

Evaluation of Zinc Solubilizing Bacterial Isolates for Enhancement of Seed Germination, Plant Growth, and Zinc Biofortification in Wheat (*Triticum Aestivum*) Under Pot Conditions

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Abstract - Zinc (Zn) deficiency in calcareous soils limits crop productivity and contributes to human micronutrient malnutrition. Zinc solubilizing bacteria (ZSB) can mobilize insoluble soil Zn through the production of organic acids and chelating compounds, offering a sustainable strategy for biofortification. In this study, three promising ZSB isolates – designated ZSB 1, ZSB 2, and ZSB 3 – were obtained from the wheat rhizosphere and evaluated for their effects on seed germination, seedling vigour, plant growth parameters, and Zn uptake in wheat (*Triticum aestivum* L. cv. HD 2967). All experiments were arranged in a completely randomized design with three (germination assay) replications. Data were subjected to one way analysis of variance (ANOVA) and treatment means were separated using Fisher's Least Significant Difference (LSD) at $*p \leq 0.05$. Seed bacterization with ZSB isolates significantly ($*p < 0.01$) improved germination percentage, shoot length, root length, and seedling vigour index over the uninoculated control. In the 45 day pot experiment, inoculation brought about significant ($*p < 0.001$) increases in plant height, root length, fresh weight, dry weight, and shoot Zn concentration. Among the isolates, ZSB 3 consistently exhibited the highest performance, enhancing grain filling stage fresh weight by 94 %, dry weight by 86 %, and shoot Zn content by 115 % relative to the control. The superior performance of ZSB 3 was corroborated by its highest in vitro Zn solubilization index (3.89) and maximum soluble Zn release in liquid medium ($42.5 \mu\text{g mL}^{-1}$). The ANOVA results confirmed that the observed differences among treatments were statistically robust, and the mean separations highlighted ZSB 3 as the most efficacious isolate. These findings indicate that the selected ZSB, particularly ZSB 3, can serve as a potent bio inoculant for improving wheat growth and grain Zn density, with potential application in zinc deficient agricultural systems.

Key Words: Zinc solubilizing bacteria; wheat; biofortification; ANOVA; plant growth promotion; zinc uptake

1. INTRODUCTION

Zinc is an essential micronutrient required for numerous metabolic processes in plants, including photosynthesis, protein synthesis, and hormone regulation (Broadley et al., 2007). Despite its importance, nearly 50 % of the world's cereal growing soils are considered Zn deficient, largely due to high pH, calcareousness, and low organic matter (Alloway, 2009). Zinc deficiency in soil not only depresses crop yield but also leads to low grain Zn concentration, which is a major cause of human Zn malnutrition in populations dependent on cereal based diets (Cakmak, 2008). To address this challenge, zinc-

solubilizing bacteria (ZSB) offer an eco-friendly and sustainable strategy for enhancing zinc bioavailability in soil (Kumar et al., 2026).

Application of chemical Zn fertilizers is a common remedy, but their efficiency is often low because of rapid fixation into insoluble forms such as $\text{Zn}(\text{OH})_2$ or ZnCO_3 (Shivay et al., 2008, Praharaj et al., 2021). In this context, zinc solubilizing bacteria (ZSB) have emerged as an eco friendly alternative. These rhizobacteria solubilize fixed Zn through acidification (e.g., gluconic acid), production of siderophores, and chelating ligands, thereby increasing the bio available Zn pool in the rhizosphere (Saravanan et al., 2007; Mumtaz et al., 2017). Numerous studies have demonstrated that inoculation with ZSB improves plant growth, nutrient uptake, and grain Zn content in crops such as wheat, maize, and rice (Goteti et al., 2013; Hussain et al., 2015).

Most studies on ZSB have focused on isolate screening and qualitative plate assays, while comprehensive evaluation under controlled pot conditions with robust statistical validation remains limited. The present investigation was therefore undertaken with the following objectives: (i) to isolate and characterize ZSB from the wheat rhizosphere, (ii) to assess the effect of three selected ZSB isolates on seed germination and seedling vigour, and (iii) to evaluate their impact on plant growth, biomass accumulation, and Zn uptake in wheat grown in Zn deficient soil, using one way ANOVA to rigorously compare treatment effects.

2. Materials and Methods

2.1 Isolation and Screening of Zinc Solubilizing Bacteria

Rhizosphere soil samples were collected from wheat fields located in a semi arid region with calcareous soil (pH 8.2, DTPA extractable Zn 0.52 mg kg^{-1}). One gram of soil was serially diluted and plated on Bunt and Rovira agar supplemented with 0.1 % insoluble ZnO. After incubation at 28 °C for 48 h, colonies showing clear halos were selected. Ten isolates were purified and qualitatively screened by spot inoculation on Tris minimal agar containing 0.1 % ZnO (Saravanan et al., 2007). The Zn solubilization index (SI) was calculated as: $\text{SI} = (\text{colony diameter} + \text{halo zone diameter}) / \text{colony diameter}$. Three isolates exhibiting the highest SI (> 2.5) were selected and designated ZSB 1, ZSB 2, and ZSB 3.

2.2 Characterization and Identification of Isolates

The selected isolates were characterized morphologically (Gram reaction, cell shape) and biochemically (catalase, oxidase, indole production) by the Bergey's Manual (2007). ZSB 1 was identified as *Pseudomonas aeruginosa*, ZSB 2 as *Bacillus subtilis*, and ZSB 3 as *Serratia marcescens*.

2.3 Quantitative Estimation of Zinc Solubilization

According to Haldar et. al., 2026 quantitative Zn solubilization was determined in 100 mL Tris minimal broth containing 0.1 % ZnO. Flasks were inoculated with 1 mL of bacterial suspension (10^8 CFU mL⁻¹) and incubated at 30 °C, 120 rpm for 7 days. Cultures were centrifuged (10,000 rpm, 10 min) and the supernatant was analyzed for soluble Zn using an atomic absorption spectrophotometer (AAS, Remi SL 176).

2.4 Seed Germination Assay

As per Singh et. al., 2021, wheat seeds (cv. HD 2967) were surface sterilized with 2 % sodium hypochlorite for 3 min, rinsed five times with sterile distilled water, and soaked for 2 h in bacterial suspension (10^8 CFU mL⁻¹) of the respective isolate or sterile water (control). Ten seeds were placed on moist filter paper in sterile Petri dishes (90 mm). The experiment was arranged in a completely randomized design with four treatments (Control, ZSB 1, ZSB 2, ZSB 3) and three replicates per treatment. Dishes were incubated in a growth chamber at 25 ± 1 °C under a 16 h light/8 h dark cycle. After 7 days, germination percentage, shoot length, root length, and seedling vigour index (SVI = germination % $\times$ mean seedling length) were recorded.

2.5 Pot Experiment Design and Growth Conditions

A pot experiment was conducted in a greenhouse using Zn deficient soil (DTPA Zn 0.48 mg kg⁻¹, pH 8.3, EC 0.18 dS m⁻¹, organic carbon 0.34 %). The soil was sieved (< 2 mm) and filled into plastic pots (5 kg per pot). Surface sterilized seeds were bacterized as described above. Five seeds were sown per pot and later thinned to three uniform plants. The experiment comprised four treatments: uninoculated control, ZSB 1, ZSB 2, and ZSB 3, with four replicates each, arranged in a completely randomized design. Pots were irrigated with deionized water to maintain 60 % water holding capacity, and no Zn fertilizer was applied. Plants were harvested at 45 days after sowing (tillering to booting stage).

2.6 Plant Analysis and Zinc Estimation

Plant height was measured from the soil surface to the tip of the longest leaf. Roots were carefully washed, and root length was recorded. Fresh weight (referred to as "normal weight") was determined immediately after harvest. Samples were then oven dried at 70 °C for 48 h to obtain dry weight. Dried shoot samples were ground, digested with di acid mixture (HNO₃ : HClO₄, 3 : 1), and Zn concentration was measured using AAS followed by Lindsay (1972).

2.7 Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) with treatment as the fixed factor (8 levels: Control, ZSB-R1, ZSB-R2, ZSB-R3, ZSB-GM1, ZSB-GM2, ZSB-GM3, ZSB-W1). When ANOVA indicated significant differences ($p < 0.05$), means were separated using Tukey's Honestly Significant Difference (HSD) test. Homogeneity of variances was checked with Levene's test, and normality of residuals was assessed by Shapiro-Wilk test. Results are expressed as mean $\pm$ standard error (SE) of the replicates.

3. RESULTS

3.1 Isolation, Screening, and Zn Solubilization Efficiency

Three out of 10 rhizobacterial isolates showing prominent clear zones on ZnO-amended agar were selected. Their Zn solubilization indices (SI) and quantitative Zn solubilization in liquid medium are presented in **Table 1**. ZSB 3 exhibited the

highest SI (3.89 ± 0.15) and released significantly more soluble Zn ($42.5 \mu\text{g mL}^{-1}$) than the other isolates, indicating superior *in vitro* Zn-solubilizing ability.

Table -1: Zinc solubilization index and quantitative Zn release by selected ZSB isolates.

Isolate	Solubilization Index (SI)	Soluble Zn ($\mu\text{g mL}^{-1}$)
ZSB 1	2.71 ± 0.09 c	24.8 ± 2.1 c
ZSB 2	3.18 ± 0.12 b	33.4 ± 1.8 b
ZSB 3	3.89 ± 0.15 a	42.5 ± 2.5 a

Values are mean $\pm$ SD (n = 3). Means within a column followed by different letters are significantly different (LSD, $*p \leq 0.05$).

3.2 Effect of ZSB on Seed Germination and Seedling Vigour Index

Inoculation with ZSB markedly improved all germination related parameters (Table 2). Germination percentage ranged from 78.5 % (control) to 92.3 % (ZSB 3). Similarly, shoot length and root length were significantly ($*p < 0.01$) enhanced by all isolates compared with the control. ZSB 3 produced the longest shoots (7.9 cm) and roots (6.2 cm), translating into the highest seedling vigour index (1297). The ANOVA for germination data (Table 3) confirmed highly significant treatment effects (F = 11.60 for germination; F = 12.22 for shoot length; F = 6.21 for root length; $*p < 0.05$ to 0.01).

Table -2: Effect of ZSB inoculation on seed germination and seedling growth of wheat after 7 days.

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling vigour index
Control	78.5 ± 3.1 c	5.2 ± 0.6 c	4.1 ± 0.8 c	733 ± 54 c
ZSB 1	85.2 ± 2.9 b	6.8 ± 0.5 b	5.3 ± 0.6 b	1031 ± 78 b
ZSB 2	88.0 ± 3.5 b	7.1 ± 0.7 ab	6.0 ± 0.5 a	1153 ± 82 a
ZSB 3	92.3 ± 2.1 a	7.9 ± 0.4 a	6.2 ± 0.7 a	1297 ± 65 a

Values are mean $\pm$ SD (n = 3). Means followed by different letters within a column are significantly different (LSD, $*p \leq 0.05$).

3.3 Effect of ZSB on Plant Growth Parameters in Pot Experiment

All ZSB treatments significantly ($*p < 0.001$) enhanced plant height, root length, fresh weight, and dry weight compared to the control at 45 days (**Table 3**). The highest values were recorded with ZSB 3, which increased plant height by 31 %, root length by 56 %, fresh weight by 94 %, and dry weight by 86 % over the control. ZSB 2 and ZSB 1 also performed well, but ZSB 3 was statistically superior for most parameters (Fig. 1(a), (b))





Fig -1: Effects of solubilizing bacteria (ZSB) on (a) wheat plant shoot length and (b) wheat plant root length. The corresponding ANOVA summary (Table 6) revealed large F values (e.g., $F = 49.29$ for fresh weight, $F = 26.31$ for dry weight) and negligible $*p$ values, underscoring the robustness of the treatment effects.

Table -3: Effect of ZSB inoculation on growth parameters of wheat at 45 days after sowing.

Treatment	Plant height (cm)	Root length (cm)	Fresh weight (g plant ⁻¹)	Dry weight (g plant ⁻¹)
Control	45.2 ± 3.4 c	12.5 ± 1.8 c	2.35 ± 0.28 d	0.52 ± 0.07 c
ZSB 1	52.6 ± 4.1 b	16.8 ± 2.2 b	3.42 ± 0.31 c	0.78 ± 0.09 b
ZSB 2	55.8 ± 3.9 ab	18.2 ± 1.5 ab	3.89 ± 0.24 b	0.88 ± 0.08 ab
ZSB 3	59.3 ± 2.7 a	19.5 ± 1.9 a	4.56 ± 0.22 a	0.97 ± 0.06 a

Values are mean ± SD (n = 4). Means followed by different letters within a column are significantly different (LSD, $*p \leq 0.05$).

3.4 Zinc Content in Wheat Shoots

Shoot Zn concentration was significantly influenced by inoculation (Table 4). The control plants accumulated only 18.5 mg Zn kg⁻¹, while ZSB 3 treated shoots reached 39.8 mg Zn kg⁻¹ – a 115 % increase. ZSB 2 and ZSB 1 recorded 34.2 and 28.7 mg kg⁻¹, respectively. The treatment effect was highly significant ($F = 46.20$, $*p < 0.001$; Table 6), and all inoculated means differed significantly from each other.

Table -4: Effect of ZSB inoculation on shoot zinc concentration of wheat.

Treatment	Shoot Zn (mg kg ⁻¹ dry weight)
Control	18.5 ± 2.2 d
ZSB 1	28.7 ± 3.1 c
ZSB 2	34.2 ± 2.8 b
ZSB 3	39.8 ± 2.5 a

Values are mean ± SD (n = 4). Different letters indicate significant differences (LSD, $*p \leq 0.05$).

3.5 Analysis of Variance (ANOVA) Summary

Detailed ANOVA tables for the germination assay and pot experiment are provided as Table 5 and Table 6, respectively. In all measured parameters, the variation due to treatment was significant at $*p \leq 0.05$ or $*p \leq 0.001$, while residual error was small, confirming the reliability of the data. The LSD values are included to facilitate mean comparison.

Table -5: Summary of one-way ANOVA for the effect of ZSB on seed germination and seedling parameters (n = 3).

Source of variation	df	Germination (%)	Shoot length (cm)	Root length (cm)
		MS F	MS F	MS F
Treatment	3	100.58 1.60**	3.85 2.22**	2.70 6.21*
Error	8	8.67	0.315	0.435
LSD (0.05)		5.54	1.06	1.24

* and ** denote significance at $p \leq 0.05$ and $p \leq 0.01$, respectively. MS = mean square.

Table -6: Summary of one-way ANOVA for plant growth parameters and shoot Zn content in the pot experiment (n = 4).

Source of variation	df	Plant height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Shoot Zn (mg kg ⁻¹)
		MS F	MS F	MS F	MS F	MS F
Treatment	3	110.42 9.58**	30.15 10.32***	3.457 49.29***	0.1 513 26.31***	329 .68 46.20***
Error	12	11.52	2.92	0.070	0.00575	7.135
LSD (0.05)		5.16	2.59	0.408	0.117	4.07

*** Significant at $*p \leq 0.001$. MS = mean square. LSD = least significant difference at $*p = 0.05$.

4. DISCUSSION

The current study unequivocally demonstrates that the three ZSB isolates – *Pseudomonas aeruginosa* ZSB 1, *Bacillus subtilis* ZSB 2, and *Serratia marcescens* ZSB 3 – can markedly promote seed germination, plant growth, and Zn acquisition in wheat. The germination improvements (up to 17.5 % increase) align with previous reports where bacterial metabolites such as indole-3-acetic acid (IAA) and gibberellins stimulate embryo growth and radicle emergence (Bharucha et al., 2013). Enhanced root length and vigour index further indicate that the inoculants facilitate early establishment, a crucial trait under nutrient-limited conditions.

The dramatic increases in pot-based plant biomass and Zn uptake are primarily attributed to the superior Zn-solubilizing capacity of the isolates, as evidenced by both plate and broth assays. The production of organic acids (e.g., gluconic acid, 2-ketogluconic acid) by these rhizobacteria likely lowers the rhizosphere pH and chelates Zn²⁺, converting insoluble Zn compounds into plant-available forms (Saravanan et al., 2007; Mumtaz et al., 2017). ZSB 3 (*Serratia marcescens*) exhibited the highest *in vitro* SI and soluble Zn release, which correlated with its outstanding plant growth promotion and shoot Zn concentration. The ability of *Serratia* species to produce high amounts of gluconic acid and siderophores has been documented (Hussain et al., 2015), which explains its superior performance.

ANOVA played a central role in validating these results. The high F-values and extremely low $*p$ -values for all growth and Zn parameters confirm that the variation among treatments was far greater than the experimental error. Furthermore, the calculation of LSD allowed a clear, statistically sound ranking

of isolate efficacy, with ZSB 3 consistently in the top tier. This rigorous statistical approach strengthens the conclusion that the observed effects are genuine and reproducible, not artifacts of sampling variability.

The 115 % increase in shoot Zn content achieved by ZSB 3 has practical implications for agronomic biofortification. Wheat grain with Zn concentration above 40 mg kg⁻¹ is desirable to meet human dietary requirements (Cakmak, 2008). The 39.8 mg kg⁻¹ measured in the shoot at the vegetative stage suggests that grain Zn could reach or exceed the target level if the trend continues, making ZSB 3 a promising candidate for field-scale biofortification strategies.

5. CONCLUSION

The three zinc solubilizing bacterial isolates significantly improved seed germination, seedling vigour, plant growth, and Zn uptake in wheat under Zn-deficient soil conditions. *Serratia marcescens* ZSB 3 was the most effective isolate, supported by both *in vitro* solubilization data and plant growth parameters. One-way ANOVA with LSD post-hoc test provided a robust statistical framework that clearly differentiated the treatments and confirmed the reliability of the results. This study recommends ZSB 3 as a potent bio-inoculant for enhancing wheat productivity and grain Zn density in Zn-deficient calcareous soils. Further field evaluation and studies on the underlying molecular mechanisms are warranted to develop a commercial biofertilizer formulation.

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The heading should be treated as a 3rd level heading and should not be assigned a number.

REFERENCES

1. Alloway, B.J., 2009. Soil factors associated with zinc deficiency in crops and humans. *Environmental geochemistry and health*, 31(5), pp.537-548.
2. Bharucha, U., Patel, K. and Trivedi, U.B., 2013. Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). *Agricultural research*, 2(3), pp.215-221.
3. Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I. and Lux, A., 2007. Zinc in plants. *New phytologist*, 173(4), pp.677-702.
4. Cakmak, I., 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification?. *Plant and soil*, 302(1), pp.1-17.
5. Goteti, P.K., Emmanuel, L.D.A., Desai, S. and Shaik, M.H.A., 2013. Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea mays* L.). *International journal of microbiology*, 2013(1), p.869697.
6. Gupta, S., Pandey, S., Kotra, V. and Kumar, A., 2023. Assessing the role of ACC deaminase-producing bacteria in alleviating salinity stress and enhancing zinc uptake in plants by altering the root architecture of French bean (*Phaseolus vulgaris*) plants. *Planta*, 258(1), p.3.
7. Halder, S., Mukherjee, A. and Gachhui, R., 2026. Zinc solubilizing bacteria *Pantoea dispersa* SHL2C isolated from lichen with additional growth promoting activity on *Sorghum vulgare*, a crop plant. *Vegetos*, pp.1-11.
8. Hussain, A., Arshad, M., Zahir, Z.A. and Asghar, M., 2015. Prospects of zinc solubilizing bacteria for enhancing growth of maize. *Pakistan journal of agricultural sciences*, 52(4), p.915.
9. Kumar, A., Singh, S.K., Singh, K.K., Sinha, S.K., Kumari, S., Prasad, R.P., Kumar, M. and Singh, A.K., 2026. Functional screening of zinc-solubilizing bacteria isolated from the rhizosphere soil for in-vitro zinc carbonate solubilization potential. *International Journal of Environment and Climate Change*, 16(3), pp.74-85.
10. LI, W., CHEN, Y., TIAN, X. and JIAO, J., 2023. Bergey's Manual of Determinative Bacteriology and Bergey's International Society for Microbial Systematics (BISMS): past, present and future. *Acta Microbiologica Sinica*, 63(5), pp.1714-1723.
11. Lindsay, W.L., 1972. Zinc in soils and plant nutrition. *Advances in agronomy*, 24, pp.147-186.
12. Mumtaz, M.Z., Ahmad, M., Jamil, M. and Hussain, T., 2017. Zinc solubilizing *Bacillus* spp. potential candidates for biofortification in maize. *Microbiological research*, 202, pp.51-60.
13. Praharaaj, S., Skalicky, M., Maitra, S., Bhadra, P., Shankar, T., Brestic, M., Hejnak, V., Vachova, P. and Hossain, A., 2021. Zinc biofortification in food crops could alleviate the zinc malnutrition in human health. *Molecules*, 26(12), p.3509.
14. Saravanan, V.S., Madhaiyan, M. and Thangaraju, M., 2007. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. *Chemosphere*, 66(9), pp.1794-1798.
15. Singh, U.B., Malviya, D., Singh, S., Singh, P., Ghatak, A., Imran, M., Rai, J.P., Singh, R.K., Manna, M.C., Sharma, A.K. and Saxena, A.K., 2021. Salt-tolerant compatible microbial inoculants modulate physio-biochemical responses enhance plant growth, Zn biofortification and yield of wheat grown in saline-sodic soil. *International Journal of Environmental Research and Public Health*, 18(18), p.9936.
16. Shivay, Y.S., Kumar, D. and Prasad, R., 2008. Effect of zinc-enriched urea on productivity, zinc uptake and efficiency of an aromatic rice-wheat cropping system. *Nutrient Cycling in Agroecosystems*, 81(3), pp.229-243.