RJM Research Journal of Microbiology

OPEN ACCESS

Zinc-Solubilization Potential of Putative Microorganisms Isolated from Tea [*Camellia sinensis* (L.) O. Kuntze] Rhizosphere

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ABSTRACT

Background and Objective: Monocropping farming practices in Northeastern tea soils are a major risk of zinc deficiency, leading to micronutrient malnutrition. Zinc Solubilizing Microorganisms (ZSMs) are important drivers in the solubilization of insoluble Zn compounds and consequently play an important role in soil fertility and nutrient allocation. The present study attempts to isolate, characterize and qualify the Zn-solubilizing microbes from the tea rhizosphere. **Materials and Methods:** Using a culture-dependent approach and dilution plate technique, the ZSMs were isolated and quantified *in vitro* from the tea rhizosphere. Standard methodologies were used to determine the effectiveness of Zn solubilization. **Results:** The 16 out of the 20 ZSM isolates were shown to have substantial halo zones. Bacteria accounted for 10 of the 16 ZSMs, whereas fungi for six. Modified Bunt and Rovira medium and 10^5 dilutions had the highest frequency of ZSM isolation (up to $6.9\pm0.7\times10^5$ CFU g^{-1} dry soil), followed by modified Pikovskayas agar ($4.6\pm1.0\times10^5$ CFU g^{-1} dry soil). The Zn solubilisation index ranged from 125-325%. Several bacteria-produced acids, as evidenced by a drop in the pH of the broth medium. When given ZnO as the source of Zn, the strains produced Indole-3-Acetic Acid (IAA) (up to 12.5 ppm by ZSB#09). The most common fungus genera were *Aspergillus* spp. and *Trichoderma* spp. Gram-positive cocci were dominant among bacteria. **Conclusion:** As zinc deficiency is common in Northeastern tea soils, utilising the potential of ZSMs could lead to improve sustainability in Zn supplementation.

KEYWORDS

Micronutrient malnutrition, monocroping farming, tea rhizosphere, Zn solubilization, sustainability, zinc solubilizing microorganisms

INTRODUCTION

Despite the advancement of several industrial and technological advancements, the tea plantation industry remains one of India's largest business sectors. Assam is the country's largest tea grower, famous for producing the country's most pungent tea as well as its liquor. Tea [*Camellia sinensis* (L). O. Kuntze] brue, requires zinc as an essential element for the proper functioning of numerous metabolic activities in the

ISSN: 1816-4935 (Print)
https://doi.org/10.3923/rjm.2022.1.13

Received: 11 Apr. 2022
Accepted: 27 Jul. 2022
Published: 01 Oct. 2022

plant such as glucose metabolism and auxin metabolism and works as an anti-oxidant[1-3]. Zinc is essential for the proper development of floral tissues, flowering, fertilisation and fruiting[4]. Plants with a zinc shortage lose biological cell membrane integrity and are unable to produce carbohydrates and phytohormones such as auxins, nucleotides, cytochromes and chlorophyll, resulting in abnormal growth and yield characteristics[5]. Monocropping agricultural practices, low availability of organic matter (mostly due to soil depletion and erotic activities), compact nature of soils, continuous use of chemical fertilisers, liming of soils etc., are believed as prime reasons for higher risk of zinc deficiency in tea soils of Assam, N.E. India, affecting crop gain and causing nutritional disorders in tea. Furthermore, because the solubility of zinc is strongly dependent on soil pH and moisture, some tea-growing locations are frequently described as zinc-deficient[6]. Zinc deficiency is anticipated to rise from 42-63% by 2025 as a result of reduced soil fertility and degradation issues as most of the marginal lands are brought under cultivation over time[7].

Micronutrient deficiency is thus an increasing issue and a cause for concern around the world, resulting in a variety of health and social problems such as mental retardation, immune system impairments and generally poor health. Inappropriate and indiscriminate use of zinc fertilisers, on the other hand, is prohibitive since it can cause difficulties in humans by impairing iron and copper absorption. As a result, solutions for transferring essential quantities of required zinc from its inorganic state to a usable form for favourable plant growth and development are urgently required. Utilizing the potential of elite zinc solubilizing microbial strains capable of converting unavailable forms of zinc into available forms would be a cost-effective and environmentally friendly way to address the zinc shortage in tea.

Being a versatile and dynamic habitat on earth, the rhizosphere represents one of the richest microecological zones of intense plant-microbe interactions[8,9]. The rhizosphere microbial communities are vigorously associated with biogeochemical cycling of nutrients, removal of toxins and production of diverse phytohormones or antibiotics etc.[10]. Rhizospheric bacteria can use a solubilization mechanism to convert an inaccessible metal into an available form[11]. Other studies isolated and screened zinc solubilizing microbes from various rhizosphere soils, establishing the potential of the rhizosphere soil environment as one of the richest niches harnessing the existence of Agriculturally Important Microbes (AIMs) like ZSMs that could be best utilised for accelerating zinc bioavailability to the growing plant[12,13].

In light of the foregoing, the current study employed a culture-dependent technique to isolate native ZSMs from tea rhizospheric soil. The ability of isolated microorganisms to produce solubilization potential *in vitro* was used to screen their zinc solubilisation capabilities[14].

MATERIALS AND METHODS

Selection of the sampling location and soil sampling: The soil was sampled primarily from the plant rhizosphere of experimental tea growing areas at Tocklai Experimental T. E., Jorhat (26°75"N, 94°22"E), Assam, India during September, 2018 to February, 2020. Plant Growth-Promoting Microorganism (PGPM) treated sites were primarily chosen for soil sampling in this study because those locations would naturally favour more diversified microbial populations with potent growth-promoting abilities. To evaluate the isolation frequency of ZSMs in tea soil, soil samples were taken from the tea rhizosphere up to a depth of 6-15 cm using a sterilised hand auger under aseptic circumstances. For the collection of soil samples, three sites at each location were randomly selected and a total of nine sampling points were chosen. To remove debris, collected soil samples were completely mixed, air-dried and sieved through a 2.0 mm sieve. From each location's composite sample, ZSMs were isolated and characterized. The soil samples were kept in a refrigerator at 4±1°C till the isolation of microorganisms was completed.

Isolation and screening of zinc solubilizing microbes from tea rhizosphere: A culture-dependent technique was used to isolate and enumerate ZSMs from tea rhizosphere soil. For this, 10.0 g of air-dried and sieved soil sample was placed in a screw cap bottle with 90 mL of Sterile Distilled Water (SDW) and incubated for 30 min in an orbital shaker with periodic shaking at 150 rpm. Following that, tenfold series dilutions were made by pouring 10 mL of the soil suspension into 90 mL of SDW[15]. Under aseptic circumstances, soil particles were allowed to settle at ambient temperature. Three different media (PKV medium (yeast extract 0.5 g L^{-1} dextrose 10.0 g L^{-1}, calcium phosphate 5.0 g L^{-1}, ammonium sulphate 0.5 g L^{-1}, potassium chloride 0.2 g L^{-1}, magnesium sulphate 0.1 g L^{-1}, manganese sulphate 0.0001 g L^{-1}, ferrous sulphate 0.0001 g L^{-1}, agar 15.0 g L^{-1} containing 0.1% insoluble zinc compounds (ZnO), modified Bunt and Rovira agar medium (glucose 20.0 g L^{-1}, peptone 1.0 g L^{-1}, yeast extract 1.0 g L^{-1}, $(NH_4)_2SO_4$ 0.5 g L^{-1}, K_2HPO_4 0.4 g L^{-1}, $MgCl_2$ 0.1 g L^{-1}, $FeCl_3$ 0.01 g L^{-1} and agar 20.0 g L^{-1}) supplemented with 0.1% zinc oxide [16] and zinc solubilizing agar (glucose 10.0 g L^{-1}, ammonium sulphate 1.0 g L^{-1}, potassium chloride 0.2 g L^{-1}, dipotassium hydrogen phosphate 0.1 g L^{-1}, magnesium sulphate 0.2 g L^{-1}, zinc oxide 1.0 g L^{-1}) were used for the isolation of ZSMs from tea soil[16,17]. In a laminar airflow chamber, 1.0 mL of the appropriate dilutions were injected into Petri dishes containing culture media under aseptic conditions. Culture plates along with microbes were allowed to grow at 25±1°C for 3-5 days. Three replicates were maintained in each case. The microorganisms were isolated and developed on media procured from Hi-Media Laboratories, Mumbai, India. Colonies surrounded by halo zones were chosen and streaked onto PKV medium plates. The plates were incubated at room temperature for up to 4 days to validate the zinc solubilizing activities.

Determination of Zn solubilization efficiency *in vitro*: Actively developing cultures of each isolated strain was spot-inoculated onto the agar plates (approx. 3 L) and incubated at 28°C for 48 hrs. The radius of the halo zone around the microbial colony was measured. For confirmation of the halo zone and to determine the zinc solubilizing capacity, streaking on culture media was done several times. For measuring the solubilization efficiency in percent and area in mm^2, the diameter of the microbial colony (B) and the halo zone around the colony (A) were measured. The formula as mentioned[18] was followed to determine the Zinc Solubilization Index (ZSI). A clear zone around a growing colony indicated zinc solubilization and was measured as ZSI. It was measured as the ratio of the total diameter (colony+halo zone) to the colony diameter.

$$\text{Zinc solubilization index (ZSI)} = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

The Zn solubilization efficiency was calculated using the formula mentioned[6]:

$$\text{Solubilization efficiency (SI) (\%)} = \frac{\text{Solubilization diameter (A)}}{\text{Diameter of the colony growth (B)}} \times 100$$

Quantitative estimation of zinc solubilizing potential (broth assay): Actively growing microbial colonies in the plate assay were selected to find out the amount of zinc solubilized in the modified Bunt and Rovira broth medium supplemented with 0.1% ZnO, $ZnCO_3$ and $ZnSO_4$, respectively[16]. For this, Zn solubilizing microbial cultures were inoculated into 100 mL Erlenmeyer flasks containing 50 mL broth medium and incubated at room temperature. Control tests were Erlenmeyer flasks with uninoculated conditions. Each of the treatments was replicated three times. After growing the cultures in the broth medium, the microbes were withdrawn after the 4th, 6th and 8th days of the incubation period for the quantitative estimation of solubilized Zn. For this, the microbial cultures were centrifuged at 15,000 rpm for 20-25 min and the supernatant was passed through a 0.2 µm membrane filter to obtain the culture filtrate containing only the soluble forms of Zn[19]. The sample was analysed spectrophotometrically for quantitative estimation of Zn solubilization.

Alterations in pH of the growth medium due to microbial inoculation: For this study, efficient strains with promising halo zones in the modified Bunt and Rovira broth medium supplemented with 0.1% ZnO were chosen[17]. Microbes were grown in flasks containing 50 mL of the same medium supplemented with 0.1% of the insoluble source of Zn (i.e., ZnO) and incubated at ambient temperatures. For comparisons and additional data analysis, an uninoculated control was kept in each case, along with three replicates. After the 4th, 6th and 8th days of microbial growth and development, the flasks were evaluated for pH. The bacterial cultures were centrifuged for 10-15 min at 15,000 rpm and then filtered using Whatman No. 01 filter paper[20]. A digital pH benchtop metre was used to determine the pH of the microbial culture filtrates.

IAA production: The effective strains were inoculated in LB broth (bacterio-peptone 10.0 g L^{-1}, yeast extract 5.0 g L^{-1}, sodium chloride 10.0 g L^{-1}, pH; 7.0) supplemented with 0.1% ZnO and cultured at 28°C for 48-72 hrs to detect the production of IAA by ZSMs. As an uninoculated control, another series of experiments was conducted without the addition of a Zn source. All of the treatments were supplemented with 0.1% tryptophan and given another seven days to incubate. After that, 2.0 mL of the microbial culture was transferred to a 2.0 mL collecting tube and centrifuged for 10 min at 8000 rpm. In a test tube, 1.0 mL of the supernatant was vigorously mixed with 2.0 mL of Salkowaski's reagent (4.5 g of $FeCl_3$ per litre in 10.8 M H_2SO_4) and incubated for 30 min at 25°C in the dark. The formation of IAA is indicated by the pink colouration in the tube. The absorbance was measured at 520 nm in a spectrophotometer and quantified using a tryptophan standard curve[21].

Characterization of zinc solubilizers: According to Rajkhowa *et al.*[22], bacterial colony features such as shape, size, appearance, pigmentation, type of bacterial cell, gram staining reactions and consistency were investigated. The spore and mycelia growth, as well as cultural and physical traits, were used to classify fungal isolates[23,24]. Pure colonies were transferred to nutrient agar slants and preserved at 4°C in the Culture Collection Laboratory (CCL), Mycology and Microbiology Department, TTRI, TRA, for further experimentations.

RESULTS AND DISCUSSION

In the present investigation, a total of 20 zinc-solubilizing microbial isolates were recovered from tea soil. Sixteen isolated strains of which ten bacteria (coded as ZSB#01, ZSB#02, ZSB#03, ZSB#04, ZSB#05, ZSB#06, ZSB#07, ZSB#08, ZSB#09, ZSB#10) and six fungi (coded as ZSF#11, ZSF#12, ZSF#13, ZSF#14, ZSF#15, ZSF#16) demonstrated their ability to solubilize zinc *in vitro*, as evidenced by the formation of halo zones under cultivation. The putative strains were pure cultured and selected based on their morphological and colony characteristics for further experimentation. The soil texture was sandy loam type with adequate internal drainage system and acidity. Figure 1a-c depicts the culture properties and creation of halo zones by zinc solubilizing microbes in a PKV agar medium. The zone of clearance (transparent halo zonation's around the microbial colony under culture) indicates Zn solubilization, *in vitro*.

Similarly, Fig. 2a-c represents microbial colony culture characterization in a modified Bunt and Rovira agar medium. The culture plates have shown transparent zones indicating the potential of isolated bacterial and fungal strains in Zn-solubilization. Zone diameter was determined for the microbes under zinc solubilization *in vitro*.

Likewise, the putative microorganisms are also able to produce Zn-solubilisation in ZSA agar medium as evidenced by halo zone formation as indicated in Fig. 3a-c. Manasa *et al.*[25] isolated and screened Zn-solubilizing bacteria from plant rhizosphere *in vitro*, establishing the idea of rhizosphere colonization with these beneficial microorganisms. Similarly, the creation of a halo zone by different microorganisms in culture plates could be attributed to acidity transport during their active growth stages[6,13,26].

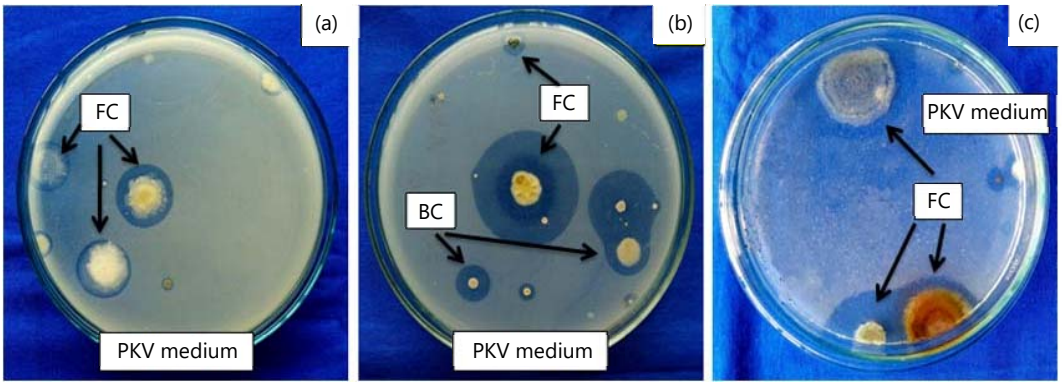

Fig. 1(a-c): Culture characteristics and halo zone formation by the isolated ZSMs in PKV agar medium, (a) Growth characteristics of fungal colonies, (b) Growth characteristics of microbial colonies (both bacteria and fungi show significant halo zones) and (c) Growth behaviour of fungal Zn-solubilizers

BC: Bacterial colony and FC: Fungal colony

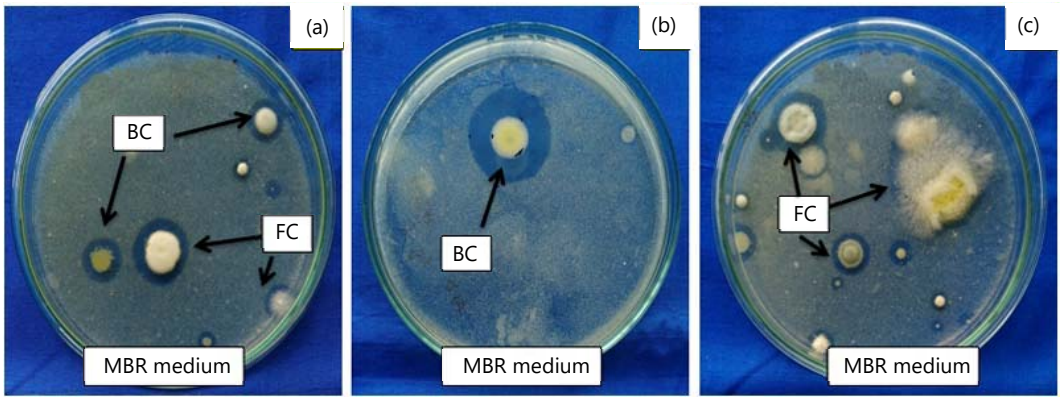

Fig. 2(a-c): Culture characteristics and halo zone formation by isolated ZSMs in modified Bunt and Rovira agar medium, (a) Growth properties and halo zone formation by microbial colonies, (b) Bacterial Zn-solubilizers on MBR medium and (c) Fungal Zn-solubilizer and formation of halo zone

BC: Bacterial colony, FC: Fungal colony, MBR medium: Modified Bunt and Rovira agar medium

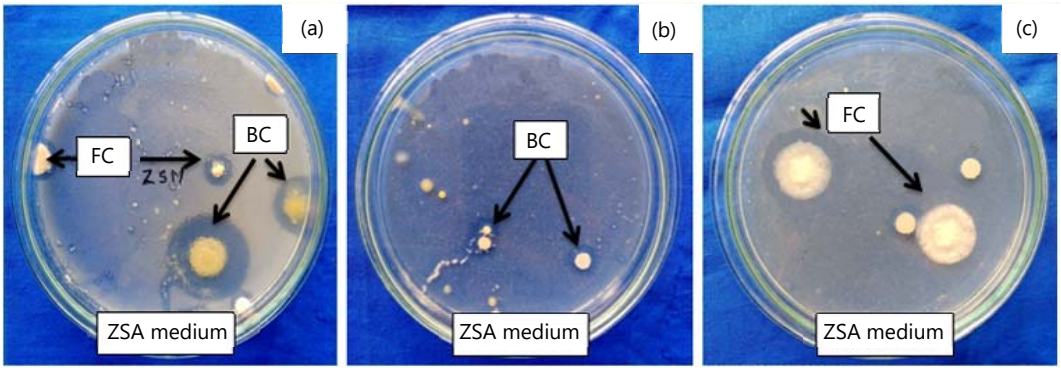

Fig. 3(a-c): Culture characteristics and halo zone formation by the isolated ZSMs in ZSA agar medium, (a) Microbial Zn-solubilizers and formation of halo zone, (b) Bacterial colonies showing Zn-solubilization and (c) Fungi growing on ZSA medium and Zn solubilization

BC: Bacterial colony, FC: Fungal colony, ZSA medium: Zinc solubilizing agar medium

Table 1: Isolation frequency of zinc solubilizing microbial isolates from tea rhizosphere using different media

	Different media		
ZSM isolates	Modified PKV medium	Modified BR medium	ZSA medium
ZSB#01	+	++	-
ZSB#02	-	++	+
ZSB#03	-	++	+
ZSB#04	++	-	-
ZSB#05	-	-	+
ZSB#06	-	+++	-
ZSB#07	++	++	+
ZSB#08	+	-	-
ZSB#09	+	+++	+
ZSB#10	+	-	+
ZSF#11	-	++	-
ZSF#12	+	-	-
ZSM#13	-	-	+
ZSM#14	-	++	+
ZSM#15	+	++	+
ZSM#16	+	+++	+

*ZSMs: Zinc solubilizing microorganisms, ZSB: Zinc solubilizing bacteria, ZSF: Zinc solubilizing fungi, +: >1-2 colonies ($1.0\pm0.9\times10^5$ cfu/g dry soil-$2.4\pm1.1\times10^5$ CFU g^{-1} dry soil), ++: >3–4 colonies ($3.0\pm1.4\times10^5$ CFU g^{-1} dry soil-$4.6\pm1.0\times10^5$ CFU g^{-1} dry soil), +++: >5-6 colonies ($5.0\pm1.1\times10^5$ CFU g^{-1} dry soil-$6.9\pm0.7\times10^5$ CFU g^{-1} dry soil)

The frequency of isolated zinc solubilizers from tea rhizosphere soil in various mediums is shown in Table 1. The isolation frequency of ZSMs was more ($3.0\pm1.4\times10^5$ CFU g^{-1} dry soil-$6.9\pm0.7\times10^5$ CFU g^{-1} dry soil) when modified Bunt and Rovira medium was used as one of the selective media to isolate ZSMs from tea soil followed by modified PKV medium ($1.0\pm0.9\times10^5$ CFU g^{-1} dry soil-$4.6\pm1.0\times10^5$ CFU g^{-1} dry soil) and ZSA medium ($1.0\pm0.9\times10^5$ CFU g^{-1} dry soil-$2.4\pm1.1\times10^5$ CFU g^{-1} dry soil) irrespective of the bacteria or fungi under culture. Bhattacharyya *et al.*[27] investigated the influence of medium variations on microorganism recovery. Changes in the nutrients in the growth medium might help microorganisms to recover from the rhizosphere soil.

The morphology of the bacterial colonies was represented in Table 2. The majority of the colonies were whitish or yellowish in appearance and the colony texture was regular, shiny and with the zone of clearance. Based on the results of the plate assay, the potent zinc solubilizing bacteria when examined for their morphological characterization using the gram staining properties, most of the bacteria were observed as Gram-negative, cocci (Table 2) in Fig. 4a-d. Additionally, ZSI also varies among bacterial isolates. The ZSB#09 showed the highest zinc solubilisation potential amongst the isolated bacteria, with ZSI values of 3.3 followed by ZSB#04 (3.2), ZSB#06 (3.1), ZSB#03 (2.5), ZSB#05 (2.1), ZSB#10 (2.0), ZSB#01 (1.8), ZSB#02 (1.6), ZSB#08 (1.4) and ZSB#07 (1.3), respectively.

Similarly, the morphological properties of the isolated Zinc Solubilizing Fungal (ZSF) strains including the colony characteristics, growth behaviour, pigmentation, media responses and solubilization potential have been shown in Table 3. Majority of the fungus exhibiting zone of clearance on Zn selective medium showed regular colony texture (Fig. 1-3), with whitish to yellowish media growth patterns. The ZSF#16 was reported as the most effective Zn solubilizer among the isolated fungus showing a ZSI value of 2.8 followed by ZSF#12 (1.9), ZSF#13 (1.8), ZSF#14 (1.7), ZSF#11 (1.3) and ZSF#15 (1.2), respectively. *Aspergillus* spp. and *Trichoderma* spp. were found to be the most effective zinc solubilizers among the fungal isolates in the present investigation.

Figure 5 depicts the zinc solubilizing potential (solubilisation efficiency) of each microbial isolate as a percentage calculated using a plate assay. The zinc solubilization potential varied between 125 and 325% depending on the zinc source underutilization. ZSB#09, ZSB#04 and ZSB#06 had the highest dissolution

Table 2: Morphology of Zinc Solubilizing Bacterial (ZSB) isolates along with *in vitro* zinc solubilization potential (solubilization index) under plate assay

Zinc Solubilizing Bacterial (ZSB) isolates	Gram staining reaction	Shape of the bacterial cell	General morphology, agar slant culture characteristics and halo zone formation	Bacterial colony diameter (B) (cm)	Diameter of the solubilization zone (A) (in cm)	Solubilization index (A/B) (cm)
ZSB#01	-	Cocci	Regular growth, submerged colony, light yellowish with the whitish edge, moderate clear zone	1.1	2.0	1.8
ZSB#02	-	Rods	Regular growth, submerged colony, orange yellow, development of the clear zone	1.0	1.6	1.6
ZSB#03	+	Cocci	Regular growth, submerged, whitish, moderate zone of clearance	0.8	1.8	2.5
ZSB#-04	+	Rods	Regular growth, yellowish white in appearance, strong zone of clearance	1.4	4.5	3.2
ZSB#05	-	Cocci	Regular growth, a brown ring with white edges, clear zone formation	1.7	3.5	2.1
ZSB#06	+	Cocci	Regular growth, brownish with a pale yellow edge, development of the clear zone	1.3	4.0	3.1
ZSB#07	-	Cocci	Smooth, light yellow in appearance, development of the clear area	0.9	1.2	1.3
ZSB#08	-	Cocci	Regular growth, shiny and white in appearance, moderate zone of clearance	0.9	1.3	1.4
ZSB#09	-	Cocci	Regular growth, smooth and deep yellow in appearance, strong halo zone	1.2	3.9	3.3
ZSB#10	+	Cocci	Smooth, shiny and white in appearance, moderate clear area	0.5	1.0	2.0

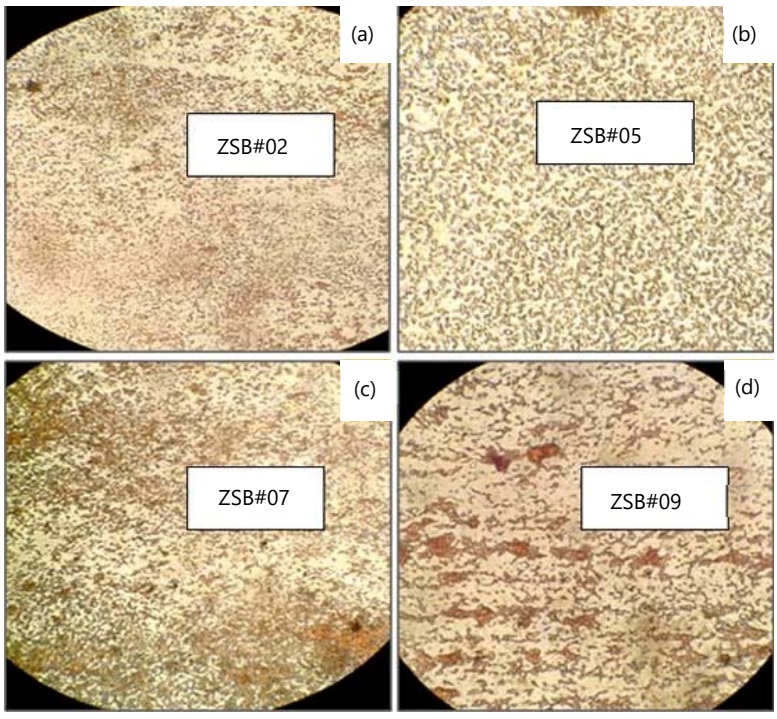

Fig. 4: Gram staining reactions of some of the isolated bacterial strains, (a) ZSB#02, (b) ZSB#05, (c) ZSB#07 and (d) ZSB#09

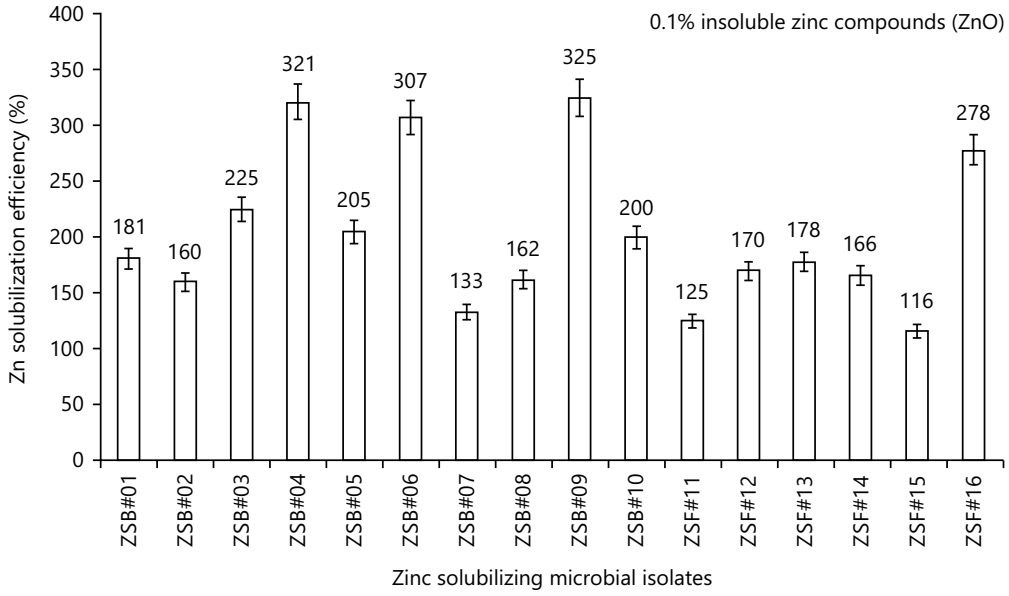

Fig. 5: *In vitro* zinc solubilizing potential of the microbial isolates (plate assay)
ZSB: Zinc solubilizing bacteria and ZSF: Zinc solubilizing fungi

Table 3: Morphology of Zinc Solubilizing Fungal (ZSF) isolates along with zinc solubilization potential (solubilization index) under plate assay *in vitro*

Zinc solubilizing fungal isolates	Morphological characteristics, growth pattern, pigmentation and halo zone formation	Diameter of fungal colony (B) (cm)	Diameter of solubilization zone (A) (cm)	Zinc solubilization index (ZSI) A/B (in cm)
ZSF#11	Cottony growth, filamentous, whitish-yellow with fuzzy edges, clear zone surrounding the colony	4.0	5.0	1.3
ZSF#12	Regular growth, submerged colony, greenish-white, fuzzy edges, moderate clear zone	1.0	1.9	1.9
ZSF#13	Regular growth, cottony, greenish-white, submerged, clear zone noticed	1.4	2.5	1.8
ZSF#14	Regular growth, whitish yellow, clear zone surrounding the colony	1.5	2.5	1.7
ZSF#15	Regular growth, shiny whitish edges with a greenish-brown ring in the centre, moderate clear zone	3.0	3.5	1.2
ZSF#16	Regular growth, cottony, brownish pale yellow, strong clear zone development	1.4	3.9	2.8

zone and solubilizing efficiency of ZnO among the isolates under observation, with 325, 321 and 307%, respectively. Even after 8 days of incubation, all of the above isolates showed their potential in zinc solubilization. Experiments on *in vitro* Zn solubilization potential of different Zinc Solubilizing Bacterial (ZSB) isolates have been made by Saravanan *et al.*[28] and recorded promising findings from bacterial isolates in Zn solubilization.

Based on the abilities in Zn solubilization under plate assay (formation of halo zones), the microbial isolates may be sorted as follows: ZSB#09 >ZSB#04 >ZSB#06 >ZSF#16, >ZSB#3 >ZSB#05 >ZSB#10 >ZSB#01 >ZSF#13 >ZSF#12, >ZSF#14 >ZSB08 >ZSB#02 >ZSB#07 >ZSF#11 >ZSF#15. The pure cultures of certain Zn solubilizing microbial isolates along with the halo zone *in vitro* have been depicted in Fig. 6a-i. The isolated strains showed varied levels of Zn solubilization potential. Yasmin *et al.*[6] conducted similar studies on the isolation of zinc solubilizing bacteria from the rhizospheric soil of an agricultural field using plate assay and were able to screen certain microorganisms with varying zinc solubilization activities as evidenced by the formation of a halo zone around the microbial colony. Variations in Zn solubilization potential among the isolates might be attributed to their isolation dynamics and variations in habitat or locations as well as their diverse abilities in solubilizing the insoluble source of mineral to its organic form.

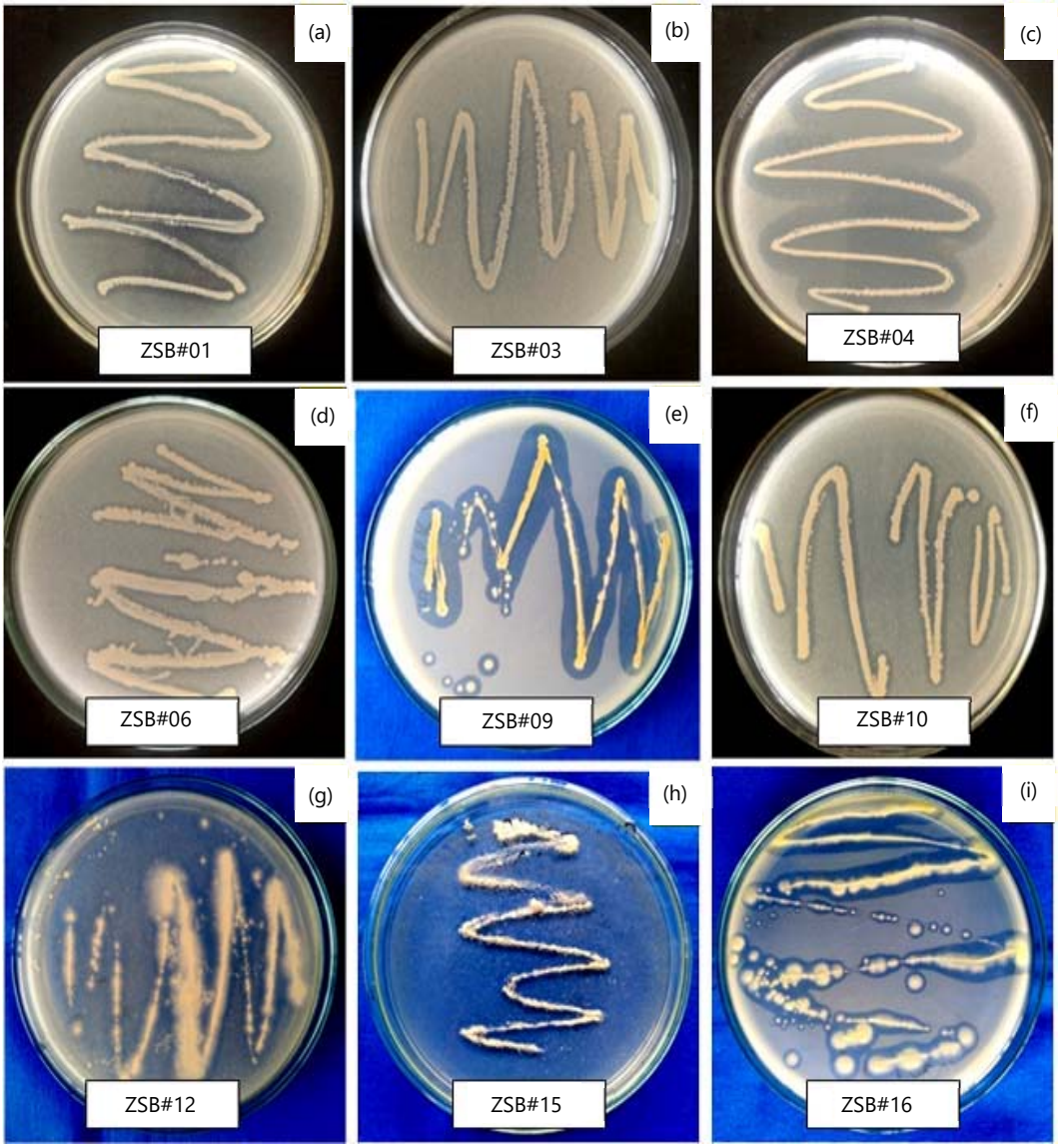

Fig. 6(a-i): Pure cultures of potent zinc solubilizers with halo zone formation under plate assay, (a) ZSB#01, (b) ZSB#03, (c) ZSB#04, (d) ZSB#6, (e) ZSB#9, (f) ZSB#10, (g) ZSF#12, (h) ZSF#15 and (i) ZSF#16

Some of the most potent microbial strains (ZSB#09 [325%], ZSB#04 [321%], ZSB#06 [307%], ZSF#16 [278%], ZSB#03 [225 %] and ZSB#05 [205%]) were also tested for their ability to grow at different time intervals (4th, 6th and 8th days of incubation, respectively) using modified Bunt and Rovira medium amended with insoluble Zn. The pH of the solution dropped as the incubation time increases from 4th-8th days after inoculation of the potent microbial strains in Table 4. After 8 days of incubation, ZSB#09 induced the greatest drop in pH from 6.7±0.45 to 4.0±0.41 followed by ZSB#04 (6.2±0.39 to 4.4±0.19), ZSB#06 (6.4±0.32 to 4.9±0.11), ZSF#16 (6.3±0.29 to 5.0±0.41), ZSB#03 (6.5±0.35 to 5.2±0.26) and ZSB#05 (6.0±0.3 to 5.3±0.19) respectively, among the microorganisms examined. The formation of organic acids and consequent acidification of the medium could explain the drop in pH of the medium[13,20]. The most essential process for heterotrophic metal solubilization was recorded to be the production of H+ and organic acids[20].

When ZnO was employed as the insoluble source of Zn, the maximum Zn solubilization efficiency after 8th days of incubation was recorded as 1.92 mg L^{-1} by ZSB#09, followed by 1.67 mg L^{-1} by ZSB#04, 1.49 mg L^{-1} by ZSB#06, 1.29 mg L^{-1} by ZSF#16, 1.10 mg L^{-1} by ZSB#03 and 1.09 mg L^{-1} by ZSB#05, respectively in Table 5. Jerlin *et al.*[20] examined the Zn solubilization efficiency of effective bacteria in

Table 4: Influence of potent zinc solubilizing microorganisms in changing the pH of the growth medium

	pH of the growth medium		
Zinc solubilizers	4 DAI	6 DAI	8 DAI
ZSB#09	6.7±0.45	5.0±0.35	4.0±0.41
ZSB#04	6.2±0.39	5.4±0.36	4.4±0.19
ZSB#06	6.4±0.32	5.7±0.18	4.9±0.11
ZSF#16	6.3±0.29	5.8±0.21	5.0±0.41
ZSB#03	6.5±0.35	5.9±0.33	5.2±0.26
ZSB#05	6.0±0.31	5.6±0.39	5.3±0.19
SEM(±)	0.267	0.193	0.294
LSD (p = 0.05)	N/A	N/A	0.939
CV (%)	7.47	5.78	10.1

*Values are the mean (±SD) of three replicates, DAI: Days after inoculation, SEM: Standard error mean (±), LSD: Least square difference and CV: Critical variance

Table 5: Quantitative zinc solubilizing potential of the potent microbial isolates in the release of Zn (mg mL^{-1}) in the modified Bunt and Rovira broth medium supplemented with 0.1% ZnO

		Quantitative Zn solubilization (mg mL^{-1})		
Zinc solubilizing microbial isolates	Zn source in the medium	4th day	6th day	8th day
ZSB#09	ZnO	1.78±0.11	1.85±0.21	1.92±0.12
ZSB#04		1.56±0.15	1.62±0.16	1.67±0.18
ZSB#06		1.32±0.17	1.39±0.04	1.49±0.04
ZSF#16		1.15±0.21	1.22±0.17	1.29±0.11
ZSB#03		0.91±0.09	0.99±0.11	1.10±0.19
ZSB#05		0.88±0.12	0.97±0.03	1.09±0.10

*Values are the mean (±SD) of three replicates

Table 6: IAA production by efficient ZSMs

Treatment details	IAA production (mg L^{-1})
Uninoculated control	0.0
ZSB#09+ZnO	13.5±0.23
ZSB#09 (sole application)	4.9±0.9
ZSB#04+ZnO	12.2±0.5
ZSB#04 (sole application)	4.4±0.22
ZSB#06+ZnO	11.9±0.19
ZSB#06 (sole application)	4.1±0.32
ZSF#16+ZnO	10.3±0.27
ZSF#16 (sole application)	3.6±0.09
ZSB#03+ZnO	9.8±0.07
ZSB#03 (sole application)	3.3±0.15
ZSB#05+ZnO	8.4±0.01
ZSB#05 (sole application)	3.1±1.1

Values are expressed as the mean (±SD)

culture broth concerning changes in the incubation period and variations in Zn sources and supply. According to the researchers, the ability of the inoculated strains to absorb the micronutrient more efficiently than the uninoculated control strains is crucial to the potent strains' Zn solubilization effectiveness.

Table 6 shows the generation of IAA by effective ZSMs in treatments with ZnO (as an inorganic Zn source) in a modified Bunt and Rovira medium supplemented with 0.1% tryptophan. All of the strains in the broth medium, together with Zn supplementation, were found to produce higher IAA than the bacteria alone (without Zn source). ZSB#09 produced the most IAA (13.5 mg L^{-1}) of all the strains tested for this test, followed by ZSB#04 (12.2 mg L^{-1}), ZSB#06 (11.9 mg L^{-1}), ZSF#16 (10.3 mg L^{-1}), ZSB#03 (9.8 mg L^{-1}) and ZSB#05 (8.4 mg L^{-1}), respectively. The increased synthesis of IAA in the microbial inoculation broth medium supplemented with an inorganic Zn source might be attributed to the induction of stimulatory effects in the microorganisms as a result of the Zn addition.

CONCLUSION

Although soils are naturally abundant in total zinc, they lack the available forms of zinc, which are critical for plant growth and development. Inorganic supplements, such as zinc sulphate are still not regarded as a cost-effective or environmentally sustainable way to address zinc deficiency in some soils. Inoculation of efficient Zinc Solubilizing Microorganisms (ZSMs) in soils, either alone or in combination with cheaper Zn sources like ZnO or $ZnCO_3$, may eventually lead to the adoption of more cost-effective and sustainable agronomic practices.

The selection, screening and use of effective ZSMs in tea have reached enormous potential for developing a biofertilizer consortium for an Integrated Nutrient Management (INM) approach. Furthermore, field testing and technological development would strengthen the concept of bio-intervention in tea ecosystem sustainability using Zn solubilizers as a cost-effective and ecologically friendly option, especially for marginal tea growers in the region.

SIGNIFICANCE STATEMENT

As micronutrient deficiency is a growing problem in North-Eastern tea plantations, the present approach of utilising the efficiency of zinc-solubilizing microbial inoculants *in vitro* could lead to the development of cost-effective, non-chemical and environmentally friendly sustainable approaches to micronutrient mineralization in tea. The method might also be used to lessen tea's reliance on chemical suppliments and to stimulate the soil's richest microecological zones of positive plant-microbe interactions. Additionlly, the putative Zn-solubilizing microbial inocula/consortium would be a valuable asset of Integrated Nutrition Management (INM) programmes in tea.

ACKNOWLEDGEMENT

For providing the research facilities to conduct the study, the authors are grateful to the Director, Tocklai Tea Research Institute (TTRI), Tea Research Association (TRA), Jorhat, Assam, India. The first and corresponding authors are also grateful to the Principals of NNS College, Titabar, Jorhat and Dhemaji College, Dhemaji, for their logistical and support assistance.

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