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Effect of Salinity Stress on Growth, Omega-3 Accumulation, and Desaturase Gene Expression in *Spirogyra* sp.

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ABSTRACT

Salinity is well known to enhance omega-3 biosynthesis in marine algae. However, the effect of salinity on freshwater algae, *Spirogyra* sp., remains unexplored. This study investigated the effect of salinity on growth, omega-3 accumulation, and desaturase genes, *SAD* (*stearoyl-ACP desaturase*), *FAD2* (*fatty acid desaturase 2*), and *FAD3* (*fatty acid desaturase 3*), expression in *Spirogyra* sp. The *Spirogyra* sp. was cultivated in Blue Green-11 (BG-11) medium for 12 days using an airlift bioreactor. Salinity stress was applied by adding NaCl in concentrations of 75 mg/L and 125 mg/L, respectively. Biomass was harvested every two days for growth-phase analysis. Omega-3 was quantified by gas chromatography mass spectrometry (GC-MS), while desaturase gene expression was analysed by quantitative reverse transcription polymerase chain reaction. Results showed that 125 mg/L NaCl enhanced growth from 14±0.0 g to 27.10±0.20 g, omega-3 accumulation from 25.87±5.77% to 31.50±14.55%, and expression of *FAD3* from 1±0.0 to 4.13 ± 0.28. Correlation analysis showed that *FAD3* expression in the exponential phase was strongly related to omega-3 accumulation (ρ = 0.735). In conclusion, cultivation of *Spirogyra* sp. with 125 mg/L NaCl improved growth and omega-3 production by enhancing desaturase gene expression, suggesting potential for large-scale use.

1. Introduction

Global demand for functional food sources continues to rise due to the high prevalence of metabolic, cardiovascular, and neurodegenerative diseases. Among various bioactive components, omega-3 fatty acids have become important because they have been proven to play a protective role in human health (Saidaiah *et al.* 2024). Omega-3 fatty acids have benefits as an anticancer agent, support brain health and protection against neurodegenerative disorders, and provide cardiovascular protection by reducing the risk of arrhythmias, hypertension, and atherosclerosis (Lau *et al.* 2020; Pifferi *et al.* 2020; Afroze *et al.* 2024). Despite widespread reports of omega-3's benefits, fish oil remains the primary source for its availability.

The use of fish oil as a source of omega-3 has several ecological concerns, such as overfishing, degradation of marine habitats, and the risk of contamination with toxic compounds (Ratteni 2020; Rastogi *et al.* 2023). Therefore, the development of alternative omega-3 sources is crucial to address the challenges of availability, sustainability, and food security.

Algae are one potential source of omega-3. The main advantage of algae as an omega-3 source is the sustainability of its cultivation. Algae require less land than terrestrial plants, grow quickly, have short life cycles, and efficiently absorb carbon dioxide (CO_2) (Winwood 2015). Although most omega-3 studies still focus on marine algae, some freshwater algae species also show similar potential. One of them is *Spirogyra* sp., an alga that is capable of absorbing carbon dioxide and converting it into lipid (Ge *et al.* 2018).

Spirogyra sp. is a filamentous green algae that is commonly found in freshwater (Wongsawad & Peeraporpisal 2015; Munir *et al.* 2015). Although abundant, its utilization is still limited, for example, only as fishing bait. In fact, *Spirogyra* sp. has the ability to absorb carbon dioxide (CO_2) and convert it into valuable

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compounds like lipids. This potential is supported by the findings of Das & Gosh (2023), who reported that *Spirogyra* has a relatively high polyunsaturated fatty acid (PUFA) content compared to other freshwater algae. Spirogyra sp. has a lipid content that ranges from 11 to 21% of its total dry weight. It is reported to range from 11 to 21% of total dry weight, with 97.7% of the FAME fraction consisting of polyunsaturated fatty acids (PUFAs) (Aminu *et al.* 2024). This high PUFA fraction indicates the potential presence of omega-3 fatty acids, such as α-linolenic acid (ALA). This potential can be optimized with the right cultivation strategies, including the cultivation system used.

To optimize the potential of *Spirogyra* sp. as a source of omega-3 fatty acids, environmental factors play an important role in triggering their accumulation. One of the most important environmental factors is salinity. Several studies have reported that salinity can increase omega-3 accumulation in some types of microalgae. For example, the addition of salinity to *Chlorella vulgaris* and *Dunaliella salina* was reported to increase ALA, EPA, and DHA levels by up to 20–300% (Rismani & Shariati 2017). Similarly, *Phaeodactylum tricornutum* showed an increase in EPA by up to 30.83–32.18% and DHA by up to 0.8–1.08% (Qiao *et al.* 2016).

The increase in omega-3 under salinity is primarily mediated through the PUFA biosynthesis pathway involving desaturase enzymes. The activity and amount of these enzymes are determined by the expression level of the genes that encode and regulate their synthesis. These genes include *Stearoyl-ACP Desaturase* (*SAD*), *Fatty Acid Desaturase* 2 (*FAD2*), and *Fatty Acid Desaturase 3* (*FAD3*). Several studies have confirmed that salinity can trigger increased expression of desaturase genes, ultimately impacting the accumulation of PUFAs, including ALA. Atikij *et al.* (2019) showed that in the microalga *Chlamydomonas reinhardtii*, salinity treatment of 100–200 mM NaCl increased the expression of *FAD2*. In *Nannochloropsis oceanica*, Wan Razali *et al.* (2022) reported that increasing salinity from 25 to 35 ppt increased the expression of *SAD* and *FAD2*, followed by an increase in MUFA and PUFA content.

Despite the recognized role of salinity in enhancing omega-3 accumulation in other marine algae, its effects on growth, omega-3 content, and desaturase gene expression in freshwater algae, particularly *Spirogyra* sp., remain largely unexplored. *Spirogyra* sp. also has potential as a sustainable alternative source of omega-3. This study aimed to investigate the effects of salinity stress on growth, omega-3 accumulation, and the expression of desaturase genes (*SAD, FAD2, FAD3*) in *Spirogyra* sp.

2. Materials and Methods

2.1. Algal Material

Spirogyra sp. filaments were collected from rice paddies in Bojong Village, Majalaya District, Bandung Regency, West Java, Indonesia (7°2′20″ S, 107°46′28″ E). Based on morphological identification, which showed unbranched filaments and spirally coiled chloroplasts (Figure 1A), the algal filaments were identified as *Spirogyra* sp., as also indicated by Takono *et al.* (2019).

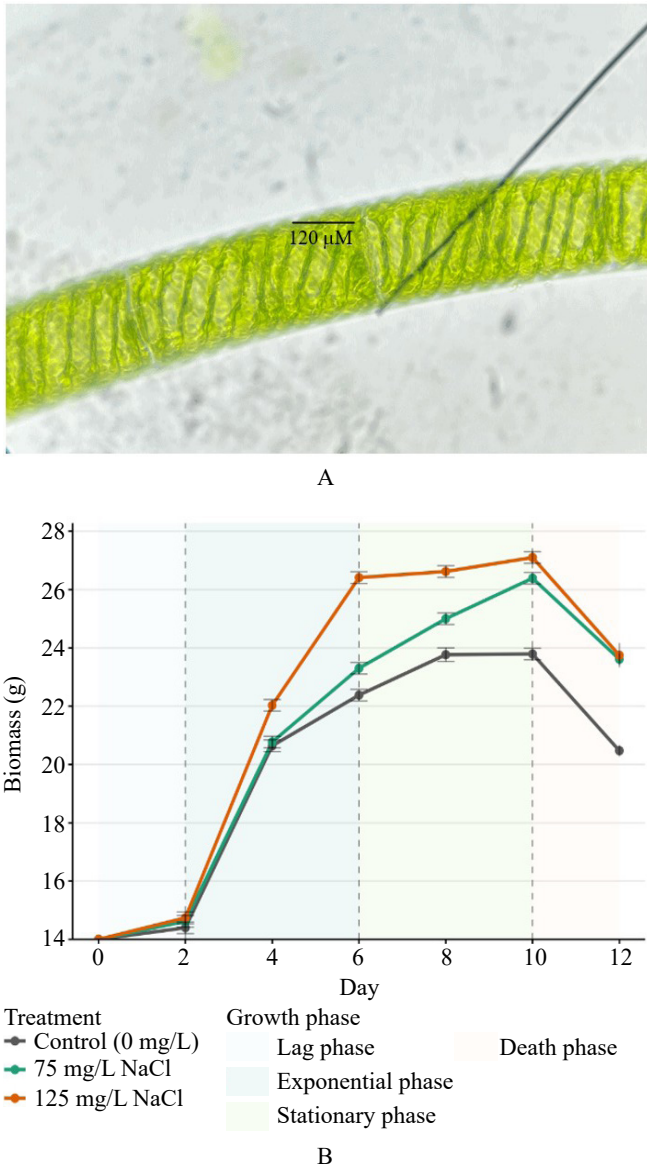


Figure 1. (A) Microscopic filament of *Spirogyra* sp., (B) growth curve of *Spirogyra* sp. cultures in an airlift bioreactor under different salinity treatments: control (0 mg/L NaCl; SpK), 75 mg/L NaCl (SpA), and 125 mg/L NaCl (SpC)

2.2. Cultivation Medium

The medium used for cultivation was BG-11 (Blue Green 11 medium) at a concentration of 25%, following the methodology used by Zafira *et al.* (2023). The medium consisted of $NaNO_3$ (93.75 mg/L), K_2HPO_4 (5 mg/L), $MgSO_4 \cdot 7H_2O$ (75 mg/L), $CaCl_2 \cdot 2H_2O$ (36 mg/L), citric acid (6 mg/L), ferric ammonium citrate (6 mg/L), Na_2EDTA (10 mg/L), Na_2CO_3 (20 mg/L), and a trace element solution containing H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $Co(NO_3)_2 \cdot 6H_2O$. The materials utilized were obtained from Sigma-Aldrich.

2.3. Cultivation and Salinity Treatment of *Spirogyra* sp.

Spirogyra sp. was cultivated according to Zafira *et al.* (2023), with modifications. The *Spirogyra* sp. was cleaned with distilled water. It was then acclimatized in 700 mL of 25% BG-11 medium in an airlift bioreactor for 5 days. After acclimatization, the *Spirogyra* sp. was transfer to fresh medium in nine airlift bioreactors under two salinity treatments: 75 and 125 mg/L NaCl. The control consisted of 25% BG-11 medium without NaCl addition. In a study reported by Putri *et al.* (2023), 75 mg/L NaCl was not the optimal concentration for enhancing omega-3 levels; therefore, this study increased the concentration of NaCl concentration to 125 mg/L. All treatments consisted of three replicates. The cultivation was performed at room temperature (25±0.5°C) with a 2:12 photoperiod (Philips TL-16W LED lamp, 2,000–2,500 lux) and an aeration rate of 0.6 L/min. During cultivation, the pH of the medium was checked and adjusted every two days to keep it within the range of 7.5±0.25.

2.4. Harvesting and Measurement of *Spirogyra* sp. Biomass

The harvesting and measurement of biomass were conducted following the modified method of Marwani *et al.* (2024). Biomass of *Spirogyra* sp. was collected every two days throughout a 12-day cultivation period (on days 2, 4, 6, 8, 10, and 12). The algal filaments were separated from the culture medium using an 80-mesh sieve, and the fresh biomass was immediately weighed to generate growth curves. Samples were harvested on days 4, 6, and 9 and preserved for RNA extraction and further analyses.

2.5. Lipid Extraction

Harvested *Spirogyra* sp. biomass was oven-dried at 50°C and used for lipid extraction. Lipids from the three treatments were extracted using a modified Bligh and Dyer method (1959). 3.5 g of dried *Spirogyra* sp. biomass, harvested in exponential, late-exponential, and stationary phases, was homogenized in 15 mL of chloroform–methanol (1:2, v/v). The mixture was centrifuged at 2,500 rpm for 7 minutes and then filtered. The residual biomass was re-extracted using 7 mL of chloroform, centrifuged, and then the upper methanol layer was removed. The chloroform fraction was filtered using Whatman No. 1 paper, and the residue was extracted with 11 mL of chloroform-methanol (9:1, v/v) and vortexed for 7 minutes. After centrifugation at 3,000 rpm for 7 minutes, the lipid-containing fraction was separated and evaporated in a 70°C water bath to remove chloroform; the residue was then oven-dried at 105°C for 15 minutes.

2.6. Transesterification and GC-MS Analysis of Omega-3 Fatty Acids

Esterification of crude lipids was carried out using boron trifluoride in methanol (BF_3–MeOH) following the method of Li *et al.* (2001). Samples were incubated at 70°C for 1 h, and fatty acid methyl esters (FAMEs) were extracted with n-hexane and deionized water. The organic layer was dried under nitrogen, dissolved in dichloromethane, and transferred to chromatography vials for GC-MS analysis.

GC-MS analysis of FAMEs was performed using a BPX70 column (120 m × 0.25 mm I.D., Agilent) with a maximum temperature of 240°C. 0.2 µL FAME was injected at 250°C using helium as the carrier gas (split ratio 10:1, constant pressure). The oven programme started at 50°C for 2 min, increased to 160°C at 20°C/min and held for 8 min, then to 220°C at 4°C/min for 5 min, and finally to 240°C at 4°C/min for 12.5 min. The transfer line was maintained at 250°C, and detection was carried out in full scan mode. Fatty acids were identified by comparing retention times with the Supelco® 37 Component FAME Mix standard and the NIST 17 mass spectral library, while omega-3 fatty acids were quantified from chromatographic peak areas.

2.7. RNA Extraction and cDNA Synthesis

Total RNA was extracted from *Spirogyra* sp. biomass at exponential, late-exponential, and stationary phases using a modified CTAB method (Murray & Thompson 1980). Biomass (500 mg) was frozen in liquid nitrogen, incubated in CTAB buffer (60°C, with β-mercaptoethanol), and RNA was purified by chloroform: isoamyl alcohol extraction, LiCl precipitation, and ethanol washes. The final pellet was resuspended in DEPC-treated water, and RNA quality was assessed by Nanodrop spectrophotometry and 1% agarose gel electrophoresis.

cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) following the manufacturer's protocol. Reverse transcription was performed at 42°C for 60 min and terminated at 70°C for 5 min. The quality of cDNA was verified by PCR amplification with GoTaq® Green Master Mix and 2% agarose gel electrophoresis.

2.8. Quantitative Polymerase Chain Reaction (qPCR) Expression Analysis

Primers for target and housekeeping genes were designed using Primer3Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Primer specificity was verified using the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). We used specific primers for desaturase genes for quantitative PCR (qPCR) (Table 1). Reactions were performed with GoTaq® qPCR Master Mix (Promega) on a QuantStudio 1 thermal cycler (Thermo Fisher). Cycling conditions were initial activation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 57°C for 15 s, and extension at 72°C for 10 s. Actin was used as the housekeeping gene, and relative expression levels were calculated using the 2^-ΔΔCT method (Livak & Schmittgen 2001).

2.8. Statistical Analysis

Statistical analyses were conducted using two-way analysis of variance (ANOVA) to compare the mean values of all experimental results, followed by Tukey's post-hoc range test with a significance level of $p<0.05$ to assess differences among treatments. Spearman's correlation analysis was also performed to evaluate the relationship between desaturase gene expression and omega-3 accumulation. IBM SPSS Statistics 25 software was employed for these analyses, and all data were presented as the mean ± SD (standard deviation) of triplicate experiments using Microsoft Excel XP.

Table 1. Specific primers for *SAD, FAD2, FAD3* and *Actin* genes used for qPCR

Primer name	Nucleotide sequences (5'--> 3')	Product size (bp)
FAD2-F	CCCACGTTGCCCATCATTTG	185
FAD2-R	GATTCCTTCCTTCCCGCCTT	185
FAD3-F	AACGTCGTCTCTCGCATCTC	135
FAD3-R	TCTTGACCTTCTGGACGCAC	135
SAD-F	TTCCTGTGCTGCTTGTCCTT	155
SAD-R	CTTCTCCGCTGTTGCTCAGA	155
Actin-F	GAGGTGCTCTTTCAGCCATC	200
Actin-R	TTGATCTTCATGGTGCTTGG	200

3. Results

3.1. Effect of Salinity on the Growth of *Spirogyra* sp.

The morphology of *Spirogyra* sp. used in this study shows unbranched filaments with spiral chloroplast arrangements (Figure 1A). The observed morphological characteristics confirmed that the sample belongs to the genus *Spirogyra*. Spiral chloroplasts and uniseriate filaments are specific features that are frequently used to identify the genus.

Growth of *Spirogyra* sp. in an airlift bioreactor with the addition of 75 mg/L and 125 mg/L NaCl and without NaCl addition (control) showed a similar growth pattern (Figure 1B). From day 0 to day 2 (lag phase), biomass increased only slightly across all treatments, ranging from 14.00 g to 14.40–14.74 g. Biomass of *Spirogyra* sp. in all treatments (75 mg/L NaCl and 125 mg/L NaCl) and the control increased significantly during the exponential phase (days 2-6). The highest enhancement was achieved in the 125 mg/L NaCl treatment, from 14.74±0.20 g to 26.41±0.20 g (79.17% increased), followed by the 75 mg/L treatment, i.e., 14.62±0.20 g to 23.30±0.20 g (59.37%), and the lowest enhancement was observed in the control, from 14.40±0.20 g to 22.38±0.20 g equal to 55.4%. From days 6–10, cultures entered the stationary phase with minimal biomass accumulation from 26.41±0.20 g to 27.10±0.20 g (2.61%) at 125 mg/L NaCl treatment, from 23.30±0.21 g to 26.39±0.20 g (13.26%) at 75 mg/L NaCl treatment, and from 22.38±0.21 g to 23.80±0.20 g (6.3%) in the control. By day 12, all treatments showed a decline in biomass (Table 2), indicating the beginning of the death phase.

In this study, higher salinity (125 mg/L NaCl) promoted greater biomass accumulation compared to

Table 2. The biomass of *Spirogyra* sp. under different salinity treatments during 12-day cultivation

Day	Biomass (g) ± SD		
	Control	75 mg/L NaCl	125 mg/L NaCl
0	14.00±0.00^{a}	14.00±0.00^{a}	14.00±0.00^{a}
2	14.40±0.21^{a}	14.62±0.20^{a}	14.74±0.16^{a}
4	20.64±0.15^{b}	20.770±0.17^{b}	22.030±0.11^{bc}
6	22.380±0.21^{bc}	23.300±0.2^{bc}	26.410±0.20^{cd}
8	23.767±0.24^{bc}	25.000±0.20^{c}	26.620±0.20^{d}
10	23.790±0.21^{bc}	26.390±0.18^{d}	27.100±0.19^{e}
12	20.473±0.21^{b}	23.610±0.20^{bc}	23.747±0.4^{bc}

Data express as a mean ± SD using two way ANOVA and Tukey's HSD test with $p<0.05$. (Data is presented as the mean from n = 3 biologival replicates for each alga sampel)

lower salinity (75 mg/L NaCl) and the control. These results indicate that salinity exerts a differential effect on *Spirogyra* sp. growth, where moderate stress (125 mg/L) enhanced biomass during the exponential and stationary phases while reducing biomass loss in the death phase.

3.2. Effect of Salinity on Omega-3 Fatty Acid Content

Treatment with 125 mg/L NaCl in the late exponential phase and in the stationary phase resulted in omega-3 contents of 25.69±5.77% and 31.50±14.55, respectively. By contrast, treatment with 75 mg/L yielded 7.16±1.69% and 0% omega-3 in the respective phases, whereas the control showed 16.89±3.86% and 25.87±3.86% (Figure 2).

These findings indicate that 125 mg/L NaCl significantly enhanced omega-3 accumulation, with the highest omega-3 proportion observed in the stationary phase. This result was consistent with the control. Conversely, 75 mg/L NaCl failed to sustain omega-3 biosynthesis from the late exponential to the stationary phase, suggesting that this concentration was insufficient to trigger metabolic adaptation under salinity stress.

3.3. Effect of Salinity on Desaturase Gene Expression

All desaturase genes (*SAD, FAD2,* and *FAD3*) showed increased expression during the late exponential phase, then declined in the stationary phase, with the most significant increase in the 125 ppm salinity treatment (Figure 3). The expression of the *SAD* gene showed a modest rise during the exponential phase, with a relative quantification (RQ) of 1.31±0.02 for the 75 mg/L NaCl treatment and 1.52±0.05 for the 125 mg/L NaCl treatment compared with the control (SpK), with RQ = 1. A more pronounced increase was noted during the late exponential phase, attaining an RQ of 3.51±0.28 (75 mg/L NaCl) and 4.13±0.28 (125 mg/L NaCl). Meanwhile, in the stationary phase, SAD expression declined to 1.19±0.04 (75 mg/L NaCl) and 1.49±0.03 (125 mg/L NaCl).

The expression pattern of *FAD2* resembles that of *SAD*. In the exponential phase, the respiratory quotient (RQ) was measured at 1.15±0.13 (75 mg/L NaCl) and

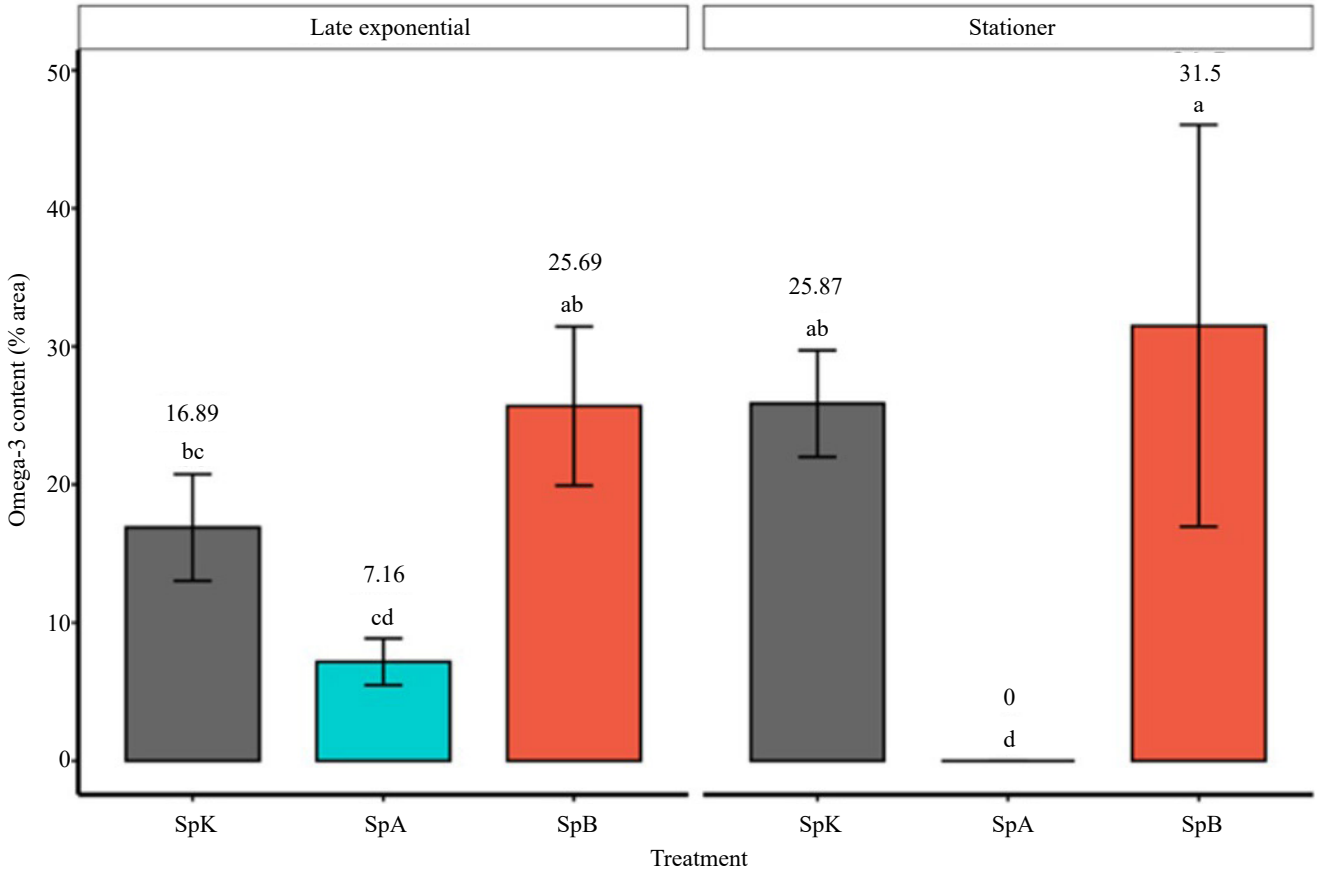


Figure 2. Omega-3 fatty acid accumulation in *Spirogyra* sp. in a medium with the addition of 75 mg/L NaCl (SpK), 125 mg/L NaCl (SpB), as well as a control, during late exponential and stationary phase

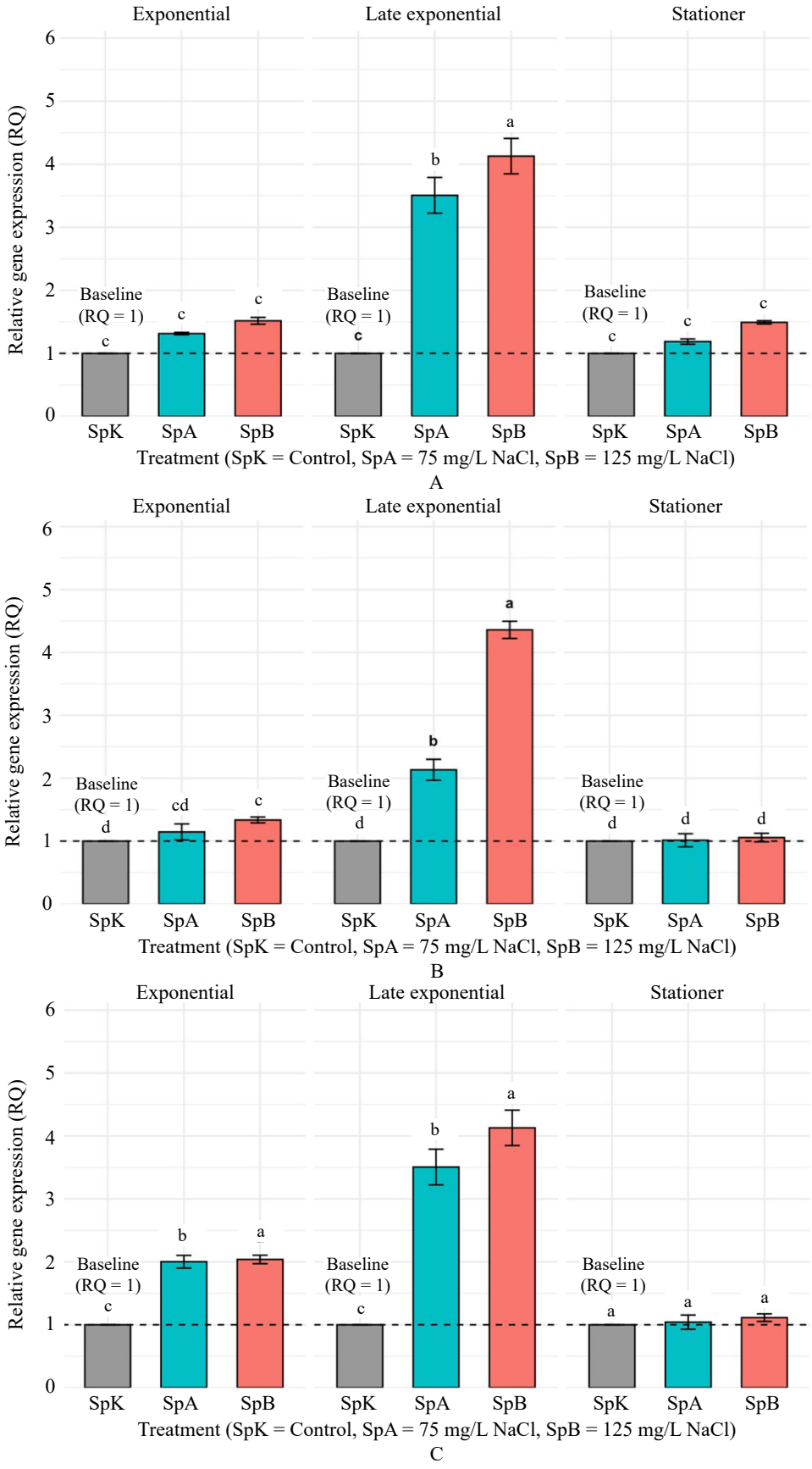


Figure 3. Relative expression levels (RQ) of desaturase genes in *Spirogyra* sp. under different salinity treatments: (A) *SAD*, (B) *FAD2*, and (C) *FAD3*. Cultures were cultivated in control (SpK), 75 mg/L NaCl (SpA), and 125 mg/L NaCl (SpB) and samples were collected during the exponential, late exponential, and stationary phases

1.34±0.05 (125 mg/L NaCl). Maximum expression was seen during the late exponential phase, with RQ values of 2.13±0.17 (75 mg/L NaCl) and 4.36±0.14 (125 mg/L NaCl). In the stationary phase, expression reverted to values approximating the control, which were 1.01±0.10 (75 mg/L NaCl) and 1.06±0.07 (125 mg/L NaCl).

The expression of FAD3 varies according to the phase and salt level. In the exponential phase, the 75 ppm treatment exhibited no significant difference from the control (RQ 1.00±0.06), whereas the 125 ppm treatment considerably rose to 1.69±0.01. During the late exponential phase, the RQ rose to 1.19±0.11 (75 ppm) and 3.82±0.11 (125 ppm). During the stationary period, the expression diminished once again, nearing the control values (1.04±0.09 at 75 ppm and 1.33±0.20 at 125 ppm).The data indicate that 125 mg/L NaCl strongly induces *SAD, FAD2,* and *FAD3* expression, particularly during the late exponential phase, whereas 75 mg/L NaCl results in only modest upregulation. This suggests that higher salinity effectively triggers desaturase-mediated responses in *Spirogyra* sp.

3.4. The Correlation Between Expression of Desaturase Genes and Omega-3 Accumulation

The Spearman correlation index of the expression level of the *FAD3* gene with ALA content was 0.73. In contrast, *FAD2* and *SAD* genes displayed moderate correlations with ALA content, with ρ values of 0.42 and 0.41, respectively. These results were consistent with the high expression level of *FAD3* (RQ = 1.69±0.01 at 125 mg/L NaCl) and the corresponding ALA content (25.69±5.77%) during the exponential phase.

4. Discussion

Salinity can influence the growth of *Spirogyra* sp., and higher salinity levels up to 125 mg/L NaCl resulted in better growth performance in this study. This increase might be related to the salinity level applied in this study, which falls within the range of mild salinity that can stimulate metabolism and promote cell division rather than inhibit growth. This phenomenon aligns with Chisti (2007), in which mild environmental stress induced adaptive responses that enhanced metabolic activity. According to Chisti (2007), the presence of Na^+ and Cl^- ions at the mild concentrations could lead to the production of compatible solutes such as proline, glycerol, and soluble sugars. Moreover, these solutes support photosynthesis and energy production, increasing carbon assimilation and cell division and ultimately affecting growth. In addition, the increased growth of *Spirogyra* sp. by applying NaCl was in agreement with previous work by Elloumi *et al.* (2020) on *Scenedesmus* sp., in which growth markedly increased after the second day of salinity stress using 10 g/L and 20 g/L NaCl. A similar positive response to mild salinity (10 g/L NaCl) was also reported by Haris (2022) in *Chlorella vulgaris*, where higher growth was observed compared to the control.

Despite its effect on growth, salinity also affected the accumulation of omega-3 fatty acids in *Spirogyra* sp. The culture treated with 125 mg/L NaCl showed the highest accumulation of omega-3 fatty acids during the stationary phase. The higher omega-3 accumulation at 125 mg/L NaCl treatment indicates that mild salinity helps the algae maintain membrane stability under osmotic stress. This finding aligns with the study by Li *et al.* (2021), which reported that moderate salinity induces osmotic stress and ionic imbalance (Na^+/K^+) within cells, leading to membrane instability and triggering adaptive responses through modifications in membrane lipid composition, including enhanced fatty acid desaturation, especially omega-3 fatty acid synthesis. A similar study by Magpayo *et al.* (2019) reported that a salinity level of 55 g/L applied to *Dunaliella* sp. cultures resulted in higher omega-3 accumulation than the control, as a mechanism to maintain membrane stability under osmotic change.

Alterations in fatty acid composition under salinity stress often reflect underlying transcriptional regulation of lipid metabolic genes (Wan Razali *et al.* 2022). The higher omega-3 accumulation observed at 125 mg/L NaCl suggests that salinity also influences the relative expression of genes involved in fatty acid desaturation. The relative expression levels of the desaturase genes (*SAD, FAD2,* and *FAD3*) in samples treated with 75 mg/L and 125 mg/L NaCl were higher than the control during the stationary phase, with the highest expression observed in the 125 mg/L NaCl treatment. The highest expression of desaturase genes at 125 mg/L NaCl suggests that this salinity level could cause osmotic stress and ionic imbalance (Na^+/K^+) within the cell, altering membrane stability and fluidity, and activating desaturase genes to modify and restore membrane stability and fluidity. The relationship between salinity and the relative expression level of desaturase genes was in accordance with Ma *et al.* (2024); adding salinity to algae cultures could trigger the activation of signalling pathways that stimulate transcription factors to increase the expression of desaturase genes, which add double bonds to fatty acid chains, thereby enhancing the formation of polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids, which play a role in maintaining membrane bilayer flexibility and stability. In agreement with this finding, previous studies on microalgae such as *Chlamydomonas reinhardtii* have

shown that salinity stress induces signalling cascades, which in turn enhance the expression of desaturase genes and promote the biosynthesis of polyunsaturated fatty acids (Zhang *et al.* 2022). Similarly, *Chlorella vulgaris* and *Nannochloropsis oceanica* demonstrated elevated expression of lipid-related genes under moderate salinity, leading to increased accumulation of long-chain ω-3 and ω-6 fatty acids (Meng *et al.* 2015; Salama *et al.* 2020).

The correlation analysis indicates that FAD3 gene expression has a strong relationship with omega-3 accumulation, suggesting that this gene plays a major role in α-linolenic acid (ALA) synthesis. The *FAD3* gene functions in converting linoleic acid into ALA, so its expression level directly influences how much ALA accumulated (Zhang *et al.* 2022). In contrast, *SAD* and *FAD2* gene expressions show only moderate correlations with ALA accumulation. This is aligned with the study of Lau *et al.* (2020), who reported that *SAD* and *FAD2* are involved in PUFA biosynthesis but have a less direct impact on ALA production. These results indicate that *FAD3* acts as the main regulator of ALA biosynthesis, while *SAD* and *FAD2* mainly support the pathway by supplying the necessary precursors. These findings suggest that mild salinity (up to 125 mg/L NaCl) enhances biomass growth (from 14.00±0.00 g to 27.10±0.20 g), omega-3 accumulation (from 25.87±5.77% to 31.50±14.55%), and *FAD3* gene expression (from 1.00±0.00 to 4.13±0.28), particularly through the upregulation of desaturase genes, with *FAD3* contributing to the elevated synthesis of omega-3 fatty acids in *Spirogyra* sp.

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