Siderophore-Mediated Growth Enhancesment in Arthrospira platensis

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Abstract

Iron availability is a critical factor influencing the growth and productivity of microalgae and cyanobacteria. In the present study, the effect of siderophore-producing bacteria, Pseudomonas lundensis and Advenella mimigardefordensis, and their crude siderophore extracts was evaluated on the growth of Arthrospira platensis. Cultures were grown in Zarrouk's medium under controlled laboratory conditions and subjected to six treatment groups, including iron-limited control, EDTA supplementation, bacterial inoculation, and siderophore addition. Growth was monitored over 7 days using optical density, wet biomass, and direct microscopic count, while iron uptake was assessed using the Ferrozine assay.

Siderophore-treated cultures exhibited significantly enhanced growth compared to EDTA and control treatments, with Pseudomonas lundensis siderophore showing the highest increase in optical density, biomass, and cell density. Two-way ANOVA revealed highly significant effects ($p < 0.0001$) of time, treatment, and their interaction on all growth parameters. Iron uptake analysis showed a marked reduction in residual iron concentration in siderophore-treated samples (~0.5–0.6 mg/L) compared to EDTA (~1.2 mg/L) and control (~1.8 mg/L), indicating superior iron chelation and uptake efficiency. Overall, the results demonstrate that siderophores significantly enhance iron bioavailability and promote growth of A. platensis, highlighting their potential application in improving microalgal cultivation and productivity.

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Introduction:

Microalgae and cyanobacteria are key primary producers in aquatic ecosystems and contribute significantly to global carbon cycling and nutrient dynamics (Araujo et al., 2022). Among them, *Arthrospira platensis* (Spirulina) is widely cultivated due to its high protein content, essential amino acids, vitamins, and bioactive compounds such as phycocyanin, making it valuable in nutraceutical, pharmaceutical, and aquaculture industries (Radzki et al., 2013).

A major limitation in Spirulina cultivation is the low bioavailability of iron, an essential micronutrient involved in photosynthesis, respiration, and enzymatic processes (Andrews et al., 2013). Under alkaline and aerobic conditions, iron predominantly exists as insoluble Fe^{3+}, restricting its uptake and consequently reducing biomass productivity (Ibisanmi et al., 2011; Herraiz-Borreguero et al., 2016).

Siderophores are low molecular weight iron-chelating compounds produced by microorganisms under iron-deficient conditions, which enhance iron solubility and availability (Miethke & Marahiel, 2007; Khelkal, 2016). In aquatic systems, siderophore-producing bacteria can indirectly support phytoplankton growth, as microalgae are capable of utilizing siderophore-bound iron through uptake or reductive mechanisms (Shaked & Lis, 2012).

Despite this potential, the application of siderophores in enhancing Spirulina cultivation remains limited. Therefore, the present study evaluates the effect of siderophore-producing bacteria, *Pseudomonas lundensis* and *Advenella mimigardefordensis*, and their crude extracts on the growth of *Arthrospira platensis* using multiple growth parameters.

Materials and Methods:

Culture Maintenance and Inoculum Preparation

Axenic cultures of *Arthrospira platensis* were maintained in Zarrouk's medium. Actively growing cultures in the exponential phase were used as inoculum. Prior to experimentation, cultures were acclimatized under standard laboratory conditions for at least 3–5 days to ensure uniform physiological state (Andersen, 2005).

Preparation of Zarrouk's medium

Zarrouk's medium was prepared using analytical-grade reagents and consisted of (per liter): sodium bicarbonate ($NaHCO_3$, 16.8 g) as the primary carbon source, sodium nitrate ($NaNO_3$, 2.5 g) as the nitrogen source, dipotassium hydrogen phosphate (K_2HPO_4, 0.5 g) as the phosphorus source, sodium chloride (NaCl, 1.0 g), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$, 0.2 g), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, 0.04 g), and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, 0.01 g) as the iron source. Additionally, 1 mL of a micronutrient solution containing trace elements such as boron, manganese, zinc, molybdenum, and copper was added. The pH of the medium was adjusted to 9.5–10.0 prior to sterilization, making it suitable for the growth of *Arthrospira platensis* (Andersen, 2005; Rajasekaran et al., 2016). For experimental treatments, EDTA was omitted to create iron-limited conditions unless specified otherwise.

Culture Conditions

Cultivation of *Arthrospira platensis* was carried out in sterile 500 mL Erlenmeyer flasks containing 250 mL of Zarrouk's medium, inoculated with 5% (v/v) actively growing culture. The cultures were maintained under controlled laboratory conditions at a temperature of 25 ± 2°C with a 12 h light/12 h dark photoperiod and a light intensity of around 4000 lux provided by white LED illumination. Continuous sterile aeration was supplied to ensure proper mixing and gas exchange, and cultures were gently agitated periodically to prevent settling of filaments and to maintain uniform growth. The experimental duration was 7 days (Andersen, 2005; Theerapisit et al., 2023).

Preparation of Siderophore Treatments

Siderophore treatments were prepared using the bacterial strains *Pseudomonas lundensis* and *Advenella mimigardefordensis*. Bacterial inocula were prepared by growing the strains in suitable broth media for 24 h, followed by adjustment of cell density to an optical density of OD_{600} = 0.1. Crude siderophore extracts were obtained from iron-deficient culture supernatants using liquid–liquid extraction methods, and were applied at a final concentration of 100 µg/mL (Rai et al., 2016). For comparison, EDTA was used as a standard chelator control at a concentration of 80 µg/mL.

Experimental Design

Six treatment groups were established:

1. Medium − EDTA (iron-limited control)
2. Medium + EDTA (80 µg/mL)
3. Medium + *Advenella mimigardefordensis* culture
4. Medium + *A. mimigardefordensis* siderophore (100 µg/mL)
5. Medium + *Pseudomonas lundensis* culture
6. Medium + *P. lundensis* siderophore (100 µg/mL)

All treatments were performed in triplicate (n = 3).

Growth Analysis

Growth of the cultures was assessed using spectrophotometric analysis, wet biomass estimation, and direct microscopic count to obtain a comprehensive evaluation of growth dynamics.

a. Optical Density (OD_{680})

For spectrophotometric analysis, optical density was measured daily at 680 nm using a UV–Vis spectrophotometer, corresponding to the absorption peak of chlorophyll *a*. A 1 mL aliquot of well-mixed culture was aseptically withdrawn, transferred into a clean cuvette, and absorbance was recorded against the respective medium blank (Adam et al., 2022; Theerapisit et al., 2023).

b. Wet Biomass Estimation

Wet biomass was determined gravimetrically by filtering 10 mL of culture through pre-weighed Whatman No.1 filter paper. The retained biomass was gently blotted to remove excess moisture and immediately weighed. Biomass accumulation was calculated as the difference between final and initial filter paper weights and expressed as mg/10 mL (Becker, 1994; Adam et al., 2022). This approach provided a direct and quantitative measure of biomass production, particularly suitable for filamentous organisms such as *Arthrospira platensis*

c. Direct Microscopic Count

Cell density was determined using a Sedgwick–Rafter counting chamber. A 1 mL aliquot of well-mixed culture was loaded into the chamber and observed under a compound microscope at 10X or 40X magnification. Cells or filaments were counted across multiple fields, and the average count was used to calculate cell density (cells/mL) using standard conversion factors (Adam et al., 2022; Theerapisit et al., 2023).

d. Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using two-way analysis of variance (ANOVA) to evaluate the effects of treatment and time, as well as their interaction on the

measured parameters. Post hoc comparisons between groups were performed using Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$.

Determination of Iron Uptake

Iron uptake was determined using the Ferrozine assay. At the end of the incubation period (day 7), 10 mL culture samples were collected and centrifuged at 10,000 rpm for 10 min to obtain clear supernatant. An aliquot (1 mL) of the supernatant was reacted with Ferrozine reagent, and where required, ferric iron (Fe^{3+}) was reduced to ferrous form prior to analysis. The reaction mixture was incubated at room temperature for 10 min, and absorbance was measured at 562 nm using a UV–Vis spectrophotometer (Stookey, 1970; Viollier et al., 2000).

Residual iron concentration was calculated using a standard curve prepared with $FeSO_4$ and expressed in mg/L. Lower absorbance values indicate higher iron uptake or chelation efficiency, whereas higher absorbance corresponds to greater residual iron in the medium.

Results and Discussion:

Effect of Siderophore-Producing Bacteria and Siderophores on Growth of *Arthrospira platensis*

Optical Density (OD_{680})

The growth of *Arthrospira platensis*, measured as optical density at 680 nm, showed a gradual and consistent increase across all treatments over the 7-day incubation period (Figure 1). However, the extent of growth varied significantly among treatments. Siderophore-associated treatments exhibited comparatively higher growth, with *Pseudomonas lundensis* siderophore showing the maximum increase, followed by *P. lundensis* bacterial culture and *Advenella mimigardefordensis* siderophore treatment. The EDTA-treated samples showed moderate growth, whereas the iron-limited control exhibited the lowest absorbance throughout the experimental period.

Two-way ANOVA revealed a highly significant effect of both time and treatment on growth. The interaction between time and treatment was also highly significant ($F(30,84) = 15.02$, $p < 0.0001$), indicating that the effect of treatments varied across different incubation periods. The row factor (time) contributed the highest proportion of variation (79.17%) and was highly significant ($F(6,84) = 1349$, $p < 0.0001$), demonstrating that growth was strongly dependent on incubation duration. The column factor (treatment) also showed a significant effect ($F(5,84) = 319.1$, $p < 0.0001$), confirming that different treatments significantly influenced Spirulina growth.

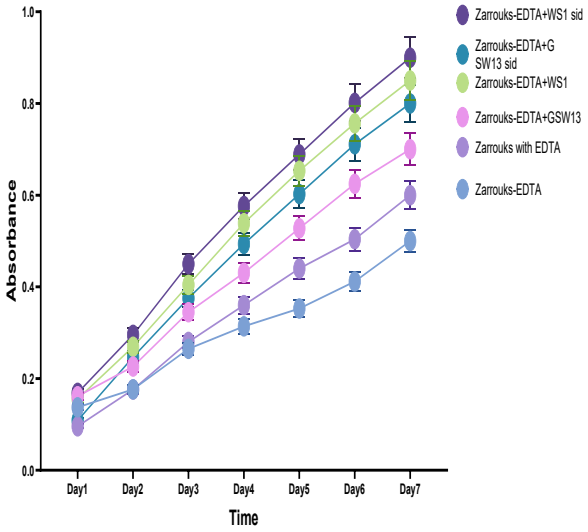

Figure 1: Effect of different treatments on the growth of *Spirulina* over a period of 7 days measured as absorbance at 630nm. Data are expressed as mean ± standard deviation (n = 3). Statistical analysis

was performed using two-way ANOVA followed by Tukey's multiple comparison test. Significant differences were observed among treatments and across time ($p < 0.0001$).

Wet Biomass

Wet biomass of *A. platensis* increased progressively across all treatments over the 7-day incubation period (Figure 2). Siderophore-associated treatments showed enhanced biomass accumulation compared to controls. The highest wet weight was recorded in the *Pseudomonas lundensis* siderophore treatment, followed by *Advenella mimigardefordensis* siderophore and EDTA-treated samples. Treatments involving bacterial cultures showed moderate improvement compared to the control.

Statistical analysis using two-way ANOVA indicated that both time and treatment had a highly significant effect on biomass production. The interaction between time and treatment was also significant ($F(30,84) = 3.229$, $p < 0.0001$), although its contribution to total variation was relatively low (1.736%). The row factor (time) contributed the largest proportion of variation (75.12%) and was highly significant ($F(6,84) = 698.9$, $p < 0.0001$), indicating that biomass accumulation was primarily dependent on incubation time. The column factor (treatment) contributed 21.64% of the variation and was also highly significant ($F(5,84) = 241.5$, $p < 0.0001$), demonstrating the influence of siderophore treatments on biomass enhancement.

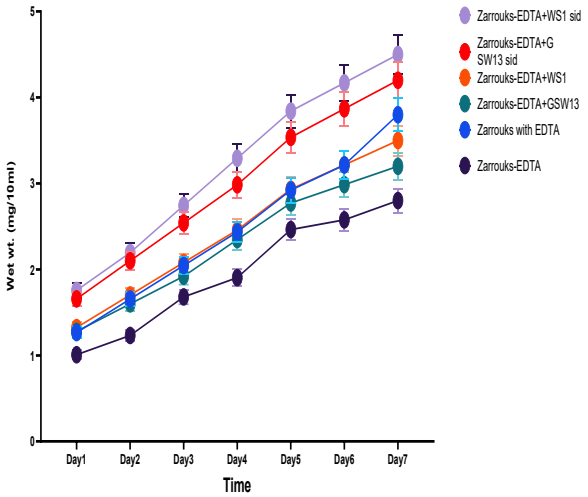

Figure 2: Effect of different treatments on the wet weight of *Spirulina* over a period of 7 days. Data are expressed as mean ± standard deviation (n = 3). Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparison test, showing significant effects of time, treatment, and their interaction ($p < 0.0001$).

Direct Microscopic Count

Cell density, determined by direct microscopic count, showed a steady increase across all treatments during the incubation period (Figure 3). Siderophore-associated treatments exhibited significantly higher cell counts, with *Pseudomonas lundensis* siderophore showing the maximum increase, followed by *P. lundensis* bacterial culture and *Advenella mimigardefordensis* siderophore treatment. EDTA-treated samples showed moderate growth, whereas the control exhibited the lowest cell density.

Two-way ANOVA revealed a highly significant effect of time, treatment, and their interaction on cell density. The interaction term was highly significant ($F(30,84) = 39.32$, $p < 0.0001$) and contributed 9.289% of the total variation, indicating that treatment effects varied substantially across time points. The row factor (time) contributed 59.99% of the variation and was highly significant ($F(6,84) = 1270$, $p < 0.0001$), confirming that cell proliferation was strongly dependent on

incubation duration. The column factor (treatment) contributed 30.06% of the variation and was also highly significant ($F(5,84) = 763.6$, $p < 0.0001$), demonstrating that siderophore treatments significantly enhanced cell growth.

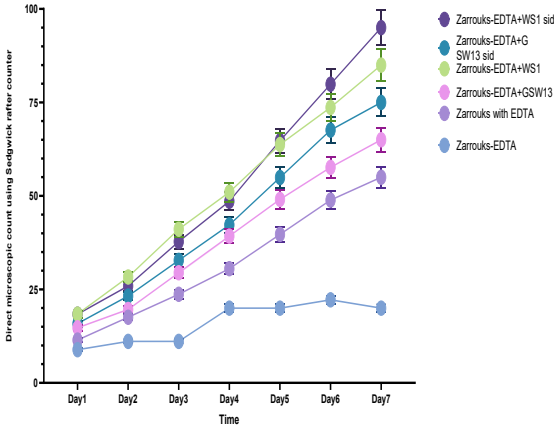

Figure 3: Effect of different treatments on the cell count of *Spirulina* over 7 days. Data are expressed as mean ± standard deviation (n = 3). Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparison test. Significant differences were observed among treatments, time points, and their interaction ($p < 0.0001$).

Determination of Iron uptake

Residual iron concentration decreased significantly across treatments compared to the iron-limited control (~1.8 mg/L), indicating enhanced iron uptake. EDTA-treated samples showed moderate reduction (~1.2 mg/L), while bacterial treatments further improved uptake (~0.7–0.9 mg/L). The lowest residual iron was observed in siderophore-treated cultures, with *Pseudomonas lundensis* siderophore (~0.5 mg/L) showing the highest uptake efficiency, followed by *Advenella mimigardefordensis* siderophore (~0.6 mg/L). Overall, siderophores were more effective than EDTA in enhancing iron uptake.

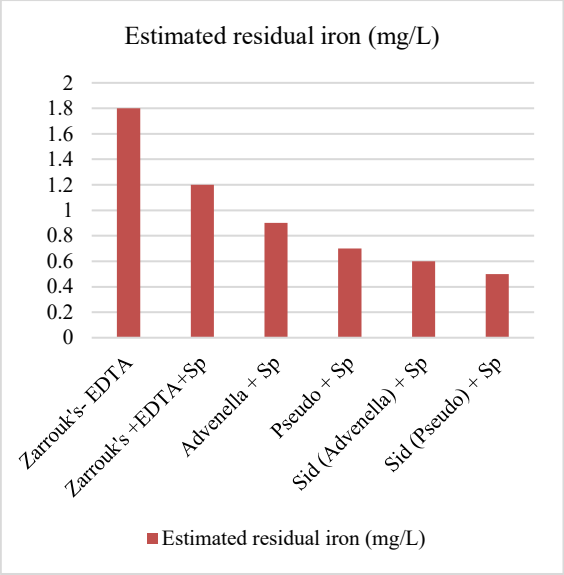

Figure 4: Effect of different treatments on residual iron concentration (mg/L) in *Arthrospira platensis* cultures after 7 days. Lower residual iron indicates higher iron uptake. Siderophore-treated samples, particularly those with *Pseudomonas lundensis*, showed the lowest residual iron compared to EDTA and control treatments. Data are expressed as mean ± SD (n = 3).

Conclusion:

The present study demonstrates that siderophore-producing bacteria and their crude extracts significantly enhance the growth and iron uptake of *Arthrospira platensis*. Among the treatments, siderophore extracts—particularly from *Pseudomonas lundensis* showed the highest effectiveness

in improving optical density, biomass accumulation, and cell density. While EDTA acted as a chemical chelator and supported moderate growth, it was consistently less effective than siderophore treatments, indicating the superiority of biologically mediated iron acquisition.
Iron uptake analysis further confirmed that siderophore treatments resulted in the lowest residual iron concentrations, reflecting enhanced iron chelation and assimilation. The strong correlation between iron uptake and growth parameters highlights the critical role of siderophores in overcoming iron limitation in algal systems.

These findings suggest that siderophores can serve as efficient bio-enhancers for improving microalgal productivity and may have potential applications in large-scale Spirulina cultivation, biotechnology, and sustainable aquaculture systems.

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