Yin *et al.*
Biotechnology for Biofuels and Bioproducts (2024) 17:135
https://doi.org/10.1186/s13068-024-02577-3

Biotechnology for Biofuels and Bioproducts

RESEARCH

Open Access

Enhancement of non-oleaginous green microalgae *Ulothrix* for bio-fixing CO_2 and producing biofuels by ARTP mutagenesis

Mingshan Yin[1], Yuliang An[2], Feng Qi[1,2*], Ruimin Mu[1,2], Guixia Ma[1,2] and Feiyong Chen[3]

Abstract

Oleaginous green microalgae are often mentioned in algae-based biodiesel industry, but most of them belong to specific genus (*Chlorella*, *Scenedesmus*, *Botryococcus* and *Desmodesmus*). Thus, the microalgal germplasm resources for biodiesel production are limited. Mutagenesis is regarded as an important technology for expanding germplasm resources. The main purpose of this study is to screen microalgae strains with high carbon dioxide tolerance and high lipid content from mutants derived from indigenous non-oleaginous green microalgae species—*Ulothrix* SDJZ-17. Two mutants with high CO_2 tolerance and high lipid content genetic stability were obtained from the mutants by high-throughput screening, named *Ulothrix* SDJZ-17-A20 and *Ulothrix* SDJZ-17-A23. In order to evaluate the potential of CO_2 fixation and biofuel production, A20 and A23 were cultured under air and 15% CO_2 (v/v) conditions, and their wild-type strains (WT) were used as controls. Under the condition of high CO_2 concentration, the growth performance and lipid production capacity of mutant strains A20 and A23 were not only significantly better than those of wild strains, but also better than those of their own cultured under air conditions. Among them, A23 obtained the highest LCE (light conversion efficiency) (14.79%), *Fv/Fm* (maximal photochemical efficiency of photosystem II) (71.04%) and biomass productivity (81.26 mg L^{-1} d^{-1}), while A20 obtained the highest lipid content (22.45%). Both mutants can be used as candidate strains for CO_2 fixation and biofuel production. By ARTP (atmospheric and room temperature plasma) mutagenesis with high-throughput screening, the mutants with higher CO_2 tolerance, photosynthetic efficiency and lipid productivity can be obtained, even if they are derived from non-oleaginous microalgae, which is of great significance for enriching the energy microalgae germplasm bank, alleviating the global warming and energy crisis.

Keywords ARTP mutagenesis, *Ulothrix*, High CO_2 tolerance, Photosynthetic efficiency, Lipid content

Background

In recent years, the burning of fossil fuels has caused varying degrees of environmental pollution, and the problem of climate change has become increasingly prominent [1, 2]. To control environmental pollution and climate change, numerous technologies for CO_2 and renewable energy fixation have been developed. Among them, algae-based biodiesel is regarded as an alternative to energy production due to its reduced dependence on fossil fuels and reduced CO_2 emissions, microalgae

*Correspondence:
Feng Qi
qifeng@sdjzu.edu.cn
[1] Engineering Technology Center of Microbial Environmental Remediation and Resource Utilization, Shandong Jianzhu University, Jinan 250101, China
[2] School of Municipal and Environmental Engineering, Shandong Jianzhu University, Jinan 250101, China
[3] Institute of Resources and Environmental Innovation, Shandong Jianzhu University, Jinan 250101, China

biofuels are clarifying the road to a sustainable future in the face of challenges and opportunities [3–7].

One of the key steps in the commercialization of algae-based biodiesel is the screening of candidate microalgae with tolerance to high concentrations of CO_2 and high lipid content. Chlorophyta (green algae) is one of the most common microalgae species for screening oleaginous microalgae. However, almost all the oleaginous oleaginous green microalgae involved in the existing research belong to Chlorococcales, and most of them belong to *Chlorella* [3], *Scenedesmus* [8], *Botryococcus* and *Desmodesmus* [9]. This indicates that despite decades of research, the microalgal germplasm resources of oleaginous green microalgae are still limited to a smaller range. Therefore, new breeding techniques are needed to expand the screening range of oleaginous microalgae.

Mutagenesis has become an important technology for microbial breeding because it can obtain germplasm characteristics that wild strains do not have in a short time [10–13]. In addition to the traditional mutation breeding methods such as chemical, ultraviolet, ultrasonic and heavy-ion-beam irradiation, atmospheric and room temperature plasma (ARTP) mutagenesis [14, 15], as a new mutation breeding method, has been widely used in recent years due to its advantages of simple equipment operation, high safety, high mutation intensity, and large mutation capacity [10, 16, 17]. However, the application of ARTP mutagenesis in the field of oleaginous microalgae breeding is rarely reported, and its application potential needs to be explored.

In this study, a set of ARTP mutagenesis high-throughput screening of algal strains with high CO_2 tolerance and oleaginous was designed. Based on this scheme, ARTP mutagenesis was performed on the wild strain of non-oleaginous green microalgae *Ulothrix*, and high CO_2 tolerance and oleaginous mutants with genetic stability were screened. The mutants and their wild-type strains were cultured in air and high CO_2 concentrations, and the content and yield of main biochemical components in biomass, light conversion efficiency (LCEs) and F_v/F_m were analyzed. The potential and application prospects of the mutants in CO_2 fixation and biofuel production were predicted.

Methods

Microalgae and culture medium

The wild microalgae strain used in this study was *Ulothrix* SDJZ-17 isolated from Yingxue Lake in Shandong Jianzhu University. *Ulothrix* is a common freshwater green microalgae in indigenous lakes of China. It is a simple unbranched filamentous body, and the lipid content of wild algae strains is usually below 10%. The improved SE medium was used for microalgae culture, and more $NaNO_3$ was added during the culture to provide a sufficient nitrogen source [18]. Table 1 lists the components in the improved SE medium, A5, and Fe-EDTA solution. A soil extract is filtered from a boiled soil solution. The pH value of SE modified medium was adjusted to 4.5 or 7. Aeration was 15% (v/v) CO_2-rich air and ambient air (0.04% CO_2 v/v) as control.

ARTP mutagenesis and algae strain screening

Based on the description of Li et al. [19], *Ulothrix* SDJZ-17 was mutagenized by ARTP-IIS atmospheric and room temperature plasma mutation breeding instruments. The concentration of *Ulothrix* SDJZ-17 in the logarithmic growth phase was adjusted to 10^6 cells/mL with phosphate buffer solution (PBS), and 10 μL of algae solution was evenly coated on the surface of the carrier. The carrier was placed in the groove of the ARTP mutation breeding instrument operating room in turn with sterile tweezers, The 2 mL EP centrifuge tube and tube containing 1 mL phosphate buffer solution were fixed. The distance D between the ARTP-IIS emission source and the plastic cover was adjusted to 2 mm, the voltage U was 120 V, the current I was 1A, the gas flow G was 5 SLM, and the mutagenesis treatment was started after 10 min. The irradiation time was set to 60 s.

After the mutagenesis was completed, the EP tube was placed on the oscillator for 1 min to shake, so that the algae liquid attached to the carrier was eluted, and then the EP tube was placed in a dark environment for 24 h to prevent the mutated microalgae from being photo-repaired.

After the completion of dark culture, the EP tube was oscillated for 1 min to mix the algae solution evenly, and the oscillating algae solution was continuously diluted with a modified SE medium with a pH of 4.5 until the

Table 1 The components of SE medium (a), A5 solution (b) and Fe-EDTA solution (c) were improved

(a)		(b)	
Component	**Improved SE medium**	**Component**	**A5 solution**
$NaNO_3$	1000 mg L^{-1}	H_3BO_3	2.86 g L^{-1}
$K_2HPO_4{\cdot}3H_2O$	75 mg L^{-1}	$MnC1_2{\cdot}4H_2O$	1.81 g L^{-1}
$MgSO_4{\cdot}7H_2O$	75 mg L^{-1}	$ZnSO_4{\cdot}4H_2O$	0.22 g L^{-1}
$CaCl_2{\cdot}2H_2O$	25 mg L^{-1}	$CuSO4{\cdot}5H_2O$	79 mg L^{-1}
KH_2PO_4	175 mg L^{-1}	$(NH)_6Mo_7O_{24}{\cdot}4H_2O$	39 mg L^{-1}
NaCl	25 mg L^{-1}	(c)	
$FeCl_3{\cdot}6H_2O$	5 mg L^{-1}	Component	Fe-EDTA
A_5 solution	2 mg L^{-1}	Na_2EDTA	10 g L^{-1}
Fe-EDTA	1 mL L^{-1}	$FeCl_3{\cdot}6H_2O$	0.81 g L^{-1}
soil solution	40 mL L^{-1}	0.1 M HCl	500 mL L^{-1}

algae cell concentration reached about 0.8/50 μL. 50 μL of diluted algae liquid and 200 μL of fresh medium with a pH value of 4.5 were placed in the micropores of a 96-well plate, and then the microplates were sealed with a Parafilm® sealing film to prevent evaporation of the liquid. The 96-well plates were placed in an artificial incubator at 25 ± 1 °C to maintain continuous light until the micropores turned green. Algae strains in all green microwells were inoculated aseptically into a 50-mL conical flask containing 30 mL freshly modified SE medium (pH 4.5) and cultured under continuous light conditions until the medium became green.

According to the neutral lipid fluorescence detection method proposed by Satpati et al. [20], the algae strains with the highest lipid content were screened from the mutants. The algae solution to be tested was diluted to 106 cells/mL, 950 μL was placed in a 1.5-mL EP tube, and 50 μL of 1 mg L^{-1} Nile red solution was added for staining. The staining was performed under dark conditions. After dyeing, the mixed liquid was transferred to a 96-well plate using a 200-μL pipette gun. After 2 min of oscillation, the fluorescence intensity at 750 nm was detected at the excitation wavelength of 528 nm and the emission wavelength of 576 nm in the microplate reader.

In order to better compare with the wild strain, the relative fluorescence intensity of the algae strain based on the standard of the fluorescence intensity of the neutral lipid of the wild strain was calculated according to Eq. (1):

$$\text{The relative fluorescence intensity} = \text{algae strain neutral lipid fluorescence intensity}/\text{wild strain neutral lipid fluorescence intensity}. \quad (1)$$

Cultivation

The wild-type or mutant cells were seeded in a photobioreactor (ID = 120 mm) containing 2.5L (working volume, V_w) fresh medium with an initial biomass concentration of approximately 72 mg L^{-1}, and then placed in an artificial climate chamber at 25 ± 1 °C. On one side of the reactor, continuous illumination was provided by a light-emitting diode (*LED*) wall, and the light intensity on the surface of the photobioreactor was 80 μmol m^{-2} s^{-1}. During the culture process, the algae water samples were collected every day, and the water samples were supplemented with deionized water and evaporated to a working volume of 2.5 L. Aeration uses air or 15% (v/v) CO_2-rich air mixed with air and pure CO_2. The flow rate of air and 15% CO_2 cylinder was adjusted to 0.2 vvm and 0.04 vvm, respectively, by using a gas mass flowmeter (Beijing Qixing Huachuang). Three parallel cultures were performed in each group.

Measurement method

The pH value of water samples was measured by PHS-3C pH meter, and the biomass concentration of water samples was measured by dry weight method. After 7 days of culture, F_v/F_m, which represents the maximum quantum yield of PSII (photosystem II-light), was measured [21]. After that, the algae solution was centrifuged at −3 °C and 4000 r/min for 10 min to form algae mud, washed twice with 0.5 M ammonium formate to desalt, and then freeze-dried and ground into powder. The high heating value (HHV) of microalgae powder was measured by an isothermal oxygen bomb calorimeter. The lipid in microalgae cells was extracted by solvent, and the total lipid contents were estimated by gravimetric method [22]. The total carbohydrate and crude protein contents in biomass were measured by the phenol–sulfuric acid method [23] and Kjeldahl's method [24], respectively.

Calculation of important parameters

The maximum biomass concentration (mg L^{-1}) obtained during culture was X_{max}.

The biomass productivity (P, mg L^{-1} d^{-1}) was calculated by Eq. (2) as an index to evaluate the biomass production potential. The overall biomass productivities for 7-day cultivation were designated as P_o, and the maximum biomass productivity (P_{max}) during cultivation was used to evaluate the potential of biomass production:

$$P = (X_t - X_0)/t, \quad (2)$$

where t (d) is the cultivated time; X_t (mg L^{-1}) is the biomass concentration on day t; X_0 (mg L^{-1}) is the initial biomass concentration.

The maximum biomass productivity in 7 days was the maximum biomass yield P_{max}, and the biomass yield at the final harvest (day 7) was the overall biomass yield P_o.

The daily specific growth rate (μ_t, d^{-1}) was calculated by Eq. (3). The maximum specific growth rate μ_{max} was used as an index to evaluate the growth potential:

$$\mu_t = (lnX_t - lnX_{t-1})/1. \quad (3)$$

X_t and X_{t-1} were the biomass concentration (mg L^{-1}) on t day and t − 1 day, respectively.

The total lipid yield (P_L, mg L^{-1} d^{-1}), total carbohydrate yield (P_C, mg L^{-1} d^{-1}) and crude protein yield (P_P, mg L^{-1} d^{-1}) were calculated by Eqs. (4, 5 and 6) to evaluate the potential of lipid, carbohydrate and protein biosynthesis:

$$P_L = P_o \times \text{total fat content}, \quad (4)$$

$$P_C = P_o \times \text{total carbohydrate content}, \quad (5)$$

$$P_{\mathrm{P}} = P_o \times \text{crude protein content.} \tag{6}$$

The light conversion efficiency (LCE, %) based on photosynthetically active radiation (PAR) was calculated by using Eq. (7):

$$\begin{aligned} \mathrm{LCE} &= (\mathrm{HHV} \times P_0 \times V_{\mathrm{w}} \times 100)/(I \times k \times \mathrm{PAR} \times A \times t) \\ &= 1.3 \times 10^{-2} \times \mathrm{HHV} \times P_0. \end{aligned} \tag{7}$$

The units of HHV and V_{w} are J mg^{-1} and L; i is the surface illumination intensity of photobioreactor (mol m^{-2} s^{-1}); the constant k for converting the illumination intensity into light energy density (W m^{-2}) is 218,800 J mol^{-1} photons. The PAR co-efficient is 48% [25]; A is the light-receiving area (m^2); t is 86,400 s per day.

Statistical analysis

One-way analysis of variance (ANOVA) with Duncan test ($p < 0.05$) was used to evaluate whether there were significant differences between the results.

Results and discussion

Screening of mutant strains

The tolerance of microalgae to high CO_2 concentration depends on its tolerance to low pH. A total of 43 viable mutants were screened by low pH medium in about 5000 micropores, named *Ulothrix* SDJZ-17-A1, SDJZ-17-A2……SDJZ-17-A43, and the wild strain was named SDJZ-17-WT. After Nile red staining of wild and mutant strains, they were detected in a fluorescence microplate reader, and the relative fluorescence intensity of each algae strain was calculated as shown in Fig. 1.

It can be seen from Fig. 1 that the fluorescence intensity of a total of 13 mutant strains exceeded that of the wild strain, and five strains with the highest fluorescence intensity were screened out, including A20 (1.73), A30 (1.47), A23 (1.39), A29 (1.29) and A6 (1.18). These five mutants can survive in a low pH medium and have high neutral lipid fluorescence intensity, and are selected as candidate algae strains for CO_2 fixation and biodiesel production.

Genetic stability screening

The selected 5 mutants were continuously cultured for 5 generations under high concentration CO_2 culture conditions, and the biomass concentration and lipid content at 7 days of harvest were detected as shown in Fig. 2. By comparing their growth curves and lipid content under (15% v/v) conditions, it can be seen that A29 cannot maintain the genetic stability of normal growth under high CO_2 concentration, and A6, A29 and A30 cannot maintain the genetic stability of oleaginous. Therefore, only A20 and A23 were retained as candidate mutant strains.

Growth characteristics

Comparing the growth curve shown in Fig. 3 with the detailed parameters shown in Table 2, it can be seen that the wild-type strain WT and the mutant strains A20 and A23 can grow normally in the air. There was no significant difference in $X_{\max}$, $\mu_{\max}$ and P among the three strains, but the $\mu_{\max}$ of A20 and A23 appeared on the second day, indicating that their adaptation period was slightly longer than that of WT, and the latter had almost no adaptation period when growing in the air. In order to overcome the problem of low CO_2 concentration in the air, almost all algae activate the key enzyme of photosynthesis (Rubisco)-ribulose-1,5-bisphosphate carboxylase/oxygenase through CO_2 concentration mechanisms (CCMs) to promote carbon assimilation [26, 27], WT, A20 and A23 are no exception. This indicates that the CCMs mechanism in A20 and A23 has not been destroyed, unlike *Chlorella* vulgaris SDEC-3M [28] and *Synechococcus* PCC7942 [29], which have high CO_2 demand characteristics due to the destruction of CCMs mechanism.

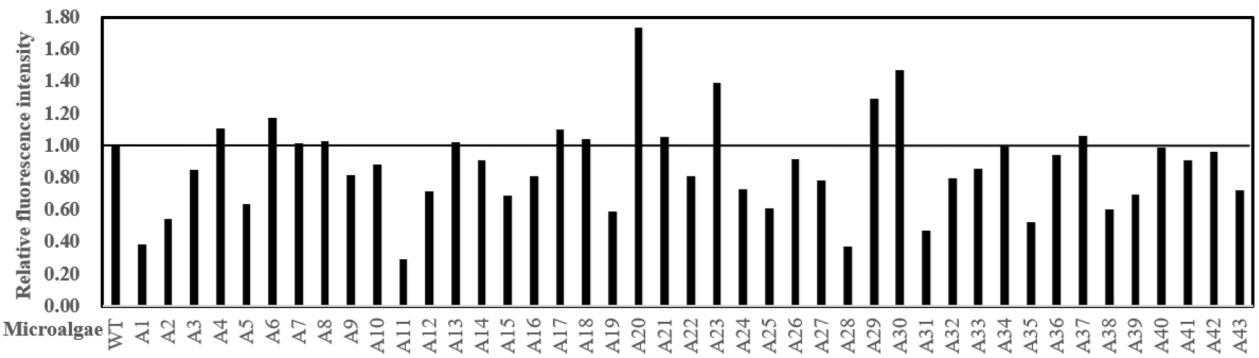

Fig. 1 *Ulothrix* SDJZ-17 wild strain (WT) and mutant strains (A1, A2,…… A43) relative neutral lipid fluorescence intensities

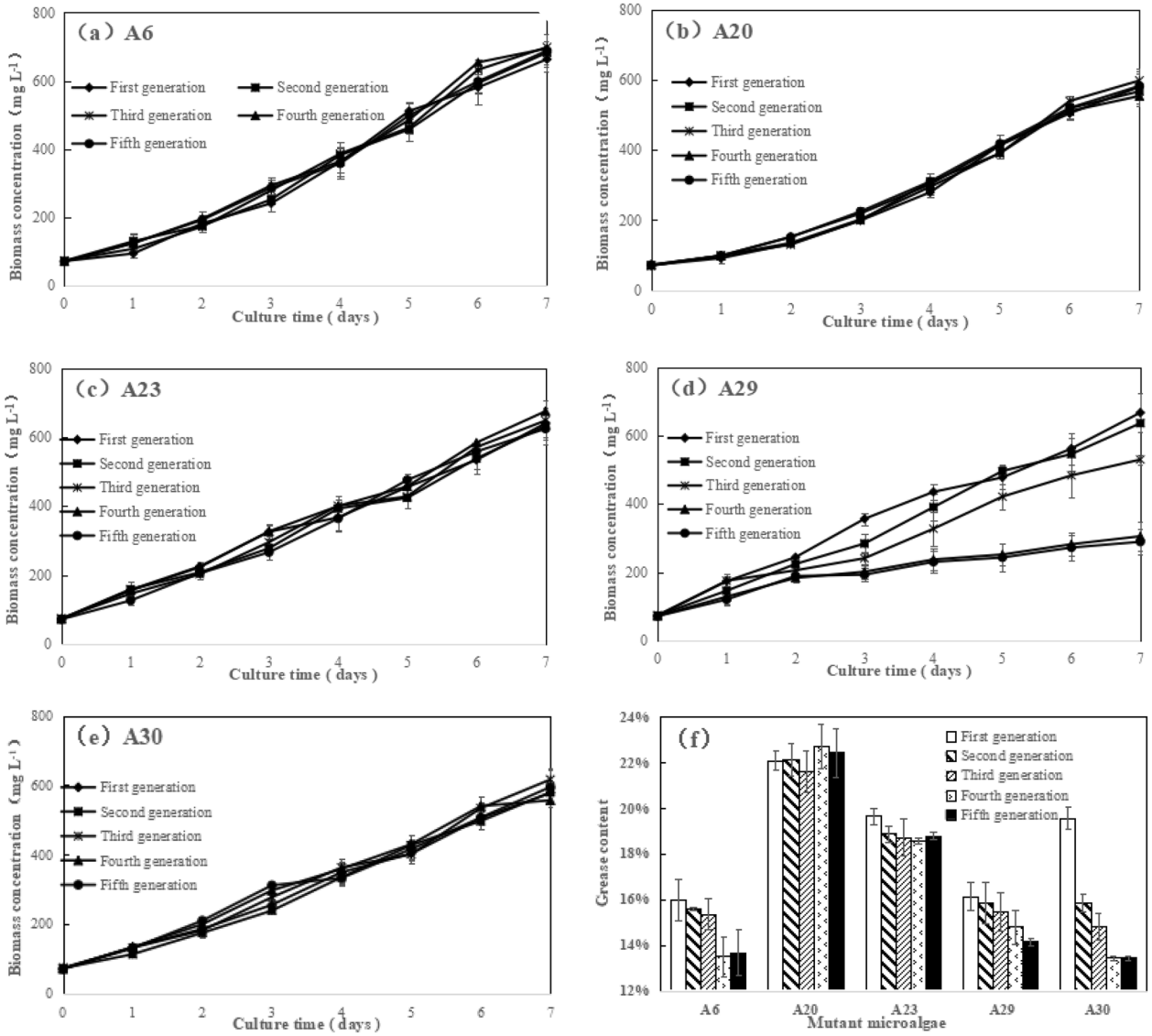

Fig. 2 Genetic stability of *Ulothrix* SDJZ-17 candidate mutants (A6, A20, A23, A29 and A30) in growth (**a**–**e**) and total lipid accumulation (**f**) under high CO_2 concentration

However, the growth of WT under 15% CO_2 was significantly worse than that in air, and X_{max}, μ_{max} and P decreased by 49.30%, 37.83% and 52.39%, respectively, μ_{max} appeared on the 4th day and required a long adaptation period. This should be caused by the decrease of enzyme activity caused by 'anesthesia' and acidification culture of microalgae cells under high CO_2 concentration (i.e., growth under low CO_2 concentration in air) [30]. Most wild Ulothrix are typically aerogenic and have low tolerance to CO_2 [31], and are therefore not generally considered as potential candidates for biomass and biofuel production.

Fortunately, the mutated mutant improved the cell's tolerance to CO_2. The X_{max}, μ_{max} and P_{max} of A20 and A23 grown under 15% CO_2 conditions were significantly increased by 152.41% and 170.69%, 63.52% and 116.81%, 181.02% and 205.80%, respectively, compared with WT, and also increased by 40.92% and 170.69%, 7.44% and 116.81%, 40.92% and 170.69%, respectively, compared with their growth under air conditions. This shows that when A20 and A23 have high CO_2 tolerance, a high concentration of CO_2 provides a sufficient carbon source, balances the increase of pH value caused by microalgae growth, and omits many benefits such as CCMs process to reserve biomass and saves energy, which promotes the

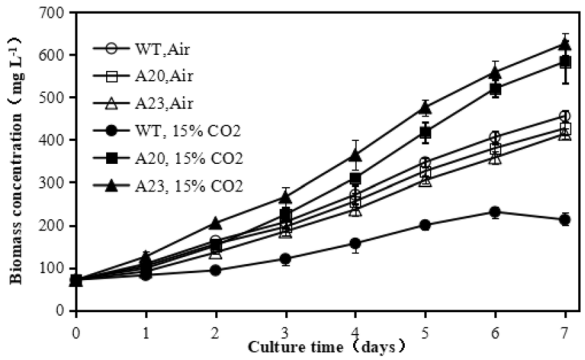

Fig. 3 Growth curves of *Ulothrix* SDJZ-17 wild type (WT) and mutant strains (A20 and A23) under air and 15% (v/v) CO_2 conditions for 7 days (each data is expressed as mean ± standard deviation from three independent cultures)

growth of microalgae[26, 28]. In fact, under the condition of high concentration of CO_2, the growth rate of high CO_2 tolerant microalgae is generally higher [26, 28, 30], and a higher culture economy is obtained. Moreover, in terms of biomass accumulation, A23 is better than A20.

It can also be seen from Table 2 that P_{max} is mostly obtained on the 6th day. Therefore, as far as biomass production is concerned, under the experimental conditions, it can be harvested on the 6th day, not the longer the culture time, the better.

Production of biochemical components

Due to the randomness of the mutation, the biochemical components of the cells may have undergone synchronous changes. Microalgae are raw materials for food, fuel and other biochemical products [32]. The content and yield of its biological components are key parameters for predicting its economic potential [32]. As shown in Fig. 4a, there were significant differences in the total lipid content data of the six groups obtained by the three *Ulothrix* strains under air and 15% CO_2 conditions. The total lipid content of A20 and A23 was significantly higher than that of WT, and the total lipid content obtained under 15% CO_2 conditions was higher than that in air. The data of Fig. 4b reflecting the yield obtained the same results. Among them, A20 obtained the highest total lipid content of 22.45 ± 1.09% under the condition of 15% CO_2, which was 138.63% and 111.96% higher than that of WT under air and 15% CO_2 conditions, respectively. In terms of yield, A20 also obtained the highest total lipid yield of 16.43 ± 2.33 mg L^{-1} d^{-1} under 15% CO_2 conditions, which was 217.98% and 668.56% higher than that of WT under air and 15% CO_2 conditions, respectively. These results indicate that the two *Ulothrix* mutants indeed enhance the tendency of lipid production, and high concentrations of CO_2 can promote the tilt of intracellular carbon and energy to lipid synthesis [28, 33, 34]. The high concentration of CO_2 adaptation and high biomass yield of the mutant further enhanced its lipid production capacity. The total lipid content and yield of some oleaginous green microalgae can reach 30% and 30 mg L^{-1} d^{-1}, respectively [35]. Although A20 has a certain gap with the data, considering that *Ulothrix* strains with lipid content exceeding 10% have never been reported, the performance of A20 in lipid accumulation is still satisfactory. Moreover, this is data under stress conditions such as no nitrogen starvation, and its lipid production potential is still expected.

It can be seen from Fig. 4a that although the lipid content of the *Ulothrix* wild strain is not high, it has rich carbohydrate and protein content. Although the mutant strain increased the lipid content at high CO_2 concentration, there was no significant difference in carbohydrate content between the mutant strain and the wild strain. Although the protein content decreased, the difference was not as obvious as the lipid content. This shows that under the pressure of mutagenesis and high concentration of CO_2, the mutant enhances the energy and carbon source required for lipid production, which is unrelated to carbohydrates, and partly reduces the energy and carbon source required for protein production. The high biomass yield of the mutant under high CO_2 concentration made it obtain higher carbohydrate yield, A20 and A23 reached 23.08 ± 2.43 mg L^{-1} d^{-1} and

Table 2 The maximum biomass concentration (X_{max}), maximum specific growth rate (μ_{max}) and maximum biomass yield (P_{max}) of Ulothrix SDJZ-17 wild type (WT) and mutants (A20 and A23) cultured in air and 15% (v/v) CO_2 for 7 days

Microalgae strain	X_{max} (mg L^{-1})		μ_{max} (d^{-1})		P_{max} (mg L^{-1} d^{-1})	
	Air	15% CO_2	Air	15% CO_2	Air	15% CO_2
WT	455.49 ± 13.6 (7)^{b}	230.95 ± 13.49 (6)^{a}	0.42 ± 0.06 (1)^{b}	0.26 ± 0.01 (4)^{a}	55.82 ± 2.49 (6)^{c}	26.57 ± 2.25 (6)^{a}
A20	426.34 ± 14.95 (7)^{b}	582.94 ± 48.93 (7)^{c}	0.4 ± 0.05 (2)^{b}	0.43 ± 0.05 (2)^{b}	51.57 ± 2.34 (6)^{bc}	74.68 ± 3.33 (6)^{d}
A23	413.66 ± 12.75 (7)b	625.14 ± 24.97 (7)^{c}	0.4 ± 0.07 (2)^{b}	0.57 ± 0.09 (1)^{c}	48.93 ± 1.82 (7)^{b}	81.26 ± 4.57 (6)^{e}

Each data represents the mean ± standard deviation from three independent cultures. Each value in the parentheses represents the time (d) when the parameter reaches its maximum. The 6 sets of data under the same parameters were marked with different letters, indicating that the results were significantly different by Duncan test ($p < 0.05$)

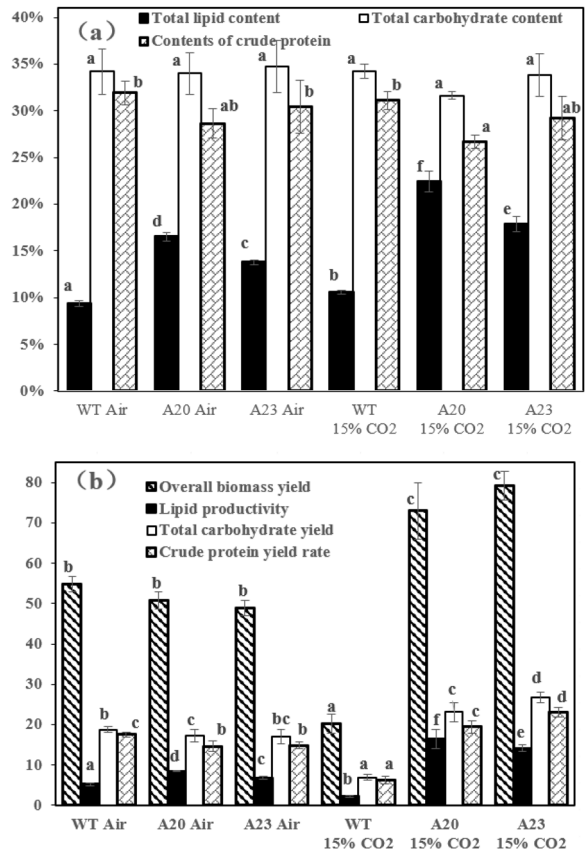

Fig. 4 Total lipid, total carbohydrate and crude protein content (**a**) and biomass, total lipid, total carbohydrate and crude protein productivities (**b**, mg L^{-1} d^{-1}) of *Ulothrix* SDJZ-17 wild-type strain (WT) and mutant strains (A20 and A23) cultured in air and 15% (v/v) CO_2 for 7 days. Each data was expressed as the mean ± standard deviation of the three independent cultures, and the same component was marked with different letters, indicating that the results were significantly different by the Duncan test ($p < 0.05$)

26.76 ± 1.29 mg L^{-1} d^{-1}, respectively. Lipids and carbohydrates are raw materials for the production of two biofuels, biodiesel and bioethanol [3, 19, 34]. Therefore, A20 and A23 are more suitable as a composite feedstock for biodiesel and bioethanol production than as a single feedstock for biodiesel.

Photosynthetic efficiency

Light conversion efficiency (LCE) and maximum PSII quantum yield (F_v/F_m) are two important parameters for analyzing photosynthetic efficiency. As shown in Table 3, there was no significant difference in LCE and F_v/F_m between WT and A20 and A23 under air conditions, and their LCE was precisely the range of LCE distribution in most microalgae—4–9% [36]. The results showed that the mutation did not change the efficiency of photosynthesis under air conditions in both wild and mutant strains of *Ulothrix* SDJZ-17. However, under the condition of 15% CO_2, the LCE and F_v/F_m of WT were significantly lower than those under air conditions, while the A20 and A23 were the opposite. Among them, A23 obtained the highest LCE (14.79 ± 0.48%) and F_v/F_m (71.04 ± 1.62%), respectively. The results obtained by A20 were slightly lower than those of A23, but there was no significant difference. The F_v/F_m value reflects the potential maximum quantum efficiency of PSII, which is related to the peripheral antenna complex. As mentioned earlier, WT had poor tolerance to high CO_2 concentration, which affected its photosynthetic efficiency. The mutant's peripheral antenna complex has undergone significant changes, resulting in high CO_2 concentration tolerance. In an environment with a high concentration of CO_2, algal cells can obtain sufficient carbon sources under low energy consumption, which greatly improves the efficiency of photosynthesis. The results showed that the effect of CO_2 concentration on the photosynthetic efficiency of SDEC-2M was more significant than that of wild strain. Therefore, CO_2 level plays an important role in improving the energy storage compounds, namely starch and lipid production of microalgae strains with high CO_2 tolerance.

In addition, it can be observed from the data in Table 3 that the HHV of the algae strains at 15% CO_2 level was higher than that in air culture, but the wild strains showed no significant difference. Previous studies have confirmed that HHV is highly correlated with lipid content. Therefore, this should be the result of algae cells cultured under high concentrations of CO_2, which accumulated more lipids.

Table 3 High calorific value (HHV), light conversion efficiency (LCE) and maximum quantum yield of PSII (F_v/F_m) of *Ulothrix* SDJZ-17 wild type (WT) and mutant strains (A20 and A23) cultured in air and 15% (v/v) CO_2 for 7 days

Microalgae strain	HHV (kJ g^{-1})		LCE (%)		F_v/F_m (%)	
	Air	15% CO_2	Air	15% CO_2	Air	15% CO_2
WT	19.19 ± 0.72^{a}	20.09 ± 0.06^{ab}	8.75 ± 0.3^{b}	3.37 ± 0.4^{a}	64.17 ± 2.53^{b}	56.74 ± 0.18^{a}
A20	21.2 ± 0.76^{b}	23.48 ± 0.85^{c}	8.94 ± 0.22^{b}	14.23 ± 1.26^{c}	64.32 ± 3.72^{b}	67.6 ± 2.79^{bc}
A23	20.09 ± 0.51^{ab}	22.5 ± 0.43^{c}	8.17 ± 0.45^{b}	14.79 ± 0.48^{c}	63.8 ± 3.46^{b}	71.04 ± 1.62^{c}

Each data represents the mean ± standard deviation from three independent cultures. The six groups of data under the same parameters were marked with different letters, indicating that the results were significantly different by the Duncan test ($p < 0.05$)

Conclusions

In this study, using ARTP mutagenesis technology with high-throughput screening, high CO_2 tolerance and oleaginous mutants A20 and A23 with genetic stability were screened from the mutants derived from non-oleaginous green microalgae *Ulothrix* SDJZ-17. The wild strain was cultured in air and 15% CO_2, and its characteristics were studied. Under the condition of 15% CO_2 concentration, the growth performance and lipid production performance of mutant strains A20 and A23 were significantly better than those of wild strains, and also better than the results of their own culture under air conditions. Under the condition of 15% CO_2, A23 obtained the highest LCE (14.79%), F_v/F_m (71.04%), biomass yield (81.26 mg L^{-1} d^{-1}) and lipid productivity (26.76 mg L^{-1} d^{-1}), while A20 obtained the highest lipid content (22.45%). The above results were much higher than the reported *Ulothrix* lipid content and yield. The above results indicate that ARTP mutagenesis can improve the CO_2 tolerance, photosynthetic efficiency and lipid yield of *Ulothrix* SDJZ-17. Therefore, although *Ulothrix* is generally not considered a candidate green microalgae for CO_2 fixation and biofuel production, this understanding can be changed by ARTP mutagenesis. This study is of great significance for enriching the energy microalgae germplasm bank and alleviating the global warming and energy crisis.

Abbreviations

ARTP	Atmospheric and room temperature plasma
SDEC	Shandong Provincial Engineering Centre on Environmental Science and Technology
ANOVA	One-way analysis of variance
PBS	Phosphate buffer solution
CCMs	CO_2 concentration mechanisms
HHV	High heating value

Acknowledgements

We are grateful for the financial assistance for this investigation provided by Shandong Top Talent Special Foundation (No.0031504), Institute of Resources and Environmental Innovation, Shandong Jianzhu University. Special thanks to Academician Chen Feiyong for the funding of this study.

Author contributions

M.Y. and Y.A. contributed equally to this work and were listed co-first authors. M.Y.: conceptualization, funding, acquisition, writing—review and editing. Y.A.: writing—original draft, validation, formal analysis, data curation. F.Q.: funding acquisition, writing—review and editing. R.M. and G.M. made a final proof of the data presented. F.C.: project administration.

Funding

This work was funded by the Shandong Top Talent Special Foundation (No. 0031504).

Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to the publication of the manuscript in *Biotechnology for Biofuels*. All authors have approved the manuscript to be published.

Competing interests

The authors declare no competing interests.

Received: 28 August 2024 Accepted: 9 October 2024
Published online: 13 November 2024

References

1. Xu J, Niehoff NM, White AJ, Werder EJ, Sandler DP. Fossil-fuel and combustion-related air pollution and hypertension in the Sister Study. Environ Pollut. 2022;315:8.
2. Xu J. Modeling of correlation between fossil fuel combustion products and atmospheric environmental pollution. Ekoloji. 2019;28(107):2255–63.
3. Moraes L, Santos LO, Costa JAV. Bioprocess strategies for enhancing biomolecules productivity in *Chlorella* fusca LEB 111 using CO_2 a carbon source. Biotechnol Prog. 2020;36(2):9.
4. Hussain F, Shah SZ, Ahmad H, Abubshait SA, Abubshait HA, Laref A, Manikandan A, Kusuma HS, Iqbal M. Microalgae an ecofriendly and sustainable wastewater treatment option: Biomass application in biofuel and bio-fertilizer production. A review. Renew Sust Energ Rev. 2021;137:10.
5. Peter AP, Khoo KS, Chew KW, Ling TC, Ho SH, Chang JS, Show PL. Microalgae for biofuels, wastewater treatment and environmental monitoring. Environ Chem Lett. 2021;19(4):2891–904.
6. Li SN, Li X, Ho SH. Microalgae as a solution of third world energy crisis for biofuels production from wastewater toward carbon neutrality: an updated review. Chemosphere. 2022;291:13.
7. Wang M, Ye XX, Bi HW, Shen ZB. Microalgae biofuels: illuminating the path to a sustainable future amidst challenges and opportunities. Biotechnol Biofuels Bioprod. 2024;17(1):24.
8. Mahesh R, Yashavanth PR, Maiti SK. Modulation of macro and micronutrients to enhance lipid production by *Scenedesmus abundans* in a flat-panel photobioreactor under outdoor natural sunlight. Biofuels-UK. 2023;14(7):687–702.
9. Pekkoh J, Ruangrit K, Aurepatipan N, Duangjana K, Sensupa S, Pumas C, Chaichana C, Pathom-aree W, Kato Y, Srinuanpan S. CO_2 to green fuel converter: photoautotrophic-cultivation of microalgae and its lipids conversion to biodiesel. Renew Energy. 2024;222:14.
10. Zhang Q, Miao RY, Feng RC, Yan JJ, Wang T, Gan Y, Zhao J, Lin JB, Gan BC. Application of atmospheric and room-temperature plasma (ARTP) to microbial breeding. Curr Issues Mol Biol. 2023;45(8):6466–84.
11. Kasai Y, Takagi S, Ota S, Ishii K, Takeshita T, Kawano S, Harayama S. Development of a CRISPR/Cas9-mediated gene-editing method to isolate a mutant of the unicellular green alga *Parachlorella kessleri* strain NIES-2152 with improved lipid productivity. Biotechnol Biofuels Bioprod. 2024;17(1):17.
12. Ma GX, Meng QY, Mu RM, Qi F, Wang XH. Obtaining mutant *Chlorella vulgaris* strains with excellent self-flocculation properties and high wastewater treatment efficiency via atmospheric and room temperature plasma technique. J Water Process Eng. 2023;53:8.
13. Hernández-Muñoz S, Pedraza-Santos ME, López PA, Gómez-Sanabria JM, Morales-García JL. Mutagenesis in the improvement of ornamental plants. Revista Chapingo Serie horticultura. 2019;25(3):151–67.
14. Mullins E, Bresson JL, Dalmay T, Dewhurst IC, Epstein MM, Firbank LG, Guerche P, Hejatko J, Moreno FJ, Naegeli H, et al. In vivo and in vitro random mutagenesis techniques in plants. Efsa J. 2021;19(11):30.
15. Yu QH, Li YC, Wu B, Hu W, He MX, Hu GQ. Novel mutagenesis and screening technologies for food microorganisms: advances and prospects. Appl Microbiol Biotechnol. 2020;104(4):1517–31.
16. Zhang X, Zhang XF, Li HP, Wang LY, Zhang C, Xing XH, Bao CY. Atmospheric and room temperature plasma (ARTP) as a new powerful mutagenesis tool. Appl Microbiol Biotechnol. 2014;98(12):5387–96.
17. Liu Y, Chen X, Wei D, Xing XH. Breeding a novel chlorophyll-deficient mutant of *Auxenochlorella pyrenoidosa* for high-quality protein

production by atmospheric room temperature plasma mutagenesis. Bioresour Technol. 2023;390:10.
18. Qi F, Pei HY, Ma GX, Zhang S, Mu RM. Improving productivity and quality of biodiesel from *Chlorella vulgaris* SDEC-3M through customized process designs. Energy Conv Manag. 2016;129:100–7.
19. Li PF, Sun X, Sun Z, Huang F, Wei WQ, Liu XS, Liu YJ, Deng LY, Cheng ZW. Biochemical and genetic changes revealing the enhanced lipid accumulation in *Desmodesmus* sp. mutated by atmospheric and room temperature plasma. Renew Energy. 2021;172:368–81.
20. Satpati GG, Mallick SK, Pal R. An alternative high-throughput staining method for detection of neutral lipids in green microalgae for biodiesel applications. Biotechnol Bioprocess Eng. 2015;20(6):1044–55.
21. Yao CH, Ai JN, Cao XP, Xue S, Zhang W. Enhancing starch production of a marine green microalga *Tetraselmis subcordiformis* through nutrient limitation. Bioresour Technol. 2012;118:438–44.
22. Song MM, Pei HY, Hu WR, Ma GX. Evaluation of the potential of 10 microalgal strains for biodiesel production. Bioresour Technol. 2013;141:245–51.
23. Hu ZB, Wang JQ, Bin DM, Liu SF, Chai XS, Kong HN: A simple method for the determination of sugars in the extraction effluents. In: 2nd international papermaking and environment conference: May 14–16 2008; Tianjin, PEOPLES R CHINA. BEIJING: China Light Industry Press; 2008: 138–141.
24. Chromy V, Vinklárková B, Sprongl L, Bittová M. The Kjeldahl method as a primary reference procedure for total protein in certified reference materials used in clinical chemistry. I. A review of Kjeldahl methods adopted by laboratory medicine. Crit Rev Anal Chem. 2015;45(2):106–11.
25. Robertson DE, Jacobson SA, Morgan F, Berry D, Church GM, Afeyan NB. A new dawn for industrial photosynthesis. Photosynth Res. 2011;107(3):269–77.
26. Huang Y, Cheng J, Lu HX, He Y, Zhou JH, Cen KF. Transcriptome and key genes expression related to carbon fixation pathways in *Chlorella* PY-ZU1 cells and their growth under high concentrations of CO_2. Biotechnol Biofuels. 2017;10:10.
27. Mackinder LCM, Chen C, Leib RD, Patena W, Blum SR, Rodman M, Ramundo S, Adams CM, Jonikas MC. A spatial interactome reveals the protein organization of the algal CO_2-concentrating mechanism. Cell. 2017;171(1):133.
28. Qi F, Pei HY, Hu WR, Mu RM, Zhang S. Characterization of a microalgal mutant for CO_2 biofixation and biofuel production. Energy Conv Manag. 2016;122:344–9.
29. Tan CL, Tao F, Xu P. Direct carbon capture for the production of high-performance biodegradable plastics by cyanobacterial cell factories. Green Chem. 2022;24(11):4470–83.
30. Shang ZX, Qi F, Mu RM, Ren Y, Ma GX, Yin MS. Enhanced potential of a *Selenastrum capricornutum* mutant for CO_2 fixation and biofuels production. Environ Prog Sustain Energy. 2022;41(5):7.
31. Munir N, Samad M, Sharif N, Haq R, Naz S. Effect of mutagens on biomass and lipid content of *Ulothrix* sp. and *Oscillatoria* sp. for improved biodiesel production. J Anim Plant Sci-JAPS. 2023;33(1):174–9.
32. Yu ZAN, Chen XL, Xia SW. The mechanism of lipids extraction from wet microalgae *Scenedesmus* sp. by ionic liquid assisted subcritical water. J Ocean Univ. 2016;15(3):549–52.
33. Braga VD, Mastrantonio DJD, Costa JAV, de Morais MG. Cultivation strategy to stimulate high carbohydrate content in *Spirulina* biomass. Bioresour Technol. 2018;269:221–6.
34. Qi F, Wu DJ, Mu RM, Zhang S, Xu XY. Characterization of a microalgal UV mutant for CO_2 biofixation and biomass production. Biomed Res Int. 2018;2018:8.
35. Danilovic BR, Cvetkovic-Rakic JM, Ciric J, Simeunovic JB, Veljkovic VB, Savic DS. The isolation and screening of microalgae for the production of oil. Hem Ind. 2017;71(1):69–74.
36. Brennan L, Owende P. Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev. 2010;14(2):557–77.

Publisher's Note