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Assessment of Zinc solubilization by Endophytic Bacteria in Legume Rhizosphere

KEYWORDS: Endophytic bacteria, Legumes, zinc solubilization

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ABSTRACT *Total of 48 endophytic bacterial isolates from soybean (43) and summer mungbean (5) rhizosphere were screened for zinc solubilizing ability in Tris- minimal medium separately amended with inorganic zinc compounds viz. zinc oxide (ZnO) and zinc phosphate Zn3(PO4)2 by plate assay method. Further zinc solubilizing isolates were assessed for morphological, biochemical and plant growth promoting (PGP) traits in vitro. Of 48 endophytic bacterial isolates, 3 and 17 were able to solubilize ZnO whereas 2 and 12 were able to solubilize Zn3(PO4)2 on Tris- minimal medium. Endophytes 1J (Klebsiella spp.) and 19D (Pseudomonas spp.) were found to be promising bacterial isolates as they solubilized both inorganic sources of zinc separately supplemented in Tris- minimal medium along with good PGP traits (P- solubilization & indole acetic acid) and can be exploited as potential biofertilizers.*

Introduction

Zinc is an essential micronutrient for microorganisms and plants. It is present on earth's crust in tune of 0.008%. Zinc has an immense role in nutrition of both eukaryotic and prokaryotic organisms as cofactor or metal activator in various enzyme systems (Hughes and Poole, 1989). Bacteria are known to immobilize metal by precipitation and adsorption. The ability to dissolve immobilized zinc viz. zinc phosphate, zinc oxide and zinc carbonate in appreciable quantity is not common feature amongst the cultivable bacteria on soil surface. Few Zn solubilizing bacterial genera viz. Thiobacillus thioxidans, Thiobacillus ferroxidans, Acinetobacter, Bacillus, Gluconacetobacter, Pseudomonas and facultative thermophilic iron oxidizers have been reported as zinc solubilizers (Saravanan et al., 2007).

The total area under Zn deficiency is about 10 Mha in India. Approximately 85% of rice-wheat cropping system is present in the Indo-Gangetic plain region and Zn is limiting factor in crop production due to alkaline and calcareous soil. Improving production from this cereal belt is therefore, vital for sustaining nutritional security and grain production in country (Singh et al., 2005). In India soybean-wheat cropping system extracts zinc from soil e.g. harvest of 6.5 ton grain/ha/yr removes 416g Zn/ha/yr in Indian soils exhibit 50% Zn deficiency which is below critical level of 1.5 ppm of available zinc (Prasad, 2010). About 75% exogenous application of zinc sources like $ZnSO_4$ get fixed in soil. Fixation of Zn in soils with pH >7.0 increases with increasing concentration of carbonates, thus become unavailable and can be reverted back to available form with Zn solubilizing microorganism (Shahab and Ahmed, 2008).

Commonly used nitrogen fixing bacteria like Rhizobium, Azospirillum and Azotobacter are not reported as zinc solubilizers but some endophytic bacteria viz. Acinetobacter, Bacillus and Pseudomonas (Saravanan et al., 2007) have been reported to solubilize zinc. Endophytic bacteria co-exist with symbiotic bacteria in nodules and they do not induce nodules and are sheltered from environmental stresses and microbial competition by the host plant. They seem to be ubiquitous in plant tissues and also isolated from leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo, 2000). Endophytic bacteria exert several beneficial effects on host plants, such as stimulation of plant growth, nitrogen fixation and induction of resistance to plant pathogens (Charenporn et al., 2010). These endophytic bacteria along with nitrogen fixing bacteria can be used as consortium in cereal based crops which can serve dual purpose of fixing nitrogen and solubilizing micronutrients like zinc. This paper aims for searching new native endophytic bacteria with plant growth promoting (PGP) traits along with Zn solubilization from mungbean and soybean rhizosphere as a possible future potential bio-inoculants.

Material and methods

Procurement of endophytic bacterial isolates:

Of total 48 endophytic bacterial isolates, 43 and 5 of soybean and summer mungbean rhizosphere, respectively were procured from culture collection centre of Pulse Microbiology Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University Ludhiana. All the isolates were maintained on nutrient agar medium at 4°C throughout the study.

Plate assay for Zn solubilization efficiency:

All the endophytic bacterial isolates were screened by plate assay for their efficiency to solubilize zinc on Tris- minimal medium supplemented separately with zinc oxide (ZnO) [1.244 /L] = 15.23mM and zinc phosphate $Zn_3(PO_4)_2$ [1.9882 g/L] = 5.0mM at a concentration equivalent to 0.1% Zn (Fasim et al., 2002). After spot inoculating plates were incubated in dark at 28°C and observed for formation of clear halo zone around bacterial growth after seven days. Zinc solubilization efficiency (SE) was calculated as described by Ramesh et al., (2014).

$$SE = \frac{\text{Diameter of solubilization halo zone}}{\text{Diameter of colony}} \times 100$$

Morphological, biochemical and Plant growth promoting (PGP) traits:

Endophytic bacterial isolates with Zn solubilization efficiency were characterized for their morphological and biochemical

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tests (Arora, 2007). Selected Zn solubilizers were assessed for functional PGP traits viz. Indole acetic acid (IAA), P- solubilization and ammonium production in vitro condition using standard methods (Spaepen et al., 2007).

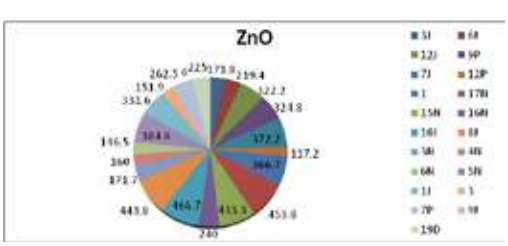
Results

Table1. Biochemical and functional characterization of endophytic bacterial isolates

Source	Isolates	IAA	P- Solubilization	Citrate Utiliza-tion	Nitrate Reduction	Oxidase	Zn solubilization		Probable Genera of Micro-organism
							ZnO	Zn_3PO_4	
Soybean	1J	+	+	+	++++	+	+	+	Klebsiella sp.
	3J	+	+	+	++++	+	+	+	Klebsiella sp.
	6J	+	+	+	++++	-	+	+	Klebsiella sp.
	7J	+	+	+	+++	+	+	-	Enterococcus sp.
	8J	+	+	+	++	-	+	+	Klebsiella sp.
	10J	+	+	+	++++	+	+	-	Klebsiella sp.
	12J	+	+	+	-	+	+	+	Klebsiella sp.
	7P	+	+	+	-	+	+	+	Bacillus sp.
	9P	+	-	+	-	-	+	+	Pseudomonas sp.
	12P	+	+	+	++++	-	+	+	-
	3N	+	+	-	++++	+	+	+	Pseudomonas sp.
	4N	+	-	-	-	-	+	+	Pseudomonas sp.
	5N	+	-	-	-	+	+	-	Bacillus sp.
	6N	+	+	-	++++	-	+	-	Pseudomonas sp.
	15N	+	-	+	-	-	+	-	-
	16N	+	+	-	++++	-	+	+	Pseudomonas aeruginosa
	17N	+	-	-	-	-	+	-	Paenibacillus sp.
Summer mung-bean	3P	-	+	+	+++	+	+	-	Pseudomonas aerogenosa
	1P	+	+	-	-	-	+	+	Klebsiella sp.
	19D	-	+	+	-	+	+	+	Pseudomonas sp.

+ (Positive) ; - (Negative) ++; (Low); +++ (Medium); ++++ (High)

1 (a)



1 (b)

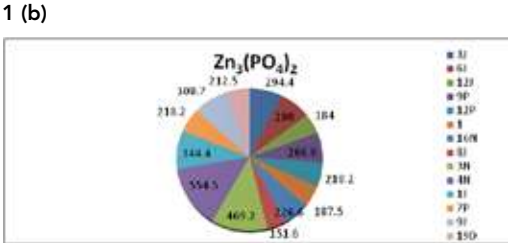


Fig 1(a & b): Zinc solubilization efficiency of different endophytic bacteria on Tris-minimal medium amended with 0.1% Zn 1(a) Zinc Oxide (ZnO); 1 (b) Zinc phosphate ($Zn_3(PO_4)_2$).

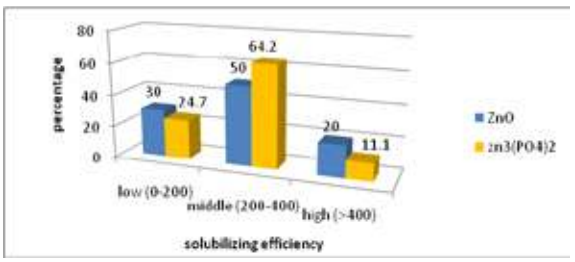


Fig 2: Percentage range of zinc solubilization efficiency of different endophytic bacterial isolates

Fig. 3: Formation of clear halo zone around endophytic bacterial colony

In plate assay, all the endophytic isolates were subjected to measure the magnitude of Zn solubilization on Tris- minimal medium amended separately with zinc oxide and zinc phosphate as inorganic source of zinc as mentioned in Table 1. Out of total 48 endophytic bacterial isolates, 3 and 17 endophytic bacterial isolates from summer mungbean and soybean rhizosphere, respectively were able to solubilize zinc oxide (insoluble) incorporated in Tris- minimal medium, respectively. Whereas, 2 and 12 endophytic bacterial isolates from summer mungbean and soybean rhizosphere, respectively were able to solubilize zinc phosphate (insoluble) supplemented in Tris- minimal medium, respectively. Solubilization efficiency of endophytic bacterial isolates were ranged from 151.6- 554 for zinc phosphate and 117.2- 466 for zinc oxide incorporated in Tris- minimal medium. Of the 21 endophytic bacterial isolates 20% and 11% categorized with highest Zn solubilization efficiency (≥ 400) whereas 50% and 64.2% were medium range Zn solubilizers (200-400) in Tris-minimal medium incorporated separately with zinc oxide and zinc phosphate, respectively as shown in Fig. 2. Highest zinc solubilization efficiency for zinc phosphate was recorded in 4N whereas 10J endophytic bacterial isolate recorded it for zinc oxide. Endophytes produced a halo zone around the bacterial colony which indicated solubilization of zinc source in Tris- minimal medium as shown in fig. 3.

In present study, 2 endophytic bacterial isolates viz. 1J (stem) and 19D (root) were identified as potent endophytic bacterial isolates with PGP traits viz. IAA, P- solubilization and NH_3 producers along with Zn solubilization. On the basis of

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morphological study isolates 1J and 19D were gram negative produced round shaped, raised colonies having shining surface, smooth margin and light yellow to off white in color. Isolate 19D produced yellow-green pigment when streaked on King's B medium and tentatively assigned to genus Pseudomonas. Biochemical characterization revealed 19D positive for oxidase, citrate and nitrate reduction and negative for IAA production. Isolate 1J was positive for IAA, oxidase, citrate utilization and nitrate reduction and probably placed in genera Klebsiella. Both these isolates produced yellow halo zone on NBRIP medium indicated positive for P- solubilization.

On the basis of PGP traits and Zn solubilization endophytic bacterial isolates 1J (stem of wild species Glycine tomentella) and 19D (root of Vigna radiata L.) can be further assessed for quantitative solubilization of Zn and after rigorous testing under field conditions can be employed as future bioinoculant for better crop production and soil health.

Discussion

Zinc deficiency is major problem that affects soil health and crop yields in agriculture. Microorganisms play an important role in solubilization of zinc in soil. Such solubilization of zinc compound mediated through production of organic acids and subsequent release of zinc in external environment and bioaccumulation of Zn inside the cells of bacterial species had been reported earlier (Fasim et al., 2002). Our results are in close agreement with the findings of Ramesh et al., (2014) who have also reported that three Bacillus aryabhattai strains MDRS7, MDRS11 and MDRS14 possessed IAA, siderophores and ammonia producing traits.

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