the supplemented CFTR1 media to maintain glucose concentration (1g/L) every two days (on days 3, 6, 9, and 12) to prevent total glucose depletion in the growth media. The sampling period during growth was carried out every day to measure OD_{560} absorbance. Cells were cultured in CFTRI medium and CFTRI with 1g/L glucose under the abovementioned condition without light. Three replicates were generated for each medium.

After that, *S. platensis* was harvested through a strainer, and the culture medium was removed. The cell pellet was washed twice with distilled water to remove excess salt and baked in the oven at 70°C until constant weight and ground into uniform powders, and the biomass was weight after 14 days of culture. The dried *S. platensis* from CFTRI with 1g/L glucose was stored for further use.

https://doi.org/10.59720/23-116

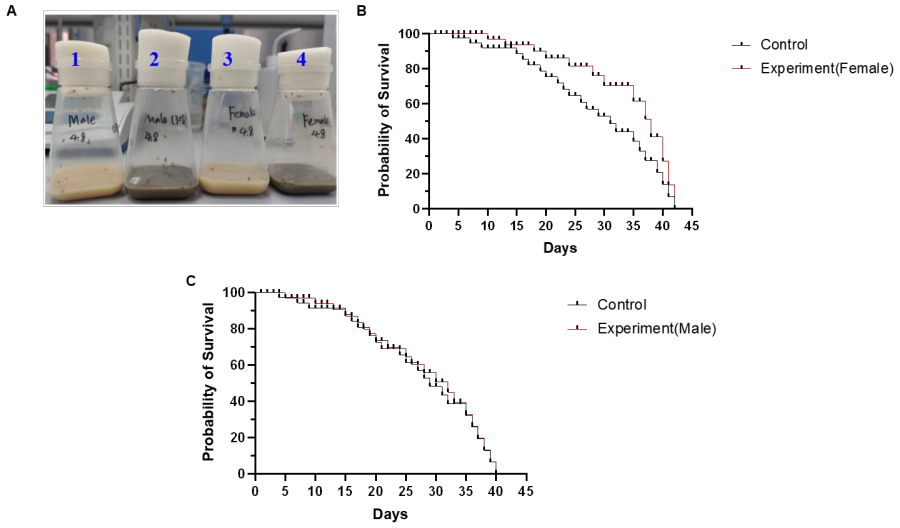

Figure 4. Effects of *S. platensis* on the lifespan of D. melanogaster. A) Fruit flies were cultured with (Flask 2, 4) and without (Flask 1, 3) Spirulina supplementation. **B, C)** Effect of *S. platensis* on lifespan of **(B)** female and **(C)** male flies was recorded (n=30 flies in each group). Survival patterns were calculated using the Kaplan-Meier method and are presented as survival curves. Log-rank (Mantel-Cox) test, p=0.0795 for contol and female groups, p=0.8725 for contol and male groups. No significance difference was seen between groups. The mean, median, minimum and maximum lifespans were determined.

Effects of *S. platensis* on the reproductive capacity and lifespan of *D. melanogaster*

Wild-type male and female flies were used. These were housed in standard fly vials containing a normal medium maintained at 25°C, 12 h L/D. Flies that eclosed within 10 hours were collected and anesthetized with CO_2. In the experimental group, 6g/L *S. platensis* powder was added to the standard medium. For the control and experimental group, 15 pairs of flies were placed in the vials for mating at a ratio of male and female 1:1, parents were removed after seven days, and offspring were counted after 14 days.

To determine the effects of *S. platensis* on longevity, flies were selected with males and females and cultured separately in the medium. Thirty flies were cultured in each vial, and three replicates were generated for males and females, respectively. The number of flies alive and dead was counted every day until all flies died, and the remaining flies were transferred to fresh vials. Survival patterns were calculated with the Kaplan-Meier method and presented as survival curves using Graphpad Prism 9.

ACKNOWLEDGMENTS

We would like to thank our school, especially Group Chairman Wuhua Zhang, for offering us the Advanced Molecular Biology Laboratory and equipment. We gratefully acknowledge School Party Branch Secretary Guangwei Fan, Principal Haiyan Yu, Principal Tao Yu, and Academic Director Ben Yao of our school for giving us the opportunity to conduct the research.

Received: June 1, 2023
Accepted: August 4, 2023
Published: November 18, 2023

REFERENCES

1. "World Population Prospects 2022: Summary of Results - Population Division." *United Nations*, www.un.org/development/desa/pd/content/World-Population-Prospects-2022. Accessed 31 May 2023.
2. "World Agriculture towards 2030/2050 - Food and Agriculture Organization." FAO *Agricultural Development Economics Division,* www.fao.org/3/ap106e/ap106e. Accessed June 2012.
3. Wu, Guoyao, et al. "Production and Supply of High-Quality Food Protein for Human Consumption: Sustainability, Challenges, and Innovations." *Annals of the New York Academy of Sciences*, vol. 1321, no. 1, 2014, https://doi.org/10.1111/nyas.12500.
4. Post, Mark J., et al. "Scientific, Sustainability and Regulatory Challenges of Cultured Meat." *Nature Food*, vol. 1, no. 7, 2020, https://doi.org/10.1038/s43016-020-0112-z.
5. Salter, Andrew M., et al. "Role of Novel Protein Sources in Sustainably Meeting Future Global Requirements." *Proceedings of the Nutrition Society*, vol. 80, no. 2, 2021, https://doi.org/10.1017/s0029665121000513.
6. Belay, Amha, et al. "Current Knowledge on Potential Health Benefits of Spirulina." *Journal of Applied Phycology*, vol. 5, no. 2, 1993, https://doi.org/10.1007/bf00004024.
7. "Scientific life: Is it good for the human body to eat spirulina?" *The Central Pcople's Government of the Pcople's Republic of China*. www.gov.cn/govweb/fwxx/kp/2007-01/17/content_498428. Accessed 12 Mar. 2023.
8. Hernández-Lepe, Marco Antonio, et al. "Hypolipidemic Effect of Arthrospira (Spirulina) Maxima Supplementation and a Systematic Physical Exercise Program in Overweight and Obese Men: A Double-Blind, Randomized, and Crossover Controlled Trial." *Marine Drugs*, vol. 17, no. 5, 2019, https://doi.org/10.3390/md17050270.
9. Hernández-Lepe, Marco Antonio, et al. "Double-Blind Randomised Controlled Trial of the Independent and Synergistic Effect of Spirulina Maxima with Exercise (ISESE) on General Fitness, Lipid Profile and Redox Status in Overweight and Obese Subjects: Study

https://doi.org/10.59720/23-116

Protocol." *BMJ Open*, vol. 7, no. 6, 2017, https://doi.org/10.1136/bmjopen-2016-013744.
10. Zhao, Yuanyuan, et al. "Optimization of Extraction Parameters of Pleurotus Eryngii Polysaccharides and Evaluation of the Hypolipidemic Effect." *RSC Advances*, vol. 10, no. 20, 2020, https://doi.org/10.1039/c9ra10991a.
11. Fabregas, Jaime, and Concepcion Herrero. "Vitamin Content of Four Marine Microalgae. Potential Use as Source of Vitamins in Nutrition." *Journal of Industrial Microbiology*, vol. 5, no. 4, 1990, https://doi.org/10.1007/bf01569683.
12. Helmy, Mohamed, et al. "Perspective: Multiomics and Machine Learning Help Unleash the Alternative Food Potential of Microalgae." *Advances in Nutrition*, vol. 14, no. 1, 2023, https://doi.org/10.1016/j.advnut.2022.11.002.
13. Wu, Qinghua, et al. "The antioxidant, immunomodulatory, and anti-inflammatory activities of spirulina: An overview." Archives of Toxicology, vol. 90, no. 8, 2016, pp. 1817–1840, https://doi.org/10.1007/s00204-016-1744-5.
14. ElFar, Omar Ashraf, et al. "Advances in delivery methods of arthrospira platensis (spirulina) for enhanced therapeutic outcomes." Bioengineered, vol. 13, no. 6, 2022, pp. 14681–14718, https://doi.org/10.1080/21655979.2022.2100863.
15. Ikeda, Isadora Kaniak, et al. "Potential Application of Spirulina in Dermatology." *Journal of Cosmetic Dermatology*, vol. 21, no. 10, 2022, https://doi.org/10.1111/jocd.14997.
16. "Functional Biscuits with PUFA-Ω3 from Isochrysis Galbana: Semantic Scholar." *Journal of the Science of Food and Agriculture.* www.semanticscholar.org/paper/Functional-biscuits-with-PUFA%E2%80%90%CF%893-from-Isochrysis-Gouveia-Coutinho/506451dd812bbe366199d89560b00f0ba75f7e73. Accessed 6 Apr. 2023.
17. Caporgno, Martín P., and Alexander Mathys. "Trends in Microalgae Incorporation into Innovative Food Products with Potential Health Benefits." *Frontiers in Nutrition*, vol. 5, 2018, https://doi.org/10.3389/fnut.2018.00058.
18. Gouveia, Luísa, et al. "Functional Biscuits with PUFA-Ω3 Fromisochrysis Galbana." *Journal of the Science of Food and Agriculture*, vol. 88, no. 5, 2008, https://doi.org/10.1002/jsfa.3166.
19. Ritala, Anneli, et al. "Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016." *Frontiers in Microbiology*, vol. 8, 2017, https://doi.org/10.3389/fmicb.2017.02009.
20. Lamela, T, and FJ Márquez-Rocha. "Phycocyanin Production in Seawater Culture of Arthrospira Maxima." *Ciencias Marinas*, vol. 26, no. 4, 2000, https://doi.org/10.7773/cm.v26i4.619.
21. "Spirulina: Growth, Physiology and Biochemistry." *Spirulina Platensis Arthrospira*, www.semanticscholar.org/paper/Spirulina%3A-Growth%2C-Physiology-and-Biochemistry-Vonshak/b4ce1aea99fb9df17f1abb6708458dd2db8b7eb2, Accessed 17 Apr 2023.
22. Wangler, Michael F., et al. "Drosophila and Genome-Wide Association Studies: A Review and Resource for the Functional Dissection of Human Complex Traits." Disease Models & Mechanisms, vol. 10, no. 2, 2017, https://doi.org/10.1242/dmm.027680.
23. Staats, Stefanie, et al. "drosophila melanogaster as a versatile model organism in food and nutrition research." Journal of Agricultural and Food Chemistry, vol. 66, no. 15, 2018, pp. 3737–3753, https://doi.org/10.1021/acs.jafc.7b05900.
24. Weinrich, Tobias, et al. "Mitochondrial function, mobility and lifespan are improved in drosophila melanogaster by extracts of 9-cis-β-carotene from Dunaliella Salina." Marine Drugs, vol. 17, no. 5, 2019, p. 279, https://doi.org/10.3390/md17050279.
25. Krogmann, David W. "Algology: *Algal Physiology and Biochemistry*." *Science*, vol. 188, no. 4193, 1975, https://doi.org/10.1126/science.188.4193.1105.a.
26. AlFadhly, Nawal K., et al. "Trends and Technological Advancements in the Possible Food Applications of Spirulina and Their Health Benefits: A Review." *Molecules*, vol. 27, no. 17, 2022, https://doi.org/10.3390/molecules27175584.
27. Borowitzka, Michael A. "M. E. Gershwin, A. Belay (Eds). Spirulina in Human Nutrition and Health." *Journal of Applied Phycology*, vol. 21, no. 6, 2009, https://doi.org/10.1007/s10811-009-9467-0.
28. "Coping with climate change : the roles of genetic resources for food and agriculture." *Animal Genetic Resources*, www.researchgate.net/publication/313566970_Coping_with_climate_change_the_roles_of_genetic_resources_for_food_and_agriculture. Accessed 19 Apr 2023.
29. Rosero-Chasoy, Gilver, et al. "Growth Kinetics and Quantification of Carbohydrate, Protein, Lipids, and Chlorophyll of Spirulina Platensis under Aqueous Conditions Using Different Carbon and Nitrogen Sources." *Bioresource Technology*, vol. 346, 2022, https://doi.org/10.1016/j.biortech.2021.126456.
30. Batista, Ana Paula, et al. "Microalgae as Functional Ingredients in Savory Food Products: Application to Wheat Crackers." *Foods*, vol. 8, no. 12, 2019, https://doi.org/10.3390/foods8120611.
31. Qiu, Shuang, et al. "Potential of Ulva Lactuca for Municipal Wastewater Bioremediation and Fly Food." *DESALINATION AND WATER TREATMENT*, vol. 91, 2017, https://doi.org/10.5004/dwt.2017.20767.