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Isolation and characterization of potential Zn solubilizing bacteria from soil and its effects on soil Zn release rates, soil available Zn and plant Zn content

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ABSTRACT

In this study, experiments were designed to isolate, characterize and evaluate an array of bacteria isolates for their Zn solubilization potential. Out of the six promising Zn solubilizing bacteria (ZnSB), ZnSB2 (*B. megaterium*, KY687496) was found to be the most potential strain owing to its enhanced Zn solubilization in vitro. In the quantitative study, the net Zn solubilized by ZnSB2 was significantly higher than those solubilized by the other ZnSB at all days of sampling. Similar effects of ZnSB2 was observed in the soil per se, wherein the rate of release of available Zn by ZnSB2 was markedly higher at all days of incubation (25.6%–40.7% of added Zn), with a peak on the 8th day. Such enhanced rates of Zn release by ZnSB2 were attributed to marked decrease in pH owing to enhanced gluconic acid production. In fact, gluconic acid production by ZnSB2 was 1884.7 ± 413.4 μg mL^{-1}, which was 35.3–69.7% greater than the other shortlisted ZnSB isolates. Further evaluation of ZnSB2 was done in the green house using turmeric as the test crop. ZnSB2 was applied either alone or in combination with chemical Zn (75% and 100% of recommended Zn). The results revealed that soil available Zn level in the treatment with 75% Zn + ZnSB2 (12.69 ± 2.96 mg kg^{-1}) was on par with the level in the treatment with 100% Zn (12.74 ± 2.63 mg kg^{-1}) at 120 days after planting, while at harvest the treatment with 75% Zn + ZnSB2 maintained 65.0% higher available Zn levels than 100% Zn. The positive effect of ZnSB2 was also manifested on rhizome yield, which was at par in the treatments with ZnSB2 + 75% Zn (154.2 g ± 36.0 pot^{-1}) and 100% Zn (177.2 ± 36.7 g pot^{-1}). Besides, the Zn concentration in the rhizome was significantly higher ($P < 0.05$) in the treatment with ZnSB2 + 75% Zn (40.5 ± 3.5 mg kg^{-1}), which was at par with 100% Zn, but was greater by 98.5% compared to control. The study indicated that ZnSB2 strain was a potential candidate for enhanced Zn dissolution in soil, which would allow reduced inorganic Zn application rates. Nonetheless, in vitro interaction studies (dual culture) suggested that this strain was seriously lacking in disease suppressing traits. But its compatibility with several plant growth promoting rhizobacteria enhanced the possibility of co-inoculation or applying ZnSB2 in a consortium mode especially in condition wherein both soil Zn solubilization and disease suppression becomes imperative.

1. Introduction

Micronutrient deficiency has become a limiting factor for crop productivity in many parts of the world. Among the micronutrient deficiencies, Zn deficiency is considered to be the most ubiquitous abiotic stress in countries like Afghanistan, Australia, Bangladesh, Brazil, China and India, Iran, Iraq, Pakistan, Philippines, Sudan, Syria, Turkey, and many parts of Europe, USA and Africa (Alloway, 2009; Cakmak et al., 1999). This is because Zn is the only micronutrient relevant to all classes of enzymes present in biological systems (Broadley et al., 2007) and almost 2800 proteins need Zn for their structural integrity and activity (Andreini et al., 2009). However, Zn deficiency in millions of hectares of agricultural soils has not only reduced crop yields but also severely hampered the nutritional quality of the crop produce causing critical nutritional and health problem in one-third of the world's human population (Hotz and Brown, 2004; Myers et al., 2015). Acid, calcareous, saline and sodic soils and coarse-textured soils prone to high weathering, besides soils subjected to intensive cropping and poor

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drainage exhibit Zn deficiency (Singh et al., 2005). Also, factors like high available P and Si, drying of upper horizons, sub-soil constraints and sometimes high fertilizer cost subscribe to Zn deficiency (Alloway, 2009).

Apparently, enhancing the available Zn pool in the soil by application of Zn containing synthetic fertilizers or organic manures becomes imperative. Unfortunately, exogenous application of chemical fertilizers alone cannot help in combating soil Zn deficiency in the long-term since 96.0–99.0% of the applied Zn is once again converted to unavailable Zn pools by precipitation to carbonates or oxides or phosphates etc. (Ma and Uren, 1997; Zhang et al., 2017). Hence, decreased use efficiency of chemical Zn fertilizers, remains an issue, especially in the long-term.

Nevertheless, a redeeming feature is that the worldwide occurrence of Zn scarcity issues in crops is not due to low levels of total Zn but is due to low solubility of Zn in soils (Cakmak, 2008). In fact, the total Zn content in soils is substantially high and exists in fixed forms such as smithsonite ($ZnCO_3$), sphalerite (ZnS), zincite (ZnO), franklinite ($ZnFe_2O_4$), wellemite (Zn_2SiO_4), and hopeite ($Zn_3(PO_4)_2{\cdot}4H_2O$), which are only sparingly soluble. Values in the literature indicate that available Zn level in soils is very low (4.0–270.0 $\mu g\,L^{-1}$) in relation to the mean total Zn level of 64.0 $mg\,kg^{-1}$ (Alloway, 2009). Reports suggest that Zn deficiency due to low amounts of bioavailable Zn is rampant in at least one-third of the cultivated soils globally (Sillanpää and Vlek, 1985). Apparently, low bioavailability not only hampers crop productivity but also markedly lowers Zn density in the harvested produce (seeds, grains, rhizomes etc.) thereby impairing nutritional quality (Cakmak and Hoffland, 2012).

Hence, a feasible alternative would be to exploit the innate capacity of certain soil microorganisms, especially bacteria and fungi, to solubilize these fixed forms of Zn to labile Zn forms for enhanced availability and subsequent uptake by plants. However, the ability to solubilize immobilized Zn (ZnO, $ZnCO_3$ or $ZnPO_4$) is not a common characteristic of cultivable bacteria and fungi in soils, though there are in vitro studies on a few genera of bacteria like *Pseudomonas* sp., *Gluconacetobacter* sp., *Thiobacillus* sp., *Bacillus* sp., *Acinetobacter* sp. etc. (Di Simine et al., 1998; Fasim et al., 2002; Hafeez et al., 2013) and fungi like *Beauveria caledonica* (Fomina et al., 2004), *Lecanicillium psalliotae* (Senthil Kumar et al., 2018) etc. capable of solubilizing Zn. Several Zn solublising bacteria (ZnSB) have been isolated from soils of tropical and temperate regions and strains of genera *Acinetobacter*, *Bacillus*, *Burkholderia*, *Gluconacetobacter*, *Pseudomonas*, *Thiobacillus* have been reported from mostly plate assays (Bapiri et al., 2012; Saravanan et al., 2007a, 2007b; Saravanan et al., 2011; Vidyashree et al., 2016).

The major objective of this study was to isolate an array of bacteria isolates and characterize them for their Zn solubilization potential and to study the Zn release mediated by the promising ZnSB in soil per se. In addition to the Zn solubilizing potential, the shortlisted ZnSB were also screened for their multi-tasking abilities that included solubilization of P, K, Si, production of IAA, NH_3, HCN, siderophore, cell wall degrading enzymes (pectinase, protease α-amylase & cellulase) etc. The effects of the most promising ZnSB on soil Zn availability and Zn uptake by turmeric were also studied. Turmeric (*Curcuma longa* L.) was used as the test crop since there are no reports on the effect of ZnSB on rhizome Zn content, though there are reports involving ZnSB on Zn biofortification in soybean (Ramesh et al., 2014), rice (Krithika and Balachandar, 2016), maize (Mumtaz et al., 2017), wheat (Shaikh and Saraf, 2017), green gram (Sharma et al., 2012) etc. Turmeric is an annual crop with duration of 7–8 months and is grown under both rainfed and irrigated conditions. It is used worldwide as a condiment, flavouring and colouring agent in the food industry besides its extensive use in the drug industry owing to its anti-viral and anti-cancer activities (Srinivasan et al., 2016).

Table 1
Physico-chemical properties of the soil samples collected from the rhizosphere of wild black pepper and wild cardamom.

	Wild black pepper (50)[a]		Wild cardamom (20)[a]	
	Range	Mean ± SD[b]	Range	Mean ± SD[b]
pH (1:2.5 H_2O)	3.3–5.2	4.3 ± 0.4	3.8–5.3	4.4 ± 0.5
Organic C ($g\,kg^{-1}$)	5.3–33.9	19.3 ± 7.2	12.0–31.0	23.0 ± 6.0
Available P ($mg\,kg^{-1}$)	3.1–34.5	10.9 ± 8.7	3.8–33.0	10.0 ± 8.7
Exchangeable K ($mg\,kg^{-1}$)	57.0–228.0	128.9 ± 36.9	73.0–195.0	112.8 ± 41.7
Available Fe ($mg\,kg^{-1}$)	21.1–61.4	33.6 ± 10.7	3.1–36.5	27.8 ± 6.5
Available Mn ($mg\,kg^{-1}$)	4.6–31.9	20.6 ± 5.4	15.4–24.8	18.6 ± 2.3
Available Zn ($mg\,kg^{-1}$)	0.6–4.7	2.1 ± 1.0	0.4–4.6	1.8 ± 0.9
Available Cu ($mg\,kg^{-1}$)	0.54–2.51	1.25 ± 0.47	0.48–3.08	1.58 ± 0.61

[a] Total number of soil samples collected from the rhizosphere of wild black pepper and wild cardamom.
[b] SD - Standard Deviation.

2. Materials and methods

2.1. Soil sampling and analyses

Soil samples (70 nos) were collected from the rhizosphere of wild pepper (*Piper nigrum* L. - 50 nos) and cardamom (*Elettaria cardamomum* (L.) Maton - 20 nos) growing in the forest areas of Idukki district (Latitude: 9° 50′ 60.00″ N; Longitude: 76° 58′ 0.01″E) of Kerala State (India). These two crops were chosen because we wanted to isolate prospective bacterial species from the rhizosphere of domesticated crops present in undisturbed soils of virgin forest sites. Virgin sites were selected because it would provide opportunity to explore undisturbed soils with more diverse bacterial communities. The soil within the confines of the space occupied by plant roots and those strongly clinging to the roots were considered to belong to the rhizosphere (Garcia et al., 2005). The soil samples were transported to the laboratory in an ice box and subsequently the plant and root debris were discarded, moisture content estimated and a chunk of each sample required for estimating the microbial parameters was stored at 4 °C. The physico-chemical properties of the soil samples are presented in Table 1.

The soil pH was measured in 1:2.5 soil: water suspension. Organic C was determined by the dichromate-oxidation method (Nelson and Sommers, 1982) and available P using the dilute acid–fluoride extractant (Kuo, 1996). Available Zn, Cu, Fe & Mn were extracted using DTPA (Lindsay and Norvell, 1978) and the concentration of these nutrients in these extracts was measured using atomic absorption spectrophotometer (AAS, Varian AA240FS).

2.2. Isolation of bacteria

This was done by serial dilution plate count wherein 1.0 g soil was transferred to a dilution tube containing 9.0 mL sterilized water (10^1) and shaken for 10 min. After shaking, the dilution tubes were kept undisturbed for 30 min to allow the suspension to stabilise. Later, 1.0 mL of the bacterial suspension from 10^1 dilution was transferred to another dilution tube containing 9.0 mL sterilized water (10^2), shaken for 10 min and allowed to stabilise for 30 min. This was continued up to 10^{10} dilution, pour-plated on Nutrient Agar (NA) and incubated at 28 °C for 72 h. The population of bacteria [colony forming units (CFU) per gram soil] was estimated from the most suitable dilution and individual bacterial colonies were sub-cultured on NA (Dinesh et al., 2015). About 70 bacterial isolates were obtained, which were cryopreserved at