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Original Article

Rhizobacteria potential in improving the availability of Zinc and maize growth

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Abstract

Zinc is an essential micronutrient for maize plants; its deficiency reduces growth and yield. The use of zinc-solubilizing rhizobacteria (ZSR) to address Zn deficiency is an eco-friendly alternative. This study aims to select ZSR potential as biofertilizers to enhance corn growth. Rhizobacterial isolates were obtained from maize rhizospheres in South Sulawesi, Indonesia, and screened for their ability to solubilize Zn in media supplemented with $Zn_3(PO_4)_2$, ZnO, and $ZnCO_3$. Capable isolates of solubilizing Zn in all types of media selected for maize growth testing. It was identified that among 46 Zn-solubilizing isolates, six isolates were active in all three media: Bn.1.7 (*Klebsiella* sp.), Bn.1.11 (*Serratia* sp. strain EB340), Btg.1.5 (*Citrobacter freundii* strain KSSN 2.2), Btg-1.6 (*Serratia marcescens* strain NPK2), Btg.2.3 (*Bacillus* sp.), and Jpt.3.7 (*Lysinibacillus* sp.). Isolates Btg.2.3 (Bacillus sp.) and Jpt.3.7 (*Lysinibacillus* sp.) demonstrated the greatest dissolution capacity and markedly increased Zn solubility in liquid media across all insoluble Zn forms and incubation periods. These isolates were applied in a pot experiment, and they significantly improved growth parameters, including plant height, fresh weight, and dry weight, compared to the control and other isolates. These findings suggest that Btg.2.3 and Jpt.3.7 have potential as biofertilizers for crop production. Furthermore, $Zn_3(PO_4)_2$-based media outperformed other media for screening rhizobacteria capable of solubilizing various forms of Zn in the soil.

Keywords: Biofertilizer, Environment friendly, Rhizobacteria, Corn, Zinc

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Introduction

Maize (*Zea mays* L.) is a strategic crop commodity in Indonesia, playing a vital role in fulfilling national food, feed, and industrial needs. In line with increasing domestic demand, national maize production continues to grow. In 2023, the maize harvest area reached 2.58 million hectares, with a productivity of 5.7 tonnes per hectare. However, national maize demand exceeds domestic production, necessitating imports to meet the deficit (ICBS, 2024). A key constraint to increasing maize yield, particularly in calcareous and light-textured soils with low organic matter, is zinc (Zn) deficiency (Alloway, 2008). As an essential micronutrient, Zinc (Zn) can regulate various metabolic activities, including protein synthesis, photosynthesis, carbohydrate metabolism, phytohormone activity, and root development (Hamzah et al., 2022). Oliveira et al. (2023) proposed that zinc (Zn) availability contributes to increased grain yield, pollen viability, and resistance to environmental stress. Stanton et al. (2022) showed that Zn deficiency caused chlorosis, leaf shrinkage, spikelet sterility, increased susceptibility to high temperatures and fungal diseases, and stunted growth, as well as reduced yield and seed Zn content.

Plants take up zinc mainly as the divalent ion Zn^{2+} and in chelated forms. However, the majority of zinc in soils exists in poorly soluble forms, making it unavailable for absorption and potentially leading to Zn deficiency (Alloway, 2008; Stanton et al., 2022). Inorganic fertilisers, such as ZnSO4 and ZnO, were effective in controlling Zn deficiency; however, they were less environmentally friendly. Excessive amounts of inorganic Zn fertilizers caused an imbalance in other nutrients, rendering them unavailable to plants, affecting growth and reducing yield (Krasilnikov et al., 2022; Petkovic et al., 2022).

The use of organic fertilisers necessitates attention to enhance nutrient use efficiency, thereby increasing crop productivity and minimising environmental impacts (Syafruddin et al., 2023, 2025). Moreover, it demonstrated that the organic fertilizer contained zinc-solubilizing rhizobacteria (ZSR), which could colonize the plant rhizosphere and convert insoluble forms of zinc into bioavailable Zn^{2+} (Upadhayay et al., 2022), and increase the growth and yield of wheat, soybean, and maize crops (Ma et al., 2018; Mumtaz et al., 2020; Sirohi et al., 2015).

Although the role of zinc-solubilizing rhizobacteria (ZSR) in improving plant Zn nutrition has been widely reported, most previous studies have focused on bacterial isolates capable of solubilizing only one insoluble Zn compound under laboratory conditions (Saravanan et al., 2007; Mumtaz et al., 2020). However, in soil, zinc exists in several poorly soluble mineral forms, such as zinc phosphate ($Zn_3(PO_4)_2$), zinc oxide (ZnO), and zinc carbonate ($ZnCO_3$), which differ in solubility and chemical stability (Alloway, 2008). Consequently, the effectiveness of ZSR in mobilizing Zn for plant uptake may depend on its ability to solubilize multiple Zn compounds in soil. Despite this importance, the identification of rhizobacterial isolates capable of simultaneously solubilizing multiple insoluble Zn forms remains limited. For example, Othman et al. (2022) reported that among 88 bacterial isolates, only nine were able to solubilize Zn from ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$. Similarly, Saravanan et al. (2007) found that some rhizobacteria could solubilize ZnO and $ZnCO_3$ but not $Zn_3(PO_4)_2$. Therefore, screening rhizobacteria capable of solubilizing multiple Zn sources is a crucial step towards developing more efficient biofertilizers. This research aims to identify rhizobacterial isolates capable of simultaneously solubilizing three major insoluble Zn compounds $Zn_3(PO_4)_2$, ZnO, and $ZnCO_3$ and to evaluate their potential to enhance maize growth. These findings are expected to contribute to the development of rhizobacteria-based biofertilizers to enhance Zn availability and maize productivity in Zn-deficient soils.

Material and Methods

Isolation of Zn-solubilizing rhizobacteria

Soil samples were collected from the rhizosphere of maize plants on the famer field growth at six sites across three districts Bone, Bantaeng, and Jeneponto in South Sulawesi, Indonesia. Discription of location in Table 1.

Healthy, pest- and disease-free maize plants, 45–60 days after planting, served as samples. Five plants from each location were randomly selected, dug to a depth of 25 cm, and cleaned of root-attached soil with a sterile knife. These subsamples were mixed to form a composite sample for each site. Approximately 100–200 g of composite soil was placed in a sterile plastic bag, stored in a cooler box, and kept at ±4 °C in the laboratory. Isolation was performed within 24–48 hours after sampling.

One gram from each soil sample was put into a dilution tube containing 9 mL of sterile distilled water (10^{-1}

dilution), and the mixture was shaken for 10 minutes. The suspension was allowed to settle for 30 minutes to ensure uniform distribution. Subsequently, 1 mL of the supernatant from the 10^{-1} dilution was transferred to another tube containing 9 mL of sterile distilled water to obtain a 10^{-2} dilution. This mixture was shaken for 10 minutes and then allowed to settle for 30 minutes. Serial dilutions were prepared to 10^{-10}, and aliquots were plated onto nutrient agar (NA) medium and incubated at 28°C for 72 hours. The population of bacteria was colony-forming units (CFU) per gram of soil, as CFU/mL, using the appropriate calculation formula as follows:

$$IP = \frac{X}{P\ x\ V}$$

X = number of colonies with dilution factors (cfu).
P = dilution factor (cfu).
V = suspension volume (ml).

Each colony was subculture by streaking onto Nutrient Agar (NA) medium to obtain pure isolates (Dinesh et al., 2018). Initial identification was carried out based on the shape, edge, elevation, and colour of the colony. The Gram reaction was determined using cultures grown on NA medium. A small portion of pure colony was collected with an inoculating needle and transferred onto a glass slide containing two drops of 3% KOH solution, which was then mixed in a circular motion for 5–10 seconds. Isolates that produced a viscous or slimy consistency were classified as Gram-negative, whereas those that remained cohesive were classified as Gram-positive. These bacterial isolates were stored at 4°C for further analysis.

Qualitative screening for Zn solubilization

Capacity of Zn-solubilization was The zinc-solubilizing capacity of the bacterial isolates was analyzed using nutrient agar medium added to basal minerals (glucose, 10 g; $(NH_4)_2SO_4$, 1 g; KCl, 0.2 g; K_2HPO_4, 0.1 g; $MgSO_4$, 0.5 g; and 1000 mL of double-distilled water, adjusted to pH 7) and supplement with 0.2% of insoluble zinc compounds ZnO, $ZnCO_3$, or $Zn_3(PO_4)_2$, autoclaving at 121°C for 20 minutes, and dropped into sterile petri dishes.

A 10 µL inoculum from a 24-hour-old bacterial culture was streaked onto the medium and incubated at 28°C for 48 hours. All assays were conducted in triplicate. The formation of a clear halo surrounding the bacterial colonies after seven days signified the ability of the isolates to solubilize Zinc. The diameter of the halo zone was recorded, and the solubilization index (SI) was determined according to the specified formula.

$$SI = \frac{\text{Diameter of clear zone (cm)+ Diameter of colony (cm)}}{Dameter\ of\ colony\ \ (cm)}$$

Quantitative test of Zn solubilization

Bacterial isolates capable of solubilizing zinc were examined for their quantitative ability to solubilize inorganic Zn in liquid medium containing $Zn_3(PO_4)_2$, ZnO, or $ZnCO_3$. The mineral medium (20 mL) contained dextrose (10 g L^{-1}) as the primary carbon source, $(NH_4)_2SO_4$ (1 g L^{-1}) as the nitrogen source, KCl (0.2 g L^{-1}), K_2HPO_4 (0.1 g L^{-1}), and $MgSO_4$ (0.2 g L^{-1}) adjusted to pH 7.0. This specified medium was chosen to promote bacterial growth while decreasing background Zn levels and preventing interaction with Zn solubilization activities. Each medium was supplemented with 100 mg L^{-1} of Zn using the relevant insoluble zinc source.

The medium was sterilized at 121°C for 20 minutes before being inoculated with 1 mL of bacterial suspension to ensure uniform starting inoculum density across treatments and then incubated at 120 rpm with continuous shaking. The shaking speed was set to ensure homogenous dispersion of insoluble Zn particles and appropriate aeration while minimizing shear stress on bacterial cells. The uninoculated medium served as a control, and all treatments were carried out four times.

Sampling was done at 1, 3, 5, and 10 days following inoculation to detect both early-stage Zn solubilization activity and progressive Zn release over an extended incubation period. At each sampling interval, cultures were centrifuged at 8000 rpm for 15 minutes to separate insoluble residues from bacterial cells. A 1 mL sample of the supernatant was diluted to 50 mL with pure water, and the dissolved Zn concentration was measured using atomic absorption spectrophotometry (AAS).

Molecular identification of selected strains

Molecular identification and taxonomic assignment of selected strains

Genomic DNA from selected Zn-solubilizing rhizobacterial isolates was extracted using the standard methodology outlined by Sambrook and Russel (2001) and subsequently purified with the Quick-DNA™ Fungal/Bacterial Miniprep Kit (D6005), in accordance with the manufacturer's guidelines. Molecular identification was conducted through partial sequencing of the 16S rRNA gene. The isolated DNA was amplified with universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTAG-3′) and 1492R (5′-TACGGYTACCTTGACGACTT-3′). MyTaq™ HS Red Mix (Bioline) was used for PCR amplification. Amplicons were resolved by electrophoresis on a 0.8% agarose gel and visualized under UV light. All selected isolates produced the predicted PCR product size of roughly 1400-1500 bp, indicating effective amplification.

The resultant 16S rRNA gene sequences were matched to reference sequences from the GenBank database using the NCBI's Basic Local Alignment Search Tool (BLASTn). Taxonomic assignment was determined using a combination of query coverage, percentage sequence similarity, and phylogenetic analysis. Sequence similarity ranging from 97.2% to 100% identity corresponds to the species level, while values between 90.3% and 99.0% indicate genus-level similarity (Hackmann, 2025). Taxonomic identification was supported by the nearest homologous sequences and their GenBank accession codes.

To validate the BLAST-based identification, phylogenetic analysis was carried out with the MEGA X program. Sequence alignments and phylogenetic trees were created using the Neighbor-Joining method, the Kimura two-parameter model, and 1000 bootstrap replications. The clustering pattern of each isolate with its nearest reference sequences was employed as an extra criterion to establish taxonomic placement.

Greenhouse experiment

The research was conducted in the greenhouse of the Research Centre for Cereal Crops, Maros, South Sulawesi, Indonesia. Soil pH value 7.0, clay loam in texture, and available Zn content 0.6 ppm (using the DTPA extraction method). A split-plot experimental design was employed. The main plot factor was the application of rhizobacteria, consisting of seven treatment levels: no bacterial isolate (control), *Klebsiella variicola* strain 145-a blue, *Lysinibacillus* sp., *Bacillus* s pp., *Serratia* sp. strain EB340, Serratia marcescens strain NPK2, and *Citrobacter freundii* strain KSSN2.2. The subplot factor was the dose of Zn fertilizer, consisting of three treatment levels: 0, 5, and 10 kg/ha, resulting in 21 treatment combinations with six replications. Each treatment included 20 plants cultivated under controlled conditions. For each treatment, 20 seeds were sown in a single row, spaced 20 cm between seeds and 75 cm between treatments. Bacterial isolates were applied via seed treatment. Zn fertilization, using ZnO, was carried out through soil application 10 days after sowing (DAS). Six plant samples were randomly selected at 60 days after planting (DAS) for measurement of plant height, fresh weight, and dry weight (g).

Data were analyzed statistically and significant differences were calculated at $P \leq 0.05$ using SPSS version 9.0.

Results

Isolation of Zn-solubilizing rhizobacteria

Soil samples as a source of Zn-solubilizing bacteria were taken from the rhizosphere of maize plants in 6 locations in 3 districts in South Sulawesi, Indonesia. Isolation results using the standard plate method obtained fifty-six rhizobacteria isolates with populations of 96×10^9 - 252×10^9 cfu/ml (Table 1).

Screening for Zn solubilization activity

Qualitative analysis using the plate

A total of 56 rhizobacterial isolates were collected from the study site (Table 1), of which 46 demonstrated zinc-solubilizing abilities, as indicated by the formation of clear halozones (Figure 1). Among these, two isolates were able to solubilize Zn only in the ZnO medium, while another two were active exclusively in the $ZnCO_3$ medium. Additionally, 36 isolates could solubilize Zn in both ZnO and $ZnCO_3$ media, whereas six isolates exhibited solubilization activity across all three media (ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$). The diameter of the halo zones, expressed as the Solubility Index (SI), varied among the isolates. On average, the halo zones measured 1.34 ± 0.15 cm with an SI of 3.92 ± 0.53 in the ZnO medium, 1.48 ± 0.17 cm with an SI of 4.52 ± 0.66 in the $ZnCO_3$ medium, and 0.93 ± 0.42 cm with an SI of 3.68 ± 0.57 in the $Zn_3(PO_4)_2$ medium (Table 2). The isolates capable of solubilizing Zn in all three media were Bn.1.7 and Bn-1.1 from Bone Regency, Btg.1.5, Btg-1.6, and Btg.2.3 from Bantaeng Regency, and Jpt.3.7 from Jeneponto Regency.

Table-1. Bacterial isolation results based on sampling location.

Location	Sample code	Coordinate	Number of isolates	Population cfu/ml
Tacipi, Bone District	Bn.1	4° 31' 20.24" S 120° 7' 0.77" E	11	209 x 10^9
Bonto Manai, Bantaeng	Btg.1	5° 33' 24.84" S 119° 54' 25.56" E	11	175 x 10^9
Bonto Jai Bantaeng	Btg.2	5° 34' 27.12" S 119° 54' 29.52" E	13	252 x 10^9
Balang Loe Jeneponto	Jpt.1	5° 39' 55.8" S 119° 47' 12.12" E	8	112 x 10^9
Kalumpang Lompoa, Jeneponto	Jpt.2	5° 36' 43.5" S 119° 37' 18" E	5	96 x 10^9
Bangkala Barat, Jeneponto	Jpt.3	5° 34' 28.2" S 119° 33' 27" E	8	133 x 10^9
Total			56	

Table-2. Halo zone and zinc solubilization index of rhizobacteria.

	Halo zone (cm)			Solubilization Index		
Isolate	ZnO	$ZnCO_3$	$Zn_3(PO_4)_2$	ZnO	$ZnCO_3$	$Zn_3(PO_4)_2$
Bn.1.1	0.30±0.10	-	-	2.61±0.35	-	-
Bn.1.4	1.43±0.15	1.87±0.15	-	3.65±0.74	4.43±0.77	-
Bn.1.5	0.53±0.06	1.13±0.06	-	2.61±0.10	3.63±0.32	-
Bn.1.6	0.26±0.06	0.30±0.10	-	2.67±0.29	2.61±0.35	-
Bn.1.7	1.4±0.10	1.4±0.20	0.70±0.17	3.38±0.54	3.23±0.21	3.00±0.0
Bn.1.8	0.27±0.06	1.57±0.15	-	2.67±0.33	5.16±1.44	-
Bn.1.9	1.63±0.25	1.4±0.10	-	4.26±0.50	3.80±0.35	-
Bn.1.11	1.50±0.20	1.70±0.10	0.83±0.50	5.21±1.27	5.69±0.57	3.83±0.74
Btg.1.1	0.30±0.26	-	-	1.56±1.34	-	-
Btg.1.2	0.37±0.06	2.16±0.0	-	5.77±1.37	4.02±0.31	-
Btg.1.3	-	1.23±0.00	-	-	4.08±0.52	-
Btg.1.4	0.40±0.10	0.57±0.21	-	2.58±0.38	2.89±0.19	-
Btg.1.5	1.46±0.21	1.47±0.15	0.80±0.26	3.74±0.13	3.97±0.29	3.18±0.35
Btg.1.6	1.53±0.15	1.53±0.15	1.03±0.46	3.0±0.12	3.37±0.46	3.81±0.37
Btg.1.7	0.76±0.06	1.17±0.25	-	2.89±0.68	2.61±1.500	-
Btg.1.8	1.17±0.15	1.30±0.17	-	2.53±0.11	3.16±0.76	-
Btg.1.10	1.63±0.11	1.43±0.06	-	4.26±1.06	5.39±0.79	-
Btg.1.11	1.07±0.46	1.10±0.26	-	2.87±0.23	4.03±0.68	-
Btg.2.1	2.27±0.25	1.87±0.15	-	3.97±0.27	4.16±0.55	-
Btg.2.2	1.47±0.58	1.50±0.10	-	5.22±1.07	4.23±0.25	-
Btg.2.3	2.07±0.15	2.4±0.26	1.17±0.61	4.89±0.28	4.83±0.73	3.80±0.62
Btg.2.14	1.43±0.23	2.15±0.15	-	3.51±0.70	4.25±0.10	-
Btg.2.5	1.37±0.15	0.27±0.57	-	4.17±0.29	3.39±0.22	-

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Btg.2.6	1.23±0.15	1.23±0.11	-	3.54±0.62	3.68±0.53	-
Btg.2.8	1.93±0.15	2.10±0.26	-	4.63±0.15	4.24±0.67	-
Btg.2.9	1.00±0.1	2.03±0.30	-	2.37±0.18	3.80±0.22	-
Btg.2.10	-	1.10±0.52	-	-	3.15±0.58	-
Btg.2.11	0.37±0.06	0.46±0.06	-	2.83±0.29	2.56±0.19	-
Btg.2.12	2.20±0.10	2.33±0.06	-	4.08±0.56	6.05±0.61	-
Btg.2.13	1.13±0.15	1.30±0.10	-	4.19±0.99	5.00±0.88	-
Jpt.1.1	1.40±0.20	1.73±0.30	-	4.23±0.25	4.83±1.04	-
Jpt.1.2	1.93±0.11	1.90±0.10	-	4.08±0.44	3.76±0.51	-
Jpt.1.3	1.03±0.15	1.63±0.21	-	2.93±0.11	3.80±0.72	-
Jpt.1.4	1.57±0.06	1.80±0.20	-	3.26±0.25	3.73±0.54	-
Jpt.1.5	1.43±0.11	1.40±0.10	-	5.11±1.02	4.89±0.72	-
Jpt.1.6	2,07±0,11	1,70±0,10	-	6,17±0,29	5,45±1,23	-
Jpt.1.7	1,87±0,21	1,83±0,30	-	7,22±0,69	6,69±1,72	-
Jpt.1.8	1,63±0,21	1,57±1,15	-	5,08±0,52	5,39±1,13	-
Jpt.2.2	1,47±0,15	1,23±0,15	-	5,11±1,09	3,33±0,42	-
Jpt.2.3	1,37±0,15	1,33±0,11	-	3,80±0,61	3,71±0,34	-
Jpt.3.2	-	0,33±0,06	-	-	2,78±1,07	-
Jpt.3.3	1,53±0,11	1,67±0,15	-	4,44±0,96	6,05±0,58	-
Jpt.3.4	1,60±0,17	1,63±0,15	-	5,83±0,29	5,11±1,83	-
Jpt.3.5	1,60±0,10	1,87±0,23	-	4,72±0,30	4,33±0,67	-
Jpt.3.6	1,47±0,15	1,33±0,15	-	3,68±0,51	4,13±0,68	-
Jpt.3.7	1.70±0.20	1.83±0.30	1.07±0.51	3.38±0.64	5.68±0.77	4.47±1.36
Average	1.34±0.15	1.48±0.17	0.93±0.42	3.92±0.53	4.52±0.66	3.68±0.57

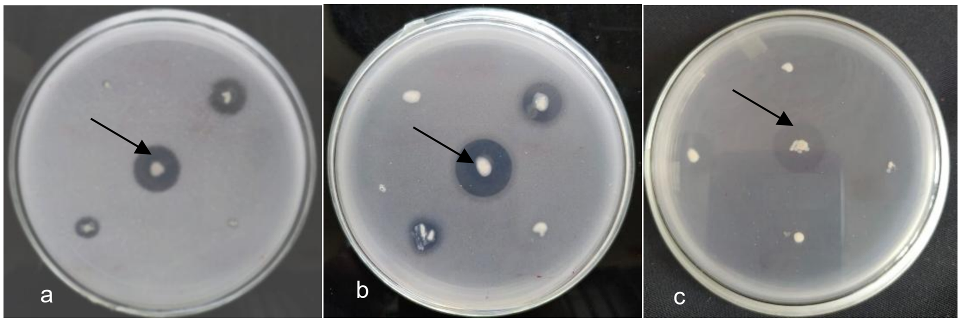

Figure-1. Bacterial isolates that form clear zones on (a) ZnO media, (b) $ZnCO_3$ and (c) $Zn_3(PO_4)_2$.

Quantitative Zn-solubility assay

Qualitative tests of bacterial isolates revealed the formation of halo zones from three different Zn sources, followed by quantitative analysis. The quantitative results indicate that Zn solubilization varies among isolates based on the media type and incubation period. The solubilization ability of isolates follows the trend ZnO > $ZnCO_3$ > $Zn_3(PO_4)_2$ in incubation media. Zn solubility increases over the first five days of incubation but declines at ten days.

Among the tested isolates, Btg.2.3 and Jpt.3.7 exhibited the highest Zn dissolution capacity. Specifically, isolate Btg.2.3 achieved maximum Zn dissolution values of 44.7, 11.5, and 10.0 ppm, whereas Jpt-37 dissolved 20.3, 10.7, and 8.4 ppm of Zn in ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$ media, respectively (Figure 2).

A significant negative correlation was observed between pH and Zn solubilization. Correlation coefficients were 0.5437 for ZnO, 0.915 for $ZnCO_3$, and 0.8682 for $Zn_3(PO_4)_2$. For each decrease of one pH unit, Zn solubility increased by 10,362 ppm in ZnO, 5,913 ppm in $ZnCO_3$, and 2,108 ppm in $Zn_3(PO_4)_2$, respectively (Figure 3).

The initial pH of the liquid medium varied with the zinc compound, pH 6.4 for ZnO, 7.5 for $ZnCO_3$ and 7.0 for $Zn_3(PO_4)_2$. Inoculation with rhizobacteria reduced solution pH, and the extent of this decrease was influenced by both the zinc form and the specific rhizobacterial isolate. The greatest average pH reduction occurred with ZnO, followed by $ZnCO_3$ and $Zn_3(PO_4)_2$. Isolates Btg. 2.3 and Bn.1.11 produced more pronounced decreases in pH compared to other isolates. For Btg. 2.3, the pH decreased to 4.2, 6.1, and 5.9, while for Bn.1.11 decreased to 5.1, 6.1, and 6.4 for ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$, respectively (Figure 4).

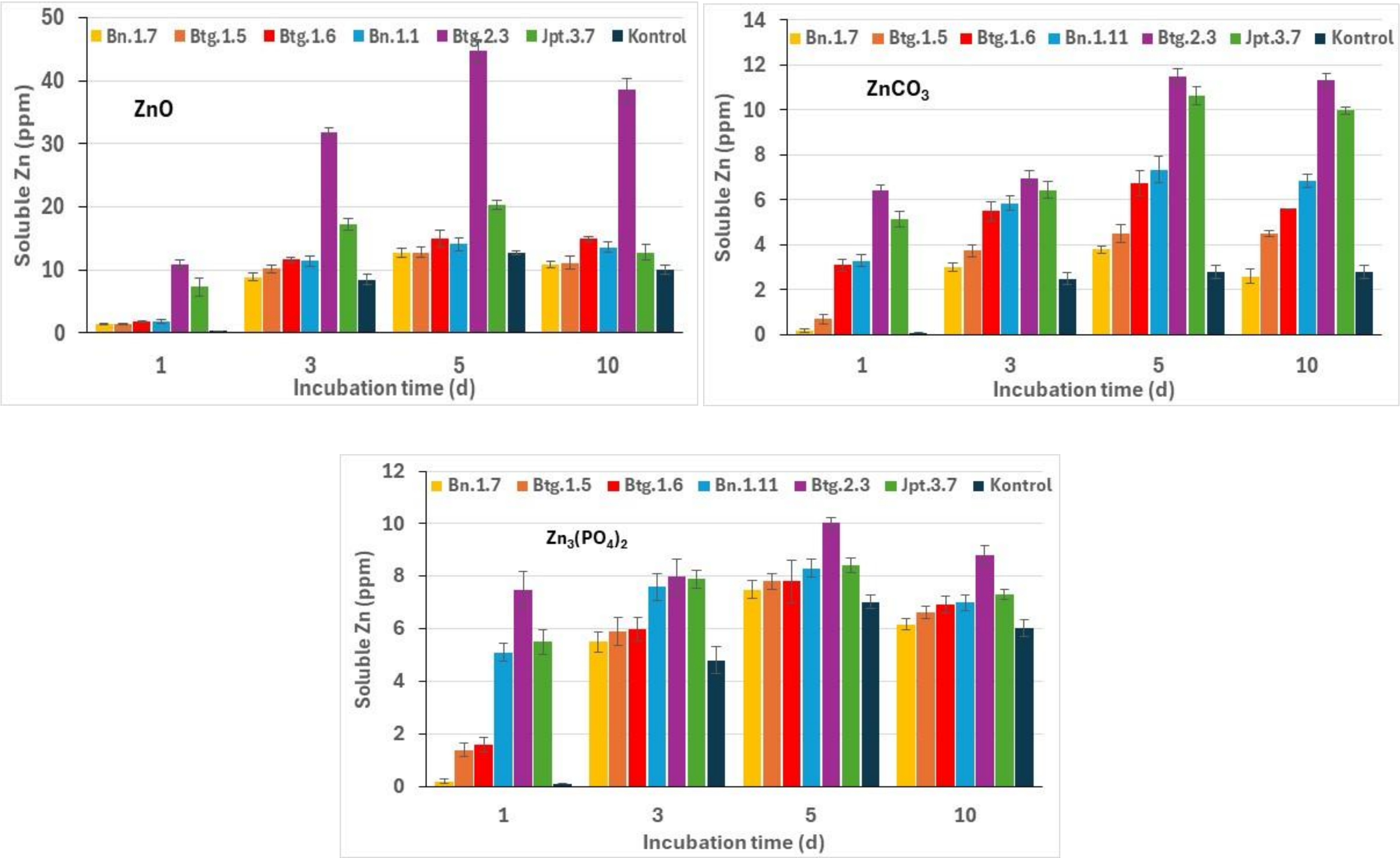

Figure-2. Zn solubilisation concentration of each rhizobacterial isolate on ZnO (top), $ZnCO_3$ (middle), and $Zn_3(PO_4)_2$ (bottom) media. Error bars represent standard deviation (n = 3).

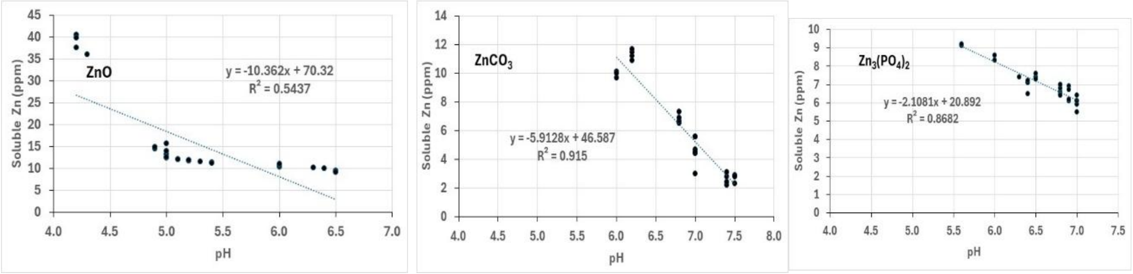

Figure-3. Correlation between pH with Zn solubilization from different source of Zn.

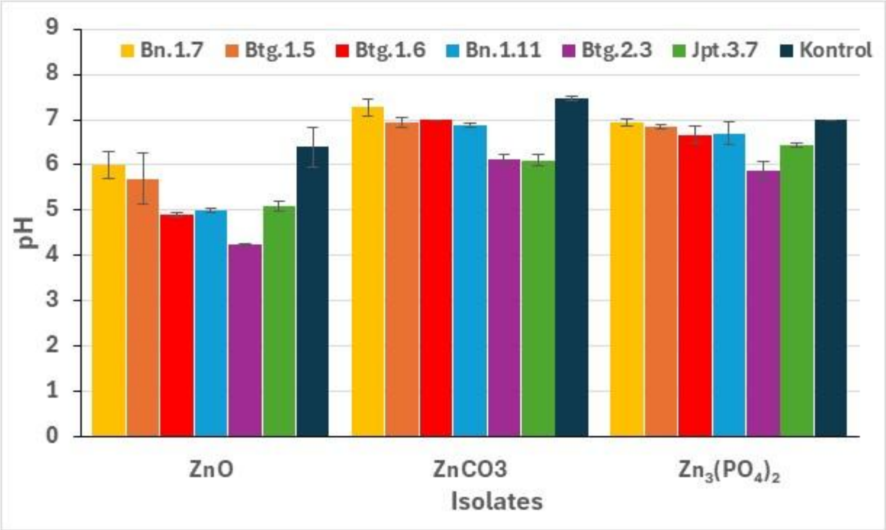

Figure-4. pH Value of each rhizobacterial isolate on ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$ Error bars represent standard deviation (n = 3).

Identification of Zn-solubilizing rhizobacteria

The six rhizobacteria isolates exhibited diverse morphological traits, as outlined in Table 3. Their colony shapes varied between irregular and round, with edges that were either complete or choppy. The colony's elevation appeared to be flat and displayed colours ranging from white to a white-reddish hue. Isolates Btg.2.3 and Jpt-37 were identified as gram-positive bacteria, while the other isolates were classified as gram-negative.

Isolates with different sources and locations in the DNA isolation results show similar bands at 1500 bp, as shown in Figure 5.

Table-3. Morphological identification of bacterial isolates.

Isolate code	Colony form	Edge of the colony	elevation	Colony colour	Gram reaction
Bn.1.7	Round	Choppy	Appear	White	Negative
Bn.1.11	Round	Complete	Appear	White reddish	Negative
Btg.1.5	Round	Choppy	Appear	White	Negative
Btg.1.16	Irregular	Choppy	Flat	White reddish	Negative
Btg.2.3	Round	Choppy	Flat	White	Positive
Jpt.3.7	Round	Complete	Flat	White	Postive

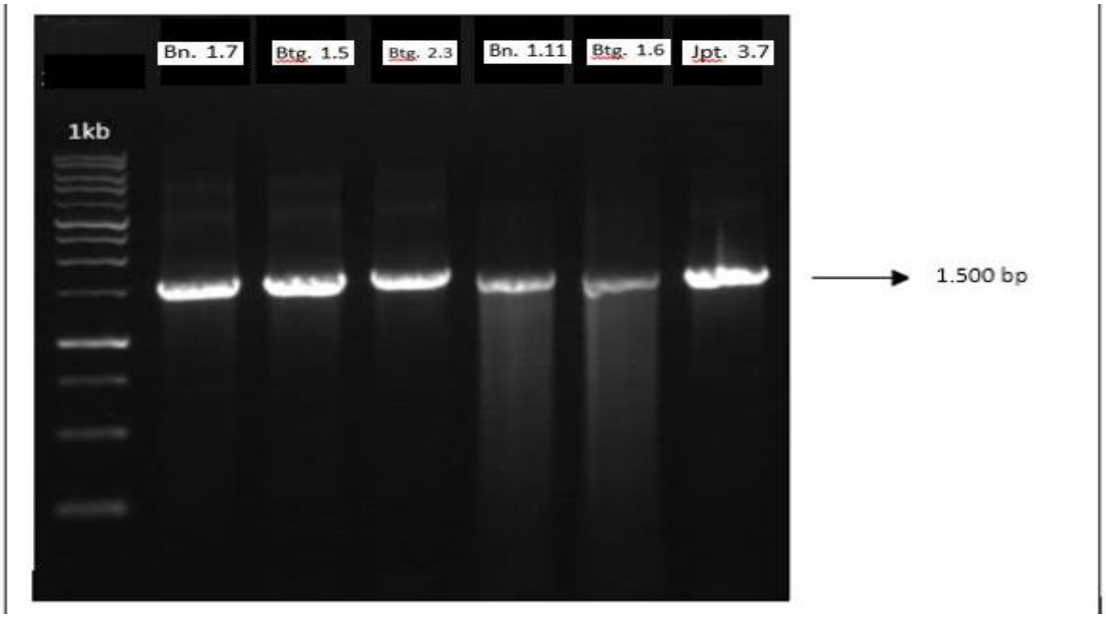

Figure-5. DNA isolation results on 0.8% agarose gel.

The results of BLAST analysis of the sequences obtained in the gene bank showed that isolate isolate Bn.1.7 has similarity with *Klebsiella* sp, isolate Bn.1.11 has the closes proximity to *Serratia* sp strain EB340, Btg.1.5 has similarity with *Citrobacter freundii* strain KSSN 2.2, isolate Btg.2.3 has similarity with *Bacillus* sp, and isolate Btg-1.6 has similarity with *Serratia marcescens*. In contrast, isolate Jpt.3.7 has similarity with *Lysinibacillus* sp Table 4 and phylogenetic (Figure 6).

Table-4. Similarity Identification Results of Isolate Sequences through NCBI.

Isolate Code	Identification	Query Cover	Similarities*	No, GenBank Accession
Bn.1.7	*Klebsiella* sp	98 %	92,10 %	MN208233,1
Bn.1.11	*Serratia_sp* stain EB340	100 %	98,94 %	MH127791,1
Btg.1.5	*Citrobacter_freundii* strain KSSN 2.2	99 %	99,63 %	KM117229,1
Btg.1.16	*Serratia_marcescens* strain NPK2	98 %	95,18 %	MN691675,1
Btg.2.3	*Bacillus*_sp	100 %	94.44 %	KT026504,1
Jpt.3.7	*Lysinibacillus* sp	100 %	92,99 %	OM142588,1

Note: *Identification of similarities based on Hackmann's 2025 criteria.

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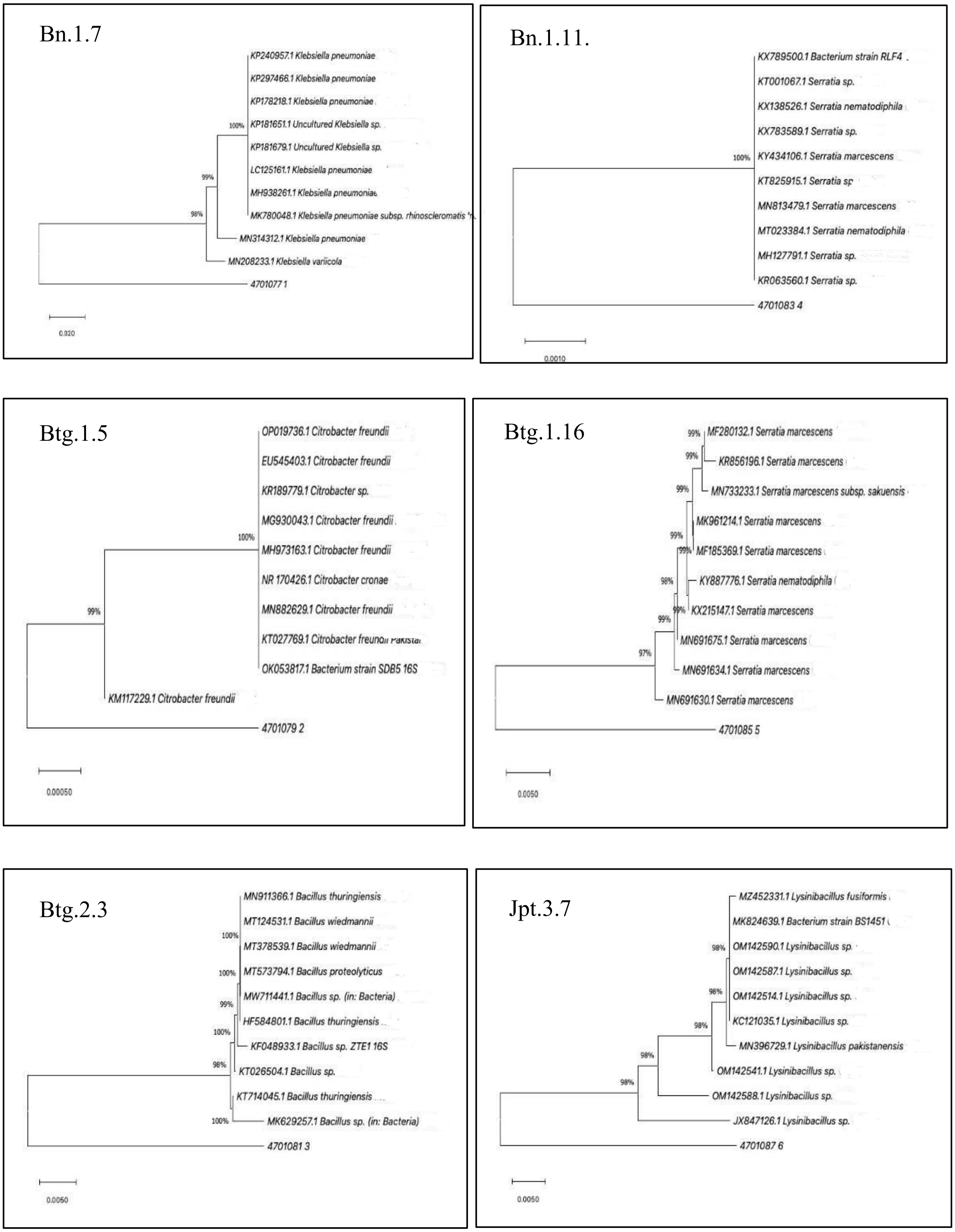

Figure-6. Phylogenetic analysis of each selected isolate using Mega X software (Neighbor Joining method, Kimura 2, Boostrap 1000x).

Plant growth promotion in pot experiments

There was a significant interaction between rhizobacterial inoculation and zinc (Zn) application on plant height. Without Zn application, the isolates Bn.1.11, Btg.2.3, and Jpt.3.7 produced taller plants than the control (without bacterial inoculation). When 5 or 10 kg/ha of Zn was applied, all With Zn application of 5 or 10 kg/ha, all three isolates resulted in increased plant height. Furthermore, inoculation with Bn.1.11, Btg.2.3, or Jpt.3.7 significantly increased plant fresh and dry weight (Tables 5, 6, and 7).

Table-5. Average plant height (cm) of maize at 60 DAS.

Zn (kg/ha)	Isolate						
	Bn.1.7	**Btg.1.5**	**Btg.1.16**	**Bn.1.11**	**Btg.2.3**	**Jpt.3.7**	**Control**
0	$217.7^{c}{}_{(q}$	$213.5^{c}{}_{(r}$	$217.3^{c}{}_{(r}$	$238.2^{b}{}_{(q}$	$250.3^{a}{}_{(q}$	$239.5^{ab}{}_{(q}$	$210.3^{c}{}_{(q}$
5	$227.0^{de}{}_{(p}$	$226.3^{de}{}_{(q}$	$233.5^{cd}{}_{(q}$	$242.0\ ^{bc}{}_{(pq}$	$258.7^{a}{}_{(p}$	$250.3^{ab}{}_{(p}$	$220.7^{e}{}_{(p}$
10	$230.5^{de}{}_{(p}$	$241.5^{cd}{}_{(p}$	$247.2^{bc}{}_{(p}$	$249.7^{bc}{}_{(p}$	$262.5^{a}{}_{(p}$	$256.8^{ab}{}_{(p}$	$223.8^{e}{}_{(p}$
Average	225.1	227.1	246.0	243.3	257.2	248.2	218.3

Note: Numbers followed by the same letter in the row (abcde) and column (pqr) mean not significantly different based on the Least Significant Difference (LSD) test at the α 0.05 level.

Table-6. Average fresh weight (g/plant) of maize plants at 60 DAS.

Zn (kg/ha)	Isolates						
	Bn.1.7	**Btg.1.5**	**Btg.1.16**	**Bn.1.11**	**Btg.2.3**	**Jpt.3.7**	**Control**
0	348.8	355.83	382.2	417.5	441.3	404.7	339.8
5	359.5	377.00	339.5	429.5	467.3	449.0	351.0
10	367.5	413.17	418.3	440.5	482.0	463.17	352.7
Average	358.6^{e}	382.0^{d}	380.0^{c}	429.2^{b}	463.5^{a}	438.8^{b}	347.8^{e}

Note: Numbers followed by the same letter in the row (abcde) and column (pqr) mean not significantly different based on the Least Significant Difference (LSD) test at the α 0.05 level.

Table-7. Average dry weight (g/plant) of maize plants at 60 DAS.

Zn (kg/ha)	Isolates						
	Bn.1.7	**Btg.1.5**	**Btg.1.16**	**Bn-1. 11**	**Btg.2.3**	**Jpt.3.7**	**Control**
0	32.33	34.67	38.50	44.50	53.50	46.83	30.17
5	34.50	36.67	41.50	49.17	60.33	53.67	31.33
10	36.33	42.33	44.50	51.33	63.00	57.33	32.83
Average	34.39^{ef}	37.89^{c}	41.50^{d}	48.33^{c}	58.94^{a}	52.61^{b}	31.44^{f}

Note: Numbers followed by the same letter in the row (abcde) and column (pqr) mean not significantly different based on the Least Significant Difference (LSD) test at the α 0.05 level.

Discussion

Zinc (Zn) deficiency is commonly found in calcareous soils or in soils subjected to intensive and excessive phosphorus (P) fertilization. Although the total Zn content in these soils may be high, it often exists in forms that are insoluble and unavailable as $ZnCO_3$, ZnO, or $Zn_3(PO_4)_2$ for plant uptake (Oliveira et al., 2023). The use of rhizobacteria holds great potential as a bio-solubilizing agent, capable of converting insoluble Zn into forms more readily accessible to plants (Rani et al., 2020). In this study, we observed that the ability of different rhizobacterial isolates to solubilize Zn varied depending on both the isolate and the type of agar medium used, as indicated by differences in halo zone formation and solubility indices. Overall, the solubilization index in qualitative tes followed the order $ZnCO_3$ > ZnO > $Zn_3(PO_4)_2$ (Table 2). However, previous research has reported varying patterns of Zn solubilization. For example, Javed et al. (2018) and Srithaworn et al. (2023) found that rhizobacteria isolated from wheat, peanut, sweet potato, and cassava rhizospheres solubilised $ZnCO_3$ more efficiently than ZnO. In contrast, studies by Sharma et al. (2014) on soybean rhizospheres and Yasmin et al. (2021) on chickpea rhizospheres observed higher solubilization efficiency in $Zn_3(PO_4)_2$ media, followed by ZnO and $ZnCO_3$. Yadav et al. (2022) showed that isolated rhizobacteria from wheat could solubilize Zn only in $Zn_3(PO_4)_2$ media, with no activity observed in ZnO or $ZnCO_3$ media. Plant species and soil conditions of isolated rhizobacteria influenced these variations across studies.

Among the 46 isolates identified as zinc solvents, only six were capable of dissolving $Zn_3(PO_4)_2$. These six isolates also demonstrated the ability to solubilize zinc from $ZnCO_3$ and ZnO. The findings suggest that $Zn_3(PO_4)_2$ is a better medium for isolating zinc-solubilizing rhizobacteria for use as Zn biofertilizers than ZnO or $ZnCO_3$, as the selected isolate can dissolve multiple forms of soil-bound zinc.

The six isolates dissolved all three media were Bn.1.7 (*Klebsiella* sp), Bn.1.11 (*Serratia sp. strain EB340*), Btg.1.5 (*Citrobacter freundii* strain KSSN 2.2), Btg-1.6 (*Serratia marcescens* strain NPK2), Btg.2.3 (*Bacillus* sp.), and Jpt.3.7 (*Lysinibacillus* sp). Several studies have reported that rhizobacteria isolated from various plants can qualitatively solubilize Zinc, as indicated by the formation of clear halo zones around bacterial colonies. These isolates can enhance zinc solubility and increase the availability of zinc nutrients in the soil. *Klebsiella* spp. isolates have been identified in association with soybean and mungbean (Sharma et al., 2014); Bacillus spp. with wheat and wild pepper (Dinesh et al., 2018; Mumtaz et al., 2020; Naseem et al., 2022); *Lysinibacillus* spp. with soybean, mungbean, and wild pepper (Dinesh et al., 2018; Sharma et al., 2014); and Serratia spp. with rice (Kulkova et al., 2024).

Typically, plants absorb only 1–5% of applied zinc fertilizer. The remaining zinc is transformed into insoluble compounds, including $Zn(OH)_2$ in high-pH soils, $ZnCO_3$ in calcium-rich alkaline soils, and $Zn_3(PO_4)_2$ in phosphate-enriched neutral to alkaline soils (Rani et al., 2020). Therefore, rhizobacteria capable of solubilising Zn from ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$ can be used to enhance zinc bioavailability and absorption, as well as the efficiency of Zn fertilization. In parallel with this research, the application of PGPR has been shown to improve uptake and nutrient use efficiency (Syafruddin et al., 2025).

The six isolates selected in the qualitative test (plate assay) were then tested in liquid media (broth assay) containing a mixture of zinc compounds: ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$. The results of this test also confirmed that more Zn was solubilized in the inoculated than in the uninoculated control, and that isolates differed in their ability to dissolve Zn across various media. A primary factor contributing to increased Zn solubility by rhizobacteria is a decrease in pH. The result of this research indicates a negative correlation between pH and Zn solubility (Figure 3). Isolates Btg.2.3 (*Bacillus* sp.) and Jpt.3.7 (*Lysinibacillus* sp. strain DGS20) consistently exhibited higher zinc solubilization than the other isolates across all incubation periods. Similarly, pH observations showed that isolates Btg.2.3 (*Bacillus* sp.) and Jpt.3.7 (*Lysinibacillus* sp. strain DGS20) experienced greater decreases in pH compared to other isolates. Zinc-solubilizing rhizobacteria (ZSR) decrease rhizosphere pH and facilitate Zn solubilization through proton extrusion (release of H^+ ions) and chelation of Zn^{2+} (Saravanan et al., 2007; Yasmin et al., 2021). In addition to acidification, ZSR increases zinc availability through several mechanisms. They secrete organic acids, such as gluconic, oxalic, lactic, and butyric acids, which chelate Zn and convert its insoluble forms into plant-available forms. ZSR also produces enzymes that decompose organic matter and release bound zinc (Masood et al., 2022; Rani et al., 2025).

The ability to dissolve Zn of the six isolates quantitatively using ZnO> $ZnCO_3$> $Zn_3(PO_4)_2$. The efficiency of rhizobacteria in solubilizing zinc (Zn) depends on the chemical properties of the zinc compound, with ZnO being more readily solubilized than $ZnCO_3$ or $Zn_3(PO_4)_2$ (Saravanan et al., 2007; Dinesh et al., 2018; Hashemnejad et al., 2021; Othman et al., 2022). The absence of strong buffering anions in ZnO permits greater pH reduction, thereby increasing zinc availability (Alloway, 2008). In contrast, $ZnCO_3$ is less efficiently solubilized because carbonate ions buffer the protons. Zn3(PO4)2 is the least soluble zinc source due to its stable crystal structure and the strong interaction between zinc and phosphate, which leads to reprecipitation and limited zinc availability (Saravanan et al., 2007; Alloway, 2008).

Subsequent evaluation of the six selected isolates on maize growth demonstrated that two isolates, Btg.2.3 (*Bacillus* sp.) and Jpt.3.7 (*Lysinibacillus* sp.), consistently promoted greater vegetative growth, including increased plant height and higher fresh and dry biomass, compared to both the uninoculated control and the other isolates (Tables 5, 6, and 7). These results confirm the superior zinc solubilization capacity of these two isolates. Consistent with these findings, previous studies have shown that bacterial isolates significantly enhance maize growth and physiological parameters relative to uninoculated controls (Hussain et al., 2015). Therefore, the application of Btg.2.3 and Jpt.3.7 represents an effective strategy for zinc solubilization and improved plant growth. Mumtaz et al. (2017) also reported that Bacillus sp. can solubilize insoluble zinc sources and promote maize growth, attributing these effects to plant growth-promoting traits that facilitate root colonization. The genus *Lysinibacillus*, formerly classified as *Bacillus* sp. within the Bacillaceae family of the Firmicutes phylum, can function as a plant growth-promoting agent and in disease control (Ahsan and Shimizu, 2021). *Lysinibacillus* can also exude the phytohormone IAA, which increases biomass and enhances corn rooting (Pantoja-Guerra et al., 2023).

Conclusion

The isolation of rhizobacteria from maize plants yielded two isolates, Btg.2.3 (*Bacillus* sp.) and Jpt.3.7 (*Lysinibacillus* sp.), both of which show potential as zinc-solubilizing biofertilizers for various insoluble forms of Zn in soil. These isolates consistently enhanced zinc solubility in ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$ agar and liquid media and improved plant growth parameters, including plant height, fresh weight, and dry weight in maize. However, further field studies in several agroecosystems, especially on soils with low Zn availability or solubility, such as calcareous soils in maize, are needed to evaluate the consistency and effectiveness of their performance.

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Contribution of Authors

Herawati, Fatmawati, Suwarti, Herniwati, Paesal & Bidhari LA: Data curation, investigation and formal analysis.
Djaenuddin N & Najamuddin E: Isolation and molecular identification.
Syafruddin, Arief R & Irmadamayanti A: Methodology, validation and writing-original draft.
Muis A, Nonci N, Pabendon MB & Yustisia: Writing review and editing.

All authors read and approved the final draft of the manuscript.

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