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Quantitative assessment of zinc solubilizing bacteria isolated from soils of western Maharashtra

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Abstract
The research was carried out at laboratory of plant pathology and Agricultural Microbiology, College of Agriculture, Pune. In the present investigation, efficient zinc solubilizing bacteria isolated from rhizospheric soils of Sangli and Satara districts of Western Maharashtra. Out of 45 samples, 9 bacterial isolates were obtained. A total of nine bacterial isolates were tested, with pH recorded on the day of inoculation and subsequently at 5, 10 and 15 days after inoculation (DAI). All isolates exhibited a gradual decline in pH over time, indicating acid production. Among the isolates, Pal-3 demonstrated the most significant drop in pH (from 7.00 to 2.99) followed by Sat-3 and Kar-2, highlighting their potential effectiveness in solubilizing zinc. Those 9 isolates were tested for their capacity to solubilize zinc *in vitro* and soluble Zn content (mg/L) was measured on the 7^{th} and 10^{th} days of incubation. Results revealed significant differences among isolates, with Pal-3 recording the highest zinc solubilization (65 mg/L on the 7^{th} day and 87 mg/L on the 10^{th} day) followed by Bor-1 and Pha-5. Based on the findings, isolates Pal-3, Bor-1 and Pha-5 emerged as the most efficient zinc solubilizers.

Keywords: Isolation, rhizospheric soil, soluble Zn content

Introduction

Zinc is recognized as one of the crucial micronutrients necessary for the proper physiological functioning, growth and successful reproduction of crop plants. It plays a significant and irreplaceable role in various metabolic processes within plants, as emphasized by Hughes and Poole (1989) [4]. In natural soil environments, zinc is commonly found in association with minerals such as sphalerite, olivine, hornblende, augite and biotite. Despite its presence in these mineral forms, the actual availability of zinc to plants largely depends on several factors, one of the most critical being the biochemical activity of rhizosphere-associated microorganisms. These beneficial soil microbes facilitate the transformation of insoluble or poorly available forms of zinc into more soluble and plant accessible forms, thereby enhancing its uptake, as supported by the findings of Singh *et al*., (2005) [9].

Although plants require zinc in relatively low quantities typically in the range of 5 to 100 mg per kilogram of plant tissue its role in both plant and microbial nutrition and physiology is profound and has been extensively studied. Zinc serves as a vital component in the functioning of numerous enzymes in both eukaryotic and prokaryotic organisms. It is often incorporated into the enzyme system as a co-factor and also functions as a metal activator essential for enzymatic activity (Parisi *et al*., 1969) [5]. Many bacterial enzymes, in particular, either possess zinc in their catalytic sites or rely on zinc for maintaining their structural stability and proper conformation. Within soil systems, zinc is involved in a dynamic equilibrium between solubilization and precipitation processes. This balance is heavily influenced by factors such as soil pH and the microbial composition of the rhizosphere. These factors, in turn, play a direct role in determining the extent to which zinc becomes accessible to plant roots for absorption (Goldstein, 1995) [3].

Zinc deficiency has been reported as a widespread issue across agricultural soils globally and it is recognized as one of the major nutritional limitations that negatively impact crop productivity. Crops such as cereals and pulses are particularly vulnerable, with wheat and rice frequently exhibiting visible symptoms of zinc deficiency. Affected plants typically display stunted growth characterized by a noticeable reduction in plant height, along with the appearance of whitish-brown lesions on leaves that eventually become necrotic. Zinc deficiency further leads to disruptions in various physiological functions, including compromised membrane integrity, decreased biosynthesis of essential compounds such as carbohydrates, cytochromes, nucleotides, auxins, and chlorophyll. Additionally, plants deficient in zinc often exhibit a heightened sensitivity to heat stress. Increased permeability of root cell membranes has also been observed under zinc-deficient conditions, which may be attributed to zinc's critical role in maintaining membrane stability and function, as noted by Parker *et al*., (1992) [6].

Microorganisms that inhabit the rhizosphere the narrow region of soil directly influenced by root secretions and associated microbial activity are generally known to synthesize and secrete auxins as secondary metabolites. This is primarily due to the abundance of organic substrates and nutrients released by plant roots in this zone, which are significantly higher than in non-rhizospheric soil environments. The nutrient-rich conditions of the rhizosphere serve as an ideal environment for microbial proliferation and metabolic activity. Several bacterial genera, including *Azospirillum*, *Pseudomonas*, *Xanthomonas* and *Rhizobium*, along with species such as *Alcaligenes faecalis*, *Enterobacter cloacae*, and *Acetobacter diazotrophicus*, have been identified as capable producers of auxins. These plant growth-promoting hormones, particularly indole-3-acetic acid (IAA), play a vital role in stimulating root elongation, cell division, and overall plant development. Furthermore, certain fungi and algae also contribute to auxin production, thereby enhancing plant establishment and vigour, as reported by Patten and Glick (1996) [7].

In addition to their known role in nutrient solubilization, zinc solubilizing bacteria (ZSB) are also capable of producing indole-3-acetic acid (IAA) and related compounds. These microbial IAA products can significantly influence plant growth by modulating root architecture, enhancing nutrient uptake and improving stress tolerance. The growth-promoting effects of such bacterial metabolites have been documented by Rajkumar *et al*., (2008) [8], who emphasized the dual role of ZSB in both micronutrient solubilization and hormonal stimulation. Moreover, the bioavailability of zinc in soils as well as its subsequent uptake and accumulation in plant tissues, is largely governed by the diversity, density and metabolic activity of microbial communities residing in the rhizosphere. The composition of these microbial populations determines the efficiency with which insoluble zinc forms are transformed into plant-accessible forms. Dotaniya and Meena (2015) [2] highlighted that fluctuations in the microbial ecosystem of the rhizosphere driven by soil conditions, crop types and environmental factors can have a direct impact on the solubility and mobility of zinc. This underscores the crucial ecological relationship between beneficial microbes and plant mineral nutrition. Hence, the inoculation of specific bacterial strains may be helpful to enhance both Zn availability and plant uptake in plant and soil. (Aketi *et al*., 2014) [1].

Material and Methods

1.Collection of soil samples: Soil samples were collected from two districts in the State of Maharashtra *viz*., Satara and Sangli. The soil samples were collected from the rhizospheric soil.

2. Isolation of Zinc solubilising bacterial strains

Serial dilution pour plate technique was used for isolation of zinc solubilising bacteria, using Bunt and Rovira medium having Zinc oxide (ZnO) as a source of insoluble zinc.

3. Quantitative zinc-solubilising assays

The ZSB isolates that showed halo zone formation further tested for their ability to release inorganic Zn in liquid medium.

4. Determination of pH

The pH of the culture filtrate in both inoculated and uninoculated samples was determined at 5th, 10th and 15th days interval after inoculation.

Result and discussion

This investigation was primarily focused on assessing a range of bacterial growth parameters and performance indicators. Additionally, it aimed to examine the potential of these microbial isolates to convert otherwise insoluble forms of zinc present in the soil into bioavailable forms that can be readily absorbed and utilized by plants.

Isolation of zinc solubilising bacteria from different crops of rhizospheric soil collected from Satara and Sangli districts of Western Maharashtra

A total of 45 rhizospheric soil samples were gathered from diverse locations in the Sangli Satara districts as well as from a variety of crops, including sugarcane, groundnut, soybean, green gram, wheat, turmeric, maize, onion, and jowar, Out of 45 soil samples only 9 isolates *viz*, Bor-1, Wat-2, Mir-1, Pal-3, Pha-5, Ash-2, Is-1, Ret-2 and Sat-3 showed better performance to zinc solubilization.

Effect on pH after 5th, 10th and 15th day of inoculation zinc solubilising isolates

The pH of the culture filtrate in both inoculated and uninoculated samples was determined at 5, 10 and 15 days interval after inoculation. The decreasing pH range over the course of 15 days indicated that bacteria were metabolizing nutrients and producing acidic byproducts, such as organic acids.

Table 1. presents the findings of the investigation, which clearly demonstrate that the initial pH of all isolates was 7.00, which is neutral. At 15 days, Pal-3 had the lowest pH (2.37) followed by Bor-1 (2.48) and Pha-5 (2.61). Is-1 (3.75) and Sat-3 (3.45) had the highest pH at 15 days. This is probably because the bacteria secreted organic acids as they grew.

According to Brazilian Journal of Microbiology (2004), Pal-3, Bor-1 and Pha-5 were the most successful in reducing pH among all of them, indicating a greater potential for mineral solubilization through acidification, particularly zinc. Conversely, isolates Sat-3, Ret-2 and Is-1 had reduced acidity, which was a sign of decreased solubilization efficiency.

These observations of decline in pH aligns with the findings reported by Sharma *et al*., (2012).

Table 1: Effect on pH after 5th, 10th and 15th day of inoculation zinc solubilising isolates

Isolate no.	Isolates name	pH			
		On inoculated day	After 5 DAI	After 10 DAI	After 15 DAI
1	Wat-1	7	6.45	5.72	3.21
2	Bor-1	7	6.35	5.58	3.08
3	Pal-3	7	6.85	5.19	2.99
4	Ash-2	7	6.94	5.82	3.45
5	sat-3	7	6.97	5.64	2.61
6	Pha-5	7	6.45	5.38	2.68
7	kar-2	7	6.93	5.7	2.37
8	Is-1	7	6.84	5.79	2.48
9	Kor-1	7	6.84	5.08	3.75

*****DAI – Days After Inoculation

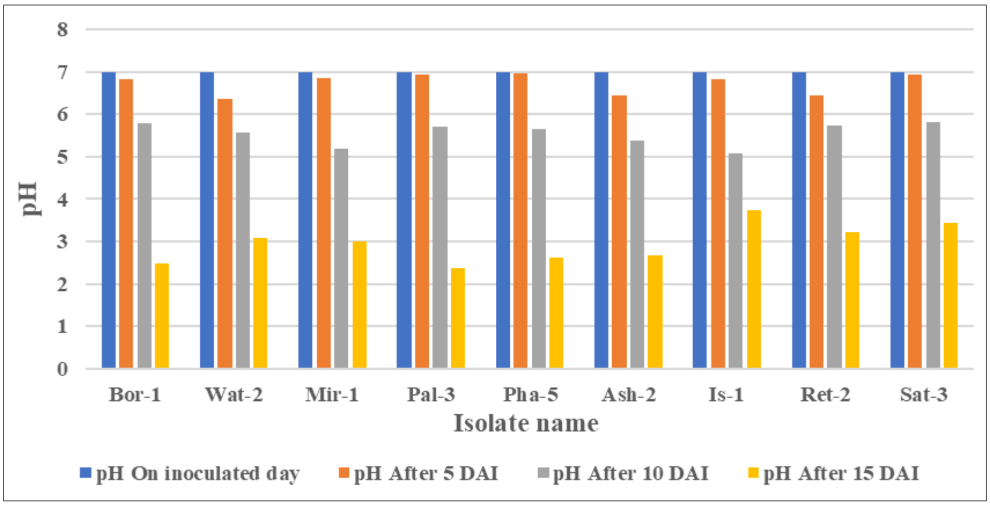

Fig 1: Effect on pH after 5th, 10th and 15th day of inoculation zinc solubilising isolates

Quantitative zinc solubilization ability of zinc solubilizing isolates

The quantity of Zn solubilized was estimated after 7th day and 10th day of inoculation, MSM broth supplemented with 0.1% insoluble Zn compound using atomic absorption spectrometer (AAS).

The ZSB isolates that were showed halo zone formation were further tested for their ability to release inorganic Zn in liquid medium. All isolates showed an increase in soluble zinc concentration from day 7 to day 10. The increase in soluble Zn quantity from the 7th to the 10th day across all isolates confirms that these bacteria are capable of solubilizing insoluble zinc compounds, likely through the production of organic acids.

The investigation's results are shown in Table 2. and unequivocally show that the highest Zn solubilization on both days was seen in Pal-3 (65 → 87 mg/L). The lowest Zn solubilization was observed in Wat-2 (39 → 43 mg/L). Pal-3, Bor-1, and Pha-5 were the top-performing isolates, with Pal-3 showing the highest solubilization efficiency, in enhancing zinc availability in soil. However it was at par with Bor-1 (79 mg/L) and Pha-5 (73 mg/L). The result of the present research are in agreement with Gontia-Mishra I. *et al.*, Frontier microbiology (2017).

Table 2: Estimation of quantitative zinc solubilization ability of zinc solubilizing isolates

Sr No.	Isolate name	Soluble Zn quantity (mg/L)	
		7th day	10th day
1	Bor-1	63	79
2	Wat-2	39	43
3	Mir-1	44	51
4	Pal-3	65	87
5	Pha-5	58	73
6	Ash-2	52	62
7	Is-1	46	48
8	Ret-2	43	46
9	Sat-3	40	47
	SE(m) ±	0.79	1.06
	CD at 1%	2.36	3.15

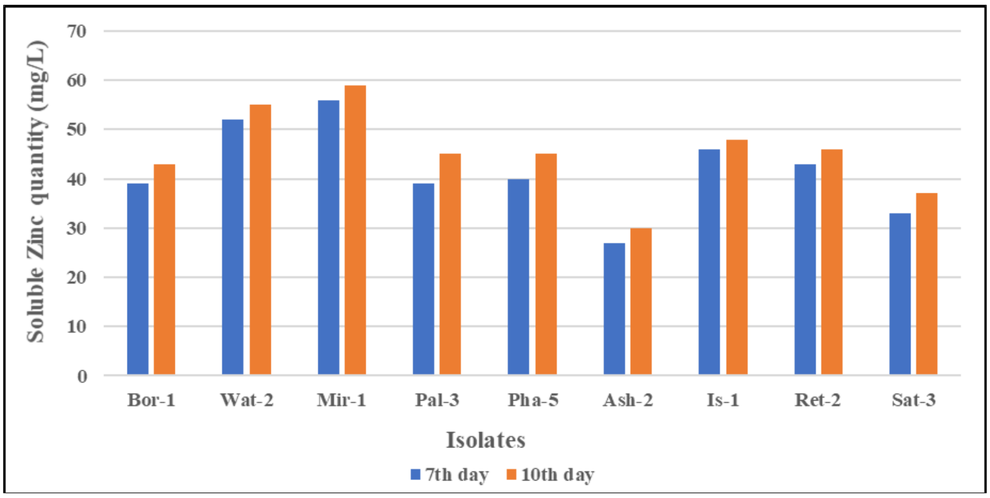

Fig. 2: Estimation of quantitative zinc solubilization ability of zinc solubilizing isolates

This trend correlates well with the pH data (from your earlier tables), as reported by Othman N. M. I. *et al.*, (2022), isolates like Pal-3 and Bor-1 also showed greater acidification (lower pH) supporting the idea that Zn solubilization is linked to acid production. Isolates like Wat-2 and Sat-3, while still active, showed comparatively lower Zn solubilization, which may be due to weaker acid production or slower metabolic activity.

Conclusion

The study found that ZSB, especially Pal-3, Bor-1 and Pha-5 had a lot of potential for releasing soluble zinc into the soil and allowing plants to use it by producing acid.

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