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Invitro Assessment of Zinc Solubilizing Potential of Bacterial Isolates

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A B S T R A C T

Zinc solubilizing ability of bacterial isolates was evaluated by using insoluble Zn compound (ZnO) in both plate and broth media assays. The total of 40 bacterial isolates were obtained from Soil Microbiology Laboratory, Department of Soil Science & Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India of which 21 showed ability of Zn solubilization on Bunt and Rovira media supplemented with 0.1% Zn source (ZnO). Remaining 19 bacterial isolates were unable to form halo zones. The diameter of halo zones formed by the bacterial isolates ranged from 0.50 to 2.7 cm. Three isolates 12, 28 and 35 could form halo zone with diameter ≥ 2.5 cm. These three bacterial isolates were named as ZSB_1, ZSB_2 and ZSB_3 respectively, and were selected for determining their zinc solubilizing capacity in broth culture assay by using AAS. In broth assay, maximum solubilization of zinc in the medium was observed on 21st day and was in the range of 29.68µg /mL to 36.08 µg /mL. Bacterial isolate ZSB_2 exhibited the maximum solubilization (36.08 µg mL^{-1}) of zinc, while the minimum (29.68 µg mL^{-1}) quantity of solubilized Zn was obtained with the inoculation of ZSB_3. Thus, the quantity of Zn solubilized by these three bacterial isolates was directly related to diameter of halo zone formed by them.

Introduction

Zinc is an essential micronutrient which is required by the plants in adequate concentration for growth and development (Sbartai *et al.,* 2011; Gurmani *et al.,* 2012 Hussain *et al.,* 2020).Zinc plays an important role in biosynthesis of many enzymes which are involved in plants metabolic reactions (Saravanan *et al.,* 2007). Zinc deficiency is the most common micronutrient deficiency occurring in more than 30% of world soils (Eshaghi *et al.,* 2019). It is estimated that about 50% of the Indian soils are zinc deficient (Ramesh *et al.,* 2014). Exogenous application of chemical zinc fertilizer in the form of zinc sulphate is used to overcome the zinc deficiency problems in the soil, but the major drawback with the application of zinc sulphate is its quick transformation into

different unavailable forms like Zn (OH) and Zn (OH_2) (Desai *et al.*, 2012).

The solubility of unavailable forms of Zn is highly dependent upon the soil physico-chemical properties (Sunithakumari *et al.*, (2016). In soil, Zn occurs in the forms of sphalerite, olivine, hornblende, augite and biotite. However, many factors are responsible for the release of zinc from these insoluble zinc compounds but rhizomicroorganisms play an important role in the transformation of such unavailable sources of Zn in to available one (Bhupinder *et al.*, 2005). These types of rhizomicroorganisms are called zinc solubilizing bacteria (Hussain *et al.*, 2020). Some of the bacterial genera viz., *Thiobacillus thioxidans, Thiobacillus ferroxidans, Acinetobacter, Bacillus, Gluconacetobacter, Pseudomonas* have been reported as zinc solubilizers (Saravanan *et al.*, 2007). These zinc solubilizers solubilize Zn through several mechanisms which include excretion of metabolites such as organic acids, proton extrusion, or production of chelating agents (Fasim *et al.*, 2002).

The aim of this study was to evaluate the zinc solubilizing ability of bacterial isolates from insoluble zinc compound. The method used was based on observing clear zones, or haloes, around colonies growing on Bunt and Rovira medium amended with the selected insoluble Zn compound.

Materials and Methods

Bacterial strains and culture conditions

Forty bacterial isolates were obtained from the Soil Microbiology Laboratory, Department of Soil Science &Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi India. All the bacterial isolates were isolated from the different parts of Varanasi region. Isolates were tested for their zinc solubilizing capacity on Bunt and Rovira medium augmented with insoluble Zn compound (ZnO). The solubilization potential of bacterial isolates were evaluated by plate and broth assay.

Plate assay

Zinc solubilizing ability of bacterial isolates was evaluated into modified Bunt and Roviramedium (Bunt and Rovira, 1955). Medium with definite chemical composition (Table 1) was augmented with 0.1% (ZnO) as an insoluble source of Zn (Sarvanan *et al* 2003; Fasim *et al.*, 2002). After that the medium was transferred in autoclave for 15–20 min at 125°C. After autoclaving, it was again transferred in petri plates which were fully sterilized in hot air oven. One loop full (10 µL) of overnight matured culture of bacterial isolates was inoculated on to the petri plates and incubated at 28℃ for a week. After a week, halo zones occurred on these bacteria inoculated petri plates. Out of forty, three bacterial isolates were selected on the basis of diameter of halo zone and named as zinc solubilizing bacteria (ZSB) and assigned serial number 1 to 3.

Broth assay

Based on the results of the plate assay, three selected bacterial isolates were again inoculated in broth assay amended with ZnO for the determination of their potential to solubilize insoluble zinc compound in liquid medium. Modified Bunt and Rovira medium was prepared, splitted in 25 mL aliquots in 50 mL Erlenmeyer flasks and 0.1% of insoluble Zn compound (ZnO) was added and steam sterilized for 30 minutes in autoclave. Then the flasks were inoculated with 0.1mL suspension of the test culture. The samples were withdrawn after 5 days, centrifuged to remove the debris and cells. One mL of this

solution was directly fed to Atomic Absorption Spectrophotometry (AAS) to determine the available zinc content.

Results and Discussion

The ability of the bacterial isolates to solubilize insoluble zinc compound was tested firstly in solid medium and then in liquid medium. The Bunt and Rovira medium was used as solid and liquid medium because it is rather a simple way to detect zinc solubilization through the formation of halo zone and amount of solubilized zinc in both the media, respectively. In plate assays, all the forty bacterial isolates were plated onto modified Bunt and Rovira agar medium containing zinc oxide (0.1% zinc) to evaluate their potential to solubilize insoluble zinc (Table 2). Nineteen bacterial isolates (isolate no. 2, 3, 4, 5, 6, 7, 10, 11, 16, 19, 21, 22, 23, 26, 31, 33, 34, 37 and 38) were unable to form halo zones. The diameter of halo zones formed by the bacterial isolates ranged from 0.50 to 2.7 cm. Isolate number 28 recorded highest solubilization zone (2.90 cm diameter) followed by isolate number 12 (2.60 cm) and isolate number 35 (2.50 cm). The lowest (0.40 cm) solubilization zone was recorded with isolate number 36. The formation of halo zones by zinc solubilizing bacteria might be due to the movement of organic acids which are secreted by these bacterial isolates (Jerlin *et al.,* 2017). Canbolat *et al.,* (2006) also reported that the formation of halo zones from the insoluble compounds might be due to the excretion of microbial metabolites. A similar study was conducted by Hussain *et al.,* (2015) who found that only 14 bacterial isolates out of 52 showed the good potential of zinc solubilization under pate assay from the insoluble source of zinc (ZnO). Halo zone formation by zinc solubilizing bacteria on basal medium augmented with ZnO was also reported by Fasim *et al.,* (2002). On the basis of their performance in halo zone formation, bacterial isolates number 12, 28 and 35 were named as ZSB1, ZSB2 and ZSB3 respectively, and were selected for determining their zinc solubilizing capacity in broth culture assay by using AAS.

All the three selected bacterial isolates were again inoculated in broth assay amended with ZnO for the determination of their potential to solubilize insoluble zinc compound in liquid medium. The results revealed that the highest solubilization of insoluble zinc was exhibited by ZSB_2 which was followed by ZSB_1 and ZSB_3 during all the intervals. Maximum solubilization of zinc in the medium was observed on 21st day (Table 3) and was in the range of 29.68 µg /mL to 36.08 µg /mL.

Table.1 Composition of Bunt and Rovira medium

S. No.	Component	Amount
1	Glucose	10.0g
2	Ammonium sulphate ($(NH_4)_2SO_4$)	1.0g
3	Potassium chloride (KCl)	0.2g
4	Potassium dihydrogen phosphate (K_2HPO_4)	0.1g
5	Magnesium sulphate ($MgSO_4.7H_2O$)	0.2g
6	Agar	1.5%
7	Zinc oxide (ZnO)	0.1%

Table.2 Diameter of holozone formed by zinc solubilizing bacterial isolates

Isolate no.	Diameter of zinc solubilization halo zone (cm)	Isolate no.	Diameter of zinc solubilization halo zone (cm)
1	1.2	21	-
2	-	22	-
3	-	23	-
4	-	24	1.2
5	-	25	1.6
6	-	26	1.6
7	-	27	-
8	1.2	28	2.9
9	1.8	29	1.5
10	-	30	0.8
11	-	31	-
12	2.6	32	1.6
13	0.5	33	-
14	1.2	34	-
15	1.2	35	2.5
16	-	36	0.4
17	1.6	37	-
18	2.1	38	-
19	-	39	1.6
20	1.2	40	2.1

Table.3 Soluble zinc content in Bunt and Rovira liquid medium supplemented with zinc oxide on 7th, 14th and 21st day after inoculation of selected zinc solubilizing bacterial isolates

Bacterial Isolates	At 7th day	At 14th day	At 21st day
ZSB_1	13.84	26.37	32.93
ZSB_2	16.74	30.07	36.08
ZSB_3	13.64	23.21	29.68

Similar result was also reported by Saravanan *et al.*, (2003) who reported that the maximum solubilization (16.4mg kg^{-1}) of Zn was achieved after 15th day of incubation. Bacterial isolate ZSB_2 exhibited the maximum solubilization (36.08 µg mL^{-1}) of zinc, while the minimum (29.68 µg mL^{-1}) quantity of solubilized Zn was obtained with the inoculation of ZSB_3.

The results indicated that all the three bacterial isolates were capable of solubilizing the insoluble zinc compound (ZnO) in varied amount in liquid medium. Similar findings were reported by saravanan *et al.*, (2003), who assessed the zinc solubilizing ability of selected bacterialisolates under plate and broth assays with ZnO and found a varied solubilizing potential among the bacterial isolates. Sunithakumari *et al.*, (2016) concluded that due to the buffering capacity of ZnO, it stimulate the efflux of organic acids which are helpful in greater solubilization of this compound. They further

concluded that the production of organic acids and H^+ is the major mechanism of metal solubilization.

In conclusion the above study revealed that only three bacterial isolates ZSB_2, ZSB_1 and ZSB_3 give good results in halo zone formation in plate assay and release of available zinc in broth assay. Inoculation of these zinc solubilizers along with insoluble/less soluble zinc compounds, like ZnO, $ZnCO_3$ and $ZnSO_4$ will lead to lot of saving in crop husbandry, besides curtailing the expenditure on agro input.

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