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Original article

Effects of ocean acidification on the growth and biochemical composition of a green alga (*Ulva fasciata*) and its associated microbiota

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ABSTRACT

In marine ecosystems, fluctuations in surface-seawater carbon dioxide (CO_2), significantly influence the whole metabolism of marine algae, especially during the early stages of macroalgal development. In this study, the response of the green alga *Ulva fasciata* for elevating ocean acidification was investigated using four levels of pCO_2 ~ 280, 550, 750 and 1050 μatm. Maximum growth rate (6.6% day^{-1}), protein (32.43 % DW) and pigment (2.9 mg/g) accumulation were observed at pCO_2-550 with an increase of ~2-fold compared to control. On the other hand, lipid and carbohydrate contents recorded their maximum production (4.23 and 46.96 %DW, respectively) at pCO_2-750 while control showed 3.70 and 42.37 %DW, respectively. SDS-PAGE showed the presence of unique bands in response to pCO_2, especially at 550 μatm. Dominant associated bacteria was shifted from *Halomonas hydrothermalis* of control *to Vibrio toranzoniae* at pCO_2-1050. These findings suggest that ocean acidification at 550 μatm might impose noticeable effects on growth, protein, pigments, and protein profile of *U. fasciata,* which could be a good source for fish farming. While, pCO_2-750 was recommended for energetic purpose, due to its high lipid and carbohydrate contents.

1. Introduction

Future climate forecasts expect the continuous raising of atmospheric CO_2 concentrations (pCO_2) due to the anthropogenic activities and exceed ~600 ppm by the year 2100 under the "business as usual" scenario (Pachauri et al., 2014). Oceans are one of the leading sinks of this CO_2 increase as they can absorb over 25 million tons of anthropogenic CO_2 daily, causing unprecedented changes to ocean chemistry (IPCC, 2007). Raised ocean CO_2 concentrations modify the speciation of dissolved inorganic carbon in seawater and reduce pH by the carbonate buffer system, along with varying abilities of macrophytes to use CO_2 and HCO_3. By the end of the millennium, the pH of the ocean surface from a pre-industrial value (8.2) to 7.4 (Caldeira and Wickett, 2003). When pH is shifted, the carbon speciation in seawater is changed, which has strong consequences for photosynthesis, respiration, and calcification metabolism.

Additionally, these same three metabolic processes themselves change the pH of the surrounding seawater of the algae. Therefore, these changes have serious pressure on algae, including macroalgae (Kinnby et al., 2021; Xiao et al., 2021) or microalgae (Pourjamshidian et al., 2019). According to the European Water Framework Directive (EWFD) 2000/60/CE, the algal community is considered an essential indicator of anthropogenic stresses in water ecosystems since it might alter the composition of their community, leading to the change or disappearance of some species (Baggini et al., 2014; Elshobary et al., 2020b; Han et al., 2020).

Seaweeds might benefit from rising CO_2 through increased photosynthesis and carbon acquisition, with subsequent greater growth rates (Aires et al., 2018; Cornwall and Hurd, 2020; Mackey et al., 2015). Unlike photosynthesis, other metabolic pro-

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cesses, such as ion homeostasis, respiration, nutrient uptake and enzyme activity, are suppressed by ocean acidification conditions (Fernández et al., 2015; Gutow et al., 2014; Hofmann et al., 2013). This shifting in algal metabolism may promote modifications in seaweed chemistry and change the dietary quality of tissue for grazers. In addition, association microbiota can also be influenced by environmental changes, with feedback results (Aires et al., 2018).

The Egyptian coastline of the Mediterranean (Elshobary et al., 2020a; Khairy and El-Sheikh, 2015; Osman et al., 2012), and Red Sea coasts (El-Shenody et al., 2019; Madkour et al., 2019), has a diverse variation of naturally-growing seaweed that could harvested throughout the year. *Ulva* or sea lettuce species are among the most plentiful representatives, being ubiquitous in coastal benthic communities around the world. Several *Ulva* species have been traditionally used for food and feed supplements for its high growth rate and high protein content suitable for food application (Kazir et al., 2019). Moreover, it also has several pharmaceuticals uses such as antimicrobial (Osman et al., 2013, 2012), antioxidant (Khairy and El-Sheikh, 2015), anticancer activities (Abou El Azm et al., 2019), biostimulants (Ashour et al., 2021; Hassan et al., 2021) and biofuel (Osman et al., 2020). Nonetheless, *Ulva* remains generally understudied, where *Ulva* is regarded as an attractive model organism for studying algal response and improvement against mutualistic interactions under stress conditions (Wichard et al., 2015).

This study aimed to evaluate the effects of pCO_2 induced ocean acidification on *Ulva fasciata*, which is widely distributed throughout the Alexandria coast, Egypt (Osman et al., 2020, 2010, 2012). *U. fasciata* has reared under control pCO_2 conditions (280 μatm) and three different pCO_2 levels (550, 750 and 1050 μatm), in order to determine how *U. fasciata* may respond to pCO_2 levels by evaluating algal specific growth, biochemical constituents, protein profile, and its associated microbiota which could pave the way to improve their applications.

2. Materials and methods

2.1. Algal sampling

Samples of the green macroalga *Ulva fasciata* were collected from the submerged rocks and substrates in the shallow water of the boulders at the sea anchor of the National Institute of Oceanography and Fisheries (NIOF) at 31°12′35.9″N 29°52′58.4″E, Alexandria, Egypt. The specimens have been washed immediately with seawater to eliminate sand and rock debris. The sample was preserved in a polyethylene bag filled with filtered seawater and transported to the laboratory in an icebox (5 min away). Seaweed was gently scrubbed with running filtered seawater (Whatman® GF/C glass microfiber filters, 0.5 μm) to clean epibiota (other seaweed, zooplankton and bivalves). All cleaned algal fronds had been blotted on towel papers to get rid of extra water and then weighed about 5 g fresh weight (FW) to start the culture experiment. The sample was adapted to lighting, temperature, and flow laboratory conditions for 48 h before evaluating its growth and biochemical composition.

2.2. Algal culture conditions

Adapted thalli of *U. fasciata* (equivalent to 5 g FW) was once positioned in 4 L plastic jars in triplicates and filled with filtered (Whatman® GF/C filters, 0.5 μm) and autoclaved seawater (salinity 30 PSU). Jars were enriched with dissolved $pCO_2 \sim 1050$, 750, 550, and control (280) μatm which organized in-stock solution then was brought in distinct concentrations to attain the desired pH values 7.2, 7.6, 7.86, 8.1 (control), respectively. Jars were covered with transparent nylon film to minimize gas exchange with the environment and subjected to the different pCO_2 levels using a flowmeter gas system that mixed ambient air with 5% CO_2 gas. The jars have been aerated gently via air blower to ensure a non-stop mixing of the water and preserve algal homogeneity with the experimental media. Alga used to be saved at a temperature of 25 ± 0.5 °C and underneath non-stop illumination through white fluorescence lamps at 200 μmol photons m^{-2} s^{-1}.

The experiment was performed at the National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt, during spring 2019. Ambient pH 8.1 global levels (280 pCO_2, control) and elevated pCO_2 levels (550, 750 and 1050 μatm) related to 7.86, 7.6, 7.2, respectively were applied using acid-base addition (±0.02 pH units) (Smithson, 2002). Acidification levels were measured in the jars continually along the day to be always controlled in the right tested pH values. Algal total biomass in DW/L was measured during the long-term CO_2 enriched seawater along 12 days culturing at three days intervals.

2.3. Growth measurement

The rate of *U. fasciata* growth was expressed as the specific growth rate (SGR) that was expressed as a percentage of daily increase or decrease in algal biomass (% / days intervals) as described in (Korzen et al., 2015). SGR was calculated using the following formula:

$$SGR=[\ln (W_t/W_0)]/t\times 100$$

Where Wt is the biomass (dry weight) in time per day culture and W_0 is the initial biomass. t is time in days

2.4. Nutritional biochemical constituents

The biochemical compositions (protein, lipid, pigments and carbohydrates contents) of *U. fasciata* cultured on the different pCO_2 concentrations were determined at the end of the exponential growth phase (9th day). About 5 g FW of algal sample was cut out and dried to a constant weight at 50 °C in an oven (approximately 0.5 g DW), ground to fine powder, and stored in a desiccator until further use.

2.4.1. Photosynthetic pigments

Three grams of *U. fasciata* fresh weight equal to 0.3 g DW were homogenized in 30 ml acetone (80%v/v) overnight in dark at 4 °C followed by centrifugation at 10,000 × g for 5 min (TDL-8 M, Luxiangyi, Hunan, China). Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoids were determined spectrophotometrically (Shimadzu UV- 2401PC, Kyoto, Japan) at wavelength 664, 470 and 450 nm, respectively, and expressed as mg/g DW according to (Lichtenthaler, 1987) and (Ismail and Osman, 2016).

2.4.2. Total carbohydrate content

Carbohydrate content was determined by a microplate phenol–sulfuric acid method (Masuko et al., 2005) and modified by Elshobary et al. (2015). Total soluble carbohydrate was measured at 490 nm against the blank and determined per DW using glucose as a standard.

2.4.3. Total lipid content

The lipid content of the macroalgal samples was measured by a solvent extraction method using Soxhlet, where petroleum ether was used as a solvent. The values are presented as a percent of the dry weight (DW) of the samples as described by (Elshobary et al., 2020a).

2.4.4. Total protein content

For protein analysis, algal powder (0.5 mg DW) were digested in 1 N NaOH for 24 h at room temperature and quantified, according to Bradford methods (Bradford, 1976) modified by (Kruger, 2009). The absorbance was measured by UV/ visible spectrophotometer at 595 nm against a blank. Bovine serum albumin was used as standard, where protein content was assessed as %DW.

2.5. Algal protein profile

The protein profile of the total soluble protein of *U. fasciata* under the four different acidification levels was detected via Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). Ten µl of protein ladder (prestained dual-color standard, 14.4–116 kDa, Bio-Rad, USA) and 15 µg of protein from each treatment were loaded onto stacking gel of 5% and separating gel of 12% acrylamide in 25 mM Tris–HCl, pH 8.3, 0.18 M Glycine and 0.1% SDS. SDS PAGE was performed using a Protean II xi cell electrophoresis unit (Biorad, Hercules, CA, USA). The separation was carried out at 180 V for 2 h. Gels were stained for 30 min with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid, followed by a distaining for 70 min with 50% (v/v) methanol and 7.5% (v/v) acetic acid. Pictures of the gels were taken with Gel documentation system (Geldoc-it, UVP, England), which was applied for data analysis using Totallab analysis software, ww.totallab.com, (Ver.1.0.1).

2.6. Isolation of the dominant associated bacteria

Within 2 h after collection, *Ulva* thallus was washed five times in autoclaved Petri dishes with sterilized and filtered (0.2 µm pore size) seawater to remove loosely attached bacteria, water was exchange after each step. Rinsed samples were placed in new sterilized Petri dishes. The whole surface of the thallus was vigorously swabbed with a sterile cotton swab. Subsequently, swab tips were spread on marine agar Zobell medium (Himedia) supplemented with 1.5% agar under aseptic conditions. The plates were incubated at 20℃ for three days, and the dominant colonies were purified on a fresh plate to obtain single colonies. Bacterial isolates were sustained at −20℃ for molecular identification.

2.7. Molecular identification of the dominant bacterial isolates

According to manufacturer protocol, DNA from bacterial isolates was obtained using GeneJET Genomic DNA Purification Kit (K0721/Thermo Fisher). PCR amplification of partial 16S rRNA gene sequences was carried out using the forward primer 27F (5′- AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′- CTACGGCTACCTTGTTACGA- 3′). Amplification protocol was carried out according to (Huo et al., 2020; Elshobary et al., 2015), using a thermal cycler (Applied Biosystems 2720, Foster City, CA, USA).

Agarose gel electrophoresis was used to detect the amplified products, which recovered and purified using E.Z.N.A.® Gel Extraction Kit (D2501-01). The purified DNA fragments have been sequenced directly by Macrogen Korea Company through *ABI* 3730XL sequencer. The sequences were blasted using the nBLAST search (http://www.ncbi.nlm.nih.gov/blast) to find the most homology isolates sequences available in the GenBank. The sequences have been aligned using Clustal W with the default parameters (MEGA X software) (www. megasoftware.net). A dendrogram was created using the neighbour-joining (NJ) algorithm based on the parameter distance (PD) using MEGA X software.

2.8. Statistical analysis

All experiments were carried out in triplicates, and results were analyzed using ANOVA. To evaluate the interactive effect of age and pCO_2 on SGR, two-way ANOVA was used with experimental age and pCO_2 as fixed factors. Whereas, one-way ANOVA was used to assess the effect of pCO_2 (fixed factor) on all biochemical components followed by Duncan and LSD (least significant difference) comparisons to determine the significance level at $P \leq 0.05$. The statistical analyses were done using SPSS software (version 23, SPSS Inc., USA), and data were presented as the mean ± SD.

3. Results

3.1. Algal growth rate

A long-term CO_2 exposure test (12 days) was performed to obtain different pCO_2 levels (280, 550, 750, and 1050 µatm) and investigate their impact on *U. fasciata* specific growth rate (SGR) (Fig. 1). In general, increasing CO_2 has enhanced the growth rate than that recorded in the control treatment. The highest value (6.6% day^{-1}) was recorded at pCO_2-550 on the 6th day with an increase of 2.7 times than control. The results also showed that the optimum SGR was obtained approximately at the 6th day of culture, where all treatments have the same exponential phase period. Otherwise, they were varied in their stationary phase durations according to their response to different pCO_2 levels. The shortest stationary phase duration was noted at the highest pCO_2 level (1050 µatm), whereas in the other treatments, it lasted till the 9th day of culture, where all the analyses in this study have been performed. The statistical analysis of two-way ANOVA indicated that the variation in pCO_2 levels, age, and interaction was significantly affected SGR (ANOVA $p < 0.001$) (Table S1).

3.2. Biochemical constituents and pigment contents of U. Fasciata

The photosynthetic pigments of *U. fasciata* were significantly affected by pCO_2 levels (ANOVA $p < 0.05$). The pigment content of *U. fasciata* thalli was increased dramatically than ambient condition. The maximum Chl *a*, Chl *b*, carotenoids and total pigments (1.95, 0.93, 0.054, 2.9 mg/g respectively) were recorded at pCO_2-550 (Fig. 2), with an increase of 107%, 55% and 1%, respectively than recorded in control. While rising pCO_2 over 550 causes a reduction in pigment content. In general, raising pCO_2 enhanced 550 µatm attained the maximum pigment production.

Total protein, lipid and carbohydrate contents of the *U. fasciata* were significantly improved by applying different pCO_2 levels (ANOVA $p < 0.05$). Higher protein accumulation was found at pCO_2-550 µatm (32.43%DW) with an increase of 1.2-fold than recoded in control. In contrast, the protein content was decreased by increasing pCO_2 levels over 550 µatm (Table 1). On the other hand, the lipid and carbohydrate contents of *U. fasciata* were improved significantly by elevated pCO_2. Their highest values (4.23 and 46.96 %DW, respectively) were recorded at pCO_2-750 (Table 1). Even though the most elevated PCO_2 of 1050 µatm recorded lipid and carbohydrate yield below 750 µatm, they were still higher than those observed in the control treatment. Table 2.

3.3. Protein profile analysis

Protein profile analysis was determined using SDS-PAGE**.** In general, the most abundant protein bands had weights of 35, 32.5, 31, 22.4 kDa. Comparing to control, different unfamiliar and unique protein bands were expressed and reflected by different pCO_2 values of *U. fasciata* culturing media (Fig. 3). Treating with

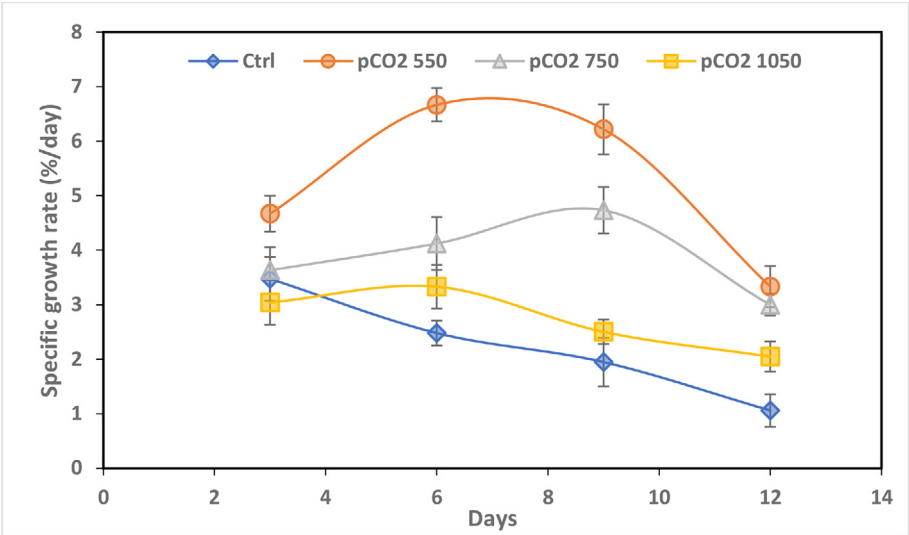

Fig. 1. Specific growth rate of *U. fasciata* (%/ day) cultured for 12 days on different pCO_2 levels.

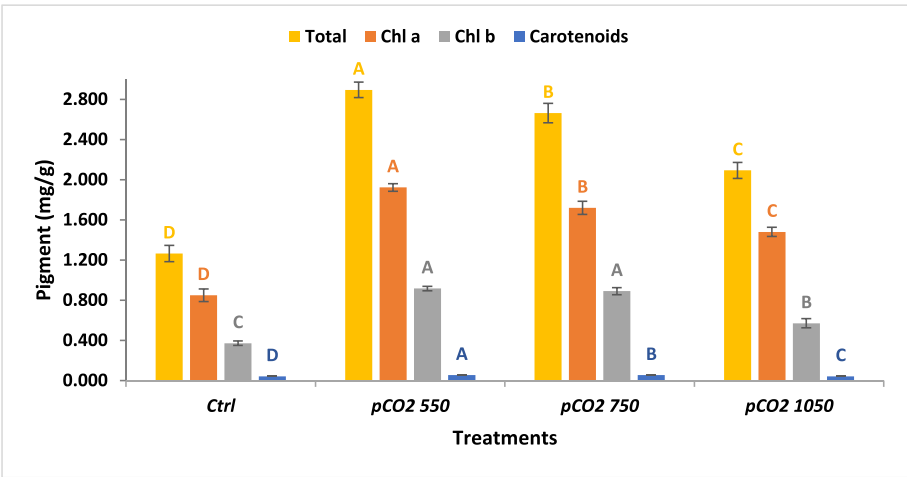

Fig. 2. Pigment content of *U. fasciata* under different pCO_2 concentrations.

Table 1
Biochemical constituents of *U. fasciata* cultured on different pCO_2 concentrations.

Treatment pCO_2	Protein (%DW)	Lipid (%DW)	Carbohydrate (%DW)
Control (pCO_2 280)	26.99 ± 0.23^c	3.70 ± 0.04^c	42.34 ± 0.21^c
pCO_2 550	32.43 ± 0.27^a	3.90 ± 0.03^b	46.04 ± 0.17^b
pCO_2 750	28.70 ± 0.48^b	4.23 ± 0.05^a	46.96 ± 0.09^a
pCO_2 1050	21.53 ± 0.20^d	3.93 ± 0.04^b	46.13 ± 0.11^b
F- value	610.00*	126.68*	559.68*
LSD	0.26	0.28	0.12

pCO_2 550 μatm reflected the highest number of bands (13 bands) with unique five bands at 59, 42, 26.5, 26 and 20 KDa. Interestingly, all pCO_2 treatments showed a unique band at 20 KDa that was not observed in control. However, the other treatment and control reflected only 11 bands but at different molecular weights. Only one unique fraction with 27 KDa was distinguished in medium with pCO_2-750. Two unique fractions with 48 and 28 KDa were reflected by pCO_2-1050 (Fig. 3).

3.4. Bacterial identification

As shown in Table (2), bacterial consortium associated with *U. fasciata* in control and different pCO_2 concentrations were identified by amplifying the 16S rDNA gene and aligned with the close related strains (Fig. 4). The dominant bacterium was identified in each treatment. The 16S rRNA gene phylogeny was inferred from

Table 2
Sequence identity of the dominate associated bacteria within *U. fasciata* thallus using a comparison with most identity GenBank accessions.

Treatments	Identified strain with the highest identity	Max Score	Total Score	Query Cover	E value	Identity %	Accession number
Control (pCO_2-280)	*Halomonas hydrothermalis*	1105	6610	100%	0.0	100.00%	AP022843
pCO_2-550	*Halomonas venusta*	761	761	100%	0.0	99.76%	MT510186
pCO_2-750	*Halomonas venusta*	981	981	100%	0.0	100.00%	MT299647
pCO_2-1050	*Vibrio campbellii*	761	761	100%	0.0	99.76%	MT510186

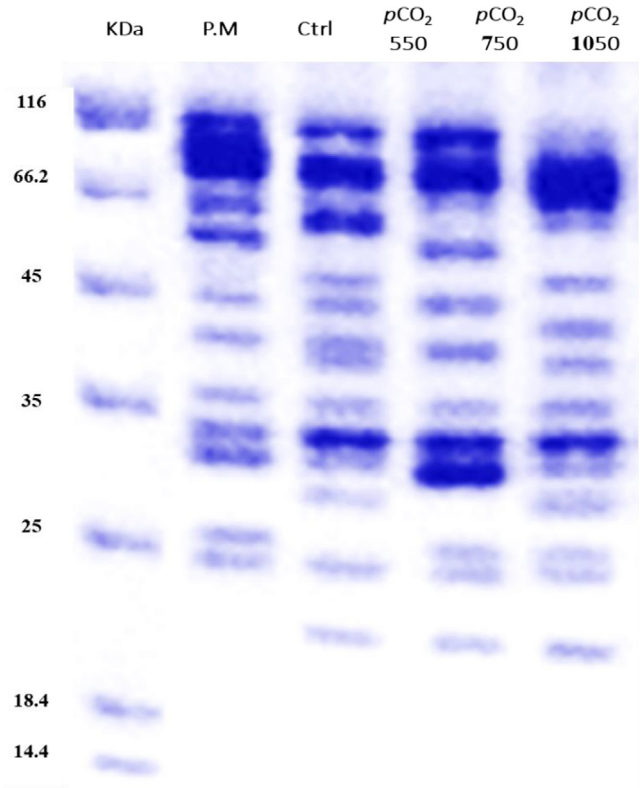

Fig. 3. Protein fingerprinting patterns for *U. fasciata* under different pCO_2. PM, protein marker.

about 500 bp nucleotide sequences (PCR-based) that originated from four predominant bacterial isolates. From the maximum neighbor joint phylogenetic tree based on the 16S rRNA gene, the 16S gene region was aligned with 16S nucleotide sequences of 29 bacterial strains in the NCBI Ribosomal RNA sequence. The phylogenetic tree consisted of two main clades the first clade of *Halomonas* sp., where the dominant strain of moderate pCO_2 level (550 and 750 μatm) was grouped with *Halomonas venusta* (MK357745, MF928305, MF928305 and LN995436) with identity percentages reached up to 99%, respectively and bootstrap of 77. While, the dominant strain of control was grouped with *Halomonas hydrothermalis* (AP022843) with 100% similarity percentage and 91 bootstraps. The second clade was grouped the dominate species of the highest pCO_2 level (1050 μatm) with *Vibrio toranzoniae* (MT510186, LR722816 and MN945290) that showed identity percentage reached to 99% and bootstrap of 92%. From the above results, pCO_2 variation has a significant effect on *U. fasciata* associated bacteria consortium.

4. Discussion

In marine ecosystems, enrichment of different CO_2 in seawater levels consequently affects the acidification of seawater, which influences marine algae metabolism. The overgrowth of *Ulva* sp. in response to elevated pCO_2 in eutrophic estuaries can be directly promoted by acidification (Young and Gobler, 2016a). However, Reidenbach et al. (2017) detected no changes in *U. australis* growth by decreasing pCO_2, which influenced the carbon and nitrogen metabolisms. Also, Chen et al. found that both lowered and increased seawater pH exert significant physiological stress on *U. lactuca* germlings (Chen et al., 2017).

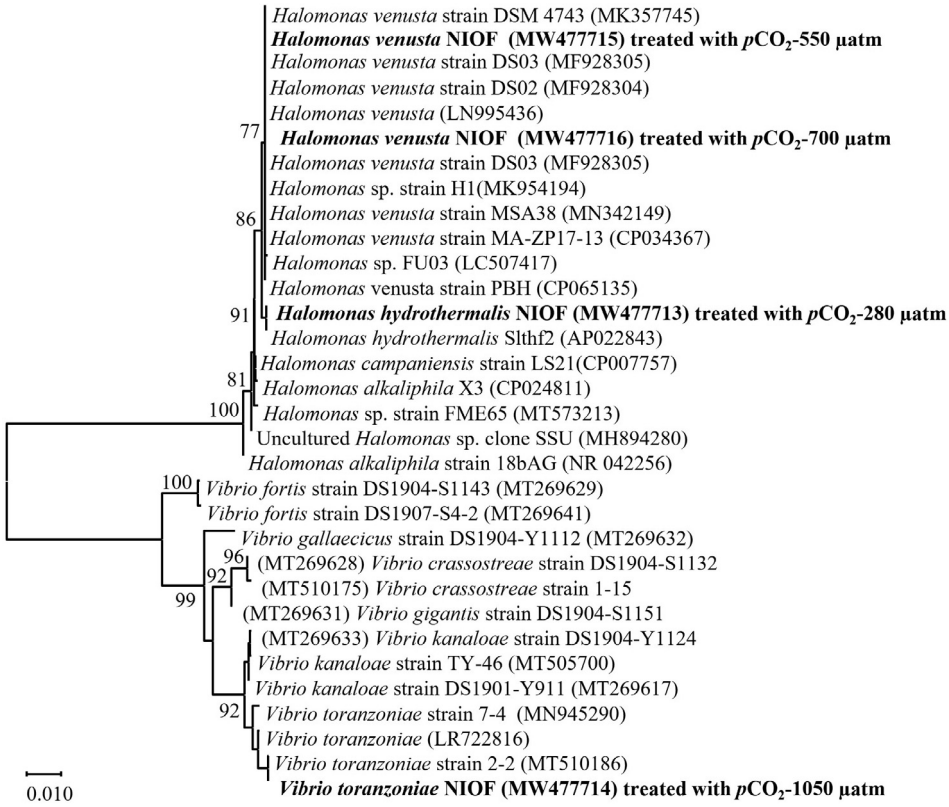

Fig. 4. Neighbour-joining (NJ) dendrogram was constructed for the dominant bacterial strains based on 16S rRNA nucleotide sequences. Bootstrap values higher than 70 are shown on the trees.

In this study, CO_2 enrichment enhanced the growth rate of *Ulva fasciata* than recorded in the control and the highest value (6.6% day^{-1}) was recorded at pCO_2-550 on the 6th day with an increase of more than double folds than control. In this regard, Young and Gobler (2016) reported that *Gracilaria* and *Ulva's* growth rates were significantly boosted by an average of 70% and 30%, respectively, beyond control treatment when exposed to raised levels of pCO_2. Furthermore, *Gracilaria* and *Ulva* show a physiological shift from near-exclusive use of HCO^{3-} to mainly CO_2 use when subjected to elevated pCO_2 via detecting $\delta^{13}C$ isotopes. This shift in carbon dependence coupled with growth rate increased in response to increased pCO_2, proposed that these seaweeds' photosynthesis depended on their inorganic carbon source (Young and Gobler, 2016a). Semesi et al. (2009) demonstrated that increasing dissolved CO_2 concentration to a specific level of ~ 26 μmol kg^{-1} caused enhancing the photosynthetic rates of *Hydrolithon* sp by 13%. Additionally, the negative impact of the higher pCO_2 on the growth of *U. fasciata* in the present study may contribute to the lowered pH. Elevated pCO_2 reduces pH at the surface of the cell, which could modify both extracellular and intracellular acid-base balance (Flynn et al., 2012). Intracellular metabolic activities, including photosynthesis and development, may be impaired by disrupted homeostasis (Z. Xu et al., 2017). A reduced growth rate caused by decreased pH was also found in different seaweed such as *Pyropia yezoensis* (G. Gao et al., 2019), *P. haitanensis* (K. Xu et al., 2017) and *U. lactuca* (Olischläger et al., 2013).

The marine macroalga *Ulva* sp. is a candidate raw biomass with a high growth rate and high protein, lipid, carbohydrate yield suitable for food application (Kazir et al., 2019; Khairy and El-Shafay, 2013). A recent study showed that pH-shift of *Ulva* sp improves the nutrient contents and gives a high grade of food applications (Harrysson et al., 2019).

Color and flavor are essential criteria in determining seaweed feed and food qualities and thus, market value. Seaweeds with a darker color and more protein are favorable by consumers and have a higher value in the market. The color of seaweeds is mainly determined by photosynthetic pigments, such as chlorophyll and carotenoids (Niwa and Harada, 2013). Regarding algal pigments, chlorophyll *a, b* and carotenoids contents of *U. fasciata* thalli were significantly differentiated at a different level of pCO_2, the maximum Chl *a*, Chl *b* and carotenoids were recorded at pCO_2-550 and declined by elevating the pCO_2 level. This finding was in accordance (G. Gao et al., 2019; K. Gao et al., 2019) who recorded that increasing pCO_2 concentration improved the color and flavor of edible red algae *Pyropia yezoensis* by boosting pigments, and amino acids contents. In brown seaweed *Sargassum vulgare*, photosynthetic pigments have improved at the acidified site than alkaline one (Kumar et al., 2020). On contrary, raising pCO_2 to 750 μatm, the pigment content was decreased. The same finding was recorded in *U. lactuca*, where both Chl *a* and Chl *b* content were reduced at 750 μatm at the end of the experiment (Olischläger et al., 2013).

Carbohydrate is the main calorific compound in seaweeds that are utilized in metabolism as a source of energy needed for respiration and other important processes (Sudhakar et al., 2019) as well as soluble carbohydrates can serve as precursors for bioactive metabolites (Kumar et al., 2020). Seaweeds carbohydrate is a valuable and sustainable source for pharmaceutical, cosmeceutical, and traditional applications (Ahmed et al., 2014) and bioethanol feedstock (Elshobary et al., 2020a; Osman et al., 2020). The results showed that the carbohydrate was the most dominant component in *U. fasciata.* This finding is agreed with results of (Osman et al., 2020) who observed that the carbohydrate content of *U. fasciata* ranged from 37.1%DW during winter to 40.46 %DW during summer, while *U. rigida* sp. showed lower trends of carbohydrates 28.6% when cultivated under fish effluents compared to the control site (Korzen et al., 2015). Different levels of pCO_2 showed a significant effect on the carbohydrate content and the highest carbohydrate content (46.96%DW) was demonstrated at 750 μatm. In a recent study, the content of fucoidan and alginate polysaccharides were higher in the algal community of acidified environment (Kumar et al., 2020). Rogers et al. (1998) and Webber et al. (1994) found that elevating CO_2 can decrease RuBisCO concentrations; however, it can lead to an increase in soluble carbohydrate content, which can increase the total carbon content of algal tissue. In contrast, pCO_2 did not affect carbohydrate content in *U. rigida* (Gao et al., 2017). These differences may be depending on acclimation ability of each species to the different degrees of pCO_2. Although there is no clear metabolic understanding of the relationship between CO_2 concentrations and cell wall carbohydrates, it has been documented that elevated CO_2 concentrations may enhance the activity of enzymes responsible for the synthesis of cell wall uronic acid, resulting in increased cell wall carbohydrate synthesis (Cheng et al., 2015). Another research discovered that under elevated acidification level, genes encoding enzymes involved in cell wall formation and structure, as well as carbon storage, were expressed at higher levels in *Sargassum vulgare* than under control conditions (Kumar et al., 2017).

In macroalgae, the lipids constitute a suitable storage material widely distributed, in different macroalgal classes (Sudhakar et al., 2019). Lipid is a calorific component that can be used for aquaculture (El-Khodary et al., 2020) or feedstock for biodiesel (Essa et al., 2018; Ashour et al., 2019; Elshobary et al., 2019; Huo et al., 2020). Despite the current study observed low lipid content (4.23 %DW), this is comparable with other reports, which showed the lipid content of macroalgae were<5% of dry weight (Elshobary et al., 2020a; Khairy and El-Shafay, 2013; Osman et al., 2020). The differences in lipid reported quantities could be due to several factors such as seasonal and geographical factors, climate change, and the development stage of the macroalgae (Osman et al., 2020). Noteworthy, the increase in ocean acidity combined with an increase in the lipid content, showing the maximum value at pCO_2-750 with a rise of 54% than that were found in control. Gao et al. (2017) observed that increasing pCO_2 up to pH 7.95 increased lipid content in *U. rigida* under the high temperature by 22.55% than low pCO_2 treatments. Gordillo et al. (2001a) detected that under different pCO_2 levels, significant changes were observed in total lipid content as well as its classes in *U. rigida*. Triglycerides accumulated at high CO_2 and under nitrogen deficient, while chloroplast-related lipids recorded an inverse response In general, high pCO_2 concentration of 1000 ppm showed a negative impact on total lipid accumulation.

Protein plays crucial roles in all algal biological processes; their activities can be described by transport and storage, enzymatic catalysis, and mechanical sustentative control (Sudhakar et al., 2019). The current study revealed that *U. fasciata* accumulated the highest protein content of 32.43%DW, at pCO_2-550 compared with high pCO_2 levels and control synchronized with the growth rate. A recent study showed that ocean acidification of *Ulva* sp. improves the protein yield to 29% by 2.3-fold higher than recorded by control and gives a high grade of food applications (Harrysson et al., 2019). Gao et al., (2017) reported that protein levels were increased in *U. rigida* in response to pH-shifting. In contrast, high CO_2 upto10,000 ppm reduced total soluble protein compared to the ambient CO_2 level of 350 ppm (Gordillo et al., 2001b, 2001a). The reduction in protein content under high CO_2 level may be attributed to the algal species tends to accumulate some biochemical such carbohydrate and lipid over the others (protein) under high CO_2 concentration. In this regard, Chen et al. observed that, carbohydrate content increased in *Pyropia haitanensis* while protein content decreased due to higher dissolved inorganic carbon in highly acidified seawater (Chen et al., 2019b). Several studies

observed the same results (Chen et al., 2019a; Duarte et al., 2016; Gao et al., 2018b, 2018a). This may be explained by increasing CO_2 concentration increases the CO_2 passive diffusion , resulting in a reduction in active transport proteins inside the seaweed's cell and allocating more energy for growth (Young and Gobler, 2016b). These results are also consistent with the SDS-PAGE which showed the largest number of unique protein bands were detected in the moderate pCO_2 level (550 μatm) and these bands reduced by increasing pCO_2 level.

The ability of seaweed to different biochemical stresses may be due to symbiosis with microbiota (Dominguez and Loret, 2019). Interactions between *Ulva* spp. and their associated bacteria have been well-characterized over the last decade, where, bacterial colonization has been defined based on 16S rRNA gene phylogeny (Wichard et al., 2015). The interconnected evolutionary history of algae and bacteria allowed a wide range of associations to be established, characterized by the coordinated exchange of nutrients and mutual support for growth factors (Cirri and Pohnert, 2019; Huo et al., 2020). Many studies stated that different compositions of bacterial communities could enable *Ulva* species to support the 'competitive lottery' theory for how symbiotic bacteria help algae in either ambient or harsh conditions (Comba-González et al., 2016; Ghaderiardakani et al., 2017; Kessler et al., 2018; Spoerner et al., 2012). As a result of acidification, the seaweed-associated bacterial community had changed, where, elevating CO_2 altered the dominant associated bacteria from *Halomonas* sp. of ambient condition (280 μatm) and moderate $pCO2^{\circ}$ (550–750 μatm) to *Vibrio toranzoniae* at the highest pCO_2 level (1050 μatm). This finding is in accordance with (Aires et al., 2018) who observed that the bacterial community of *Sargassum muticum* was changed, where Oceanospirillales and Vibrionales significantly increased their abundance in acidified conditions. Vibrionales, usually associated with diseased seaweeds, proposing that acidification may facilitate opportunistic/pathogenic bacteria. Moreover, Alpha diversity of total bacteria communities and Cyanobacteria communities was significantly variated among different pH/CO_2 sites (Taylor et al., 2014). Coral microbiomes contribute to seaweed adaptation to environmental change, especially pH/CO_2 levels (Biagi et al., 2020).

5. Conclusion

From the above results, it could be concluded that a considerable influence of pCO_2 on the whole performance of *U. fasciata,* including growth rate, protein, pigment, lipid and carbohydrate, and associated microbiota. Ocean acidification at pCO_2-550 μatm is the optimum concentration to improving growth, protein and pigment contents and protein profile which could be a good source for alimentary fish source. While elevating ocean acidification to pCO_2-750 μatm could be preferable for bioenergy production by stimulating energetic compounds of lipid and carbohydrate.

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.

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Appendix A. Supplementary data

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