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Impact of Zinc Solubilizing Bacteria and Microbial Consortia on Growth and Yield of Rice (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rice (*Oryza sativa* L.) is a staple food for over half the world's population, particularly in Asia. This study explores the potential of Zn-solubilizing microbes to enhance micronutrient content in rice, aiming to address these deficiencies sustainably. Beneficial free-living soil bacteria, specifically plant growth-promoting rhizobacteria (PGPR), were investigated for their role in improving plant health and yield. Twenty-two bacterial isolates were screened for Zn solubilization, with *Enterobacter hormaechei* identified as the most promising. Field experiments with rice varieties PD 26 and NDR 359 involved treatmentssuch asT1 (Control), T2 (ZnSB1*: Enterobacter hormaechei* MT507226.1), T3 (Consortium1: *Pantoearodasii* MZ397586 + *Seratiamarcesecens* MW843567),

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and T4 (Consortium2: *Enterobacter hormaechei* MT507226.1 + *Pantoearodasii* MZ397586 + *Seratiamarcesecens* MW843567). with individual and consortia of Zn-solubilizing bacteria. Significant improvements were shown in plant height, leaf area index (LAI), total chlorophyll, total dry matter (TDM), and grain yield. Results showed that Consortium1 (T3) significantly increased plant height by 7.94% for PD26 and 10.16% for NDR 359. Leaf Area Index (LAI) also improved notably under Consortium1, with increases of 15.56% for PD26 and 24.39% for NDR 359. Total chlorophyll content was highest under Consortium1 for PD26 (36.63% increase) and under Consortium2 for NDR 359 (31.41% increase). Total dry matter (TDM) showed substantial gains, especially in NDR 359 with Consortium2 treatment, achieving a 36.51% increase. Grain yield increased significantly across all treatments, with Consortium1 showing the highest yields: 12.26% for PD26 and 23.01% for NDR 359.Correlation analysis indicated strong positive relationships between plant height, TDM, and grain yield, underscoring the importance of these parameters in determining crop productivity. The findings suggest that microbial consortia, particularly Consortium1, can effectively replace traditional zinc fertilizers, enhancing sustainable agriculture by promoting plant growth and yield. These results are consistent with recent studies on the role of plant growth-promoting rhizobacteria (PGPR) in improving nutrient uptake and crop performance.

Keywords: Zinc solubilizing bacteria; Rice; yield; PGPR.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population, particularly in Asia [1]. Despite its significance in global food security, rice is inherently low in essential micronutrients such as zinc (Zn) and iron (Fe), leading to widespread deficiencies among rice-dependent populations. Micronutrient malnutrition, often referred to as "hidden hunger," affects billions of people worldwide, resulting in severe health issues such as impaired cognitive development, weakened immune systems, and increased susceptibility to infections [2]. In context to the human andhealth of other organisms, Zn is required in trace amounts to support proper physiological functions such as cell division, cell growth, wound healing, and the breakdown of carbohydrates; recognized as a vital mineral for overall well-being [3]. However, the availability of Zn in soils is diminishing due to factors like low organic matter, excessive fertilization, inadequate recycling of crop residues, cultivation of high-yielding crop varieties, and intensive cropping patterns [4]. Addressing the issue of Zn deficiency, there is a rising focus on micronutrient biofortification of staple grain crops in developing nations, aiming to enhance nutritional quality and combat widespread deficiencies [5]. Beneficial free-living soil bacteria, specifically plant growth-promoting rhizobacteria (PGPR), have shown promise in improving plant health and bolstering yield [6]. PGPR fulfill multifaceted roles in sustainable agriculture as they reside within the rhizosphere, encompassing root surfaces and establishing symbiotic relationships with plant roots to enhance overall plant growth and health [7]. The solubilization of metal salts constitutes a significant trait of PGPR, facilitating the mobilization of compounds accessible to plants. Various PGPR, including strains from genera such as *Serratia, Bacillus, Pseudomonas*, have been identified as effective Zn solubilizers, augmenting plant growth by colonizing the rhizosphere and converting complex Zn compounds into simpler forms accessible to plants [8,9]. Study by Ali et al., [10] in field experiments was conducted to evaluate the impact of treatment combinations (control (without Zn and bacterial inoculation), 4, 8, 12, 16 and 20 kg Zn ha^{-1} were applied to soil without and with inoculation of zinc-solubilizing bacteria to the seed of wheat cultivar, i.e., Wadaan-17 and Zincol-16). Results showed that zinc-solubilizing bacteria in conjunction with zinc sulfate significantly ($P \leq 0.05$) increased the yield by 61%. Among the treatment combinations, inoculation of Zn-solubilizing bacteria in conjunction with 8 kg Zn ha^{-1} substantially boosted the yield and yield attributes of wheat crop under field conditions. Also, Unnikrishnan and Karayi, [11] found that there was an increase in plant height, leaf area, number of grains per panicle and grain yield per plant on inoculation with *Phanerochaeteconcrescens KS7* in two selected varieties of rice grown in zinc deficient soil.The utilization of PGPR presents a promising and environmentally friendly approach, serving as a viable substitute for chemical fertilizers, pesticides, and supplements, contributing to sustainable agricultural practices and promoting soil health.

The present study aims to evaluate the effects of Zinc Solubilizing Bacteria and microbial consortia on rice plant growth parameters, including plant height, Leaf Area Index (LAI), total chlorophyll, total dry matter (TDM), and grain yield. By assessing these parameters, the study seeks to provide insights into the potential of ZSB and microbial consortia as sustainable alternatives to improve Zn content in rice cultivation.

2. MATERIALS AND METHODS

The bacterial culture employed in this investigation was obtained from rhizospheric soil samples of field-grown rice at the vegetative stage collected from Pantnagar (29.0369° N, 79.4472° E), Udham Singh Nagar District, Uttarakhand, India. Soil samples were collected in triplicate by uprooting the plant and carefully collecting the soil adhered to roots, followed by mixing the soil to make a composite sample. Twenty-two isolates were initially screened for their Zn solubilization capabilities, leading to the identification of the most promising isolate, TRR2, was chosen for subsequent experiments. Utilizing 16S rRNA sequencing, the selected isolate was identified as *Enterobacter hormaechei*. Reference strains (*Pantoearodasii* MZ397586 and *Seratiamarcesecens* MW843567) were collected from the Department of Microbiology, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology Pantnagar. Further characterization of this strain included a study of its PGPR attributes, followed by a field experiment conducted on the staple crop rice (*Oryza sativa* L.) at the Dr. Norman E. Borlaug Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

2.1 Seed and Seedling Bacterization and Field Experiment

Seeds of cultivable rice varieties (PD 26 and NDR 359) underwent surface sterilization using 0.1% mercuric chloride solution for 3 min followed by 70% ethanol for 3 min and were then rinsed eight times with sterile distilled water as described by Prathap et al. [12]. Subsequently, the seeds were treated with an overnight grown bacterial inoculum having an optical density (10^5 to 10^6 Colony Forming Unit) along with 0.5% carboxymethylcellulose (CMC) to provide adhesiveness. Seeds were sown, and after 21 days, the seedlings were treated with bacterial inoculation in the same manner as the seed bacterization.

2.2 Measurement of Plant Height, LAI, Total Dry Matter, Total Chlorophyll and Grain Yield

At the time of flowering, plant height was measured using a measuring tape from the base at soil level to the highest point of the plant on a representative sample. Leaf Area Index (LAI) was determined by collecting all leaves from the sampled plants, measuring their area with a leaf area meter, and calculating LAI as the total leaf area per unit ground area. For Total dry matter (TDM), the entire above-ground portion of the plants was harvested, dried in an oven at 65°C until a constant weight, and weighed. At the time of flowering, total chlorophyll content was determined using the DMSO (dimethyl sulfoxide) method. Fresh leaf samples were collected and cut into small pieces, with 0.1 g of the leaf tissue placed in a test tube containing 10 ml of DMSO. The test tubes were incubated in a water bath at 65°C for 30 minutes to extract the chlorophyll. After incubation, the solution was cooled to room temperature, and the absorbance was measured at 663 nm and 645 nm using a spectrophotometer. Total chlorophyll content was calculated using the Arnon’s [13] formula:

$$\text{Total chlorophyll (mg/g)} = \frac{(20.2\text{X A665} + 8.02\text{ XA649})\text{X V}}{\text{Weight (g)X 1000}}$$

At the time of harvest, grain yield was determined by harvesting grain from a representative sample area of 1 m² within each plot. The harvested grain was dried to a constant

Table 1. Treatment Details

T1	Control
T2	ZnSB1 (*Enterobacter hormaechei*MT507226.1)
T3	Consortium1 (*Pantoearodasii* MZ397586 + *Seratiamarcesecens* MW843567)
T4	Consortium2 (*Enterobacter hormaechei*MT507226.1+*Pantoearodasii* MZ397586 + *Seratiamarcesecens* MW843567)

moisture content, then weighed to determine the yield in grams per square meter (g/m^2). To calculate the grain yield per hectare (t/ha), the yield in g/m^2 was converted using the formula:

$$\text{Yield (t/ha)} = \text{Yield (g/m}^2\text{)} \times 0.01$$

This conversion factor accounts for the scaling from square meters to hectares and grams to metric tons.

2.3 Statistical Analysis

Based on the experimental design, the data from the plant study were meticulously analysed in triplicate and subjected to Analysis of Variance (ANOVA). Statistical analysis was executed utilizing the Microsoft Excel to accurately quantify and evaluate the sources of variation. While graphs were plotted by the help of Origine Pro. Subsequently, treatment means were compared at a significance level of 5% to ascertain the presence of any significant differences.

3. RESULTS

3.1 Effect of Bacterial Strains on Rice Plant Height and Leaf Area Index

3.1.1 Plant height (cm)

The effect of different treatments on plant height is presented in Table 2. The plant height for the PD26 variety under the control treatment (T1) was 118.32±1.77 cm, whereas the NDR 359 variety measured 85.12±0.74 cm. Treatment with ZnSB1 (T2) resulted in an increase in plant height to 120.36±1.80 cm for PD26, which is a 1.69% increase compared to the control, and to 88.66±0.77 cm for NDR 359, a 4.00% increase. Consortium1 treatment (T3) showed the highest increase, with plant heights of 128.52±1.92 cm for PD26 (7.94% increase) and 94.74±0.82 cm for NDR 359 (10.16% increase). Consortium2 treatment (T4) resulted in plant heights of 120.36±1.80 cm for PD26 (1.70% increase) and 90.19±0.78 cm for NDR 359 (5.62% increase).

3.1.2 Leaf Area Index (LAI)

The Leaf Area Index (LAI) results also shown in Table 2. Under control conditions (T1), the LAI was 3.75±0.15 for PD26 and 3.10±0.18 for NDR 359. Treatment with ZnSB1 (T2) resulted in LAI values of 3.80±0.16 for PD26 (1.30% increase) and 3.50±0.20 for NDR 359 (11.43% increase). Consortium1 treatment (T3) significantly increased the LAI to 4.44±0.18 for PD26 (15.56% increase) and 4.10±0.24 for NDR 359 (24.39% increase). Consortium2 treatment (T4) produced LAI values of 4.04±0.17 for PD26 (7.32% increase) and 3.90±0.22 for NDR 359 (20.51% increase).

3.2 Effect of Bacterial Strain on Total Chlorophyll, Total Dry Matter and Grain Yield

3.2.1 Total chlorophyll content (mg/g FW)

The total chlorophyll content showed significant increases across the different treatments for both PD26 and NDR 359 varieties. For PD26, the control group (T1) recorded a total chlorophyll content of 2.76±0.08 mg/g FW, with percentage increases in treatments T2 (ZnSB1), T3 (Consortium1), and T4 (Consortium2) of 10.99%, 36.63%, and 33.87%, resulting in chlorophyll contents of 3.10±0.10, 4.36±0.14, and 4.17±0.12 respectively. For NDR 359, the control group's chlorophyll content was 2.59±0.06 mg/g FW, with percentage increases in T2, T3, and T4 of 11.70%, 29.57%, and 31.41%, resulting in chlorophyll contents of 2.94±0.06, 3.68±0.08, and 3.78±0.08 respectively. These results indicated that the T3 (Consortium1) treatment led to the highest increase in chlorophyll content for PD26, while T4 (Consortium2) showed the highest increase for NDR 359.

3.2.2 Total dry matter (g/m^2)

The total dry matter showed notable increases across the different treatments. For the PD26 variety, the control group (T1) had a TDM of 825.44±15.19, while the T2, T3, and T4 treatments saw percentage increases of 4.71%, 9.17%, and 8.78%, resulting in TDM values of 866.22±24.11, 908.78±47.11, and 904.89±20.78 respectively. In the NDR 359 variety, the control group recorded a TDM of 395.89±29.41, with T2, T3, and T4 treatments showing percentage increases of 15.50%, 20.40%, and 36.51%, leading to TDM values of 468.44±50.34, 497.33±21.94, and 623.56±55.05 respectively. These results indicate that the T4 (Consortium2) treatment significantly boosted TDM in the NDR 359 variety, demonstrating its potential effectiveness in enhancing dry matter accumulation.

Table 2. Effect of bacterial strains on rice plant height and leaf area index

Treatment		Plant Height(cm)		LAI	
		PD26	NDR 359	PD26	NDR 359
T1	Control	118.32±1.77^{b} (0.00)	85.12±0.74^{e} (0.00)	3.75±0.15^{bc} (0.00)	3.10±0.18^{d} (0.00)
T2	ZnSB1	120.36±1.80^{b} (1.69)	88.66±0.77^{de} (4.00)	3.80±0.16^{bc} (1.30)	3.50±0.20^{cd} (11.43)
T3	Consortium1	128.52±1.92^{a} (7.94)	94.74±0.82^{c} (10.16)	4.44±0.18^{a} (15.56)	4.10±0.24^{ab}(24.39)
T4	Consortium2	120.36±1.80^{b} (1.70)	90.19±0.78^{d} (5.62)	4.04±0.17^{abc} (7.32)	3.90±0.22^{abc} (20.51)

**Each value is the mean of three replicates. Different letter(s) within the column indicate significant differences in the mean for interaction, Variety x treatments (Fisher LSD test, $p < 0.05$). ± indicates the standard error of the mean. Values in the brackets indicate a percent increase over the control*

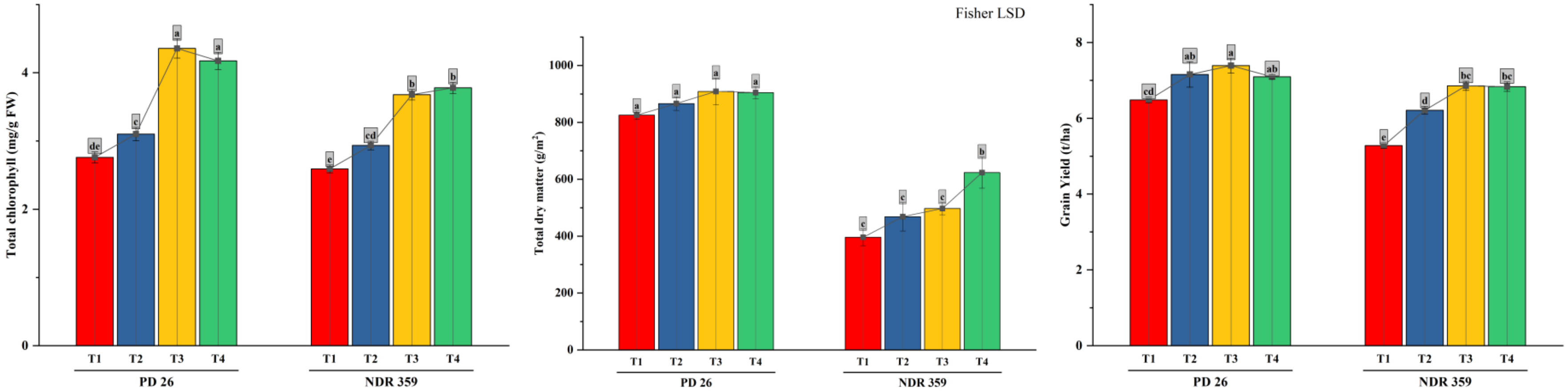

Fig. 1. Effect of bacterial strains on, total chlorophyll, total dry matter and grain yield

3.2.3 Grain yield (t/ha)

The grain yield significantly increased across various treatments for both PD26 and NDR 359 varieties. For the PD26 variety, the control group (T1) had a grain yield of 6.48±0.07 t/ha, with treatments T2 (ZnSB1), T3 (Consortium1), and T4 (Consortium2) resulting in yields of 7.16±0.34, 7.39±0.19, and 7.09±0.06 t/ha, showing percentage increases of 9.39%, 12.26%, and 8.61% respectively. For the NDR 359 variety, the control group recorded a grain yield of 5.28±0.07 t/ha, while treatments T2, T3, and T4 increased yields to 6.21±0.10, 6.86±0.12, and 6.83±0.12 t/ha, representing percentage increases of 15.03%, 23.01%, and 22.76% respectively. These results indicate significant enhancements in grain yield for both varieties with the various treatments, particularly with the T3 (Consortium1) treatment demonstrating the highest increase in both cases.

3.3 Correlation Analysis among Parameters Affected by Bacterial Treatment

The correlation matrix in the provided Fig. 2. illustrates the relationships between several agronomic traits: plant height, leaf area index (LAI), total chlorophyll (Total chl), total dry matter (TDM), and grain yield. The analysis reveals that plant height has a strong positive correlation with TDM (0.93) and a moderate positive correlation with grain yield (0.65). LAI shows a moderate positive correlation with total chlorophyll (0.72) and grain yield (0.68), while also maintaining a weaker positive correlation with plant height (0.53) and TDM (0.49). Total chlorophyll is moderately positively correlated with LAI (0.72) and grain yield (0.72), with weaker positive correlations observed with TDM (0.47) and plant height (0.45). TDM displays a very strong positive correlation with plant height (0.93) and a moderate positive correlation with grain yield (0.68). Grain yield itself is moderately positively correlated with total chlorophyll (0.72), LAI (0.68), plant height (0.65), and TDM (0.68). The colour gradient and ellipses in the matrix provide a visual representation of these correlations, with red indicating positive correlations and the intensity of the colour reflecting the strength of these relationships.

4. DISCUSSION

The results of this study clearly demonstrate the positive impact of Zinc Solubilizing Bacteria

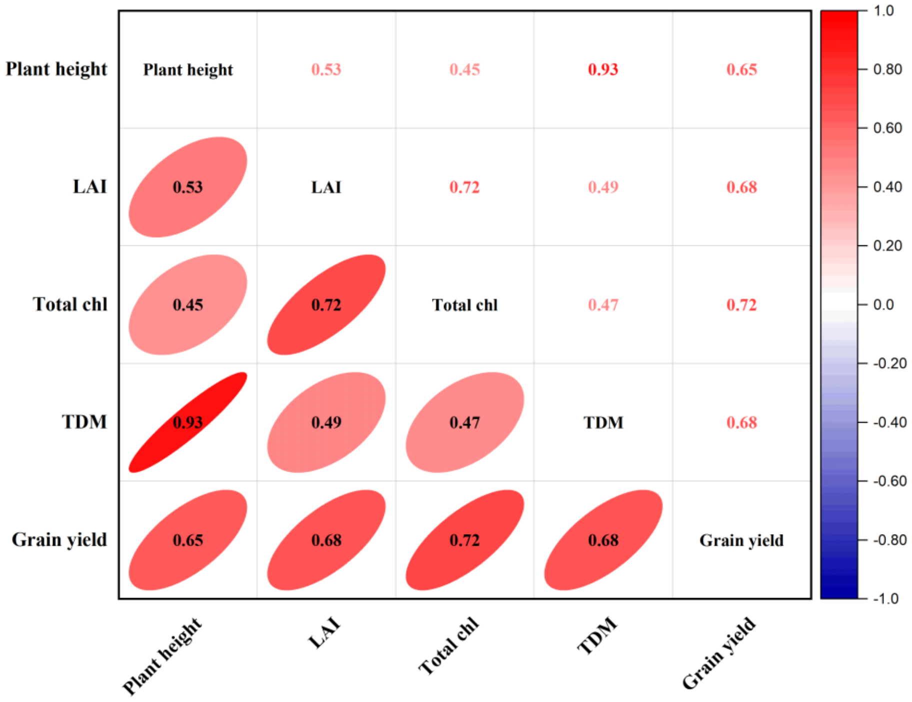

Fig. 2. The correlation analysis between plant height, Leaf Area Index (LAI), total chlorophyll, total dry matter (TDM), and grain yield

(ZnSB) and microbial consortia on various growth parameters and the yield of rice. The application of ZnSB1 (T2) and microbial consortia (T3 and T4) (Pantoearodasii + Seratiamarcesecens, Enterobacter hormaechei + Pantoearodasii + Seratiamarcesecens) resulted in significant increases in plant height, Leaf Area Index (LAI), total chlorophyll, total dry matter (TDM), and grain yield for both PD26 and NDR 359 rice varieties. Notably, the Consortium1 (T3) treatment showed the highest improvements across all parameters, indicating a synergistic effect among the microbes.These treatments are thought to stimulate the plant's root system, improving the absorption of essential nutrients from the soil. This can lead to better growth and higher yields. This enhancement can be attributed to the improved solubilization and availability of zinc and other nutrients, which are crucial for various physiological processes such as photosynthesis, protein synthesis, and overall plant growth [14,15]. These findings align with recent studies highlighting the role of beneficial microbes in promoting plant growth through enhanced nutrient uptake and hormonal regulation [16].

The observed improvements in plant height and TDM were strongly correlated with higher grain yields, underscoring the importance of these growth parameters in determining overall productivity. The increase in LAI and total chlorophyll further suggests enhanced photosynthetic capacity, contributing to better biomass accumulation and yield [17]. These results support the potential of using ZnSB and microbial consortia as sustainable alternatives to traditional zinc fertilizers, providing a holistic approach to improving rice cultivation [18]. The significant yield gains achieved with these treatments indicate their practical applicability in enhancing food security and agricultural sustainability.The treatments might promote the development of stronger and more resilient plant structures, including roots, stems, and leaves, contributing to overall plant vigor and the ability to support higher yields. Future research should focus on understanding the long-term effects of these microbial treatments on soil health and exploring their potential in other crop systems (Nabi, 2023; Ali et al., 2024).

5. CONCLUSION

In conclusion, the study reveals that the Consortium1 (T3) and Consortium2 (T4) treatments substantially improve various growth parameters and overall productivity of PD26 and NDR 359 plant varieties. These treatments significantly increased plant height, Leaf Area Index (LAI), total chlorophyll content, total dry matter (TDM), and grain yield. The strong positive correlations among these parameters indicated that improvements in plant height and TDM are particularly influential in boosting grain yield. The findings are consistent with recent research demonstrating the efficacy of microbial consortium and bio-fertilizer treatments in enhancing plant growth and yield. Therefore, Consortium1 and Consortium2 treatments present a promising strategy for improving crop performance and agricultural productivity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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