

Research Paper

Salinity Stress as a Metabolic Modulator in *Fischerella* sp. FS 18: Impacts on Biomass, Antibacterial Activity, and Biochemical ProfilingRahil Sadat Rahimhosseini¹, Aryan Sateei^{1,2*}, Shadman Shokravi¹, Mehdi Ebadi^{2,3}, Hamid Reza Pordeli¹

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ABSTRACT

Background: The rise of multidrug-resistant (MDR) pathogens has intensified the search for new bioactive compounds from natural sources. Cyanobacteria, particularly the genus *Fischerella*, are promising producers of secondary metabolites, and environmental factors, such as salinity, can modulate their metabolic output.

Materials and Methods: This study investigated how distinct salinity stress regimes modulate growth, antibacterial activity, and metabolic output in *Fischerella* sp. FS 18. Cultures were assigned to four treatments: untreated control, 24-h pre-exposure to sodium chloride (NaCl) (Pre-24), 96-h pre-exposure (Pre-96), and chronic NaCl exposure for 44 days. Antibacterial activity of ethanolic, propanolic, and butanolic extracts was evaluated against *Bacillus cereus* and *Pseudomonas aeruginosa* using disk diffusion. Metabolic profiling was performed using gas chromatography-mass spectrometry (GC-MS).

Results: Control and Pre-24 supported the highest biomass (6.89 ± 0.42 and 6.66 ± 0.26 g/L), while prolonged or continuous salinity significantly impaired growth (Pre-96: 4.27 ± 0.31 g/L; NaCl: 3.82 ± 0.28 g/L). The antibacterial activity was observed only in butanolic extracts against *B. cereus*, with inhibition zones ranging from 10.0 ± 0.0 mm to 13.0 ± 0.6 mm. Both strains were resistant to beta-lactam antibiotics but susceptible to the butanolic extract. Gas chromatography-mass spectrometry (GC-MS) revealed treatment-dependent biochemical variation, with the greatest chemical richness in Pre-96 (81 compounds). Bis(2-ethylhexyl) phthalate was predominant across treatments (28.61-60.74%).

Conclusion: Salinity acts as a metabolic modulator in *Fischerella* sp. FS 18, generating a trade-off between biomass production and chemical diversity. Short-term salinity priming may enrich bioactive metabolite profiles with limited growth penalty. Immunological effects were not examined and remain speculative.

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Introduction

Algae are recognized as prolific sources of bioactive secondary metabolites, offering a vast chemical repertoire for pharmacological and industrial applications. Their extraordinary metabolic plasticity enables the synthesis of diverse specialized compounds, including alkaloids, terpenoids, and phenolics, which serve various ecological functions [1-3]. Among the diverse taxa, the genus *Fischerella* has emerged as a particularly promising candidate in biotechnology due to its unique metabolic pathways, making it valuable for the discovery of novel bioactive agents [4, 5].

The global healthcare landscape faces an unprecedented crisis due to the rapid emergence of multidrug-resistant (MDR) pathogens. As conventional antibiotics lose efficacy, the search for new antimicrobial scaffolds has become a priority. Natural products from algae represent a critical frontier, as many organisms have evolved complex chemical defense mechanisms. The ability of *Fischerella* to produce bioactive extracts suggests its potential as a reservoir for new antibacterial agents capable of circumventing existing resistance mechanisms [4].

Environmental fluctuations frequently modulate the metabolic capacity of algae. Salinity is one of the most impactful environmental factors affecting the physiological and metabolic integrity of aquatic organisms. When exposed to salinity stress, algae must overcome osmotic imbalance and ionic toxicity, triggering complex molecular and physiological responses, including the production of reactive oxygen species (ROS) and activation of antioxidant defense mechanisms [6-8].

The response to salinity can lead to significant “metabolic reprogramming.” Photoautotrophic organisms, such as *Fischerella*, employ sophisticated adaptive strategies to maintain homeostasis under fluctuating osmotic conditions [3]. These adaptive responses can manifest as “metabolic memory”, where the biochemical profile is fundamentally altered following prior stress exposure. While continuous salinity causes growth inhibition, transient or controlled salinity regimes may serve as metabolic modulators, stimulating secondary metabolite diversification without causing irreversible physiological damage [1].

The rationale for selecting 1% sodium chloride (NaCl) concentration was based on previous optimization studies on *Fischerella* sp. FS 18 and related cyanobacteria, demonstrating that this concentration induces measurable stress responses without causing complete growth inhibition [4, 11, 12].

Research on algal acclimation has revealed that pretreatment strategies significantly influence photosynthetic performance and growth. Amirlatifi et al. [11] demonstrated that *Calothrix* sp. ISC 65 exhibits enhanced growth and pigment production under 1% salinity combined with low CO₂ and irradiance. Further work [12] showed that salinity pretreatment (160 mM NaCl for 96 h) in *Calothrix* sp. FS 65 can eliminate the lag phase and increase phycobiliprotein components by over 60%. Abbasi et al. [13] found that pH pretreatment in *Fischerella* sp. FS 18 affected phycobilisomes and altered photosystem ratios. Collectively, these findings underscore the species-specific and condition-dependent nature of cyanobacterial responses, with potential biotechnological applications [13-15].

Despite the recognized importance of environmental modulation, the specific impacts of different salinity exposure regimes—ranging from short-term pre-treatment to continuous exposure—on antibacterial efficacy and chemical richness of *Fischerella* remain insufficiently understood. Therefore, this study aimed to systematically evaluate the effects of four distinct salinity treatment regimes on growth performance, antibacterial activity, and biochemical profile of *Fischerella* sp. FS 18. By characterizing the relationship between salinity duration and metabolic output, this study sought to provide insights into optimal cultivation strategies for maximizing high-value bioactive metabolites.

Materials and Methods

Experimental design and treatments

To evaluate the impact of salinity stress, four distinct treatment groups were established:

1. Control: Cultivation in standard BG11 medium without salt addition.
2. Pre-24: 24-hour salinity pre-treatment (NaCl 1%) followed by standard conditions.
3. Pre-96: 96-hour salinity pre-treatment (NaCl 1%) followed by standard conditions.
4. NaCl: Continuous exposure to salinity (NaCl 1%) throughout the 44-day cultivation period.

Algal strain and culture conditions

The alga *Fischerella* sp. FS 18 was obtained from the Algae Collection of the Institute of Medicinal Plants,

Shahid Beheshti, Iran. The strain was maintained in BG11 medium and cultured in 500 mL Erlenmeyer flasks. The cultures were incubated for 44 days under a constant and continuous light intensity of 50 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at room temperature. To maintain specific experimental conditions, the cultures were grown without artificial aeration.

Growth measurement

Following the 44-day cultivation period, the contents of each algal suspension were harvested by centrifugation. The pellet was weighed to determine the total fresh biomass yield per culture.

Extraction procedure

The harvested fresh biomass was immediately subjected to a sequential extraction protocol to isolate compounds with varying polarities. First, the biomass was extracted with 50 mL of 96% ethanol (polar solvent) for a week at 4 °C. The mixture was centrifuged at $4000 \times g$ for 15 min, and the supernatant (ethanolic extract) was collected. The remaining pellet was then subjected to a second extraction with 20 mL of propanol (intermediate polarity) for a week at 4 °C. After centrifugation under the same conditions, the supernatant (propanolic extract) was collected. Finally, the residual pellet was extracted with 20 mL of butanol (less polar solvent) for a week at 4 °C. The supernatant (butanolic extract) was collected via centrifugation. All supernatants were concentrated under reduced pressure using a rotary evaporator. The dried extracts were then redissolved in 5 mL of 10% dimethyl sulfoxide (DMSO) [14, 15].

Bacterial strains

Antimicrobial activity of the extracts was evaluated against two bacterial strains: the gram-positive bacterium *Bacillus cereus* PTCC 1154 and the gram-negative bacterium *Pseudomonas aeruginosa* PTCC 1430. Both strains were obtained from the Microbial Culture Collection of the Iranian Research Organization for Science and Technology (IROST). The strain identities were verified via standard biochemical tests and 16S rRNA sequencing and were officially confirmed by IROST. Bacterial cultures were maintained on Mueller–Hinton agar (MHA; Merck, Germany) at 37 °C and subcultured every 48 h for routine use [14].

For comparative reference, *P. aeruginosa* PTCC 1430 was included as a standard gram-negative test organism. Similarly, *B. cereus* PTCC 1154 was used as a representative gram-positive strain for antibacterial screening.

Preparation of bacterial cultures

Bacterial suspensions were adjusted to the turbidity of a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. The standardized suspensions were then uniformly inoculated onto the surface of Mueller–Hinton agar plates (Ibresco, Italy).

Disk diffusion assay

The antimicrobial activity was screened using the standard disk diffusion method, according to [Clinical and Laboratory Standards Institute \(CLSI\) 2021 guidelines \[14\]](#). Sterile paper disks (6 mm diameter) were impregnated with 20 μL of each extract solution. The disks were then allowed to dry under a laminar flow hood for 15–20 minutes to ensure complete solvent evaporation. Subsequently, the disks were aseptically placed on the surface of inoculated Mueller–Hinton agar plates. A negative control disk was prepared by impregnating a disk with 20 μL of 10% DMSO, which confirmed the absence of any inhibitory effect from the solvent alone. Standard antibiotic disks (Pad Tan Teb Company, Iran) containing penicillin G (10 units), ampicillin (10 μg), penicillin V (10 units), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), and cefixime (10 μg) were used as positive controls. The plates were incubated at 37 °C for 24 h, after which the diameters of the inhibition zones were measured in millimeters. Antimicrobial activity was screened using the standard disk diffusion method, according to [CLSI \(2021\) guidelines \[14–18\]](#).

Gas chromatography-mass spectrometry (GC-MS) biochemical profiling

To minimize the risk of plasticizer contamination, all extractions and sample preparations were performed exclusively using glassware. Solvent blanks (pure solvents processed identically to samples) were also analyzed under the same GC-MS conditions to monitor for background contamination.

For biochemical analysis, a composite sample was prepared to ensure representativeness of the treatment groups. The composite sample was derived from five individual sub-samples of equal mass for each treatment, which were homogenized prior to extraction and subsequent GC-MS analysis. The chemical composition of the

extracts was analyzed using an Agilent 7890B gas chromatograph coupled with an Agilent 5977A mass selective detector (GC-MS system). Separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The operating conditions were as follows: injector temperature, 250 °C; carrier gas, helium, at a constant flow rate of 1 mL/min; ionization mode, electron impact (EI) at 70 eV. The oven temperature program was set as follows: initial temperature 60 °C (held for 5 min), increased to 300 °C at a rate of 5 °C min⁻¹, and finally held at 300 °C for 10 min. The total GC run time was 65 min. Sample injection volume was 1 µL in splitless mode. The constituents were identified by comparing their mass spectra with those stored in the National Institute of Standards and Technology library. The relative percentage of each compound was calculated based on the peak area integration using Agilent MassHunter software [19].

Statistical analysis

Data regarding three independent replicates for biomass production and antibacterial activity were analyzed using one-way analysis of variance (ANOVA). Prior to ANOVA, normality of data distribution was verified using the Shapiro–Wilk test, and homogeneity of variances was confirmed using Levene’s test. Differences between means were considered statistically significant at $P < 0.05$.

and were assessed using post-hoc comparisons by the Scheffe test. All statistical analyses were performed using SPSS software (version 26). Figures were prepared using Excel (version 2022).

Results

Growth performance

The growth of *Fischerella* sp. FS 18 (measured in g/L) was evaluated across four treatments after 44 days of cultivation. The highest biomass production was observed in the control group, followed closely by the Pre-24 treatment. Both the Pre-96 and NaCl treatments showed lower growth values compared to the control and Pre-24 groups (Figure 1).

Statistically, the Pre-24 treatment is marked with the letter ‘a’, indicating no significant difference from the control group (which lacks a label). The NaCl treatment is marked with ‘b’, and the Pre-96 treatment is marked with ‘c’, indicating that these two groups are significantly different from each other and from the other treatments. Error bars represent the standard deviation/error for each data point.

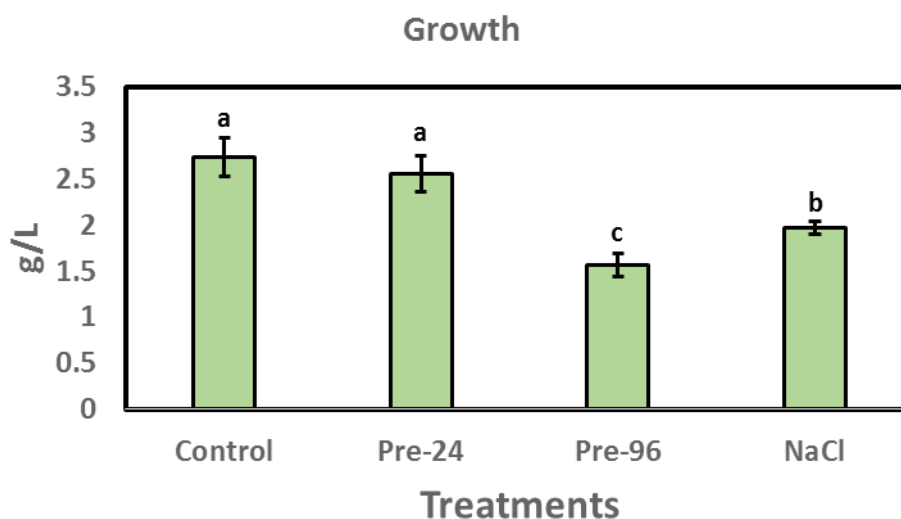


Figure 1. Effect of different salinity treatments and pre-treatments on the growth (biomass yield) of *Fischerella* sp. FS 18 microalgae after 44 days of cultivation

Treatments: Control (salt-free medium), Pre-24 (24 h pre-treatment with 1% NaCl), Pre-96 (96 h pre-treatment with 1% NaCl), and NaCl (continuous exposure to 1% NaCl). Data are presented as Mean ± SD from three independent replicates (n=3), with error bars representing SD. Different letters above the bars indicate statistically significant differences between treatments at $P \leq 0.05$. The negative control (10% DMSO) showed no inhibition zone (0 mm) against both bacterial strains (data not shown).

Treatments: Control (salt-free medium), Pre-24 (24 h pre-treatment with 1% NaCl followed by transfer to salt-free medium), Pre-96 (96 h pre-treatment with 1% NaCl followed by transfer to salt-free medium), and NaCl (continuous exposure to 1% NaCl). Data are presented as the Mean $\pm$ SE. Different letters above the bars indicate statistically significant differences between treatments at the $P \leq 0.05$ level.

Antibacterial activity and antibiotic resistance profile

The antibacterial activity of *Fischerella* sp. FS 18 extracts (butanolic, ethanolic, and propanolic) obtained from different salinity treatments (Control, Pre-24, Pre-96, and NaCl) were tested against two bacterial species: the gram-positive *B. cereus* and the Gram-negative *Pseudomonas aeruginosa*.

Antibiotic susceptibility profile

Both *B. cereus* and *P. aeruginosa* exhibited resistance to all seven tested commercial antibiotics, penicillin V, penicillin G, ampicillin, cefotaxime, ceftriaxone, ceftazidime, and cefixime, according to the criteria and guidelines of the CLSI 2021. The diameters of the inhibition zones formed around the antibiotic disks were smaller than the reference values established by CLSI (Table 1) [19-22].

Antibacterial activity of the *Fischerella* sp. FS 18 extracts

The ethanolic and propanolic extracts of *Fischerella* sp. FS 18 showed no zone of inhibition against either *B. cereus* or *P. aeruginosa* under any of the tested conditions. Similarly, the butanolic extract exhibited no antibacterial activity against *P. aeruginosa*. The negative

control (10% dimethyl sulfoxide [DMSO]) consistently produced no inhibition zone (0 mm) against both bacterial strains, confirming that the solvent itself had no antibacterial activity at the concentration used.

The lack of activity observed in the ethanolic and propanolic extracts suggests that the bioactive compound(s) responsible for the antibacterial effect are likely mid-to-non-polar in nature and are optimally extracted using butanol, which has appropriate polarity characteristics for recovering such metabolites [23, 24]. This solvent-dependent activity pattern is consistent with previous studies showing that n-butanol often facilitates the recovery of a broader spectrum of active secondary metabolites than shorter-chain alcohols, such as ethanol or propanol.

Furthermore, the absence of activity against *P. aeruginosa* (gram-negative) across all extracts, including the butanolic fraction, is likely attributable to the structural characteristics of the gram-negative cell envelope. Unlike gram-positive bacteria such as *B. cereus*, Gram-negative bacteria possess an additional outer membrane rich in lipopolysaccharides (LPS) that acts as a formidable permeability barrier, limiting the access of many hydrophobic and macromolecular bioactive compounds to their intracellular targets [14, 19-22]. This differential susceptibility is a well-documented phenomenon in antimicrobial research. Further, it supports the hypothesis that the active compound(s) in the butanolic extract are mid-to-non-polar in nature, which may be able to penetrate the thick peptidoglycan layer of gram-positive bacteria but are hindered by the outer membrane of gram-negative organisms.

Notably, the tested bacterial strains exhibited MDR to all beta-lactam antibiotics examined (Table 1); however, the butanolic extract of *Fischerella* sp. FS 18 retained

Table 1. Antibiotic resistance profile of the tested bacterial strains against selected commercial antibiotics

Antibiotics	<i>B. cereus</i>	<i>P. aeruginosa</i>
Penicillin V	R	R
Penicillin G	R	R
Ampicillin	R	R
Cefotaxime	R	R
Ceftriaxone	R	R
Ceftazidime	R	R
Cefixime	R	R

R: Resistance of the bacteria to the respective antibiotic according to the disc diffusion assay.

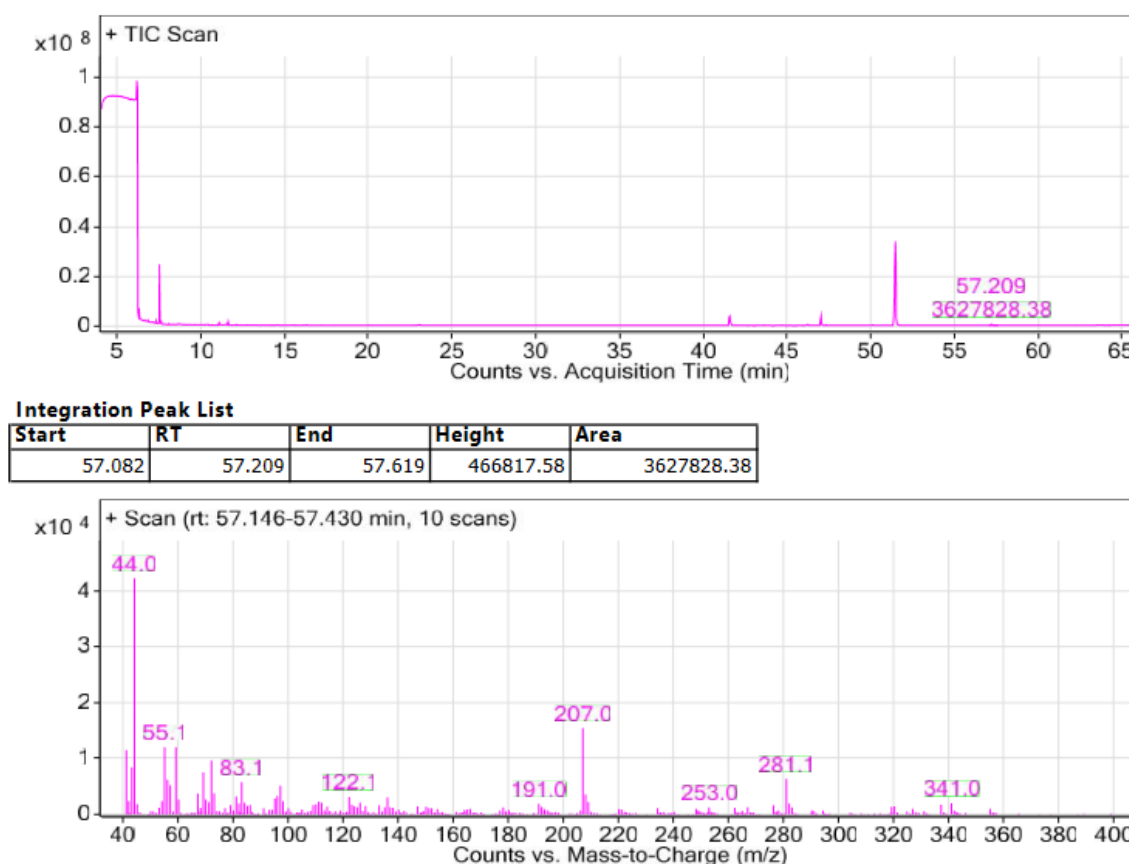


Figure 2. GC-MS profile of the prominent phthalate derivative identified in the extract of *Fischerella* sp. FS 18, grown under Pre-24 treatment

Note: Top: Total ion chromatogram (TIC) scan displaying the elution profile, highlighting the target compound peak at retention time (RT) of 51.47 minutes (corresponding to bis(2-ethylhexyl) phthalate, DEHP); Middle: Integration peak parameter table specifying the start/end times, peak height, and peak area; Bottom: Mass spectrum scan (m/z range of 40–400) at RT = 51.146–51.430 minutes illustrating the characteristic ion fragmentation pattern of the identified phthalate ester (di(2-ethylhexyl) phthalate, DEHP)

antibacterial activity against *B. cereus*. This observation highlights the potential of this cyanobacterial extract as a source of alternative therapeutic agents against MDR gram-positive pathogens.

GC-MS analysis

A comprehensive GC-MS profiling of the butanolic extracts identified a total of 62, 69, 81, and 61 chemical constituents for the control, Pre-24, Pre-96, and NaCl treatments, respectively. While numerous compounds were detected, only those with a relative abundance of $\geq 1\%$ were considered major components and are detailed in Tables 2, 3, 4, and 5.

GC-MS analysis of the butanolic extract under control conditions

Seven major compounds (with individual abundances $\geq 1\%$) were identified. Other minor compounds, each constituting less than 1% of the total peak area, collectively accounted for 10.09% of the extract composition. The dominant compound in the control extract was bis (2-ethylhexyl) phthalate, which was detected at a retention time (RT) of 51.47 minutes and accounted for 60.17% of the total peak area. The second most abundant compound was diethoxydimethylsilane at RT 7.55 minutes with a relative abundance of 14.21%. Other detected compounds in smaller proportions included hexadecanoic acid, butyl ester at RT 41.59 min (6.41%); octadecanoic acid, butyl ester at RT 47.038 min (4.93%); 3-pentanol, 2,2-dimethyl at RT 6.32 min (1.94%); dibutoxy(dimethyl)silane at RT 11.64 min (1.19%); trans-13-docosenamide at RT 57.20 min (1.06%).

Table 2. Chemical compounds identified in the butanolic extract of *Fischerella* sp. FS 18 microalgae under control conditions (salt-free) using GC-MS analysis

Control					
Compound	RT (min)	CAS Number	Formula	Qual (%)	Area (%)
Bis(2-ethylhexyl) phthalate	51.47	117-81-7	C ₂₄ H ₃₈ O ₄	94	60.17
Silane, diethoxydimethyl-	7.55	78-62-6	C ₆ H ₁₆ O ₂ Si	89	14.21
Hexadecanoic acid, butyl ester	41.59	111-06-8	C ₂₀ H ₄₀ O ₂	93	6.41
Octadecanoic acid, butyl ester	47.038	123-95-5	C ₂₂ H ₄₄ O ₂	95	4.93
3-Pentanol, 2,2-dimethyl	6.32	594-84-3	C ₇ H ₁₆ O	90	1.94
Dibutoxy(dimethyl)silane	11.64	1591-02-2	C ₁₀ H ₂₄ O ₂ Si	88	1.19
trans-13-Docosenamide (Erucamide)	57.2	112-84-5	C ₂₂ H ₄₃ NO	90	1.06
Other compounds					10.09

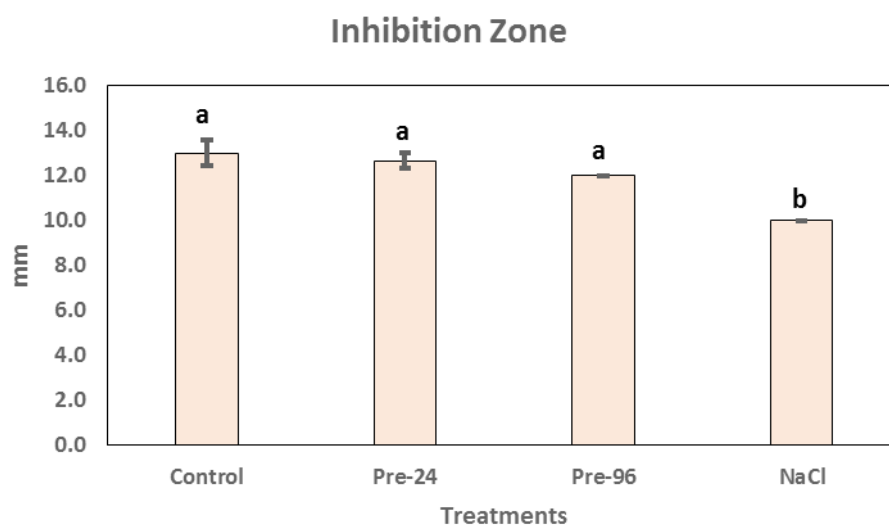
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Abbreviations: RT: Retention time (minutes); %: Relative percentage of the compound based on peak area; GC-MS: Gas chromatography-mass spectrometry.

Note: Other compounds represent a mixture of compounds, each with a relative abundance of less than 1%.

The GC-MS profile of the butanolic extract of *Fischerella* sp. FS 18 subjected to the Pre-24 salinity treatment is presented in Table 3. Seven major chemical compounds with relative abundances of $\geq 1\%$ were identified, while minor compounds (each $< 1\%$) collectively constituted 12.11% of the total peak area. The main component identified in this treatment was bis (2-ethylhexyl) phthalate at an RT of 50.51 minutes, accounting for 53.80% of the extract (Table 3 and Figure 2). The second most prominent compound was 1,3-propanediol, TBDMS de-

rivative at RT 7.58 minutes with a relative abundance of 16.82%. The remaining major compounds identified in this extract were hexadecanoic acid, butyl ester at RT 41.59 min (6.57%); octadecanoic acid, butyl ester at RT 47.037 min (5.47%); 1-butanol, 2-methyl- RT 6.38 min (3.05%); trans-(2-chlorovinyl)dimethylethoxysilane at RT 11.10 min (1.17%); 3-pentanol, 2,4-dimethyl- at RT 6.88 min (1.01%).



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Figure 3. Antibacterial activity of the butanolic extract of *Fischerella* sp. FS 18 microalgae cultured under different salinity treatments against *B. cereus*, measured by the zone of inhibition (mm)

Table 3. Chemical compounds identified in the butanolic extract of *Fischerella* sp. FS 18 microalgae under Pre-24 conditions (24 h salt pre-treatment) using GC-MS analysis

Pre-24					
Compound	RT (min)	CAS Number	Formula	Qual (%)	Area (%)
Bis(2-ethylhexyl) phthalate	50.51	117-81-7	C ₂₄ H ₃₈ O ₄	94	53.8
1,3-propanediol, TBDMS derivative	7.58	55745-90-9	C ₉ H ₂₂ O ₂ Si	91	16.82
Hexadecanoic acid, butyl ester	41.59	111-06-8	C ₂₀ H ₄₀ O ₂	95	6.57
Octadecanoic acid, butyl ester	47.037	123-95-5	C ₂₂ H ₄₄ O ₂	94	5.47
1-butanol, 2-methyl-	6.38	137-32-6	C ₅ H ₁₂ O	93	3.05
Trans-(2-chlorovinyl)dimethylethoxysilane	11.1	5356-84-3	C ₆ H ₁₃ ClOSi	90	1.17
3-pentanol, 2,4-dimethyl-	6.88	1620-98-0	C ₇ H ₁₆ O	91	1.01
Other compounds					12.11

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Abbreviations: RT: Retention time (minutes); %: Relative percentage of the compound based on peak area; GC-MS: Gas chromatography-mass spectrometry.

Note: Other compounds represent a mixture of compounds, each with a relative abundance of less than 1%.

GC-MS analysis of the butanolic extract under Pre-96 treatment

The chemical profile of the butanolic extract of *Fischerella* sp. FS 18 after 96 hours of salt pre-treatment

(Pre-96) was determined by GC-MS analysis (Table 4). Eight major compounds with relative abundances $\geq 1\%$ were identified, while minor compounds (each $< 1\%$) accounted for 10.69% of the total peak area. In this treatment, diethoxydimethylsilane was the most abundant

Table 4. Chemical compounds identified in the butanolic extract of *Fischerella* sp. FS 18 microalgae under Pre-96 conditions (96 h salt pre-treatment) using GC-MS analysis

Pre-96					
Compound	RT (min)	CAS Number	Formula	Qual (%)	Area (%)
Silane, diethoxydimethyl- (diethoxydimethylsilane)	7.61	78-62-6	C ₆ H ₁₆ O ₂ Si	89	30.98
Bis(2-ethylhexyl) phthalate	51.44	117-81-7	C ₂₄ H ₃₈ O ₄	94	28.61
2,2-dimethyl-3-hydroxypropionaldehyde (hydroxypivalaldehyde)	5.37	597-31-9	C ₅ H ₁₀ O ₂	90	9.71
Hexadecanoic acid, butyl ester	41.59	111-06-8	C ₂₀ H ₄₀ O ₂	93	8.72
Octadecanoic acid, butyl ester	47.039	123-95-5	C ₂₂ H ₄₄ O ₂	94	6.66
3-pentanol, 2,2-dimethyl-	6.38	594-84-3	C ₇ H ₁₆ O	93	1.86
Silane, dimethylisobutoxybutoxy-	11.67	18132-64-4	C ₁₀ H ₂₄ O ₂ Si	90	1.39
Trans-(2-Chlorovinyl)dimethylethoxysilane	11.1	5356-84-3	C ₆ H ₁₃ ClOSi	89	1.38
Other compounds					10.69

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Abbreviations: RT: Retention time (minutes); %: Relative percentage of the compound based on peak area; GC-MS: Gas chromatography-mass spectrometry.

Note: Other compounds represent a mixture of compounds, each with a relative abundance of less than 1%.

Table 5. Chemical compounds identified in the butanolic extract of *Fischerella* sp. FS 18 microalgae under continuous NaCl conditions (1% salt treatment) using GC-MS analysis

NaCl (1%)					
Compound	RT (min)	CAS Number	Formula	Qual (%)	Area (%)
Bis(2-ethylhexyl) phthalate	51.51	117-81-7	C ₂₄ H ₃₈ O ₄	96	60.74
Silane, diethoxydimethyl-	7.55	78-62-6	C ₆ H ₁₆ O ₂ Si	89	15.2
Hexadecanoic acid, butyl ester	41.59	111-06-8	C ₂₀ H ₄₀ O ₂	92	5.36
1-Butanol, 2-methyl-, (S)-	6.32	1565-80-6	C ₅ H ₁₂ O	94	5.03
Octadecanoic acid, butyl ester	47.03	123-95-5	C ₂₂ H ₄₄ O ₂	95	4.1
Dibutoxy(dimethyl)silane	11.64	1591-02-2	C ₁₀ H ₂₄ O ₂ Si	88	1.02
Other compounds					8.55

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Abbreviations: RT: Retention time (minutes); %: relative percentage of the compound based on peak area; NaCl: Sodium chloride; GC-MS: Gas chromatography-mass spectrometry.

Note: Other compounds represent a mixture of compounds, each with a relative abundance of less than 1%.

compound, detected at an RT of 7.61 minutes and representing 30.98% of the total extract. The second most prominent compound was bis (2-ethylhexyl) phthalate at RT 51.44 minutes with a relative abundance of 28.61%. Other major components identified in the Pre-96 extract included:

2,2-dimethyl-3-hydroxypropionaldehyde at RT 5.37 min (9.71%); hexadecanoic acid, butyl ester at RT 41.59 min (8.72%); octadecanoic acid, butyl ester at RT 47.039 min (6.66%); 3-pentanol, 2,2-dimethyl- at RT 6.38 min (1.86%); silane, dimethylisobutoxybutoxy at RT 11.67 min (1.39%); trans-(2-chlorovinyl)dimethylethoxysilane at RT 11.10 min (1.38%).

GC-MS analysis of the butanolic extract under continuous NaCl treatment

The chemical composition of the butanolic extract of *Fischerella* sp. FS 18 cultured under continuous salinity stress (NaCl) was characterized using GC-MS (Table 5). Six major chemical compounds with relative abundances $\geq 1\%$ were identified, with minor constituents (each $< 1\%$) accounting for 8.55% of the total peak area.

The dominant component in this treatment was bis (2-ethylhexyl) phthalate at an RT of 51.51 minutes, constituting 60.74% of the total peak area. The second most abundant compound was diethoxydimethylsilane at RT 7.55 minutes, with a relative abundance of 15.20%. The remaining major compounds detected in the extract were: Hexadecanoic acid, butyl ester at RT 41.59 min (5.36%);

1-butanol, 2-methyl-, (S)- at RT 6.32 min (5.03%); octadecanoic acid, butyl ester at RT 47.03 min (4.10%); dibutoxy(dimethyl)silane at RT 11.64 min (1.02%).

Discussion

Impact of salinity stress and pre-treatments on *Fischerella* sp. FS 18 growth dynamics

In this study, the salinity regime clearly structured the growth outcome of *Fischerella* sp. FS 18 after 44 days. The salt-free control and the 24 h salt pre-treatment (Pre-24; followed by cultivation in salt-free medium) produced the highest biomass levels, whereas longer exposure history (Pre-96) and continuous salinity (NaCl) were associated with lower biomass at the endpoint. Such treatment-dependent growth patterns are consistent with the general physiological response of cyanophytes and microalgae to osmotic stress: elevated external salinity imposes an immediate water efflux and turgor reduction, requiring rapid metabolic reallocation to restore osmotic balance and ion homeostasis, often at the expense of cell division and carbon partitioning to growth [3, 15]. A widely reported mechanism underlying salinity-related growth limitation is the energetic and reductant costs of acclimation. Cells must actively manage cytosolic ion composition—particularly avoiding toxic accumulation of Na⁺ while maintaining adequate K⁺—and simultaneously adjust osmolarity through the synthesis/accumulation of compatible solutes (e.g. glucosylglycerol, trehalose, sucrose, glycine betaine). These processes require ATP and carbon skeletons and

can divert photosynthate away from biomass production, especially under sustained salt exposure [3, 16]. In cyanophytes, salinity can also perturb photosynthetic electron transport and pigment organization, promoting oxidative stress if excitation energy exceeds downstream metabolic capacity; antioxidant and repair demands further increase maintenance costs and can contribute to reduced net growth under prolonged or continuous stress [2, 15]. The comparison between short (Pre-24) and longer (Pre-96) salt pre-treatments suggests that exposure duration prior to recovery in salt-free medium can determine whether acclimation responses are largely reversible or become more persistent. In many photosynthetic microorganisms, brief stress can trigger transient acclimation (e.g. induction of protective solutes and stress proteins) that may subside after returning to favorable conditions, allowing growth to rebound. By contrast, longer exposure can lead to deeper remodeling of metabolism and cellular composition (e.g. membrane lipid restructuring, sustained stress-protein expression, altered carbon allocation), which may not fully revert over the subsequent culture period, leaving a measurable legacy effect on biomass accumulation [3, 17]. This “stress memory” framing has been used in plant and microbial contexts to describe how prior stress history influences later performance even after the stressor is removed [18]. From an experimental standpoint, the endpoint biomass differences observed after 44 days align with an expectation that continuous salinity (NaCl) maintains a persistent energetic burden for osmotic/ionic regulation, while pre-treatments create a time-limited burden followed by a recovery phase whose effectiveness depends on stress duration. Similar patterns—reduced growth under continuous salinity and partial recovery after stress relief—have been widely documented across cyanophytes and other algae, although the magnitude and direction can be strongly strain- and condition-dependent [3, 17, 15]. Collectively, the growth results support the idea that short salinity exposure can be tolerated with limited long-term penalty, whereas longer pre-exposure or chronic salinity is associated with lower final biomass under the conditions tested.

Antibacterial potential of *Fischerella* sp. FS 18 butanolic extract against MDR bacteria

The rise in MDR bacterial pathogens, as demonstrated by the resistance of the *B. cereus* and *P. aeruginosa* strains in this study, poses a significant global threat to public health [14, 19]. The screening of the *Fischerella* sp. FS 18 butanolic extract against these resistant isolates revealed selective antimicrobial activity. Based on the CLSI [14], bacterial resistance against biological

extracts, regarding the disk diffusion assay, was defined as inhibition zones <9 mm, moderate sensitivity as 9–12 mm, and sensitivity as >12 mm. Consequently, in the present study, *B. cereus* showed sensitivity to Control and Pre-24, and moderate sensitivity to Pre-96 and NaCl algal extracts (Figure 3). Nevertheless, the extracts failed to show activity against the gram-negative *P. aeruginosa* (Table 1). This observation is consistent with established trends in antimicrobial research, where gram-negative bacteria often exhibit higher intrinsic resistance to external bioactive agents due to the presence of an outer membrane rich in lipopolysaccharides (LPS), which acts as a formidable permeability barrier to many secondary metabolites [20]. The successful inhibition of *B. cereus* by the butanolic extract, particularly in the face of widespread resistance to commercial antibiotics, such as Penicillin V, G, and various cephalosporins (cefotaxime, ceftriaxone, etc.), highlights the potential of the blue-green algae metabolites as alternative therapeutic agents. Blue-green algae, including *Fischerella* species, are recognized as prolific producers of diverse bioactive secondary metabolites, including fatty acids, alkaloids, and cyclic peptides, which often possess unique modes of action distinct from conventional antibiotics [14, 1, 9, 20–22]. The fact that the resistance profile of the test strains included multiple classes of cell-wall synthesis inhibitors suggests that the *Fischerella* sp. FS 18 metabolites likely exert their antibacterial effect through different targets or pathways. The specificity of the extract efficacy is further underscored by the solvent-dependency observed in this study. The finding that only the butanolic extract exhibited antimicrobial activity—while ethanolic and propanolic extracts were ineffective—suggests that the bioactive compounds are likely mid-to-non-polar in nature and are optimally extracted using the solvent properties of n-butanol. Solvent polarity plays a critical role in the extraction efficiency of bioactive compounds from algal biomass, and studies have consistently shown that butanol often facilitates the recovery of a broader spectrum of active secondary metabolites than shorter-chain alcohols, such as ethanol [23, 24]. Furthermore, the variations in the inhibition zones among the salinity treatments suggest that environmental factors modulate the production of these bioactive metabolites. While the continuous NaCl treatment resulted in a smaller inhibition zone compared to the control, the fact that activity was maintained across all pre-treatments suggests that the antimicrobial synthesis in *Fischerella* sp. FS 18 is robust, though potentially sensitive to the metabolic flux induced by salt stress. Future efforts aimed at identifying the specific molecules responsible for this activity, particularly the phthalates and fatty acid esters identified in

the GC-MS analysis, will be crucial to understanding the pharmacological potential of this microalga.

Salinity-induced changes in the chemical profile of *Fischerella* sp. FS 18

GC-MS analysis showed that salinity treatment altered the chemical composition of *Fischerella* sp. FS 18 extracts, both in the number of detected constituents and in their relative abundances. The total number of identified compounds increased from 62 in the control to 69 in Pre-24 and 81 in Pre-96, then declined to 61 under continuous NaCl treatment. This pattern suggests that short- to moderate-term salt exposure may stimulate broader metabolic diversification, whereas prolonged continuous salinity may restrict overall metabolite production to a narrower set of dominant compounds. In cyanophytes, environmental stress is well known to reshape secondary metabolism by redirecting carbon flux, modifying membrane composition, and activating protective biochemical pathways that support acclimation and survival [25, 26]. In the present study, regarding previous researchers [27, 28] several measures were taken to address the contamination concern. All extractions were performed using glassware, and solvent blanks were analyzed under identical GC-MS conditions; no phthalate peaks were detected in the blanks, supporting the biological origin of the detected compounds. Furthermore, the treatment-dependent variation in DEHP abundance (ranging from 28.61% to 60.74%) was inconsistent with a constant background contamination and instead suggested a biologically regulated response to salinity stress.

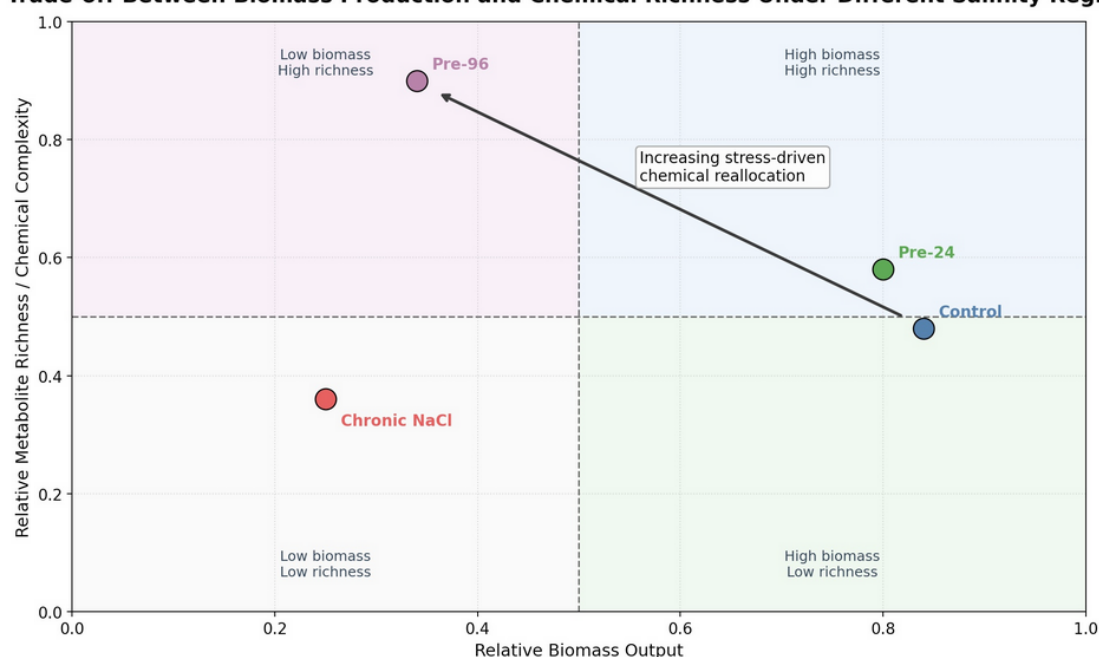
Nevertheless, this contamination-centered interpretation should not be generalized uncritically. Our previous findings showed that in *Euphorbia trigona*, treatment with the auxin naphthalene acetic acid elicited a remarkable metabolic reprogramming, increasing the content of bis(2-ethylhexyl) phthalate from 9.59% in control plants to 49.31% of the total extractable compounds, alongside increases in other bioactive fatty acids and terpenoids [29, 30]. This five-fold increase demonstrated that phthalate accumulation is a biologically regulated response to exogenous elicitors rather than passive environmental uptake. Similarly, we reported that in agave americana. Urea fertilizer treatment caused outstanding changes in the content of bis(2-ethylhexyl) phthalate, methyl palmitate, and methyl stearate [31]. Collectively, these findings across multiple species and treatments confirm that phthalates and related esters are endogenously produced plant metabolites whose profiles are dynamically altered by specific hormonal and nutritional elicitors, providing compelling evidence against their

exclusive characterization as anthropogenic pollutants. Babu and Wu (2010) [1] and Gharajeh et al. (2025) [32] provided direct evidence that several freshwater algae, including cyanophytes, can synthesize phthalate esters de novo, and that their release may increase under stress conditions. Accordingly, the occurrence of phthalate-related compounds in the GC-MS profile of *Fischerella* sp. FS 18 cannot be attributed solely to contamination and may, at least in part, reflect a genuine metabolic response to salinity stress and/or resource limitation (e.g. nutrient and CO₂ depletion during prolonged cultivation without aeration and without nutrient replenishment).

Beyond the major common compounds, the salinity treatments appeared to influence the balance of minor and intermediate constituents. The increase in compound richness under Pre-24 and especially Pre-96 may reflect stress-induced synthesis or accumulation of metabolites associated with osmotic adjustment, antioxidative defense, lipid remodeling, or degradation of primary metabolites into smaller derivatives detectable by GC-MS. Salt stress commonly affects fatty acid composition, hydrocarbon production, ester formation, and low-molecular-weight organic compounds in blue-green algae, as part of broader efforts to maintain membrane stability and cellular homeostasis [33, 26]. Such changes do not necessarily imply enhanced total productivity, because a stressed culture may generate a more chemically diverse extract even while exhibiting lower biomass. The quadrant map supports a metabolic trade-off interpretation, where pre-exposure duration appears to tune the balance between growth and diversified metabolite output (Figure 4).

The distinct profile observed in the continuous NaCl treatment is also biologically plausible. Under chronic salinity, cells often shift from flexible acclimatory responses toward a more constrained survival-oriented metabolic state, in which energy is prioritized for maintenance rather than broad biosynthetic output. This can reduce the diversity of detectable compounds and increase the relative prominence of a smaller number of constituents [3, 15]. In contrast, pre-treated cultures that were later returned to salt-free conditions may retain a metabolic imprint of earlier stress, allowing detection of a wider spectrum of compounds at harvest. Taken together, the GC-MS results indicate that salinity history influenced not only how much *Fischerella* sp. FS 18 grew, but also partitioned metabolism into chemically distinct outputs.

Trade-off Between Biomass Production and Chemical Richness Under Different Salinity Regimes



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Figure 4. Trade-off landscape between biomass and metabolite richness under distinct salinity regimes in *Fischerella* sp. FS 18

Note: Treatments were positioned in a quadrant space defined by biomass (g/L, x-axis) and metabolite richness/chemical diversity (number of detected compounds, y-axis). Data points represent actual values: Control (6.89 g/L, 62 compounds), Pre-24 (6.66 g/L, 69 compounds), Pre-96 (4.27 g/L, 81 compounds), and NaCl (3.82 g/L, 61 compounds). The distribution highlights a pattern in which Pre-24 aligns with relatively high biomass, Pre-96 aligns with elevated chemical diversity, and chronic salinity maps to reduced growth and diminished metabolite richness. This visualization summarizes the inferred allocation shift from growth-associated metabolism toward diversified secondary metabolite profiles under priming-like stress exposure.

Relationship between salinity stress and antibacterial metabolite production

An important outcome of this study was that salinity stress influenced not only growth and chemical composition, but also the antibacterial performance of *Fischerella* sp. FS 18 extracts. Although antibacterial activity against *B. cereus* was detected in all butanolic extracts, the inhibition zones varied among treatments, indicating that salt history modulated either the quantity, the composition, or the extractability of active metabolites. This finding is consistent with the broader concept that environmental stress can alter secondary metabolite biosynthesis in cyanophytes by redirecting metabolic fluxes toward protective or adaptive compounds, some of which may also possess antimicrobial properties [34, 35]. In photosynthetic microorganisms, salinity commonly triggers a cascade of physiological adjustments, including osmolyte synthesis, lipid remodeling, antioxidant defense, and changes in carbon allocation. Because secondary metabolites often arise from the same precursor pools used in central metabolism, any stress-induced shift in resource partitioning can directly affect

the production of bioactive compounds. Under moderate or transient stress, the induction of defense-related pathways may enhance synthesis of certain antimicrobial metabolites. However, under prolonged or severe stress, the energetic demands of survival and homeostasis may reduce the overall biosynthetic capacity for secondary products, or favor compounds primarily involved in stress tolerance rather than antibacterial defense [3, 15]. This framework may explain why antibacterial activity was retained but varied across treatments in the present study. The pre-treatment groups are particularly interesting in this regard. Since Pre-24 and Pre-96 cultures were only temporarily exposed to salt before being returned to normal medium, any subsequent antibacterial activity suggests that prior salinity exposure left a measurable metabolic legacy. Such a legacy effect may reflect persistent changes in enzymatic expression, precursor availability, or regulatory networks controlling secondary metabolism. Similar “stress memory” responses have been described in plants and microorganisms, where earlier stress episodes modify later metabolic behavior even after the stressor is removed [18]. In *Fischerella* sp.

FS 18, this may have enabled the cultures to preserve or reconfigure metabolite production patterns that still supported antibacterial activity at harvest. At the same time, caution is needed when inferring direct causation between individual GC-MS compounds and antimicrobial effects. The antibacterial activity of algal extracts frequently results from synergistic interactions among multiple compounds rather than from a single dominant constituent [36]. Moreover, the most abundant compound in the current GC-MS profiles, bis(2-ethylhexyl) phthalate, may not necessarily be the sole or even the principal antibacterial agent, especially given ongoing debate about its biological versus exogenous origin. Therefore, the observed relationship between salinity treatment and antibacterial activity was best interpreted at the extract level: salinity altered the overall biochemical landscape of *Fischerella* sp. FS 18, and these compositional shifts were associated with measurable differences in antibacterial efficacy against *B. cereus*.

Contribution of salinity to metabolic reprogramming and stress-induced plasticity in *Fischerella* sp. FS 18

The combined growth, antibacterial, and GC-MS findings indicated that *Fischerella* sp. FS 18 responds to salinity through a coordinated reprogramming of its metabolic networks. Such reprogramming reflects the intrinsic plasticity of cyanophytes, enabling them to adjust carbon allocation, energy distribution, and biosynthetic priorities in response to environmental constraints. In the present study, salinity exposure—whether transient (Pre-24, Pre-96) or continuous (NaCl)—appeared to activate metabolic pathways associated with osmotic adjustment, membrane remodeling, and stress defense, all of which can influence both primary and secondary metabolism [26, 3]. A central feature of salinity-driven metabolic plasticity is the energetic trade-off between growth and stress acclimation. Cellular resources must be diverted toward compatible solute synthesis, ion transport, chaperone production, and repair of stress-induced molecular damage. These adjustments may reduce carbon availability for growth pathways while simultaneously stimulating the synthesis of certain stress-associated metabolites, including fatty acid derivatives, esters, and phenolic compounds. This dual effect—reduced biomass but expanded chemical diversity—was reflected here by lower growth under Pre-96 and NaCl, yet a larger number of metabolites detected in the GC-MS profiles of the pre-treated samples, especially Pre-96. Evidence from other cyanophytes and microalgae suggests that stress can reorganize metabolite pools by modifying flux through key pathways such as the tricarboxylic acid (TCA) cycle,

glycolysis, fatty acid biosynthesis, and polyketide/peptide pathways [34, 15]. In *Fischerella* sp. FS 18, many of the identified metabolites belong to classes known to fluctuate under stress, including long-chain fatty acids, alcohols, esters, and phthalate-like compounds. These shifts may serve physiological roles in stress tolerance, such as stabilizing membranes, scavenging reactive oxygen species, or modulating the fluidity and permeability of cellular structures. Even minor compounds (<1% abundance) may play critical signaling or protective functions that do not require high concentration. Most notably, the pre-treatment groups displayed metabolic profiles that differed substantially from the control despite being grown for weeks after stress removal. Such long-lasting alterations suggest a degree of metabolic “memory” or imprinting. In microbial systems, this phenomenon emerges when prior stress exposure permanently or semi-permanently modifies gene expression patterns, enzyme pools, redox balance, or membrane composition, resulting in modified metabolic states even after return to optimal conditions [18]. The higher number of compounds in Pre-96 than in Pre-24 supports the idea that longer stress exposure leads to deeper remodeling and a more persistent metabolic shift. However, the continuous salt group (NaCl) showed the lowest metabolite richness among the stressed treatments. This aligns with reports that chronic salt exposure can constrain metabolic diversity, as cells prioritize essential survival functions over secondary metabolism when resources remain limited for extended periods [33]. In this scenario, metabolic flexibility becomes narrowed. Instead of producing a broad suite of compounds, the culture maintains a more minimal metabolic profile dominated by a few stable or stress-resistant constituents. Overall, the data indicate that salinity acts as a strong ecological and biochemical driver by reorganizing *Fischerella* sp. FS 18 metabolism and thereby influencing growth, chemical diversity, and antibacterial activity. The results emphasize that even brief stress episodes can induce lasting metabolic changes, while continuous stress narrows metabolic options. Such plasticity highlights the potential of controlled stress modulation as a tool to enhance or tailor the production of valuable metabolites in blue-green algae.

Potential immunological relevance of salinity-induced metabolic reprogramming

The observed metabolic trade-off between biomass accumulation and chemical richness, particularly the shift toward a more diverse metabolite profile in the Pre-96h regime (Figure 4), holds significant implications beyond algal physiology, potentially extending into the realm of

innate immunity and immunoregulation. In cyanobacteria, such as *Fischerella* sp., salinity-induced stress is inherently linked to ROS dynamics and cellular redox signaling [7, 14, 19-23]. From a translational perspective, such redox-driven metabolic reprogramming can generate bioactive secondary metabolites, including fatty acids, phenolics, and alkaloids, which interact with mammalian immune pathways. While our study primarily identified antibacterial activity against *B. cereus* in the butanolic fraction, the broader chemical diversification observed under pre-exposure regimes suggests a possible enrichment of immunomodulatory scaffolds. Many cyanobacterial metabolites act as ligands or modulators for redox-sensitive signaling hubs, such as the nuclear factor erythroid 2-related factor 2 or nuclear factor-kappa B pathways, which are central to regulating inflammatory responses [8, 14, 19-23]. Therefore, the metabolic imprinting induced by salinity priming may serve as a bioprocessing strategy to enhance the yield of compounds capable of modulating innate immune cell activation or cytokine production. However, confirming whether these salinity-modulated extracts possess specific anti-inflammatory or immunoregulatory properties—such as the suppression of pro-inflammatory mediators (e.g. tumor necrosis factor- α [TNF- α], interleukin-6 [IL-6]) or the induction of regulatory phenotypes—requires systematic validation in macrophage or lymphocyte models. Integrating these findings into an “infection-inflammation” axis provides a framework for future studies to evaluate *Fischerella* sp. FS 18 as a source of novel immunotherapeutic leads.

Biotechnological significance of salinity modulation in *Fischerella* sp. FS 18

The findings of this study suggest that salinity modulation can be considered a practical strategy for directing the physiological and biochemical output of *Fischerella* sp. FS 18 toward specific biotechnological goals. By altering the salt exposure regime, it was possible to influence biomass formation, antibacterial activity, and metabolite composition simultaneously. This is particularly relevant in cyanophyte biotechnology, where culture conditions are often manipulated not only to maximize growth, but also to enhance the accumulation of target bioactive compounds. In this context, salinity acts as a controllable environmental factor capable of shifting *Fischerella* sp. FS 18 from a primarily growth-oriented state toward a more stress-responsive metabolic condition with altered secondary metabolite production [3, 26]. From a production perspective, the results indicate that different salinity regimes may serve different industrial purposes. The control and Pre-24 treatments, which supported higher biomass accumulation, may be more suitable when the

goal is bulk biomass production or when metabolite yield depends largely on culture density. In contrast, the Pre-96 treatment, despite lower biomass, generated the highest number of detected compounds, suggesting that moderate prior stress may enhance chemical complexity and potentially broaden the range of recoverable metabolites. The continuous NaCl treatment, while less favorable for growth and metabolite richness, still maintained detectable antibacterial activity, showing that even under chronic osmotic stress, *Fischerella* sp. FS 18 retains the capacity to produce bioactive substances. This type of growth-versus-metabolite trade-off is common in algal biotechnology and is often exploited through two-stage cultivation systems, where biomass is first maximized under favorable conditions, and stress is then applied to stimulate desired product formation [37, 38]. The selective activity of the butanolic extract against *B. cereus* also adds to the applied value of the organism. Blue-green algae are increasingly explored as renewable sources of antimicrobial compounds, especially in the context of rising antibiotic resistance and demand for new natural-product scaffolds [34]. Although the present study did not identify the exact active principle, it demonstrated that *Fischerella* sp. FS 18 can produce solvent-extractable antibacterial metabolites under both normal and salt-modulated conditions. This opens the possibility of optimizing culture stress to improve either potency or chemical diversity before downstream purification and characterization. In addition, the persistence of treatment-specific metabolic patterns after transient salt exposure has practical importance. This suggests that permanent exposure to saline medium may not be necessary to obtain altered metabolite profiles; instead, a short pre-treatment may be sufficient to induce desirable biochemical changes while avoiding the full growth penalty of continuous stress. Such an approach could reduce production costs, simplify medium management, and improve overall process efficiency. In applied terms, transient salinity priming may represent a more economical and scalable strategy than chronic salt cultivation, particularly if future studies confirm enhanced yields of target antimicrobial or industrial metabolites. Overall, this study supports the view that salinity is not merely a stress factor limiting cyanophyte growth, but also a useful regulatory tool for metabolic engineering at the cultivation level. By carefully adjusting the duration and continuity of salt exposure, it may be possible to balance productivity and metabolite quality in *Fischerella* sp. FS 18. Future work should focus on quantifying specific target compounds, determining productivity on a per-culture-volume basis, and validating biological activities of purified fractions to translate these observations into a robust biotechnological platform.

Limitations of the study

This study had several limitations. First, antibacterial screening was performed only against two bacterial strains (one gram-positive and one gram-negative); therefore, the broader spectrum of activity remains to be determined. Second, although the butanolic extract showed promising activity, the specific compound(s) responsible for the antibacterial effect were not purified or identified; bioassay-guided fractionation is required to confirm the active principle. Third, the immunological relevance discussed in this paper is speculative and based on indirect evidence; direct validation in cell-based or animal models is required. Fourth, while solvent blanks and glassware were used to minimize contamination, the possibility of trace phthalate contamination from other sources cannot be entirely excluded. Finally, the study was conducted under laboratory conditions, and scalability to larger production systems requires further investigation.

Conclusion

The salinity regime markedly influences biomass production, antibacterial activity, and metabolite composition in *Fischerella* sp. FS 18. Our findings indicate that while sustained osmotic stress imposes a physiological cost on growth, short-term salinity priming can effectively modulate metabolic output. This duration-dependent response highlights a trade-off between primary metabolism (growth) and secondary metabolic diversification. Antibacterial assays showed selective inhibitory activity in butanolic extracts against *B. cereus*, supporting the potential of *Fischerella* sp. FS 18 as a source of bioactive metabolites against gram-positive targets. GC-MS profiling further confirmed that salinity regimes, particularly the 96-h pre-treatment, induce broader chemical richness. Although bis(2-ethylhexyl) phthalate was the dominant constituent, its biological origin remains to be confirmed through more rigorous control measures. In summary, salinity serves as both a growth-limiting factor and a metabolic modulator. Controlled salinity exposure, particularly through short-term priming, may be utilized to enrich metabolite profiles for future bioactivity screening. Beyond antibacterial properties, the identified metabolic shifts may have broader relevance for redox-related signaling and immunological research in the future; however, direct immunomodulatory effects were not investigated in the present study and require experimental validation. Future work should focus on bioassay-guided fractionation and the purification of active compounds to fully characterize their therapeutic and immunomodulatory potential.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization, supervision, review and editing: Shadman Shokravi and Aryan Sateei; Methodology: Aryan Sateei, Hamidreza Pordeli, Mehdi Ebadi; Investigation and data analysis: Aryan Sateei and Rahil Sadat Rahimhosseini; Writing the original draft: Aryan Sateei; Data collection: Rahil Sadat Rahimhosseini and Aryan Sateei.

Conflicts of interest

The authors declared no conflict of interest.

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