Algal Research 74 (2023) 103203



Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal





Isolation and selection of microalgae capable of stimulating rice plant development and seed production

Thainá Inês Lamb [a,c], Emilio Berghahn [a], Fernanda Miyagi Pita [c,d], Leonardo de Oliveira Neves [b], Édina Aparecida dos Reis Blasi [a,c], Jamili Seibel Hofstetter [b,c], Mariana Dammann [c], Luiz Carlos Oliveira da Silva [b], Giseli Buffon [c], Anja Dullius [c], Camille Eichelberger Granada [a], Raul Antonio Sperotto [a,b,e,*]

[a] Graduate Program in Biotechnology, Universidade do Vale do Taquari - Univates, Lajeado, Brazil
[b] Life Sciences Area, Universidade do Vale do Taquari - Univates, Lajeado, Brazil
[c] Syntalgae Research and Development, Lajeado, Brazil
[d] Universidad Científica del Sur, Lima, Peru
[e] Graduate Program in Plant Physiology, Universidade Federal de Pelotas, Pelotas, Brazil

ARTICLE INFO

Keywords:
Biofertilizer
Chlamydomonas
Chlorella
Desmodesmus
Microalgae
Rice

ABSTRACT

The growing global demand for food, in addition to the environmental impacts of agricultural intensification and climate change, reinforce the need for sustainable alternatives to maintain and increase agricultural productivity. Microalgae are widely available renewable resources for application in agricultural environments. There is a vast biodiversity of microalgae species, and their versatile metabolism produces a great diversity of compounds that favor the capture and supply of nutrients, improve soil characteristics, and stimulate plant growth. In this study, we evaluated the production of indole compounds, exopolysaccharides, siderophores, and the development capacity of microalgae strains of the genera *Chlamydomonas* sp., *Chlorella* sp. and *Desmodesmus* sp. isolated from different Brazilian States. Microalgae that produced a greater range of compounds and developed more quickly were selected for the production of aqueous extracts from their biomass in order to evaluate their potential as a biostimulant for rice (*Oryza sativa* L.) plants. Seed inoculation and foliar application of extracts stimulated plant development. Shoot length in seedlings 7 days after germination was increased after the application of Syn 16 aqueous extract at concentration of 0.5 g/L. Shoots of 30-day-old plants increased in length with the application of Syn 16 and Syn 90 strains, regardless of the tested concentration, while root length was increased by Syn 16 (at both concentrations - 0.1 and 0.5 g/L) and Syn 90 (0.1 g/L). Regarding shoot dry weight, increases of up to 36 % were detected with the Syn 16 strain at concentration of 0.5 g/L. With the Syn 90 strain at a concentration of 0.5 g/L, the increase was 27 %. Furthermore, root dry weight was 43 % and 35 % higher than control plants after the application of Syn 16 and Syn 90, respectively (both at 0.5 g/L concentration). At the maturity stage, application of Syn 46 strain (0.5 g/L concentration) increased 11 % the number of full seeds per panicle when compared to control condition. Our results demonstrate the ability of microalgae extracts in stimulating the development and seed production of rice plants.

1. Introduction

Global agriculture has been continuously improved, and the introduction of new technologies is essential to meet the growing food demand. However, the environmental costs of these advances are significant, causing soil depletion, groundwater contamination and air pollution, which pose serious threats to global health [1]. Considering this pressing reality, in addition to population growth, it is essential to look for innovative alternatives that aim to achieve agricultural sustainability, encompassing issues such as environmental health, social equality and profits.

Agricultural sustainability is based on the principle of meeting present needs without compromising future generations [2]. Sustainable agricultural practices strive to expand the natural resource base,

* Corresponding author at: Graduate Program in Biotechnology, Universidade do Vale do Taquari - Univates, Lajeado, Brazil.
E-mail address: rasperotto@univates.br (R.A. Sperotto).

https://doi.org/10.1016/j.algal.2023.103203
Received 9 March 2023; Received in revised form 9 July 2023; Accepted 11 July 2023
Available online 13 July 2023
2211-9264/© 2023 Elsevier B.V. All rights reserved.

decrease the use of chemicals, improve soil fertility and crop productivity, while protecting and preserving the environment. In this regard, biofertilizers and biostimulants are products of animal, plant or microbial origin that allow plants to develop and produce at their maximum potential, in addition to providing protection against biotic and abiotic stresses [3], without causing environmental damage [4,5].

In this scenario, microalgae are an alternative to traditional chemical fertilizers, either used in isolation or in association with other biological products [6]. Microalgae are sources of several substances that can be used in sustainable agriculture. They are considered multifunctional microorganisms, as they are capable of supplying nitrogen and carbon for assimilation by plants. In addition, they can enhance the development of cultures through the production of phytohormones (auxins, gibberellins and cytokinins), solubilization of macro and micronutrients, release of exopolysaccharides (source of organic carbon and soil aggregation/stabilization), in addition to having a positive influence in the soil's microbial diversity [3,6,8,9]. These compounds can improve plant response against environmental stress and pathogen attacks, in addition to favoring essential metabolic processes in plant development.

There are several methodologies for applying microalgae in agriculture, such as foliar application, directly in the soil or in seeds [3]. It has already been shown that microalgae inoculation in irrigation water can enhance growth in rice (*Oryza sativa*) plants [10]. However, developments in the use of microalgae as fertilizers in the agricultural industry need to improve further [11]. Therefore, the objective of this work was to isolate and select microalgae capable of acting as biostimulants in rice plants. Thus, specific methodologies were used for the isolation of the genera *Scenedesmus* spp., *Chlorella* spp. and *Chlamydomonas* spp.. The production of bioactive compounds and the generation time of the isolates (an important factor for scaling up industrial production) were evaluated *in vitro*, and the selected isolates were subsequently inoculated in rice plants to verify the biostimulant/biofertilizer effect.

2. Materials and methods

2.1. Microalgae collection and isolation

The microalgae were isolated from water samples obtained from interconnected ponds, lakes and dams in four Brazilian States (Rio Grande do Sul, Paraná, Santa Catarina and Ceará). Samples were first enriched in liquid culture medium and subsequently cultured in BBM agar (*Bold's Basal Medium* - [12]), BG-11 (*Blue-Green algae medium* - [13,14]), or TAP (*Tris-Acetate-Phosphate medium* - [15]). In order to inhibit the growth of fungi and bacteria, carbendazim (1 μg/mL) and streptomycin (25 μg/mL) were added. The microalgae cultures were incubated at 25 °C with a photoperiod of 16 h of light at an irradiance of 55 μmol/m^2/s. The formed microalgae colonies were purified and preserved in a standard culture medium containing agar.

2.2. Strain selection and identification

The morphological characteristics of axenic microalgae were evaluated by optical microscopy (Nikon Eclipse Ei and Nikon Eclipse E200). Cells that had the characteristic shape of the genera *Scenedesmus* (ovoid, being single or aggregated cells, with spines or ornamentation), *Chlorella* (spherical, approximately 0–10 μm in diameter and without flagellum), and *Chlamydomonas* (large ovoid cells with two flagella), were selected and identified by sequencing the *18S rRNA* gene and *ITS* (*internal transcribed spacer*) regions. In order to achieve this, the genomic DNA of the microalgae was isolated using the PureLink™ Plant Total DNA Purification Kit (Invitrogen), following the manufacturer's recommendations. The obtained DNA was used as a template for the amplification of the *18S rRNA* gene using primers P2 (5′-CTGGTTGATTCTGCCAGT-3′) and P4 (5′-TGATCCTTCYGCAGGTTCAC-3′) [16]. PCR was performed using about 10 ng of DNA, 500 nM of each primer, 250 nM dNTP, 1.5 mM $MgCl_2$ and 1 unit of Platinum™ *Taq* DNA Polymerase (Invitrogen). The parameters used in the thermal cycle were denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min, followed by a final extension of 10 min at 72 °C [17]. In order to amplify the *ITS* regions, primers ITS1*f* (5′-AGGAGAAGTCGTAACAAGGT-3′), ITS2*r* (5′-GCTGCGTTCTTCATCGATGC-3′), ITS3*f* (5′-GCATCGATGAAGAACGCAGC-3′), and ITS4*r* (5′-TCCTCCGCTTATTGATATGC-3′) [18] were used. The conditions applied for the PCR were denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, followed by a final 10 min extension at 72 °C [19]. The obtained PCR products were sequenced by the Sanger method and compared with the sequences available in the National Center for Biotechnology Information (NCBI) database, using the BLASTN tool (2.0.5). The sequences obtained were deposited at the NCBI and are available via accession numbers OQ473921, OQ473922, OQ564605-OQ564626, and OQ564627-OQ564649.

2.3. Microalgae cultivation and growth curve

The microalgae were inoculated into 10 mL of BBM, BG11, and TAP liquid media, and then incubated in a growth chamber (Marconi) with a light/dark cycle of 16 h/8 h, and light irradiance of 80–120 μmol/m^2/s at 25 °C for 3 days, with vortexing once a day. After 2 days, 10 mL of the microalgae culture were inoculated into a new broth supplemented with glucose (10 g/L), for a final volume of 50 mL. The flasks were incubated under the same conditions of light and temperature mentioned above, however, with agitation at 170 rpm. Growth was monitored by cell counting in a Fuchs-Rosenthal chamber [20]. When the cultures reached the intermediate exponential phase, a 10 mL aliquot of the inoculum (5 % v/v) was transferred to a 500 mL volume, exposed to photosynthetically active radiation (PAR, 400–700 nm) and light irradiance of 150 μmol/m^2/s, 16 h/8 h light/dark at 25 °C with agitation. The cell counts and the determination of the optical density of the microalgae culture were made from the inoculation of the microalgae in a volume of 1 L. The growth of the culture was determined every 24 h, over 5 days. The 48 h growth time was used to calculate the generation time using a methodology adapted from Schlegel [21]. With this methodology, the number of divisions per hour is first calculated (Eq. (1)).

$$v\,(h) = \frac{log\ N - log\ N0}{log2^{*}(t - t0)} \quad (1)$$

$v\ (h)$ = division rate per hour.
$N0$ and $t0$ = starting count and time, respectively.
N = obtained count at each evaluated time.
t = evaluated time.

Subsequently, the generation time is calculated (Eq. (2)).

$$g(h) = 1/v(h) \quad (2)$$

2.4. Production of exopolysaccharides, indole compounds and siderophores

The production of exopolysaccharides was determined by a methodology adapted from Sasaki [22]. The microalgae were cultivated for 7 days in the ideal agar for their growth. A small amount of the cells was then inoculated in liquid medium and stained with India ink 50 % (v/v). The presence of extracellular polysaccharides was visualized by phase contrast microscopy under an optical microscope. The production of indole compounds was evaluated using the colorimetric test developed by Glickmann and Dessaux [23]. The strains were cultured for 7 days at 25 °C with constant agitation at 170 rpm in King B culture medium (glycerol 15 g, peptone 20 g, K_2HPO_4 1.15 g, $MgSO_4{\cdot}7H_2O$ 1.5 g,

tryptophan 0.5 g, distilled water enough amount to 1 L) [24]. After this period, the culture was centrifuged at 10,000 rpm for 10 min, and 50 μL of cell-free supernatant was added to 50 μL of Salkowsky's reagent ($FeCl_3$ 120 g/L + H_2SO_4 421 mL/L) and incubated in the dark for 30 min. The reading of the colorimetric reaction was performed on a Spectramax spectrophotometer (Molecular Device), at a wavelength of 520 nm. The obtained readings were compared to those obtained in a standard curve with known concentrations of indole-butyric acid (0 to 200 μg/mL). The produced siderophores were evaluated using 50 μL of the same supernatant described above, with the addition of 50 μL of CAS (Chromium Azurol S) reagent [25], incubated for 30 min in the dark at room temperature. The colorimetric reading was performed in a spectrophotometer at 630 nm, using only King B broth with CAS solution as a control.

2.5. Preparation of microalgal extract and plant material

Four strains of microalgae were selected based on the results of exopolysaccharide determination, production of indole compounds and siderophores, and generation time. These four strains were used to evaluate biostimulant effect in rice plants, in the germinative, vegetative, and reproductive stages. The generated microalgal biomass after 7 days of growth was centrifuged at 5000 rpm for 30 min, and the pellet was collected and dried in an oven with air circulation at 40 °C (adapted from Navarro-López et al. [26]). The microalgae extracts were prepared with cells lysed in distilled water, both for rice seed inoculation and foliar application.

Rice seeds (*Oryza sativa* ssp. *indica* cv. IRGA 421) were disinfected, placed in containers with two sheets of germitest paper and incubated at 25 °C for 7 days for germination. Nine treatments were evaluated: control condition (seeds moistened with 10 mL of distilled water), and eight treatments (four selected microalgae isolates, at two cell concentrations - 0.1 and 0.5 g/L) (Fig. 1a and b). The seeds treated with the microalgae isolates were maintained in contact with the aqueous extract for 24 h.

2.6. Determination of the germination index

The germination index (GI) was determined according to the methodology described by Navarro-López et al. [26] (Eq. (3)), where G represents the number of germinated seeds and L represents the root length in the treatments. G_W and L_W represent the same parameters in the control condition (adapted from [26]).

$$GI\,(\%) = \frac{G \times L}{G_W \times L_W} \times 100 \tag{3}$$

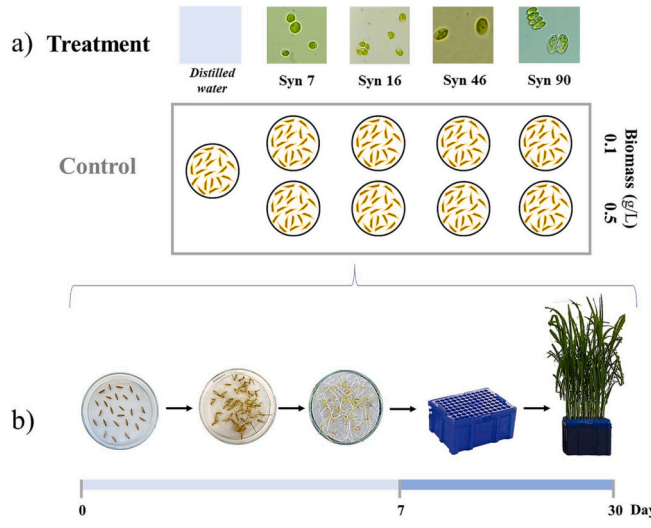


Fig. 1. (a) Experimental design to evaluate the stimulating effect in the germination stage. (b) Process from germination to the establishment of adult plants. Light blue: germination chamber phase; dark blue: growth room phase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.7. Evaluation of the biostimulant effect in rice plants at the vegetative phase

After 7 days of germination, approximately 25 plants were used to determine root and shoot length using a ruler and digital caliper (DIGIMESS-100.172). Another 25 plants were transferred to a hydroponic system with nutrient solution [27] and kept for another 23 days in a growth room with controlled lighting and temperature (25 °C and photoperiod 16 h/8 h light/dark). Leaf spraying of 1.5 mL of microalgal solution (0.1 or 0.5 g/L) was carried out on plants with 20 days of development, with the aid of an airbrush (Model 977,055 - Worker). At 30 days, root and shoot length were evaluated, in addition to chlorophyll concentration. Subsequently, the plants were dried for 5 days at 60 °C, to determine the dry weight.

2.8. Chlorophyll quantification

Total chlorophyll concentration was determined for the plants after 30 days of growth by the Falker chlorophyll index using a chlorophyll meter (ClorofiLOG CFL1030 - FALKER).

2.9. Evaluation of the biostimulant effect in rice plants at the reproductive phase

In an independent experiment, 20 rice plants of each condition were grown as described above. Thirty-day-old plants were transferred to greenhouse conditions (plastic buckets containing 5 L of non-sterile sand:organic compost (3:7, w/w) and water). When necessary, the moisture was maintained with water addition. In this experiment we tested 50 % (3.75 g NPK (4–17-27), 2.5 g urea at the tillering initiation, and 1.25 g urea at the booting stage) and 100 % of the recommended fertilization dose (doubling NPK and urea amounts), and 20-day-old plants were only treated with 0.5 g/L microalgal solution. At the full maturity stage, the seed production was evaluated.

2.10. Statistical analysis

All data were submitted to the Shapiro-Wilks normality test. Data that demonstrated normal distribution were compared by One-Way ANOVA followed by Scott-Knott ($p < 0.05$ or $p < 0.01$). Data that did not demonstrate normal distribution were compared by Kruskal-Wallis followed by Bonferroni ($p < 0.05$ or $p < 0.01$), using the Infostat software version 2017.1.2. Principal component analysis (PCA) was performed using the Past3 software, and included the results of germination index (%), shoot and root length at 7 and 30 days, shoot and root dry weight, and concentration of total chlorophyll in the nine evaluated treatments.

3. Results and discussion

3.1. Strain selection and identification

Among the microalgae species with potential application in agriculture, the genera *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Euglena*, *Dunaliella* and *Nannochloropsis* are the most investigated, and some are already used as commercial biostimulants [4]. In this work, 30 isolates of axenic microalgae were obtained. According to morphological characteristics, 13 isolates belonged to the genera of interest (Fig. 2). These isolates were evaluated to determine the production of biostimulant compounds, evaluation of generation time, and molecular identification

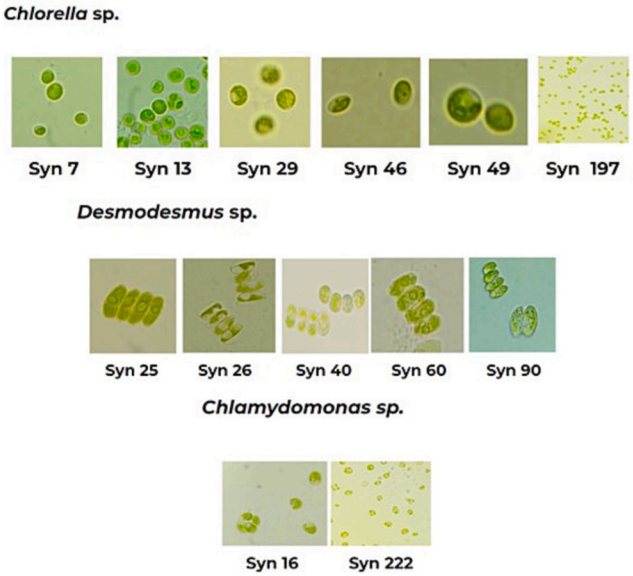


Fig. 2. Optical microscopy images of the isolated strains, separated by their respective genera. 1000× magnification.

of the genus (Table 1).

Sequencing of the *18S rRNA* and *ITS* markers showed that isolates with morphological characteristics similar to the genus *Scenedesmus* belonged to the genus *Desmodesmus*. This genus of microalgae was classified as a subgenus until 1999. Taxonomists showed that *Scenedesmus* lacked spines, while the new genus *Desmodesmus* presented said characteristic. However, many works still present these organisms under the old classification, which makes scientific research with strains of *Desmodesmus* more difficult [28,29]. This genus is characterized by the presence of unicellular individuals or in groups (coenobia), in addition to having a fourth layer of sporolenin in the cell wall, which enables the formation of spines and other ornamentations (ribs, warts, denticles or rosettes - [30]). In our work, most isolates belong to the genus *Chlorella*, followed by *Desmodesmus* and *Chlamydomonas* (Table 1). Fig. 2 shows the morphological characteristics of the isolated microalgae. *Chlorella* cells are spherical, approximately 0–10 μm in diameter, without flagellum, and the cell wall is rigid and thin [31]. Cells of the genus *Chlamydomonas* are large ovoids, mobile by two isocontus flagella inserted apically from two dilated zones, with intraplastid stigma and contractile vacuoles in the apical zone [32].

Table 1
Molecular identification and biochemical characterization of the 13 isolated microalgae strains. Generation time of isolated microalgae strains was calculated after 48 h of growth.

Strain	Genus	Production of indolic compounds (μg/mL)	Production of siderophores (%)	Generation time (h)
Syn 7	*Chlorella* sp.	1.44	-	8.49
Syn 13	*Chlorella* sp.	1.70	1.60	13.72
Syn 16	*Chlamydomonas* sp.	52.55	5.06	9.24
Syn 25	*Desmodesmus* sp.	0.91	2.04	17.40
Syn 26	*Desmodesmus* sp.	1.44	4.24	52.67
Syn 29	*Chlorella* sp.	1.70	1.65	14.20
Syn 40	*Desmodesmus* sp.	1.17	5.67	14.41
Syn 46	*Chlorella* sp.	2.22	12.76	10.60
Syn 49	*Chlorella* sp.	2.75	12.49	13.61
Syn 60	*Desmodesmus* sp.	0.13	0.72	15.04
Syn 90	*Desmodesmus* sp.	14.81	4.46	9.60
Syn 197	*Chlorella* sp.	0.13	1.27	17.95
Syn 222	*Chlamydomonas* sp.	4.06	14.14	9.32

(-) non-producer.

The identified genera in this work are common in freshwater environments. A study by Bonett et al. [33] isolated a strain of *Desmodesmus* sp. and several strains of the genus *Chlorella* spp. of lakes in the region of Minas Gerais, Brazil. In another study carried out in the same region, isolates of *Chlorococcum* sp., *Coelastrum* sp., *Monoraphidium caribeum* and *Nitzschia* sp. were identified [34]. Microalgae from freshwater sources in the Amazon Forest, Cerrado and Pantanal Biomes were collected by Hadi et al. [35]. Biodiversity was assessed through the analysis of *Ribulose Bisphosphate Carboxylase Large subunit* (*rbcL*) and *ITS* markers, and the authors identified the same genera found in the present work.

The bioactive metabolites produced by *Chlorella* sp., *Desmodesmus* sp. and *Chlamydomonas* sp., as well as the effects they have on plants, are extremely diverse [4]. Studies have shown that these microalgae produce phytohormones such as auxins, gibberellins, cytokinins, abscisic and jasmonic acids [36], in addition to polyamines [37], polysaccharides [38], amino acids and carotenoids [39]. The physiological effects comprise the control of cell division, promotion of protein and chlorophyll synthesis, elongation of stems and roots, initiation of seed germination, growth of buds, fruits and flowers, in addition to tolerance to biotic and abiotic stresses [4,40].

3.2. Production of exopolysaccharides, indole compounds and siderophores

Considering their ability to produce bioactive molecules such as fatty acids, osmolytes, phytohormones and polysaccharides, microalgae can be exploited in agriculture as a renewable bioresource to produce plant biostimulants [3,41]. Thus, the selected microalgae after microscopic evaluation were evaluated for their ability to produce bioactive compounds. In this work, the production of exopolysaccharides (EPS) by microalgae was verified by the formation of a clear halo around the cells (Fig. 3). EPS are carbohydrates produced and excreted by microalgae that differ from cell wall polysaccharides and intracellular starch in that they have a more complex chemical structure, often with 9–12 different monosaccharides and several non-glycosylated substituents. Depending on the species of microalgae, the synthesis of EPS can occur in the exponential or stationary growth phase [42]. EPS can be released into the culture medium, accumulate inside the cells, or be connected to the cell wall of microalgae, forming a capsule-like structure [43,44]. Syn 16, 29 and 90 strains showed the highest amount of these compounds, with a capsule-like cluster with a thick, viscous layer associated with the cell surface (Fig. 3). Sasaki et al. [22] also visualized this thick layer around the cells of *Parachlorella* sp. BX1.5 cultured in different culture media. The production of polysaccharides is closely associated with the amount of nitrogen available in the culture medium [45]. Mahesh et al. [46] induced EPS synthesis in microalgae cultivated with nitrogen reduction and found that deprivation of this nutrient resulted in maximum production (236 mg/L) and yield (37 mg/g) of EPS produced by *Scenedesmus abundans*. The two-fold increase in N and P concentrations in the *Chlorella* sp. promoted a reduction in the concentration of EPS [47]. Studies show that an increase in the C/N ratio can also trigger an increase in EPS production [42,48,49]. However, this may vary according to the microalgae species. The increase in $NaNO_3$ concentration from 100 to 422 mg/L in the *Chlamydomonas reinhardtii* culture medium increased the EPS production from 382 to 628 mg/L [50]. Changes in the

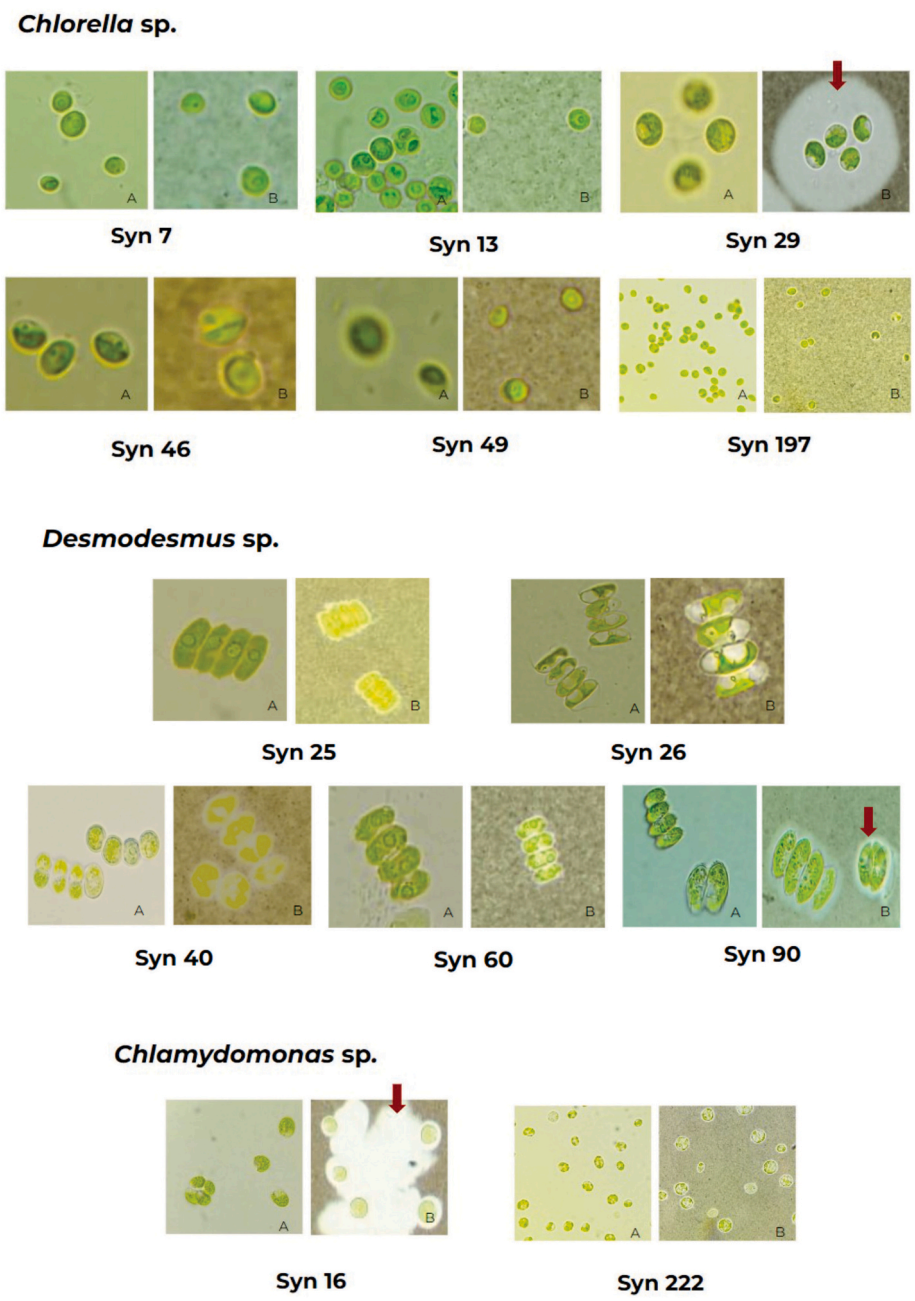


Fig. 3. Optical microscopy images of the isolated strains. (a) Cells at 1000× magnification. (b) Cells at 1000× magnification stained with India ink. The red arrows point to the presence of exopolysaccharides (EPS). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

formulation of microalgae culture media can favor the production of EPS, which can add even more value to products applied in agriculture. These compounds can provide organic C for the development of beneficial microorganisms, leading to the formation of biofilms in the rhizosphere. This association can help solubilize nutrients in the rhizosphere and trigger signaling cascades to activate the plant's defense response against biotic and abiotic stresses [7,51]. In addition, EPS can increase soil organic C, helping to prevent erosion and improving its structure [52].

Another evaluation of the potential production of biostimulant compounds was related to the concentration of indole compounds in the culture supernatant. The Syn 90 (*Desmodesmus* sp.) and Syn 16 (*Chlamydomonas* sp.) strains stood out in relation to the others, producing 14.81 μg/mL and 52.55 μg/mL of indole compounds (Table 1). The presence of auxins in cells, extracts and in supernatants of microalgae cultures vary greatly depending on the studied microalgae isolates. Among the indole compounds is the indole acetic acid (IAA), which plays an important role in the development of chlorophyll organisms, in addition to indole-3-butyric acid (IBA), indole-3-propionic acid (IPK) and indole-3-acetamide (IAM), compounds already found in 46 species of microalgae belonging to the Cyanophyta and Chlorophyta divisions [53]. Indole compounds from cultures of cyanobacteria belonging to the genera *Anabaena* and *Nostoc* were extracted, and IBA production was detected in the range of 140 to 2146 ng/g of fresh weight, in addition to small amounts of IAA (2.2 to 9.9 ng/g of fresh weight) [54]. Recently, an increase of 11 % in the production of microalgal biomass and of 16 % in the concentration of lipids was verified when 10 mg/L of IAA was added [55]. In addition, the authors pointed out that this can be an important

tool for improving microalgae cultivation, increasing the produced biomass and the amount of compounds of interest. Lu and Xu [56] highlight the importance of manipulating hormone levels in the culture medium as a way to improve microalgal development, with IAA being responsible for increasing growth rate and tolerance to stresses such as drought and salinity. Thus, the phytohormones produced by microalgae can be applied to stimulate plant growth. Do et al. [57] demonstrated that the *Chlorella sorokiniana* TH01 strain is capable of endogenously producing phytohormones. The effect of the extract of this strain was evaluated in seeds, and an advance in the germination of rice and tomato was verified, which germinated in just 48 and 24 h, respectively. It is noted that algae capable of producing IAA and excreting it in the supernatant can be interesting tools to stimulate plant growth. Therefore, the evaluation of the production of indole compounds carried out in our study was important for the selection of microalgae aiming at a biostimulant effect. An effect analogous to the phytohormone auxin was detected after application of aqueous extract of the microalgae *Micractinium pusillum* on the development of mung bean roots. The effect of extract application was compared to IBA standards tested (0.3–0.7 mg/L), promoting plant growth through increased elongation and cell division [58–60].

Another evaluation of the potential production of biostimulant compounds was related to the ability to make Fe available through the production of siderophores. Only the Syn 46, 49 and 222 isolates showed the ability to produce low siderophore concentrations (Table 1). Studies on siderophores in microalgae are scarce, since most authors describe the production of these compounds in microalgae cultures in consortium with bacteria, showing mutual benefits through this association [61]. However, in our study, the cultivation of all analyzed microalgae was axenic, and a low production of siderophores was detected. The schizokinen (SK) compound, a hydroxamic acid functionally related to a citric acid and resulting from the formal condensation of the primary amino group of N-(3-aminopropyl)-N-hydroxyacetamide with the carboxy groups at positions 1 and 3 of citric acid, was isolated from a culture of the cyanobacterium *Anabaena* sp. with low Fe concentration [62]. SK is a siderophore produced by the species *Bacillus megaterium* and *Anabaena* sp. and plays a role as a siderophore and bacterial metabolite. Akers et al. [63] also isolated SK in soils where rice was grown, a fact associated with the colonization of cyanobacteria from the *Nostocaceae* family, which are common inhabitants of flooded rice fields. It is suggested that the presence of SK results from the degradation or displacement of SK produced by algae during the flood period. Thus, further studies are needed to evaluate the production of siderophores by green microalgae, since these compounds have a high affinity for insoluble Fe molecules, removing Fe from environmental stocks and increasing its solubility, thus making it available for the plants [61].

3.3. Microalgae growth curve

For a sustainable production, we first need to overcome challenges in the production of microalgae. One of the most challenging and crucial issues is enhancing microalgae growth rate [64]. The isolates studied in this work showed different generation times, ranging from 8.49 to 52.67 h (Table 1). According to Thangavel et al. [65], the generation time varies according to the microalgae species, and the shortest generation time when evaluating eight different types of algae was found for cyanobacterium *Tolypothrix* sp. PBGA1 (1.0 ± 0.12 days). Whereas microalgae double in an average time of 26 h [66], *Chlamydomonas reinhardtii* can be considered a species with a relatively fast doubling time (around 8–12 h) [67].

Considering the strains that presented the best results in the evaluations of production of biostimulant compounds and generation time, we selected strains Syn 90 (*Desmodesmus*), Syn 16 (*Chlamydomonas*), and two strains of the genus *Chlorella* (Syn 46, which showed higher levels of siderophores; and Syn 7, which had the shortest generation time).

3.4. Evaluation of the biostimulant effect at the germinative, vegetative, and reproductive stages

3.4.1. Germination index (GI)

No increase in seed germination index (GI) was detected in any of the treatments (Fig. 4). However, treatments inoculated with Syn 7, Syn 16 and Syn 46 reduced the GI at both tested concentrations (0.1 and 0.5 g/L). Ferreira et al. [68] evaluated the GI in cucumber, barley, wheat, soybean, watercress and tomato seeds inoculated with *Synechocystis* sp. (PCC 6803), *Tetradesmus obliquus* (ACOI 204/07), *Chlorella protothecoides* (strain 25) and *Chlorella vulgaris* (INETI 58). In general, it was observed that all studied microalgae showed a positive effect on the GI of the tested seeds, with emphasis on *C. vulgaris*, which presented the most promising results in cucumber, wheat and tomato seeds; and *T. obliquus*, which presented better results in barley and watercress seeds. This study evaluated the aqueous extract of lysed microalgae cells, which can increase the concentration of bioactive molecules naturally present inside the cells. In another study, high germination rates were verified in watercress seeds by inoculation of intact cells of microalgae biomass [26]. According to the authors, intact biomass already has a high concentration of amino acids and/or polyamines, phytohormones and other bioactive compounds that influence physiological processes. When the biomass is subjected to cell disruption, the concentrations of these compounds can reach even higher levels, which may result in the inhibition of seed germination. The biostimulant potential of *Chlorella vulgaris* extracts (Beijerinck, CCAP 211/11C) and *Scenedesmus quadricauda* was evaluated in beet seeds [69]. Considering the amount of organic carbon (C_{org}) in the extracts, it was found that the lowest concentrations (1 and 2 mg C_{org}/L) promoted the highest germination rates, in addition to increasing root length. The effect of the aqueous extract of the seaweed *Sargassum wightii* Greville was also studied in seeds of *Triticum aestivum* var. Pusa Gold [70]. The application of the extract at a 20 % concentration stimulated germination, in addition to providing greater growth and seed yield.

Our results allow us to infer that the GI was not affected only by the Syn 90 strain (*Desmodesmus* sp.) (Fig. 4). This fact may be associated with the concentration of bioactive compounds, but it is not possible to confirm such a statement without evaluating the concentration of bioactive compounds in the extract. It should be noted that even at the highest concentration (0.5 g/L) the result did not differ from the lowest tested concentration (0.1 g/L), suggesting that the different compositions, and not just the concentration of the bioactive compounds, are related to the different effect on the GI.

3.4.2. Physiological and biochemical parameters at the vegetative stage

Root and shoot length were checked in seedlings 7 days after germination (Fig. 5a and c) and 30 days after germination (Fig. 5b and d). The shoot length of rice seedlings 7 days after germination was higher in the treatment with the microalgae Syn 16 at both tested concentrations (0.1 and 0.5 g/L). The application of Syn 7 and Syn 46 at a concentration of 0.5 g/L reduced seedling length, which did not exceed 4 cm. In the other treatments, there was no change when compared to the control condition (Fig. 5a). Root length 7 days after germination showed a reduction with the inoculation of Syn 7, 16 and 46 strains, regardless of the applied concentration (Fig. 5c). The Syn 90 strain showed results similar to the control condition. The shoot length of plants 30 days after germination increased with the application of Syn 16 and Syn 90 strains, regardless of the tested concentration (Fig. 5b). The roots of the 30-day-old plants showed higher length with the application of the strain Syn 16 (at both concentrations) and Syn 90 (at a concentration of 0.1 g/L) (Fig. 5d). The application of strains Syn 7 and Syn 46 did not differ from the control condition (Fig. 5b and d), regardless of the tested concentration. It was verified that extracts of microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda* (both at 1 and 2 mg C_{org}/L) applied in hydroponic medium increased the total length and surface area of sugar beet roots 36 h after treatment [71]. It was also

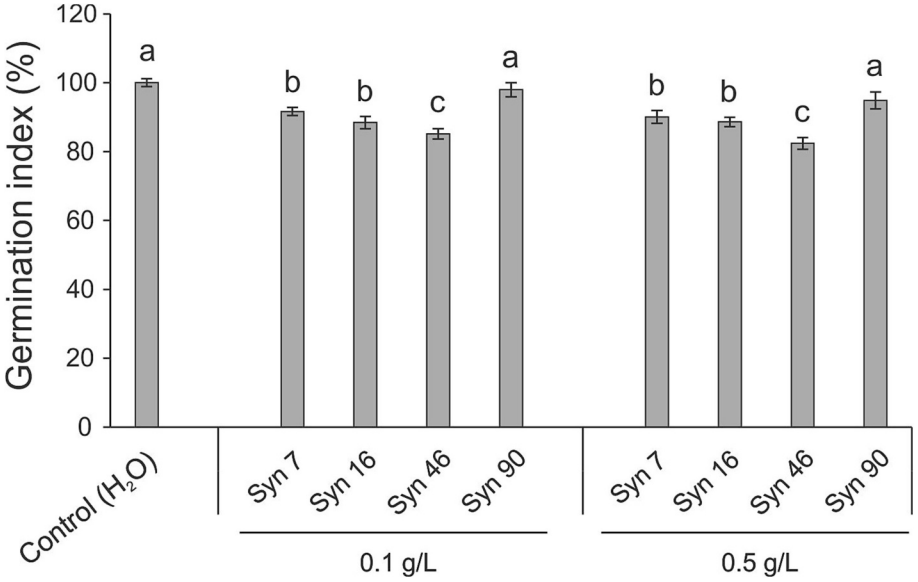


Fig. 4. Effect of microalgae extract application (0.1 and 0.5 g/L) on rice seed germination index, 7 days after seed soaking. Different letters indicate statistically significant difference (One-Way ANOVA followed by Scott-Knott - $p < 0.05$).

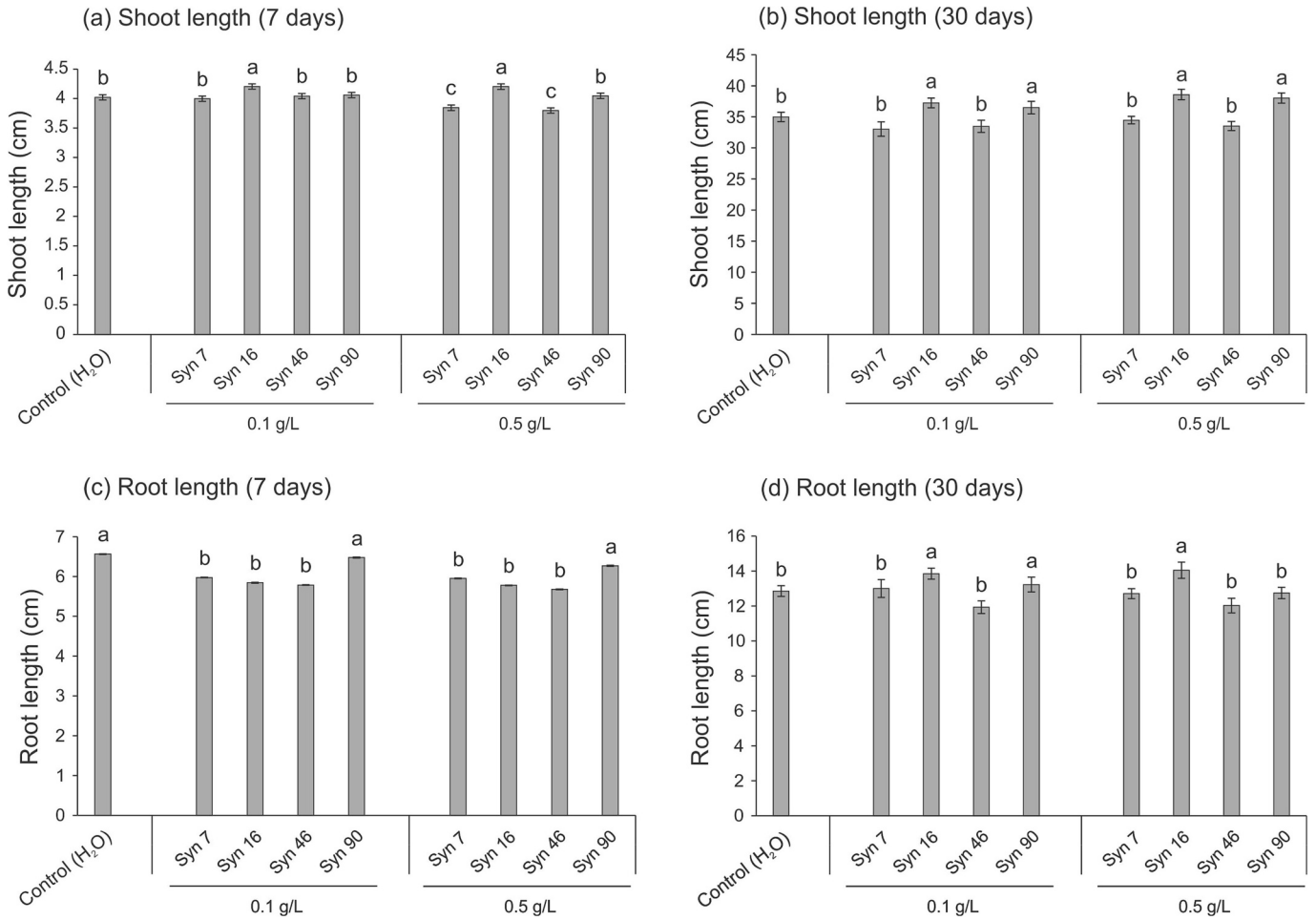


Fig. 5. Effect of microalgae extract application (0.1 and 0.5 g/L) on shoot (a) and root (c) length of rice plants 7 days after germination. Different letters indicate statistically significant difference (One-Way ANOVA followed by Scott-Knott - $p < 0.01$). Effect of microalgae extract application (0.1 and 0.5 g/L) on shoot (b) and root (d) length of rice plants 30 days after germination. Different letters indicate statistically significant difference (One-Way ANOVA followed by Scott-Knott - $p < 0.05$).

seen an increase in the expression of genes related to the acquisition of nutrients, certainly improving the growth and vigor of the plants. These findings indicate the biostimulating effects promoted by microalgae, as observed in our study.

The dry weight of roots and shoots of plants after 30 days of cultivation was also investigated (Fig. 6). The Syn 16 strain proved to be the most efficient, generating the highest values of shoot (Fig. 6a) and root (Fig. 6b) dry weight, regardless of the tested concentration. The dry weight of shoots increased by 22 and 36 % with the application of the Syn 16 strain at concentrations of 0.1 and 0.5 g/L, respectively (Fig. 6a). Also, root dry weight increased by 38 and 43 % with the application of the two Syn 16 concentrations (Fig. 6b). Another strain that proved to be efficient was Syn 90, whose application at a concentration of 0.5 g/L increased the dry weight of shoots and roots by 27 and 36 %, respectively, when compared to the control condition (Fig. 6a and b). In a similar study, the foliar application of hydrolyzed extracts of *Scenedesmus almeriensis* (CCAP 276/24) and *Arthrospira platensis* (strain BioVeg-F025) increased 49 % the dry weight of the roots and 20 % the dry weight of the flowers of *Petunia* × *hybrida* compared to control plants [36].

Regarding total chlorophyll concentration, there was no significant difference in any of the treatments, regardless of concentration (data not shown). Fresh cells of the microalgae *Chlorella vulgaris* S45 were applied to Swiss chard, causing an increase in the concentration of chlorophyll and carotenoids, quantified 7 days after the second foliar application [72]. According to the authors, the response of the plants is generally faster in the application via foliar spraying since the foliar absorption and translocation of nutrients/solutes is quite fast. In our study, the determination of the chlorophyll concentration was carried out 10 days after the foliar spraying. Thus, it would be interesting to verify the chlorophyll concentration a few days earlier.

In general, our results agree with previous studies. The effect of soil application of extracts obtained by acid hydrolysis of microalgae of the genus *Chlorella* sp., *Chlamydomonas* sp., *Aphanothece* sp., *Scenedesmus* sp., *Tetraselmis* sp., *Porphyridium* sp., *Arthrospira platensis*, *Spirulina maxima*, *Dunaliela salina*, *Nannochloropsis gaditana* and *Isochrysis galbana* was verified in tomato plants with 40 days of cultivation [73]. The application of the extract increased the length/dry weight of roots and shoots, chlorophyll concentration, and absorption of NPK. The higher absorption of P and K was related to the increased root size, while the increases in N and chlorophyll were related to shoot and root dry weights. These inoculated plants also accumulated higher levels of fatty acids (palmitic, linolenic and stearic), as well as pyridine-3-carboxamide, an active form of vitamin B3 amide. These results indicate a stimulus in biosynthetic pathways that lead to the production of lipids and plant hormones. The biostimulant capacity of the strains Syn 16 and Syn 90 in rice plants during both germinative and vegetative stages evidences the presence of bioactive compounds in the extract.

The produced biostimulant compounds differ between algal species and isolates. Therefore, the combination of different species of microalgae can potentiate the effects on plants [4,74]. The biofertilizing potential of the microalgae *Chlorella* sp. (KP972095) and *Scenedesmus* sp. (KR025877) was evaluated individually and in consortium, when applied in tomato cultivation soil [75]. This inoculant stimulated plant development, increased shoot and root fresh and dry weight, improved N, P, K, Ca, Mg and Fe composition, and increased productivity (174 % compared to control plants). Another study inoculated the biomass of microalgae *Scenedesmus* sp. in the rice cultivation soil, at doses of 50 and 100 % of the N recommendation [76]. The rice plants did not show any impairment in development, and the general cost of production was lower using microalgal biomass. In addition to economic viability, the combination of fertilizers (chemical and organic) resulted in an increased grain production/quality compared to isolated treatments at an equivalent dose.

Another factor that contributes to enhancing the effects provided by biostimulants in plants is the form of application, since foliar application is more effective in conditions of high relative humidity and when the stomata are open, facilitating the permeability and absorption of the formulation [7,77]. In a study carried out by Garcia-Gonzalez and Sommerfeld [78], it was verified that tomato seeds treated with the aqueous extract of *Acutodesmus dimorphus* (LARB-0414) at concentrations >0.75 g/mL germinate two days earlier than the control group. The aqueous extracts were also applied as foliar fertilizers on tomato plants, with the 50 % extract (3.75 g/mL) resulting in increased plant height and number of flowers/branches per plant. The authors also applied dry biomass (50 and 100 g) to the cultivation substrate, and found an increase in the number of branches/flowers in comparison with the control group, and with the treatments where the biofertilizer was applied to the substrate at the time of transplanting. These results suggest that early application is necessary for biomass to be decomposed and nutrients to become available for plant uptake.

In order to visualize the combined effect of all evaluated characteristics, principal component analysis (PCA) was performed. This analysis explained 87.57 % of the total variability, with PC 1 explaining 64.04 %, and PC 2 explaining 23.53 % (Fig. 7). PCA revealed that the germination index and root length 7 days after germination are related to the Syn 90 application at both concentrations. This analysis also demonstrated that the root/shoot dry weight, the shoot length 7 and 30 days after germination, as well as the root length 30 days after germination, are related to the Syn 16 application at both concentrations. Therefore, we can say

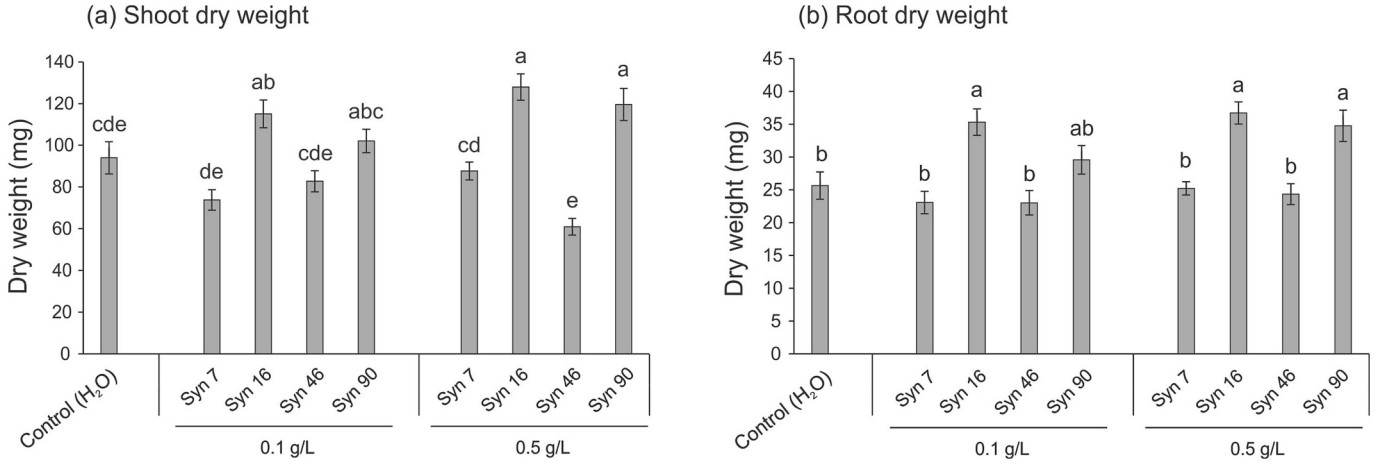


Fig. 6. Effect of microalgae extract application (0.1 and 0.5 g/L) on shoot (a) and root (b) dry weight of rice plants 30 days after germination. Different letters indicate statistically significant difference (One-Way ANOVA followed by Scott-Knott - $p < 0.01$).

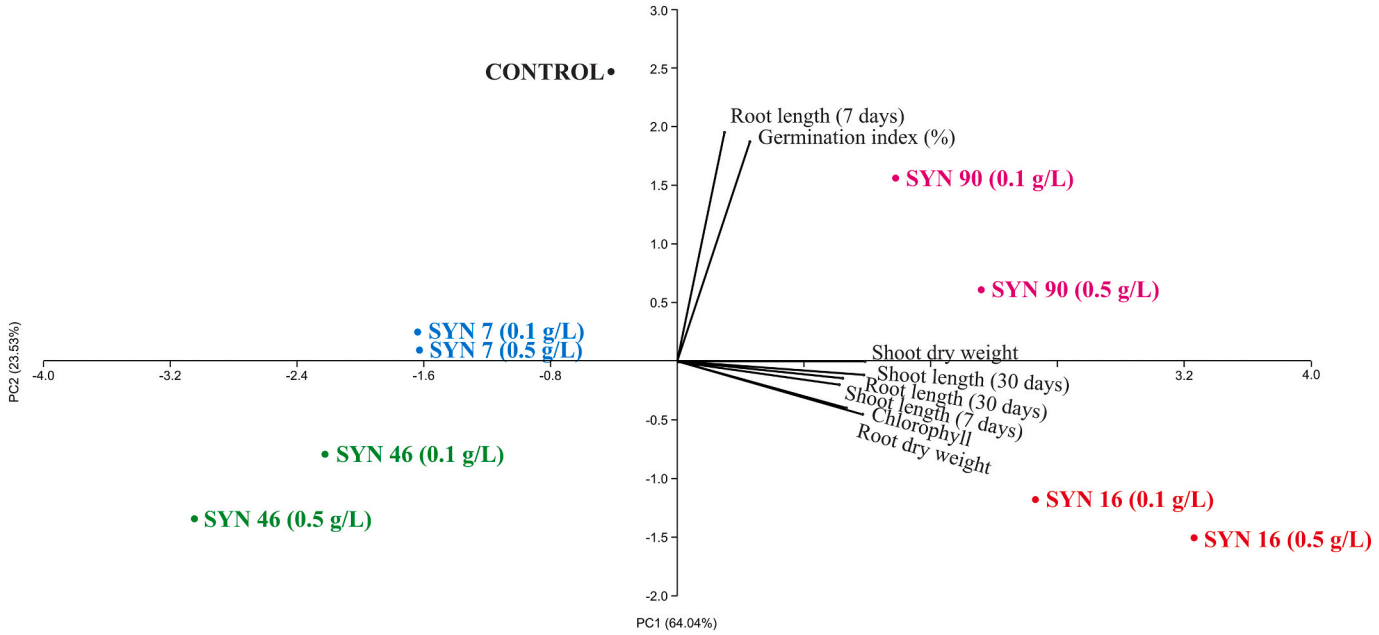


Fig. 7. Principal component analysis (PCA) relating the variables germination index, shoot and root length (7 and 30 days after germination), total chlorophyll concentration, and shoot and root dry weight (30 days after germination) with the treatments Syn 7, Syn 16, Syn 46 and Syn 90, at concentrations 0.1 and 0.5 g/L.

that these two microalgae (Syn 90 and Syn 16) showed more promising results in seed germination and growth promotion of rice plants.

3.4.3. *Seed production*

The seed production was checked in rice plants at the full maturity stage. Using 50 % of the recommended fertilization dose, the number of seeds per panicle was 11 % higher in rice plants treated with the strain Syn 46 when compared with the control condition (Fig. 8). On the other hand, Syn 7 application decreased the number of full seeds per panicle. Other agronomic parameters, as percentage of full seeds per plant, weight of 1000 full seeds, and seed length presented no change when the strains were compared to control condition (data not shown). Using the full fertilization dose, we were not able to detect beneficial effects with the application of strains (data not shown). This result is not unexpected, since it is already known that fully nourished plants do not take advantage of the potential use of microorganisms/biofertilizers in such conditions [79].

It is interesting to note that the most effective strains in the vegetative stage (Syn 16 and Syn 90) did not present beneficial effects in seed production, evidencing that different species/strains can stimulate different plant responses at distinct developmental stages. This is an important question when considering the use of more than one organism to formulate a biostimulant product, as a consortium [80]. As far as we know, this is the first report of increased seed production in rice plants treated with microalgae strains, evidencing the innovative character of this study.

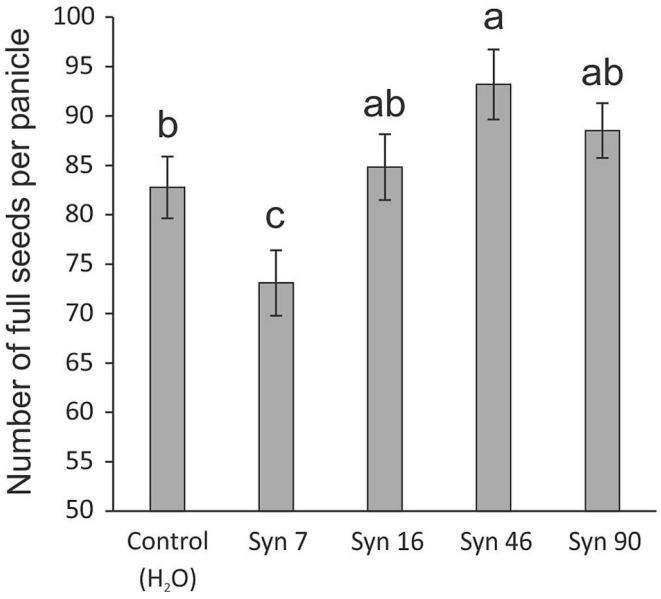


Fig. 8. Effect of microalgae extract application (0.5 g/L) on the number of seeds per panicle of rice plants at the full maturity stage. Different letters indicate statistically significant difference (Kruskal-Wallis followed by Bonferroni - $p < 0.05$).

4. Conclusions

In general, our results show that the inoculation of rice seeds, as well as the foliar spraying with the aqueous extract of microalgae of the genus *Chlamydomonas* sp. Syn 16, *Desmodesmus* sp. Syn 90, and *Chlorella* sp. Syn 46 provided several beneficial effects on development, stimulating rice seed germination, plant growth, and seed production. This indicates that extracts from these microalgae have bioactive compounds capable of causing a biostimulating effect, resulting in more vigorous plants. Our efforts are now focused on identifying the bioactive compounds of these microalgae that can stimulate plant development and yield, as well as on elucidating the role of these compounds in inducing tolerance to environmental stresses.

CRediT authorship contribution statement

Thainá Inês Lamb: Investigation, Writing - original draft. **Emílio Berghahn**: Investigation. **Fernanda Miyagi Pita**: Investigation. **Leonardo de Oliveira Neves**: Investigation. **Édina Aparecida dos Reis Blasi**: Investigation. **Jamili Seibel Hofstetter**: Investigation. **Mariana Dammann**: Investigation. **Luiz Carlos Oliveira da Silva**: Investigation. **Giseli Buffon**: Conceptualization, Writing - review & editing. **Anja Dullius**: Conceptualization, Writing - review & editing. **Camille Eichelberger Granada**: Conceptualization, Writing - review & editing.

Raul Antonio Sperotto: Conceptualization, Writing - review & editing. All authors read and approved the final form of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors would like to acknowledge Universidade do Vale do Taquari - Univates, Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (Fapergs), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Syntalgae Research and Development for providing financial support. The authors also thank Liane Terezinha Dorneles (Instituto Rio-Grandense do Arroz - IRGA) for technical support.

Ethical consent

No conflicts, informed consent, or human or animal rights are applicable to this study.

References

[1] S. Brodt, J. Six, G. Feenstra, C. Ingels, D. Campbell, Sustainable agriculture, Nat. Educ. Knowl. 3 (2011) 10, https://doi.org/10.1007/978-94-007-0394-0.
[2] C. Deeming, in: C. Deeming (Ed.), The Struggle for Social Sustainability, 2021, https://doi.org/10.2307/j.ctv1ktbds4.
[3] D. Ronga, E. Biazzi, K. Parati, D. Carminati, E. Carminati, A. Tava, Microalgal biostimulants and biofertilisers in crop productions, Agronomy 9 (4) (2019) 192, https://doi.org/10.3390/agronomy9040192.
[4] R.V. Kapoore, E.E. Wood, C.A. Llewellyn, Algae biostimulants: a critical look at microalgal biostimulants for sustainable agricultural practices, Biotechnol. Adv. 49 (2021), 107754, https://doi.org/10.1016/j.biotechadv.2021.107754.
[5] S. Kvantaliani, Producción de Biofertilizantes a Partir de Microalgas Cultivadas En Aguas Residuales, Universitat Politécnica de Catalunya, 2022 (Tesis de Master).
[6] M.L. Ortiz-Moreno, K.X. Sandoval-Parra, L.V. Solarte-Murillo, Chlorella, ¿Un Potencial Biofertilizante? Orinoquia 23 (2) (2019) https://doi.org/10.22579/20112629.582.
[7] P. Chiaiese, G. Corrado, G. Colla, M.C. Kyriacou, Y. Rouphael, Renewable sources of plant biostimulation: microalgae as a sustainable means to improve crop performance, Front. Plant Sci. 9 (2018) 1782, https://doi.org/10.3389/fpls.2018.01782.
[8] S. Araujo-Abad, Y. Collahuazo-Reinoso, Producción de Biofertilizantes a Partir de Microalgas, CEDAMAZ 9 (2) (2019) 81–87.
[9] P.D. Álvarez-Díaz, J. Ruiz, Z. Arbib, J. Barragán, M.C. Garrido-Pérez, J.A. Perales, Freshwater microalgae selection for simultaneous wastewater nutrient removal and lipid production, Algal Res. 24 (2017) 477–485, https://doi.org/10.1016/j.algal.2017.02.006.
[10] M. Jochum, L.P. Moncayo, Y.-K. Jo, Microalgal cultivation for biofertilization in rice plants using a vertical semi-closed airlift photobioreactor, PLoS One 13 (9) (2018), e0203456, https://doi.org/10.1371/journal.pone.0203456.
[11] M.M. Murata, L.R. Ito Morioka, J.B. Da Silva Marques, A. Bosso, H.H. Suguimoto, What do patents tell us about microalgae in agriculture? AMB Express 11 (1) (2021) https://doi.org/10.1186/s13568-021-01315-4.
[12] H.W. Nichols, H.C. Bold, *Trichosarcina polymorpha* Gen. et Sp. Nov, J. Phycol. 1 (1965) 34–38, https://doi.org/10.1111/j.1529-8817.1965.tb04552.x.
[13] M.M. Allen, Simple conditions for growth of unicellular blue-green algae on plates, J. Phycol. 4 (1) (1968) 1–4, https://doi.org/10.1111/j.1529-8817.1968.tb04667.x.
[14] R. Rippka, R.Y. Stanier, J. Deruelles, M. Herdman, J.B. Waterbury, Generic assignments, strain histories and properties of pure cultures of Cyanobacteria, Microbiology 111 (1) (1979) 1–61, https://doi.org/10.1099/00221287-111-1-1.
[15] D.S. Gorman, R.P. Levine, Cytochrome F and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardi*, Proc. Natl. Acad. Sci. 54 (6) (1965) 1665–1669, https://doi.org/10.1073/pnas.54.6.1665.
[16] D. Asker, T.S. Awad, Isolation and characterization of a novel lutein-producing marine microalga using high throughput screening, Food Res. Int. 116 (2019) 660–667, https://doi.org/10.1016/j.foodres.2018.08.093.
[17] S.Y. Moon-van der Staay, G.W.M. van der Staay, L. Guillou, D. Vaulot, H. Claustre, L.K. Medlin, Abundance and diversity of Prymnesiophytes in the picoplankton community from the equatorial Pacific Ocean inferred from 18S rDNA sequences, Limnol. Oceanogr. 45 (1) (2000) 98–109, https://doi.org/10.4319/lo.2000.45.1.0098.
[18] T.J. White, T.D. Bruns, S.B. Lee, J.W. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: M.A. Innis, D.H. Gelfand, J. J. Sninsky, T.J. White (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press, New York, 1990, pp. 315–322, https://doi.org/10.1016/B978-0-12-372180-8.50042-1.
[19] S. Radha, A.A. Fathima, S. Iyappan, M. Ramya, Direct colony PCR for rapid identification of varied microalgae from freshwater environment, J. Appl. Phycol. 25 (2) (2012) 609–613, https://doi.org/10.1007/s10811-012-9895-0.
[20] J.C. de Queiroz, A.C. de M. Ferreira, A.C.A. da Costa, The growth of *Monoraphidium* sp. and *Scenedesmus* sp. cells in the presence of thorium, Sci. World J. 2012 (2012) 1–8, https://doi.org/10.1100/2012/592721.
[21] H. Schlegel, General Microbiology, Cambridge University Press, 1993.
[22] M. Sasaki, A. Takagi, S. Ota, S. Kawano, D. Sasaki, M. Asayama, Coproduction of lipids and extracellular polysaccharides from the novel green alga *Parachlorella* sp. BX1.5 depending on cultivation conditions, Biotechnol. Rep. 25 (2020), e00392, https://doi.org/10.1016/j.btre.2019.e00392.
[23] E. Glickmann, Y. Dessaux, A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria, Appl. Environ. Microbiol. 61 (2) (1995) 793–796, https://doi.org/10.1128/aem.61.2.793-796.1995.
[24] E.O. King, M.K. Ward, D.E. Raney, Two simple media for the demonstration of pyocyanin and fluorescin, J. Lab. Clin. Med. 44 (2) (1954) 301–307.
[25] B. Schwyn, J.B. Neilands, Universal chemical assay for the detection and determination of siderophores, Anal. Biochem. 160 (1) (1987) 47–56, https://doi.org/10.1016/0003-2697(87)90612-9.
[26] E. Navarro-López, A. Ruíz-Nieto, A. Ferreira, F.G. Acién, L. Gouveia, Biostimulant potential of *Scenedesmus obliquus* grown in brewery wastewater, Molecules 25 (3) (2020) 664, https://doi.org/10.3390/molecules25030664.
[27] F.K. Ricachenevsky, R.A. Sperotto, P.K. Menguer, E.R. Sperb, K.L. Lopes, J.P. Fett, Zinc-induced facilitatoR-like family in plants: lineage-specific expansion in monocotyledons and conserved genomic and expression features among rice (*Oryza sativa*) paralogs, BMC Plant Biol. 11 (1) (2011), https://doi.org/10.1186/1471-2229-11-20.
[28] M. Lürling, Phenotypic plasticity in the green algae *Desmodesmus* and *Scenedesmus* with special reference to the induction of defensive morphology, Ann. Limnol. Int. J. Limnol. 39 (2) (2003) 85–101, https://doi.org/10.1051/limn/2003014.
[29] G.S. Hentschke, L.C. Torgan, *Desmodesmus* e *Scenedesmus* (Scenedesmaceae, Sphaeropleales, Chlorophyceae) em Ambientes Aquáticos na Planície Costeira do Rio Grande do Sul, Brasil, Rodriguésia 61 (4) (2010) 585–601, https://doi.org/10.1590/2175-7860201061403.
[30] E. Hegewald, New combinations in the genus *Desmodesmus* (Chlorophyceae, Scenedesmaceae), Algol. Stud./Archiv für Hydrobiologie, Supplement Volumes 96 (2000) 1–18, https://doi.org/10.1127/algol_stud/96/2000/1.
[31] C. Safi, B. Zebib, O. Merah, P.-Y. Pontalier, C. Vaca-Garcia, Morphology, composition, production, processing and applications of *Chlorella vulgaris*: a review, Renew. Sust. Energ. Rev. 35 (2014) 265–278, https://doi.org/10.1016/j.rser.2014.04.007.
[32] M.C. de Q. Mendes, A.A.C. Gonzalez, M. Menezes, J.M. de C. Nunes, S. Pereira, I. A. Nascimento, Coleção de microalgas de ambientes dulciaquícolas naturais da Bahia, Brasil, como potencial fonte para a produção de biocombustíveis: uma abordagem taxonômica, Acta Bot. Bras. 26 (3) (2012) 691–696, https://doi.org/10.1590/s0102-33062012000300019.
[33] J.E.A. Bonett, P. de Sousa Geraldino, P.G. Cardoso, F. de Freitas Coelho, W. F. Duarte, Isolation of freshwater microalgae and outdoor cultivation using cheese whey as substrate, Biocatalysis Agric. Biotechnol. 29 (2020), 101799, https://doi.org/10.1016/j.bcab.2020.101799.
[34] A. Alves da Silva, G. Graciano Fonseca, Bioprospection of freshwater microalgae from Bonito, MS, Brazil, Int. J. Biodivers. Conserv. 10 (3) (2018) 117–121, https://doi.org/10.5897/IJBC2017.1102.
[35] S.I.I.A. Hadi, H. Santana, P.P.M. Brunale, T.G. Gomes, M.D. Oliveira, A. Matthiensen, M.E.C. Oliveira, F.C.P. Silva, B.S.A.F. Brasil, DNA barcoding green microalgae isolated from neotropical inland waters, PLoS One 11 (2) (2016), e0149284, https://doi.org/10.1371/journal.pone.0149284.
[36] B.M. Plaza, C. Gómez-Serrano, F.G. Acién-Fernández, S. Jimenez-Becker, Effect of microalgae hydrolysate foliar application (*Arthrospira platensis* and *Scenedesmus* sp.) on Petunia × hybrida growth, J. Appl. Phycol. 30 (4) (2018) 2359–2365, https://doi.org/10.1007/s10811-018-1427-0.
[37] A. Piotrowska-Niczyporuk, A. Bajguz, E. Zambrzycka, B. Godlewska-Żyłkiewicz, Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris* (Chlorophyceae), Plant Physiol. Biochem. 52 (2012) 52–65, https://doi.org/10.1016/j.plaphy.2011.11.009.
[38] A. Rahman, S. Kumar, A. Bafana, S. Dahoumane, C. Jeffryes, Individual and combined effects of extracellular polymeric substances and whole cell components of *Chlamydomonas reinhardtii* on silver nanoparticle synthesis and stability, Molecules 24 (5) (2019) 956, https://doi.org/10.3390/molecules24050956.
[39] Y. Ren, H. Sun, J. Deng, J. Huang, F. Chen, Carotenoid production from microalgae: biosynthesis, salinity responses and novel biotechnologies, Mar. Drugs 19 (12) (2021) 713, https://doi.org/10.3390/md19120713.
[40] T.N.-D. Cao, H. Mukhtar, L.-T. Le, D.P.-H. Tran, M.T.T. Ngo, M.-D.-T. Pham, T.-B. Nguyen, T.-K.-Q. Vo, X.-T. Bui, Roles of microalgae-based biofertilizer in sustainability of green agriculture and food-water-energy security nexus, Sci. Total Environ. 870 (2023), 161927, https://doi.org/10.1016/j.scitotenv.2023.161927.

[41] M. Chanda, N. Merghoub, E.L. Arroussi, H., Microalgae polysaccharides: the new sustainable bioactive products for the development of plant bio-stimulants? World J. Microbiol. Biotechnol. 35 (11) (2019) https://doi.org/10.1007/s11274-019-2745-3.

[42] C. Delattre, G. Pierre, C. Laroche, P. Michaud, Production, extraction and characterization of microalgal and cyanobacterial exopolysaccharides, Biotechnol. Adv. 34 (7) (2016) 1159–1179, https://doi.org/10.1016/j.biotechadv.2016.08.001.

[43] D. Cruz, V. Vasconcelos, G. Pierre, P. Michaud, C. Delattre, Exopolysaccharides from Cyanobacteria: strategies for bioprocess development, Appl. Sci. 10 (11) (2020) 3763, https://doi.org/10.3390/app10113763.

[44] O. Babich, E. Budenkova, E. Kashirskikh, V. Dolganyuk, S. Ivanova, A. Prosekov, V. Anokhova, A. Andreeva, S. Sukhikh, Study of the polysaccharide production by the microalga *Vischeria punctata* in relation to cultivation conditions, Life 12 (10) (2022) 1614, https://doi.org/10.3390/life12101614.

[45] M.G. Morais, T.D. Santos, L. Moraes, B.S. Vaz, E.G. Morais, J.A.V. Costa, Exopolysaccharides from microalgae: production in a biorefinery framework and potential applications, Bioresour. Technol. Rep. 18 (2022), 101006, https://doi.org/10.1016/j.biteb.2022.101006.

[46] R. Mahesh, V.R. Naira, S.K. Maiti, Concomitant production of fatty acid methyl ester (biodiesel) and exopolysaccharides using efficient harvesting technology in flat panel photobioreactor with special Sparging system via *Scenedesmus abundans*, Bioresour. Technol. 278 (2019) 231–241, https://doi.org/10.1016/j.biortech.2019.01.091.

[47] B. Cheirsilp, Y.I. Mandik, P. Prasertsan, Evaluation of optimal conditions for cultivation of marine *Chlorella* sp. as potential sources of lipids, exopolymeric substances and pigments, Aquac. Int. 24 (1) (2015) 313–326, https://doi.org/10.1007/s10499-015-9927-2.

[48] S. Pereira, A. Zille, E. Micheletti, P. Moradas-Ferreira, R. De Philippis, P. Tamagnini, Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly, FEMS Microbiol. Rev. 33 (5) (2009) 917–941, https://doi.org/10.1111/j.1574-6976.2009.00183.x.

[49] S. Li, L. Ji, C. Chen, S. Zhao, M. Sun, Z. Gao, H. Wu, J. Fan, Efficient accumulation of high-value bioactive substances by carbon to nitrogen ratio regulation in marine microalgae *Porphyridium purpureum*, Bioresour. Technol. 309 (2020), 123362, https://doi.org/10.1016/j.biortech.2020.123362.

[50] A. Bafana, Characterization and optimization of production of exopolysaccharide from *Chlamydomonas reinhardtii*, Carbohydr. Polym. 95 (2) (2013) 746–752, https://doi.org/10.1016/j.carbpol.2013.02.016.

[51] I. Michalak, K. Chojnacka, A. Saeid, Plant growth biostimulants, dietary feed supplements and cosmetics formulated with supercritical CO_2 algal extracts, Molecules 22 (1) (2017) 66, https://doi.org/10.3390/molecules22010066.

[52] S. Guo, P. Wang, X. Wang, M. Zou, C. Liu, J. Hao, Microalgae as biofertilizer in modern agriculture, Microalgae Biotechnol. Food Health High Value Prod. (2020) 397–411, https://doi.org/10.1007/978-981-15-0169-2_12.

[53] E.A. Romanenko, I.V. Kosakovskaya, P.A. Romanenko, Phytohormones of microalgae: biological role and involvement in the regulation of physiological processes. Pt I. Auxins, abscisic acid, ethylene, Int. J. Algae 17 (3) (2015) 275–289, https://doi.org/10.1615/interjalgae.v17.i3.80.

[54] M.S. Hashtroudi, A. Ghassempour, H. Riahi, Z. Shariatmadari, M. Khanjir, Endogenous auxins in plant growth-promoting Cyanobacteria—*Anabaena vaginicola* and *Nostoc calcicola*, J. Appl. Phycol. 25 (2) (2012) 379–386, https://doi.org/10.1007/s10811-012-9872-7.

[55] W. Chang, Y. Li, Y. Qu, Y. Liu, G. Zhang, Y. Zhao, S. Liu, Mixotrophic cultivation of microalgae to enhance the biomass and lipid production with synergistic effect of red light and phytohormone IAA, Renew. Energy 187 (2022) 819–828, https://doi.org/10.1016/j.renene.2022.01.108.

[56] Y. Lu, J. Xu, Phytohormones in microalgae: a new opportunity for microalgal biotechnology? Trends Plant Sci. 20 (5) (2015) 273–282, https://doi.org/10.1016/j.tplants.2015.01.006.

[57] T.C.V. Do, D.T. Tran, T.G. Le, Q.T. Nguyen, Characterization of endogenous auxins and gibberellins produced by *Chlorella sorokiniana* TH01 under phototrophic and mixtrophic cultivation modes toward applications in microalgal biorefinery and crop research, J. Chem. 2020 (2020), e4910621, https://doi.org/10.1155/2020/4910621.

[58] J. Masojídek, C. Gómez-Serrano, K. Ranglová, B. Cicchi, Á. Encinas Bogeat, J. A. Câmara Manoel, A. Sanches Zurano, A.M. Silva Benavides, M. Barceló-Villalobos, V.A. Robles Carnero, V. Ördög, J.L. Gómez Pinchetti, L. Vörös, Z. Arbib, F. Rogalla, G. Torzillo, F. Lopez Figueroa, F.G. Acién-Fernández, Photosynthesis monitoring in microalgae cultures grown on municipal wastewater as a nutrient source in large-scale outdoor bioreactors, Biology 11 (10) (2022) 1380, https://doi.org/10.3390/biology11101380.

[59] Y. Zhao, Auxin biosynthesis and its role in plant development, Annu. Rev. Plant Biol. 61 (1) (2010) 49–64, https://doi.org/10.1146/annurev-arplant-042809-112308.

[60] C. Keswani, S.P. Singh, L. Cueto, C. García-Estrada, S. Mezaache-Aichour, T. R. Glare, R. Borriss, S.P. Singh, M.A. Blázquez, E. Sansinenea, Auxins of microbial origin and their use in agriculture, Appl. Microbiol. Biotechnol. 104 (20) (2020) 8549–8565, https://doi.org/10.1007/s00253-020-10890-8.

[61] L.M. González-González, de-Bashan, L. E., Toward the enhancement of microalgal metabolite production through microalgae–bacteria consortia, Biology 10 (4) (2021) 282, https://doi.org/10.3390/biology10040282.

[62] F.B. Simpson, J.B. Neilands, Siderochromes in Cyanophyceae: isolation and characterization of Schizokinen from *Anabaena* sp, J. Phycol. 12 (1) (1976) 44–48, https://doi.org/10.1111/j.1529-8817.1976.tb02824.x.

[63] H.A. Akers, Isolation of the siderophore schizokinen from soil of rice fields, Appl. Environ. Microbiol. 45 (5) (1983) 1704–1706, https://doi.org/10.1128/aem.45.5.1704-1706.1983.

[64] M.I. Khan, J.H. Shin, J.D. Kim, The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products, Microb. Cell Factories 17 (2018) 36.

[65] K. Thangavel, P. Radha Krishnan, S. Nagaiah, S. Kuppusamy, S. Chinnasamy, J. S. Rajadorai, G. Nellaiappan Olaganathan, B. Dananjeyan, Growth and metabolic characteristics of oleaginous microalgal isolates from Nilgiri Biosphere Reserve of India, BMC Microbiol. 18 (1) (2018), https://doi.org/10.1186/s12866-017-1144-x.

[66] J. Liu, J. Huang, F. Chen, Microalgae as feedstocks for biodiesel production, Biodiesel Feedstocks Process. Technol. (2011) 58, https://doi.org/10.5772/25600.

[67] V. Calatrava, M. Tejada-Jimenez, E. Sanz-Luque, E. Fernandez, A. Galvan, A. Llamas, *Chlamydomonas reinhardtii*, a reference organism to study algal–microbial interactions: why can't they be friends? Plants 12 (4) (2023) 788, https://doi.org/10.3390/plants12040788.

[68] A. Ferreira, L. Melkonyan, S. Carapinha, B. Ribeiro, D. Figueiredo, G. Avetisova, L. Gouveia, Biostimulant and biopesticide potential of microalgae growing in piggery wastewater, Environ. Adv. 4 (2021), 100062, https://doi.org/10.1016/j.envadv.2021.100062.

[69] I. Puglisi, E. La Bella, E.I. Rovetto, A.R. Lo Piero, A. Baglieri, Biostimulant effect and biochemical response in lettuce seedlings treated with a *Scenedesmus quadricauda* extract, Plants 9 (1) (2020) 123, https://doi.org/10.3390/plants9010123.

[70] G. Kumar, D. Sahoo, Effect of seaweed liquid extract on growth and yield of *Triticum aestivum* var. Pusa Gold, J. Appl. Phycol. 23 (2) (2011) 251–255, https://doi.org/10.1007/s10811-011-9660-9.

[71] V. Barone, A. Baglieri, P. Stevanato, C. Broccanello, G. Bertoldo, M. Bertaggia, M. Cagnin, D. Pizzeghello, V.M.C. Moliterni, G. Mandolino, F. Fornasier, A. Squartini, S. Nardi, G. Concheri, Root morphological and molecular responses induced by microalgae extracts in sugar beet (*Beta vulgaris* L.), J. Appl. Phycol. 30 (2) (2017) 1061–1071, https://doi.org/10.1007/s10811-017-1283-3.

[72] T. Hajnal-Jafari, V. Seman, D. Stamenov, S. Đurić, Effect of *Chlorella vulgaris* on growth and photosynthetic pigment content in Swiss chard (*Beta vulgaris* L. subsp. cicla), Pol. J. Microbiol. 69 (2) (2020) 235–238, https://doi.org/10.33073/pjm-2020-023.

[73] C. Mutale-joan, B. Redouane, E. Najib, K. Yassine, K. Lyamlouli, S. Laila, Y. Zeroual, E.A. Hicham, Screening of microalgae liquid extracts for their bio stimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L, Sci. Rep. 10 (1) (2020), https://doi.org/10.1038/s41598-020-59840-4.

[74] A.L. Alvarez, S.L. Weyers, H.M. Goemann, B.M. Peyton, R.D. Gardner, Microalgae, soil and plants: a critical review of microalgae as renewable resources for agriculture, Algal Res. 54 (2021), 102200, https://doi.org/10.1016/j.algal.2021.102200.

[75] S. Silambarasan, P. Logeswari, R. Sivaramakrishnan, A. Incharoensakdi, P. Cornejo, B. Kamaraj, N.T.L. Chi, Removal of nutrients from domestic wastewater by microalgae coupled to lipid augmentation for biodiesel production and influence of deoiled algal biomass as biofertilizer for *Solanum lycopersicum* cultivation, Chemosphere 268 (2021), 129323, https://doi.org/10.1016/j.chemosphere.2020.129323.

[76] M. Nayak, D.K. Swain, R. Sen, Strategic valorization of de-oiled microalgal biomass waste as biofertilizer for sustainable and improved agriculture of rice (*Oryza sativa* L.) crop, Sci. Total Environ. 682 (2019) 475–484, https://doi.org/10.1016/j.scitotenv.2019.05.123.

[77] J.C.A. Braun, L.M. Colla, Use of microalgae for the development of biofertilizers and biostimulants, BioEnergy Res. (2022), https://doi.org/10.1007/s12155-022-10456-8.

[78] J. Garcia-Gonzalez, M. Sommerfeld, Biofertilizer and biostimulant properties of the microalga *Acutodesmus dimorphus*, J. Appl. Phycol. 28 (2) (2015) 1051–1061, https://doi.org/10.1007/s10811-015-0625-2.

[79] P. Chen, Q. Wei, Y. Yao, J. Wei, L. Qiu, B. Zhang, H. Liu, Inoculation with *Azorhizobium caulinodans* ORS571 enhances plant growth and salt tolerance of switchgrass (*Panicum virgatum* L.) seedlings, Biotechnol. Biofuels Bioprod. 16 (1) (2023) 35, https://doi.org/10.1186/s13068-023-02286-3.

[80] M.S. Brzezinska, J. Świątczak, A. Wojciechowska, A. Burkowska-But, A. Kalwasińska, Consortium of plant growth-promoting rhizobacteria enhances oilseed rape (*Brassica napus* L.) growth under normal and saline conditions, Arch. Microbiol. 204 (7) (2022) 393, https://doi.org/10.1007/s00203-022-03018-1.