

# Plant Growth Promoting Response of Zinc Solubilizing Bacteria *Bacillus tequilensis*

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## ABSTRACT

Zinc is a nutritive element for the growth of the plant, but its bioavailability in soil is frequently restricted. This study aimed to isolate, characterize, and optimize zinc solubilization by bacterial isolates of the rhizosphere of Finger Millet (*Eleusine coracana* L.) grown in Kaprada Taluka, Valsad District, Gujarat, India. A total 25 bacteria were isolated; from these isolates, 10 exhibited significant zinc solubilization on ZnO-supplemented Bunt and Rovira agar medium. The most potent isolate ZB9, showed the highest zinc solubilization potential (182  $\mu\text{L L}^{-1}$ ) with a reduction in pH to 4.8. Furthermore, other plant growth-promoting (PGP) traits as indole acetic acid production (43  $\mu\text{g/mL}$ ), phosphate solubilization (92  $\mu\text{g/mL}$ ), hydrogen cyanide and ammonia production, were assessed. Optimization studies determined the ideal conditions for maximum zinc solubilization, including 0.1% ZnO, dextrose as a source of carbon, ammonium sulfate as a source of nitrogen, pH 6.0, incubation temperature 35°C, and 0.2% NaCl salinity. Molecular level identification through 16S rRNA sequencing of the isolate confirmed ZB9 as belonging to the *Bacillus tequilensis*. This study highlights the potential of ZB9 as a biofertilizer to improve zinc bioavailability and enhance plant growth and nutrient quality in Finger Millet cultivation.

**Keywords:** Finger Millet Rhizosphere, Zinc Solubilization, Phosphate solubilization, IAA, HCN, and Ammonia production.

### Highlights:

- Zinc Solubilizing Bacteria isolated from Finger Millet Rhizospheric Soil in Valsad, Gujarat.
- AAS analysis revealed Quantitative Zinc solubilization.
- Zinc-solubilizing bacteria can produce Phytohormone (Indole Acetic Acid).
- Bacterial isolate has the capability towards Plant Growth Promotion (Phosphate solubilization, HCN, and Ammonia production).
- Optimized environmental condition for Zinc solubilization was evaluated.

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## INTRODUCTION

Increased food production, approximately two billion people suffer from "hidden hunger" micronutrient deficiencies that affect health and development. Micronutrients (including minor elements and minerals) are essential for the human body in minor amounts, although their absence has devastating consequences. Deficiencies of these essential micronutrients contribute to chronic diseases, abnormal growth, reduced immune function, and reduced cognitive and physical development, collectively described as "hidden hunger" as reported by the World Health Organization (WHO) (Kadapa *et al.*, 2023).

The United Nations' General Assembly (UNGA) declared year 2023 as "International Year of Millets" by accepting the proposal on the Government of India (Malhotra, 2023). In spite of climatic condition change, resilient crops like finger millet (*Eleusine coracana* L.) play a role in validating nutritional security for growing populations. As a nutrient cereal, finger millet supports crop diversification and climate change mitigation due to its adaptability to diverse growing conditions (Babu *et al.*, 2025).

Zinc (Zn) is an essential micronutrient that performs a major role in diverse physiological and biochemical activities in plants that including auxin production, enzyme activation, protein synthesis, and chlorophyll synthesis (Broadley *et al.*, 2007; Alloway, 2008). Globally, Zn deficiency has become the most widespread problem influencing agricultural soils (Cakmak, 2008; White and Broadley, 2011). This deficiency not only restricts crop development and productivity but also influences the nutritional quality of food crops, contributing to human Zn

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malnutrition, particularly in developing countries where cereal-based diets are prevalent (Prasad, 2014).

The conventional method to addressing Zn deficiency in agriculture has been through the application of chemical Zn fertilizers (zinc sulfate). However, chemical fertilizers encounter several limitations, including minimum bio-availability (generally approximately 5% of applied Zn is used by plants), potential environmental contamination through leaching and runoff, and their high costs (Ghasemi *et al.*, 2013; Rehman *et al.*, 2018). Moreover, in alkaline and calcareous soils which compose approximately 30% of the world's cultivated lands, applied Zn rapidly transforms into insoluble forms (e.g.,  $\text{ZnCO}_3$ ,  $\text{Zn(OH)}_2$ ), making it unavailable for plant uptake (Alloway, 2008). These challenges have stimulated interest in developing more sustainable and efficient alternatives for Zn management in agricultural systems.

In this circumstance, microbial Zn solubilization has emerged as a promising sustainable approach to improve Zn bioavailability in soils (Saravanan *et al.*, 2007; Kamran *et al.*, 2017). Certain rhizospheric microorganisms, particularly zinc solubilizing bacteria (ZSB), possess the prominent efficiency to convert insoluble Zn compounds into bioavailable forms through various mechanisms, including acidification, chelation, and redox reactions (Fasim *et al.*, 2002; Mumtaz *et al.*, 2017). These rhizospheric bacteria produce organic acids which dissolve mineral Zn compounds, while certain strains also produce siderophores that can chelate Zn ions (Ghosh *et al.*, 2019). The present study aims to isolate, characterize, identify, and optimize the zinc solubilization by bacterial isolates from Finger millet rhizospheric soil and evaluate their potency towards plant growth promotion.

## MATERIALS AND METHODS

### Soil Sample Collection

Five various soil samples were collected from the rhizospheric soil of *Eleusine coracana* L. crop from five different locations (20.38699° N, 73.21908° E; 20.38697° N, 73.21909° E; 20.38698° N, 73.21917° E; 20.38933° N, 73.22042° E; 20.3904° N, 73.22042° E), Kaprada Taluka of Valsad District Gujarat India.

### Characterization of the Soil sample

The pH and Electric Conductivity of the soil samples were determined by using a pH meter (Schofield and Taylor, 1955) and a conductivity meter (Rhoades *et al.*, 1989), respectively. Organic Carbon content was measured by adopting the modified Walkley and Black method (Walkley and Black, 1934). Level of nitrogen in soil was measured by the Kjeldahl method (Kjeldahl, 1883). Phosphorus was analyzed by use of the vanadomolybdate method (Olsen, 1954); potassium was by flame photometer (Jackson, 1973), and micronutrients such as available Cu, Zn, Mn, and Fe were estimated by DTPA method (diethylene triamine penta acetic acid method) with use of atomic absorption spectrophotometer (Lindsay and Norvell, 1978).

### Isolation of Zinc-Solubilizing Bacteria

One-gram soil was suspended in nine mL of water and vigorously mixed for two minutes. The sample was serially diluted and plated on a nutrient agar (NA) medium, then incubated at 37 ± 2°C for 3-5 days (Othman *et al.*, 2022).

### Zinc Solubilization: Qualitative and Quantitative Analysis

The isolates were inoculated on Bunt and Rovira agar plate containing insoluble zinc (ZnO), and then incubated at 37 ± 2°C for seven days. The zinc solubilizer was identified by the occurrence of a clear zone surrounding the colonies. The bacterial isolates were primarily characterized by colony characters, biochemical test, and vancomycin test (Bhatt and Maheshwari, 2020). The zinc solubilizing isolates were quantitatively determined for the solubilization of zinc from insoluble zinc (ZnO) using an Atomic Absorption Spectrophotometer and measurement of pH. The isolate was inoculated in 100 mL of Bunt and Rovira Broth (0.1% ZnO) and then incubated at 37 °C ± 2 °C in a shaker at 120 rpm

for 15 days along with their control. After an incubation period, the broths were centrifuged at 8000 rpm for 15 minutes and then the supernatant was separated and collected for estimation of pH and Atomic Absorption Spectrophotometric analysis (Ahmad *et al.*, 2021; Suriyachadkun *et al.*, 2022).

### Screening for Plant Growth-Promoting (PGP) traits

#### *Indole 3 Acetic Acid (IAA) Production: Qualitative and Quantitative Analysis*

The efficiency of zinc-solubilizing bacterial isolates to produce IAA was estimated by Salkowski's method. All isolated colonies were inoculated to the nutrient broth medium with 0.1% L-L-tryptophan and then incubated in dark conditions at room temperature for four days. The cultured medium was centrifuged at 10000 rpm for 10 minutes. Two mL of Salkowski's reagent was mixed with one mL of the culture supernatant in a fresh tube incubated in the dark conditions at room temperature for 20–30 minutes. A pink to reddish color development indicates the IAA production by the bacterial isolates strain.

Measurement of IAA in supernatant was done by using a colorimetric assay. IAA amount was estimated using the standard graph of known concentrations of IAA between ranged of 10-100 µg/ml (Dawwam *et al.*, 2013).

#### *Phosphate Solubilization: Qualitative and Quantitative Analysis*

The efficiency of zinc-solubilizing isolates to solubilize phosphate was measured using tricalcium phosphate as an insoluble phosphate source. Spot inoculation of the isolate onto Pikovskaya's agar plate medium and then incubated at 30°C for 48 to 72 hours. Phosphate solubilization was confirmed by the clear zone surrounding the bacterial colonies, which was attributed to the secretion of the organic acids that facilitate the dissolution of tricalcium phosphate.

The bacterial isolate was quantitatively determined for the solubilization of phosphate in a liquid medium. The isolated culture was inoculated in 100 ml Pikovskaya Broth medium and then incubated at 25 ± 2 °C in an incubator shaker at 120 rpm along with its control for 48 hrs. After incubation, the broth was separated by centrifuging at 10000 rpm for 10 minutes, and available phosphorous content in the broth was measured by the vanadomolybdate method. The soluble phosphorus content was assessed by using a standard curve of known concentration of KH<sub>2</sub>PO<sub>4</sub> between the range of 100-500 µg/ml (Mohamed *et al.*, 2019; Suriyachadkun *et al.*, 2022).

#### *Hydrogen Cyanide (HCN) and Ammonia Production*

The isolates were grown on a nutrient agar added with glycine. The Whatman filter paper was immersed in 0.5% picric acid and 1% Sodium carbonate and was kept in the upper lid on a plate. The plate was incubated at 28 ± 2 °C for 48h. The results showed the appearance of a deep yellow-to-brown color (Cappucino and Sherman, 1992; Kumar *et al.*, 2020).

For measuring the ammonia production, bacterial isolates were inoculated in 4% peptone broth and then incubated for seven days at 30 ± 2 °C. After that added the Nessler's reagent. The formation of brown-yellow colour displays ammonia production (Suriyachadkun *et al.*, 2022; Chaudhary *et al.*, 2023).

## Molecular Identification and DNA Sequencing of Selected Effective Isolates

For molecular characterization, a single bacterial isolate exhibiting the highest zinc solubilization efficiency and various plant growth-promoting (PGP) traits was selected. The chosen isolate demonstrated significant resistance to heavy metals and strong PGP attributes. Molecular level identification was performed based on 16S rRNA gene amplification by using primers 27F and 1492R specific to bacteria (Hyder *et al.*, 2020; Shreshtha *et al.*, 2024). Sequence was conducted using the Codon Gene Aligner, and the obtained sequences were compared by the NCBI 16S ribosomal RNA database using BLASTn. After BLAST, the obtained sequence was aligned by Clustal X. The MEGA 12 software was used to phylogenetic analysis of gene sequences. The neighbors-joining method was used to infer the phylogenetic tree (Saitou and Nei, 1987; Inokuma *et al.*, 2004; Shreshtha *et al.*, 2024) and bootstrap support was generated based on 1000 replicates (Felsenstein, 1985; Tamura *et al.*, 2004).

## Optimization of Growth Conditions for Zinc Solubilization

### *Influence of Zinc Oxide Concentration on Zinc Solubilization Efficiency*

The impact of varying concentrations of zinc oxide on zinc solubilization efficiency was assessed by incorporating different levels of zinc oxide (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) into a broth medium. Every treatment was conducted in triplicate, with inoculated flasks incubated at 30°C in the shaker for 14 days at 120 rpm. The supernatant from the centrifuged broth was analyzed using an Atomic Absorption Spectrophotometer (AAS) (Fasim *et al.*, 2002).

### *Influence of Different Carbon Sources on Zinc Solubilization Efficiency*

To determine the effect of carbon sources on zinc solubilization, the carbon source in the Bunt and Rovira medium was substituted with dextrose, fructose, sucrose, starch, carboxymethyl cellulose (CMC), and xylose. The zinc concentration was maintained at 0.1% ZnO. Culture inoculated medium was incubated at 30 ± 2°C in a shaker at 120 rpm for 14 days. Following incubation, the supernatant from the centrifuged broth was subjected to analysis using AAS (Fasim *et al.*, 2002).

### *Influence of Different Nitrogen Sources on Zinc Solubilization Efficiency*

The influence of various nitrogen sources, such as ammonium sulfate, urea, casein, and sodium nitrate, on zinc solubilization was investigated. The broth medium was prepared by substituting different nitrogen sources. Inoculated medium was incubated at 30 ± 2°C in the shaker at 120 rpm for 14 days. The supernatant from the centrifuged broth was analyzed for available zinc using AAS (Fasim *et al.*, 2002).

### *Influence of Temperature on Zinc Solubilization Efficiency*

The role of temperature in zinc solubilization was examined by using the optimized media composition with 0.1% zinc, dextrose, and ammonium sulfate as nutrient source. The culture inoculated broths were incubated at various temperatures (15, 20, 25, 30, 35, 40, 45, 50, and 55 ± 2°C) in the shaker at 120 rpm

for 14 days. The supernatant from the centrifuged broth was analyzed using AAS (Fasim *et al.*, 2002).

### *Influence of pH on Zinc Solubilization Efficiency*

To estimate optimal pH for zinc solubilization, isolates were cultured in media with the previously optimized conditions, while the pH varied from 5.0 to 8.0 (in increments of 0.5). Inoculated culture media were incubated in a shaker at 120 rpm for 14 days. After incubation, the supernatant from the centrifuged broth was analyzed using AAS to quantify the available zinc content (Fasim *et al.*, 2002).

### *Influence of Salinity on Zinc Solubilization Efficiency*

The impact of different salinity levels on zinc solubilization was evaluated by maintaining a consistent media composition while varying the sodium chloride (NaCl) concentration (0.2%, 0.4%, 0.6%, 0.8%, and 1%). Inoculated media were incubated in a shaker at 120 rpm for 14 days. The supernatant obtained by centrifugation was analyzed using AAS (Fasim *et al.*, 2002).

## RESULTS AND DISCUSSION

### Chemical characteristics of Soil samples

Five diverse Soil samples were collected from various sites of Kaprada Taluka of Valsad District, Gujarat India for isolation of PGPR and analyzed for chemical properties and nutrient availability. The Table 1 showing the chemical characteristics of soil samples. The pH of soil sampling sites ranged between 5.5 and 5.9 (slightly acidic conditions). Electrical conductivity (E.C.) values were low, ranging from 0.14 to 0.18 milliohms cm<sup>-1</sup>. The content of organic carbon (O.C.) varied, with the highest observed at 2.5% and the lowest at 0.85%. The nitrogen (N) concentration was between 0.07% and 0.22%. Available phosphorus (P<sub>2</sub>O<sub>5</sub>) measured in the range of 1.18 to 5.88 mg g<sup>-1</sup>, while Available potassium (K<sub>2</sub>O) levels fluctuated across samples. Nutrient availability was measured by micronutrient analysis, which revealed variable concentrations of zinc, copper, iron, and manganese, with iron and zinc availability ranging from 39.0 to 108.5 mg kg<sup>-1</sup> and 0.84 to 1.96 mg kg<sup>-1</sup>, respectively. These results provide insight into the soil's fertility status. The chemical properties of soil samples were analyzed, revealing significant variations across different locations. The soils exhibited acidic pH levels, specific electric conductivity, and particular levels of total organic carbon and other nutrients, which play a critical role in rate-limiting processes like organic matter degradation and nutrient cycling in soil ecosystems (Alloway, 2008).

### Isolation of Zinc-Solubilizing Bacteria

#### *Qualitative Analysis of Zinc Solubilization*

To assess Zn solubilization efficiency, isolates were screened using an agar plate assay, a widely adopted qualitative method for identifying Zn-solubilizing microorganisms. Bacteria capable of forming clear zones on Bunt and Rovira agar media supplemented with insoluble Zn compounds (0.1% ZnO) were confirmed as Zinc Solubilizing Bacteria. (Khande *et al.*, 2017). A total 25 bacterial isolates were isolated from the collected

**Table 1:** Chemical characteristics of Soil samples

Parameters	Site 1	Site 2	Site 3	Site 4	Site 5
pH	5.80	5.50	5.90	5.80	5.70
E.C. (milliohms/cm)	0.16	0.14	0.18	0.17	0.17
Organic Carbon (%)	1.62	2.50	0.85	1.21	1.57
Available Nitrogen (%)	0.14	0.22	0.07	0.10	0.14
Available Phosphorous (mg/g)	3.92	1.96	5.88	1.18	4.31
Available Potassium (mg/g)	189.10	145.25	135.00	168.50	167.65
Available Cu (mg/kg)	3.16	1.94	1.43	1.68	2.06
Available Zn (mg/kg)	1.54	0.84	1.50	1.96	1.02
Available Mn (mg/kg)	42.50	36.50	35.00	78.50	31.00
Available Fe (mg/kg)	75.00	72.50	39.00	108.50	50.00

rhizospheric soil. The isolates were further screened for zinc solubilizing capability on the Bunt and Rovira medium contains 0.1% ZnO as an insoluble zinc source (Devi *et al.*, 2016). Out of these isolates, 10 bacterial isolates showed solubilization of zinc oxide which was observed by a clear zone of solubilization surrounding the colony (Gupta *et al.*, 2015). The colony characteristics of zinc solubilizing isolates were observed based on various properties like size, shape, margin, surface texture, elevation, opacity, and pigmentation. Each isolate was rod-shaped, gram-positive and sensitive to vancomycin. The uniform rod-shaped morphology and chain-forming arrangement suggested phylogenetic relatedness (Gupta *et al.*, 2015). The biochemical tests showed positive results in indole and ammonia production, nitrate reduction test, making them as promising plant growth promoters.

#### Quantitative Analysis of Zinc solubilization

The release of various organic acids enhances zinc solubilization and gives an acidic pH. The solubilization of zinc oxide (insoluble form) to solubilized zinc form was measured by available zinc concentration through AAS (atomic absorption spectrophotometer) and pH changes due to organic acid production (Gontia-Mishra *et al.*, 2017). Table 2 shows that every isolate exhibited acidic pH as compared with the control, which represents the solubilization mechanism. Among the isolates, ZB9 exhibited the highest zinc solubilization ( $182 \mu\text{L L}^{-1}$ ) at an acidic pH (4.8). The quantitative analysis of zinc solubilization revealed significant strain variability, with isolate ZB9 showing maximum solubilization along with the lowest pH. This correlation between zinc solubilization and pH reduction indicates the organic acid-mediated zinc solubilization mechanism.

#### Screening for Plant Growth-Promoting (PGP) traits

##### IAA Production by Zinc Solubilizing Isolates

IAA is one of the members of a group of phytohormones, commonly known to be the common auxin. Fig. 1 illustrates the IAA production of zinc solubilizing isolates. All isolates were positive for IAA production, but among those isolates, ZB9 and ZB8 isolates were given maximum IAA production ( $43 \mu\text{g/ml}$  and

$41 \mu\text{g/ml}$ ) after 4 days. The isolates ZB6, ZB3, ZB1, ZB5, ZB10, ZB4, and ZB7 produced IAA, ranging between 39 to  $13 \mu\text{g/ml}$ , while the lowest amount of IAA produced by ZB2 ( $7 \mu\text{g/ml}$ ) same as the control ( $7 \mu\text{g/ml}$ ). The plant growth-promoting properties were particularly noteworthy, including all isolates produced IAA as an effective PGPR strain (Khalid and Aftab, 2020),

##### Phosphate Solubilization by Zinc Solubilizing Isolates

Phosphorus is a vital macronutrient that all living organisms require. As shown in Fig. 2, all bacterial isolates were efficient to solubilize phosphate but among those, the ZB9 isolate exhibited maximum solubilization ( $92 \mu\text{g/ml}$ ) after 48 hrs. followed by ZB1 ( $89 \mu\text{g/ml}$ ), ZB10 ( $83 \mu\text{g/ml}$ ), ZB6 ( $82 \mu\text{g/ml}$ ), ZB3 ( $76 \mu\text{g/ml}$ ), ZB7 ( $73 \mu\text{g/ml}$ ), ZB5 ( $66 \mu\text{g/ml}$ ), ZB8 ( $65 \mu\text{g/ml}$ ), ZB2 ( $64 \mu\text{g/ml}$ ), and lowest Phosphate solubilization was occur in ZB4 ( $18 \mu\text{g/ml}$ ) similar to control ( $18 \mu\text{g/ml}$ ). Phosphate solubilization correlated with zinc solubilization efficiency, suggesting shared acidic metabolite pathways (Wang *et al.*, 2020),

##### HCN and Ammonia Production by Zinc Solubilizing Isolates

All isolates were positive for HCN and Ammonia production. As per Table 3, Isolates ZB6 and ZB8 produced maximum HCN, isolates ZB2 and ZB4 produced minimum HCN, while the remaining isolates produced moderate HCN Table 3. The ZB2, ZB4, ZB6, and ZB9 produced maximum ammonia; ZB5, ZB7, and ZB10 produce minimum ammonia, while others produced moderate levels of ammonia. The hydrogen cyanide and ammonia production by isolated bacteria indicates their efficacy to promote plant overall health. These findings have implications for the development of bio-stimulation strategies in agriculture, aiming to enhance plant growth and protect against various soil-borne pathogens (Saharan and Verma, 2015).

##### Optimization of Zinc Solubilization

The efficient bacterial isolate ZB9 showing the highest zinc solubilization qualitatively and quantitatively in all different sources of zinc was further taken for growth optimization including physical and chemical parameters like different zinc concentrations, carbon source, nitrogen source, pH, incubation temperature, and salinity.

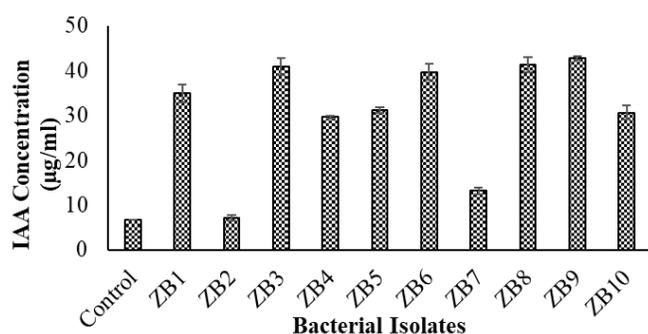
**Table 2:** Quantitative Analysis and Change in pH during zinc solubilization broth assay

Isolates	Zinc concentration ( $\mu\text{L L}^{-1}$ )	pH
Control	11.73 $\pm$ 1.3	7.0 $\pm$ 0.00
ZB1	106.67 $\pm$ 0.47	5.9 $\pm$ 0.04
ZB2	115.00 $\pm$ 0.81	5.6 $\pm$ 0.05
ZB3	121.67 $\pm$ 1.69	5.5 $\pm$ 0.14
ZB4	177.00 $\pm$ 2.1	5.1 $\pm$ 0.08
ZB5	161.66 $\pm$ 1.24	5.4 $\pm$ 0.05
ZB6	110.00 $\pm$ 0.81	5.6 $\pm$ 0.06
ZB7	146.00 $\pm$ 2.1	5.3 $\pm$ 0.05
ZB8	98.00 $\pm$ 0.81	5.7 $\pm$ 0.09
ZB9	182.00 $\pm$ 1.6	4.8 $\pm$ 0.12
ZB10	166.00 $\pm$ 0.81	5.2 $\pm$ 0.05

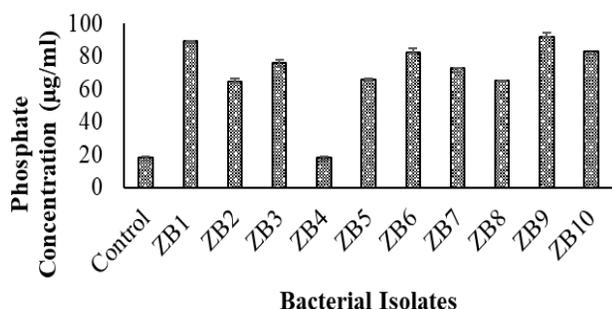
**Table 3:** HCN and Ammonia Production by Zinc solubilizing isolates

Bacterial isolates	HCN production	Ammonia production
ZB1	++	++
ZB2	+	+++
ZB3	++	++
ZB4	+	+++
ZB5	++	+
ZB6	+++	+++
ZB7	++	+
ZB8	+++	++
ZB9	++	+++
ZB10	++	+

+: minimum production; ++: moderate production; +++: maximum production.



**Fig 1:** IAA Production by Zinc Solubilizing isolates



**Fig 2:** Phosphate solubilization by Zinc solubilizing isolates

*Influence of Various Concentrations of Zinc on Zinc Solubilization Efficiency*

The effect of different concentrations of zinc oxide at different concentrations of Zn in Bunt and Rovira broth medium (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) was studied. The highest zinc solubilization was observed in 0.1% ZnO concentration by ZB9 (166  $\mu\text{L L}^{-1}$ ). The media with 0.2% of concentration shows a slight decrease in the solubilization of zinc by isolate ZB9 (101  $\mu\text{L L}^{-1}$ ), followed by 0.3% of concentration (44  $\mu\text{L L}^{-1}$ ), 0.4% concentration (42  $\mu\text{L L}^{-1}$ ), 0.5% concentration (34  $\mu\text{L L}^{-1}$ ) which was higher than the control (10  $\mu\text{L L}^{-1}$ ). Fig. 3 summarizes that subsequent fall

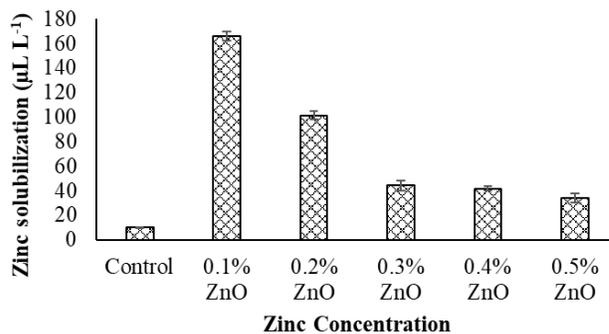
was observed in the zinc solubilization as the concentration of Zn was increased in the broth medium by the isolate because of heavy metal toxicity.

*Influence of Various Carbon Sources on Zinc Solubilization Efficiency*

Zinc solubilization efficiency varies with different carbon sources, the maximum zinc solubilization was showed in dextrose as a carbon source (193  $\mu\text{L L}^{-1}$ ), followed by sucrose (180  $\mu\text{L L}^{-1}$ ). Fig. 4 lists the comparative solubilization of zinc with different carbon sources. The reduction in the solubilization was found in fructose (95  $\mu\text{L L}^{-1}$ ) and starch (73  $\mu\text{L L}^{-1}$ ). Further minimum solubilization was occurred in xylose (45  $\mu\text{L L}^{-1}$ ) and CMC (39  $\mu\text{L L}^{-1}$ ), while in the control (10  $\mu\text{L L}^{-1}$ ).

*Influence of Various Nitrogen source on Zinc Solubilization Efficiency*

To determine the role of nitrogen on zinc solubilization, different nitrogen sources were used in the study such as  $(\text{NH}_4)_2\text{SO}_4$ , Casein, Urea, and  $\text{NaNO}_3$  as source of nitrogen (0.05%) in Bunt and Rovira broth medium with the ideal condition of ZnO (0.1%) and dextrose (0.5%) as the source of carbon in the medium. As shown in Fig. 5, the finding displays that the ideal nitrogen source for maximum zinc solubilization was found to be



**Fig 3:** Influence of Various Concentrations of Zinc on Zinc Solubilization

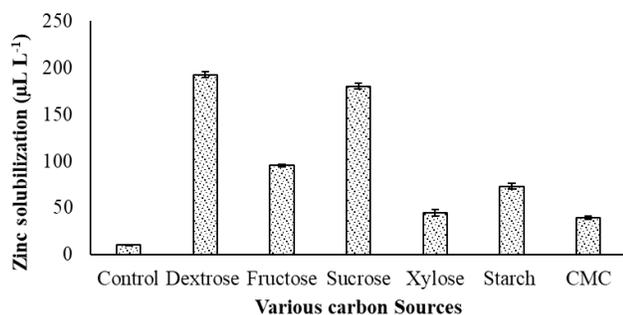


Fig 4: Influence of Various Carbon sources on Zinc Solubilization

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> where the maximum zinc solubilization was occurred by ZB9 (194 µL L<sup>-1</sup>), followed by Urea (84 µL L<sup>-1</sup>), NaNO<sub>3</sub> (45 µL L<sup>-1</sup>), and Casein (34 µL L<sup>-1</sup>). All nitrogen sources solubilized higher amount of zinc as compared to control.

#### Influence of Different Temperatures on Zinc Solubilization Efficiency

To study of various temperature effects on the zinc solubilization was performed by the use of temperature range from 15 to 55 ±2°C. The growth and zinc solubilization under range of temperature was shown in Fig. 6. The highest solubilization of insoluble ZnO into the available form by ZB9 at 35 ±2°C and at 25 ± 2°C were 186 µL L<sup>-1</sup>, respectively. Slightly decreased at 30 ± 2°C was 184 µL L<sup>-1</sup>, whereas below and above that range the zinc solubilization was reduced at 40, 45 and 50 ±2°C and no noticeable change was occurred at 15, 20 and 55 ±2°C same as control (Fig. 6).

#### Influence of pH on Zinc Solubilization Efficiency

To analyze the optimum pH for maximum zinc solubilization by the isolate ZB9, optimal medium composition and suitable temperature were used at different pH 5, 5.5, 6, 6.5, 7, 7.5, and 8. The Fig. 7 displays the zinc solubilization efficiency with varying pH. The maximum solubilization was analyzed with pH 6 (194 µL L<sup>-1</sup>), followed by pH 6.5 (172 µL L<sup>-1</sup>), pH 5.5 (91 µL L<sup>-1</sup>), pH 7 (75 µL L<sup>-1</sup>), pH 5 (46 µL L<sup>-1</sup>) and pH 7.5 (32 µL L<sup>-1</sup>) and no noticeable change was occurred with pH 8, similar to control (Fig. 7).

#### Influence of Salinity on Zinc Solubilization Efficiency

The tolerance level of salinity was evaluated by preparing optimal medium composition and other conditions with varying concentrations of NaCl (0.2 %, 0.4 %, 0.6 %, 0.8 %, and 1 %). As per Fig. 8, the result displays zinc solubilization in different saline concentrations and found that bacterial isolate ZB9 was able to tolerate an adequate level of salinity from 0.2 % to 0.6% NaCl. The 83µL L<sup>-1</sup>, 43µL L<sup>-1</sup>, and 30µL L<sup>-1</sup> solubilization occurred in 0.2%, 0.4%, and 0.6% NaCl concentrations which was higher than control (Fig. 8).

Optimization studies revealed Dextrose as the superior carbon source and ammonium sulfate as optimal nitrogen source supporting previous findings on *Bacillus* Spp carbon and nitrogen source preferences, maximum solubilization occurs at 0.1% zinc concentration with inhibition above 0.3% due to metal toxicity and Optimum solubilization was performed at pH 6.0 and 35°C matching typical soil conditions (Zhang *et al.*, 2024).

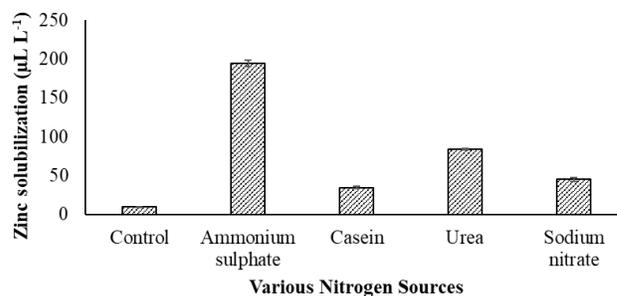


Fig 5: Influence of Various Nitrogen Sources on Zinc Solubilization

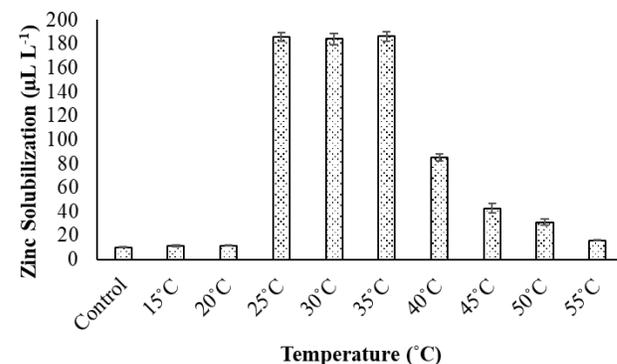


Fig 6: Influence of Different Temperatures on Zinc Solubilization

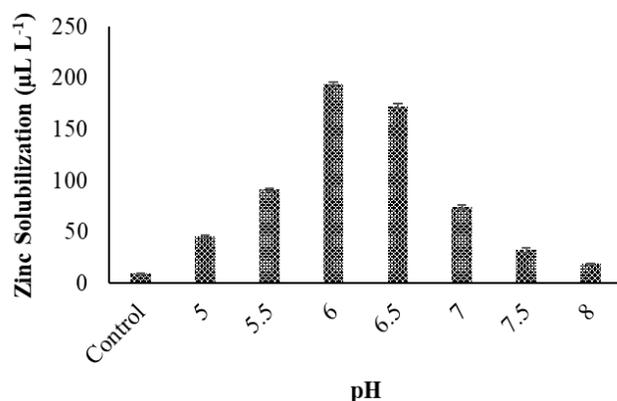


Fig 7: Influence of pH on Zinc Solubilization

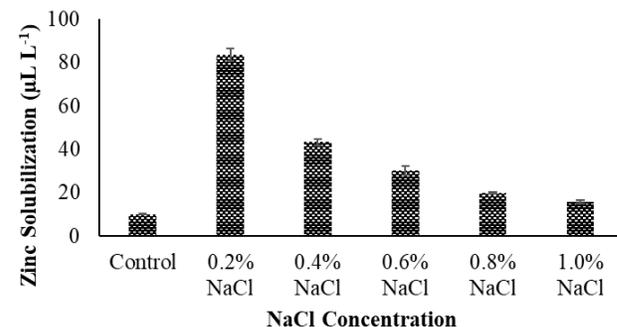
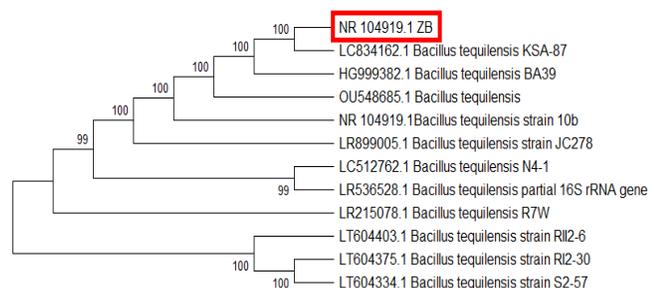


Fig 8: Influence of Salinity on Zinc Solubilization



**Fig 9:** Phylogenetic neighbor-joining trees based on 16S rRNA gene sequencing of *Bacillus tequilensis*

### Molecular identification and DNA sequencing of selected effective isolate

Evaluation of 16s rRNA gene sequence of an isolated bacterial strain was sequenced, aligned and analyzed using a combination of NCBI Gene Bank databases. The Fig. 9 showing the relationship of isolate to closely related *Bacillus* species. The sequence of bacterial isolate ZB9 (ZB) was identified as *Bacillus tequilensis* based on 16s rRNA sequence and submitted in gene bank with accession number: NR\_104919.1. The molecular identification of ZB9 as *Bacillus tequilensis* plays the role of a bioinoculant that enhances zinc bioavailability and promotes the growth of plants. The combination of efficient zinc solubilization with multiple PGP properties and environmental stress tolerance capacity, *Bacillus tequilensis* a promising bioinoculant for zinc-deficient soils (Othman *et al.*, 2022).

### CONCLUSION

This study confirmed the potent isolated bacterial strain from Finger millet rhizospheric soil can be used as a zinc solubilizer. Based on molecular study, the best zinc solubilizing isolate was confirmed as *Bacillus tequilensis*. This zinc solubilizing bacterial strain exhibited multiple plant growth-promoting attributes, including phosphate solubilization and the production of indole-3-acetic acid (IAA), hydrogen cyanide (HCN), and ammonia. Furthermore, its ability to thrive under varying environmental conditions suggesting its resilience and suitability for agricultural application as a sustainable bioinoculant. These attributes collectively indicate that *Bacillus tequilensis* could serve as an effective bioinoculant for. Its multifunctional traits also support its use in the development of eco-friendly biofertilizers aimed at sustainable agricultural practices. Additionally, further validation should focus on pot and field conditions to evaluate its efficiency towards zinc solubilization and plant growth promotion.

### AUTHOR CONTRIBUTIONS

Vasudha Jadav: Soil samples collection, all the analysis, investigations, and drafting of the paper. Dr. Niraj Sheth: Supervision in all analysis and paper drafting. Dr. D. Srinivas Murty: Methodology and designing the experiments. Dr. Prateek Shilpkar: Reviewing and editing.

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### CONFLICT OF INTERESTS

The authors declared no conflict of interest.

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