

# Zinc Solubilizing Rhizobacteria Associated with Sugarcane from South Gujarat Region

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**Abstract:** Zinc is a one of the eight essential micronutrients required for the normal healthy growth and reproduction of crop plants. Zinc deficiency in an agricultural soil is well-studied and wide spread problem worldwide. This study on zinc solubilization by bacteria has an immense importance in zinc nutrition to plant particularly in fields having alkaline soils as observed in present study. Among 309 isolates, 141 isolates were positive for zinc solubilization. Considering the quantitative aspects solubilization efficiency of bacterial isolates was ranged from (116-366) for 0.1% zinc oxide incorporated in the medium. Based on solubilization efficiency five isolates with highest Zn solubilization efficiency were chosen for multiple plant growth promoting traits from which BM/F1/ER/8, NFM/F2/RP/15, and KBM/F4/RH/5 have multiple plant growth promoting traits can be exploited as a potential biofertilizers.

**Keywords:** Zinc solubilization, Zinc oxide, Biofertilizer.

## 1. Introduction

The use of microorganisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture (Freitas *et al.*, 2007). Rhizosphere is the place where very important and intensive interactions take place between plants, soil, microorganisms and soil micro fauna, influenced by compounds exuded by roots, and microorganisms feeding on its compounds (Antoun and Prevost, 2006)

In eukaryotic and prokaryotic organisms, the role of Zinc in the nutrition, physiology and especially its importance for activity of many enzymes is widely studied (Hughes and Poole, 1989; Clarke and Berg, 1998). Few zinc solubilizing viz, *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, *Acinetobacter*, *Bacillus*, *Glucanacetobacter*, *Pseudomonas* and facultative thermophilic iron oxidizers have been reported as a zinc solubilizers (Saravanan *et al.*, 2007). 50% of agricultural soils contain low level of available zinc, mainly due to high soil pH, low soil moisture and low organic matter (Alloway, 2008; Cakmak 2008). Root cell membrane permeability is increased under Zn deficiency, which might be related to the function of Zn in cell membranes (Parker *et al.*, 1992). Exogenous application of zinc fertilizer has been applied to various crops and this causes transformation of about 96-99 percent of applied available zinc to various unavailable forms. This unavailable zinc can be reverted back to available form by inoculating a bacterial strain capable of zinc solubilizing potential (Saravanan *et al.*, 2003). Fixation of zinc in soil with pH > 7.0 increases with increasing concentration of carbonates and thus become unavailable form with Zn solubilizing microorganism (Shahad and Ahmed, 2008). Since zinc is a limiting factor in crop production in alkaline soils of the world. This study on zinc solubilization by bacteria has an immense importance in zinc nutrition to plant particularly in fields having alkaline soils as possible future potential bio-inoculants.

## 2. Material and Methods

### Sample Collection

The root adhering samples of five sugarcane plants were collected from the agricultural field of Kamrej, Abrama, Chalthan and Maroli, India. The sample were collected in aseptic bags and immediately transported to lab for further process (Zarrin *et al.*, 2009).

### Isolation of Rhizobacteria and Endophytes

The isolation of rhizobacteria, were carried out from bulk soil, rhizospheric soil, rhizoplane and endorhizospheric sites using 100 µL of diluted suspension from each sites were spreaded on different media viz. king's B medium (KBM), ashby's mannitol agar (AMA), nitrogen free medium(NFM), yeast extract mannitol agar (YMA), azospirillum medium (AZ), pikovskaya medium (PM), bacillus medium(BM).

### Zinc solubilization assay

Zinc solubilization assay was performed for all isolates on to the modified Pikovskaya medium (Pikovskaya, 1948) containing 0.1% insoluble zinc compound (ZNO). All the plates were incubated for 48 h at 28°C. Further the Zinc solubilization efficiency (SE) of the isolates was calculated and determined as mentioned by Ramesh *et al.*, (2014)

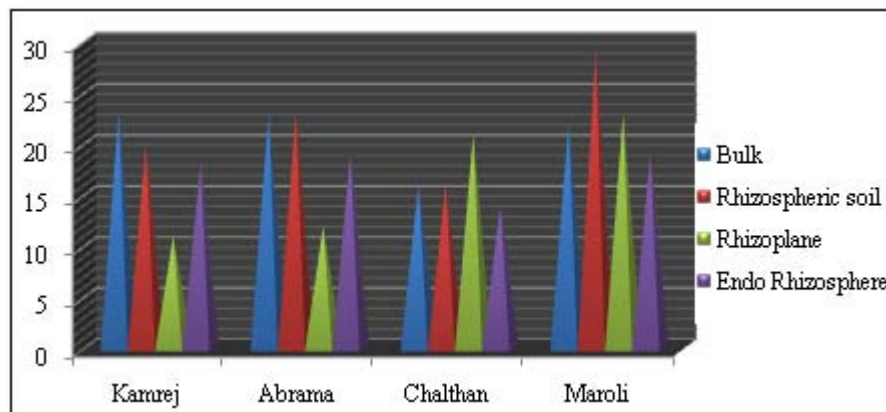
**SE = Diameter of solubilization halo zone / diameter of colony × 100**

## 3. Result and Discussion

Zinc solubilization has been studied and evaluated nearly in crop plants like cereals (cakmak, 2008) Maize (Shruthi P., 2013, Preveen kumar *et al.*, 2013), Soybean (Poonam S. *et al.*, 2014). While comparing all the four locations, the Maroli field has a highest number of isolates (85) from all the sites. The rhizospheric soil has highest number of isolates compare to rhizoplane and endorhizosphere (Fig.1). Reyes *et al.*, 2006 have also reported that rhizospheric soil possesses higher number of bacteria as compared to non-rhizospheric soil. Which prove the necessity of bacteria for solubilization of nutrients present in bulk soil and make it available in more amounts in rhizospheric soil and finally available to

the plant at rhizoplane and endorhizosphere for more physiological activities. In the present study role of bacteria in zinc solubilizing activity have been studied where more number of such isolates found from Maroli field with alkaline soil. This is in agreement with the finding of

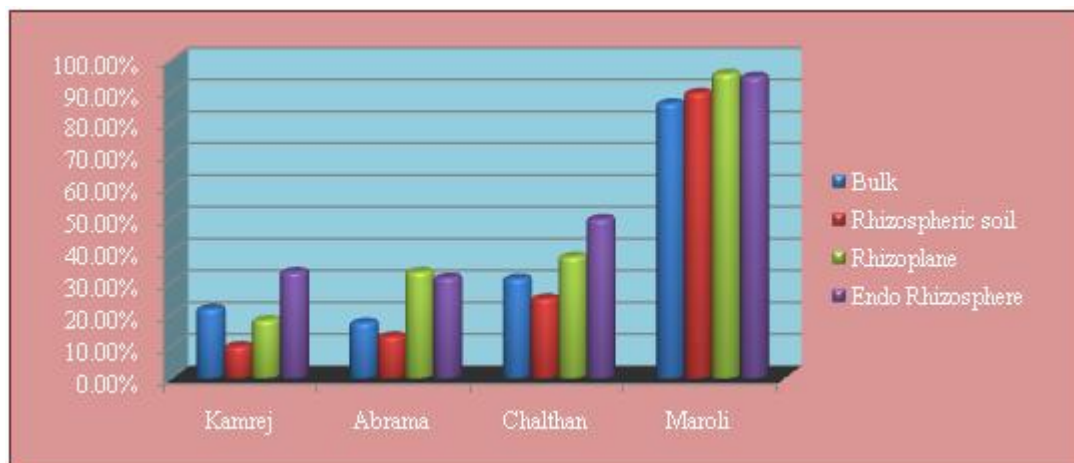
(Azadeh B.*et al.*, 2012) who finds the zinc solubilizing potential by fluorescent Pseudomonads from the alkaline soil.



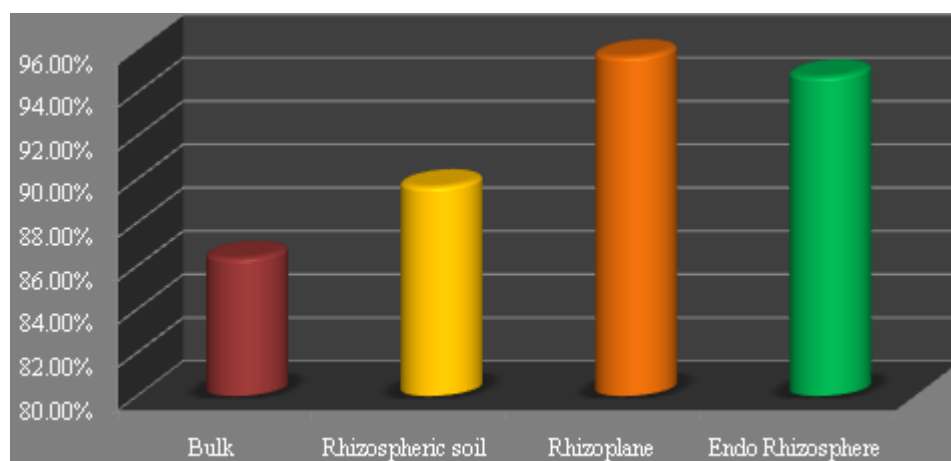
**Figure 1:** Total No. of isolates from diverse sites.

Zinc is an important micronutrient required for many enzymatic activities by bacteria as well as plant. Plant cannot solubilize zinc, which is solubilize by plant growth promoting bacteria present in soil as well as endophytes in root. As mention above such isolates are found at Maroli

field and in particular from site of rhizoplane (Fig.3) which support the role of bacteria and supplying such trace elements after solubization to the plant for the growth.



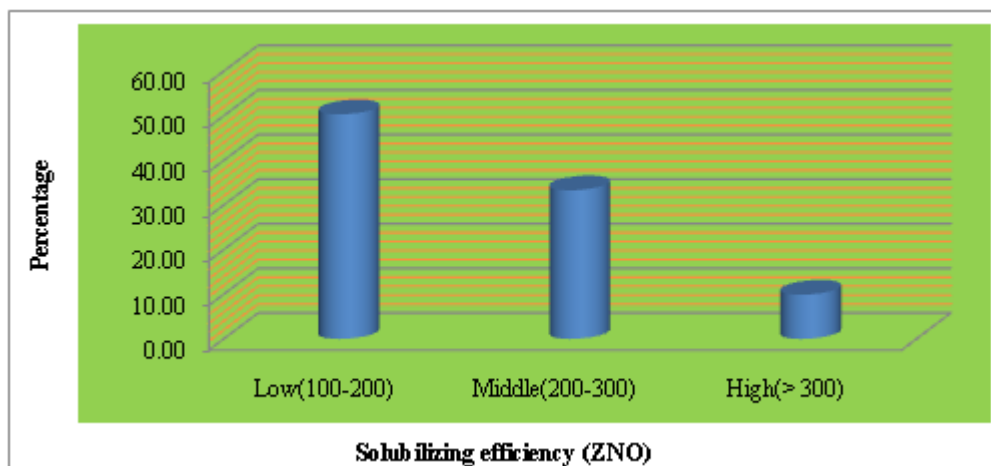
**Figure 2:** Zinc solubilizing isolates from South Gujarat region



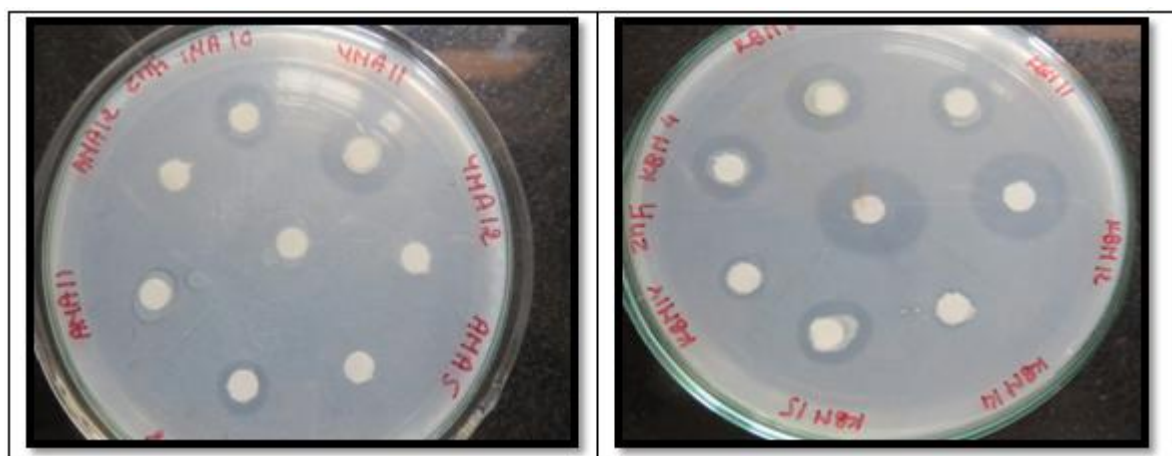
**Figure 3:** Zinc solubilizing isolates from diverse site of plant

In plate assay, all positive isolates were subjected to measure the magnitude of Zn solubilization with zinc oxide as an inorganic source of zinc. Total of 309 isolates 141 isolates were positive for zinc solubilization. Solubilization efficiency of bacterial isolates were ranged from (116-366) for zinc oxide incorporated in the medium. Of the 141 bacterial isolates 9.92% categorized with highest Zn solubilization efficiency (>300) whereas 33.33% were medium range Zn solubilizers (200-300) (Fig.4). Similar to

our study, Poonam et al., (2014) had observed zinc solubilization efficiency of bacterial isolates from legume rhizosphere were ranged from (117.2-466) for zinc oxide incorporated in the medium and 11% categorized with highest Zn solubilization efficiency and 50% were medium range Zn solubilizers. Bacterial isolates produce a halo zone around the bacterial colony which indicated solubilization of zinc oxide in fig.5.



**Figure 4:** Percentage range of zinc solubilizing efficiency of bacterial isolates



**Figure 5:** Formation of clear halo zone around bacterial colony

**Table 1:** Multiple plant growth promoting traits of maximum zinc solubilizing efficiency

S. No.	Isolate No.	Phosphate solubilizing bacteria	Potassium solubilizing bacteria	Zinc solubilizing bacteria	Nitrogen fixing bacteria
1	AZ/F1/ER/3	-	-	++++	+
2	BM/F1/ER/8	+++	++	++++	+
3	NFM/F2/RP/15	++++	++++	++++	+
4	KBM/F4/RH/5	++++	++++	++++	+
5	PM/F4/RH/7	-	+++	++++	+

Based on solubilization efficiency five isolates (Table 1.) with highest Zn solubilization efficiency (366) were chosen for multiple plant growth promoting traits viz. Nitrogen fixation, Phosphate solubilization, Potassium solubilization and Zinc solubilization, from which 3 isolates having maximum positive plant growth promoting potentials.

#### 4. Conclusion

Based on present study highest Zn solubilization efficiency of bacterial isolates were chosen for multiple plant growth promoting traits, from which BM/F1/ER/8, NFM/F2/RP/15, and KBM/F4/RH/5 have highest positive plant growth promoting potentials. Such combined inoculation of zinc solubilizing bacterial cultures can be further explores as effective bio-inoculants as they possess a multiple positive

traits useful for improving plant productivity for substituting chemical fertilizers and also to correct nutrient deficiency in sugarcane for sustainable agriculture.

## 5. Acknowledgement

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