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EFFICIENCY OF ZINC-SOLUBILIZNG BACTERIA FOR *IN VITRO* ZINC SOLUBILIZATION AND ITS EFFECTS ON IAA RICE PRODUCTION

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Abstract

Zinc solubilizing efficiency of zinc-solubilizing bacteria (ZSB) were studied for its *in vitro* zinc solubilization, bacterial population, pH and IAA production. Four bacterial isolates from lowland rice soils, namely TM56 (*Acinetobacter* sp.), TM23 (*Serratia* sp.), TM9 (*Serratia* sp.) and BM13 (*Serratia* sp.) were found to solubilize different forms of insoluble zinc. TM56 was found to be significantly better for zinc carbonate and zinc oxide solubilization, followed by TM23 for zinc phosphate. The highest pH decreased occurred in zinc oxide and zinc phosphate, while zinc carbonate was lowest. Growth of bacteria was also significantly affected by different insoluble zinc in the modified liquid salts medium. Under gnotobiotic condition, TM56 showed the highest IAA production with the presence of zinc source.

Introduction

Zinc is an essential micronutrient for plant growth. It is an important constituent of various metabolic enzymes and its poor mobility in plants suggests the need for a constant supply of available zinc for optimum plant growth. Deficiency symptoms of zinc include premature yellowing and drying of leaf tips and leaf margin occurs in sugarcane, rice and coffee. Exogenous application of soluble zinc sources, like fertilizer application, has been advocated in various crops (Bapiri *et al*. 2012). However, zinc fertilizers like zinc sulfate which in turn transformed into different insoluble forms especially in submerged soil condition, soil chemical reactions and totally unavailable in the environment within seven days of application (Hafeez *et al*. 2016). Several bacteria associated with roots, have the ability to increase plant growth and productivity by increasing the supply of mineral nutrients of low mobility in the soil like zinc and it is called zinc solubilizing bacteria (ZSB) (Gandhi and Muralidharan 2016). Zinc solubilization can be achieved by a range of mechanisms through excretion of metabolites such as organic acids, proton extrusion, or production of chelating agents (Vaid *et al*. 2014, Goteti *et al*. 2013, Nahas 1996). Organic acid production by microbial isolates has been reported to be a major mechanism of solubilization (Shakeel *et al.* 2015, Fasim *et al*. 2002, Nguyen *et al*. 1992). It is apparent from the zinc solubilization data that the solubilization potential and its biochemical properties like indole acetic acid (IAA) and pH during solubilization were varied (Shahab and Ahmed 2008, Saravanan *et al.* 2003). The objective of this study was to determine the efficiency of zinc-solubilizing bacteria for *in vitro* zinc solubilization and its effects on IAA rice production.

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Materials and Methods

This experiment was conducted at Soil Microbiology Lab, Universiti Putra Malaysia for *in vitro* zinc solubilization efficiency. A total of four isolates, TM56 (*Acinetobacter* sp.), TM23 (*Serratia* sp.), TM9 (*Serratia* sp.) and BM13 (*Serratia* sp.) that previously isolated from lowland rice soil were grown in 200 ml of modified liquid mineral salts medium consisting of glucose: 10.0; $(NH_4)_2SO_4$: 1.0; KCl: 0.2; K_2HPO_4: 0.1; $MgSO_4$:0.2; pH: 7.0; powdered agar - agar : 15.0 g) and supplemented with 0.1% of three insoluble zinc sources i.e. zinc oxide, zinc carbonate and zinc phosphate (Saravanan *et al*. 2003). The isolates were inoculated in respective liquid salt medium were incubated at 28ºC in a shaker incubator at 120 rpm (Goteti *et al.* 2013). About one ml of each sample was filtered through 0.22 µm membrane filter after centrifugation and were analyzed using Atomic Absorbance Spectrophotometer (AAS). Percentage of the amount of zinc solubilization = amount solubilized/total amount zinc × 100% (Murphy and Riley 1962).The flasks were then sampled for pH determination at 0, 6, 12, 24,48 hrs of incubation similar to sampling for ZSB population. Exactly 2 ml of samples were taken at every sampling. Bacterial population were measured using drop plate method (Somasegaran and Hoben 1985).

For IAA production, gnotobiotic experiment was conducted in growth chamber. Better ZSB isolates (TM56 and TM9) were selected from previous experiment and were grown for 48 - 72 hrs in nutrient broth (NB), respectively. About 10^9cfu/ml live bacterial were used to inoculate plants in each planting unit. No bacteria were inoculated for control treatment. The axenic eight germinated seeds of 5 days olds rice plantlets were placed in a sterile 2 liter glass tube on stainless steel sieve. Fifty ml of sterile zinc free plant growth culture medium were used for each planting unit. Composition of the medium (L^{-1}) KH_2PO_4, 1.5 g; K_2HPO_4, 0.33 g; K_2SO_4, 0.2 g; ferric citrate, 13 mg, $CaCl_2.2H_20$, 0.4 g; $MgCl_2$, 0.4 g; $Na_2MoO_4.2H_2O$, 2 mg; H_3BO_3, 3 mg; $MnSO_4.H_2O$, 2 mg; $CuSO_4.5H_2O$, 0.1 mg and two different conditions of Zn (with Zn and without Zn). IAA production was determined at 21 days of plant growth following method of Gordon and Weber (1951).

Experimental results were subjected to one way analysis using the PROC ANOVA functions of the SAS statistical programme (SAS 9.4). Treatment comparisons were deemed significant at $p < 0.05$.

Results and Discussion

TM56 showed the highest zinc oxide solubilization ability (20.3%) and zinc carbonate solubilization (9.03%) (Table 1). However, TM23 showed the highest zinc phopshate solubilization ability (15.99%). Most of the isolates were able to solubilize zinc carbonate but with lower amount compared to zinc oxide and zinc phosphate. Low amount of zinc carbonate solubilization by bacterial isolates were found even though the bacteria still growing after 6 hours to 48 hrs. Varied zinc solubilization among isolates might be due to differences in genomics, plasmid properties of strain and habitat of the strain (Vaid *et al*. 2014).

The result showed that pH decreased by isolates from initial value 6.65 to 2.07 in media with zinc oxide after 48 hrs of incubation period (Fig. 1a). In media with zinc phosphate there were lower pH decreased with initial value 6.20 to 2.59 (Fig. 1b). While, in media with zinc carbonate, pH also decreased from 6.65 to 2.07 (Fig. 1c). This might be due to the production of organic acid during zinc solubilization process. The process contribute to lower the pH of the broth. Previous work found that, pH decreased was due to production of gluconic acid by isolates and dissolution of the zinc carbonate and zinc oxide (Simine and Gadd 1998, Vaid *et al.* 2014).

Table 1. Zinc solubilization ability by bacterial isolates after 48 hours of incubation.

Bacterial isolates	Zinc oxide		Zinc carbonate		Zinc phosphate	
	Soluble zinc					
	ppm	Percentage of solubilized zinc	ppm	Percentage of solubilized zinc	ppm	Percentage of solubilized zinc
TM56	40.60a	20.3	18.05a	9.03	15.72b	7.86
TM9	20.63b	10.3	9.71b	4.86	13.17b	6.59
TM23	12.20c	6.1	13.17c	6.58	31.98a	15.99
TM13	10.21d	5.1	7.67ab	3.83	21.49c	10.74

Means in each column followed by the same letters are not significantly different according to Tukey's HSD at $p<0.05$.

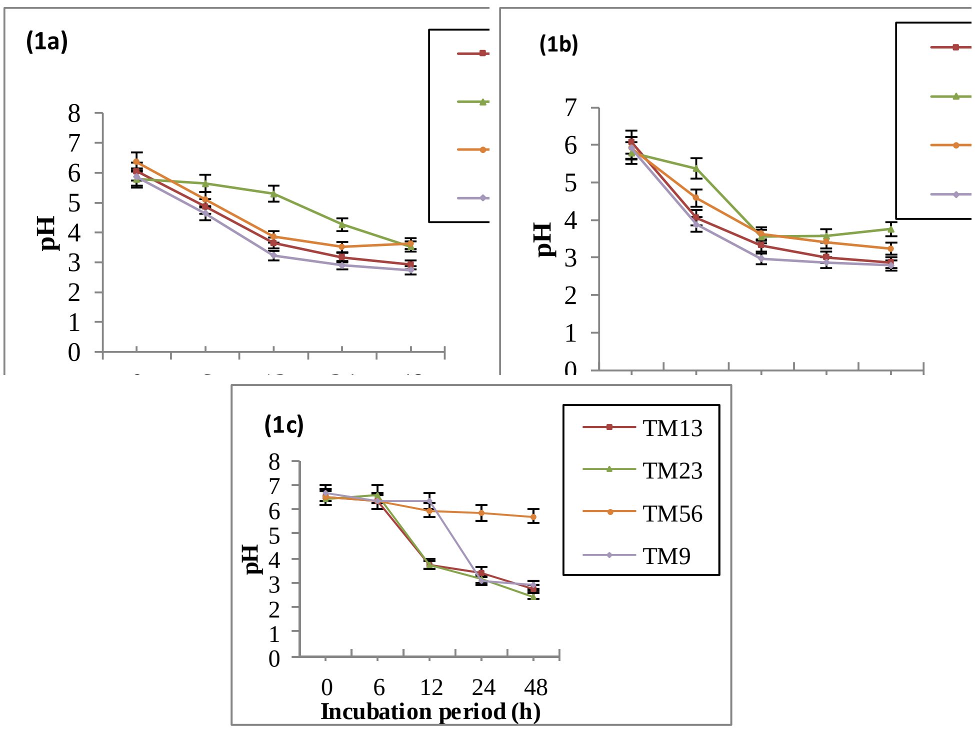

Fig. 1. Effects of bacterial strains on pH in media supplemented with (1a) zinc oxide, (1b) zinc carbonate and (1c) zinc phosphate .

Growth of bacterial isolates were significantly affected by different insoluble zinc in the media (Fig. 2). Initially, bacterial population was higher in media with zinc phosphate, followed by zinc oxide and zinc carbonate. In all media, TM23 were found significantly lowest compared to the other isolates (Fig. 2a,c). For zinc carbonate, TM9 was significantly highest followed by TM56 and TM13 (Fig. 2b). While for zinc oxide, TM13 was significantly highest followed by TM9 and TM56. This might be due to zinc tolerance potential which varied among the organisms and might be due to bacterial spore that can help them to overcome with certain amount of solubilized zinc (Saravanan *et al*. 2003).

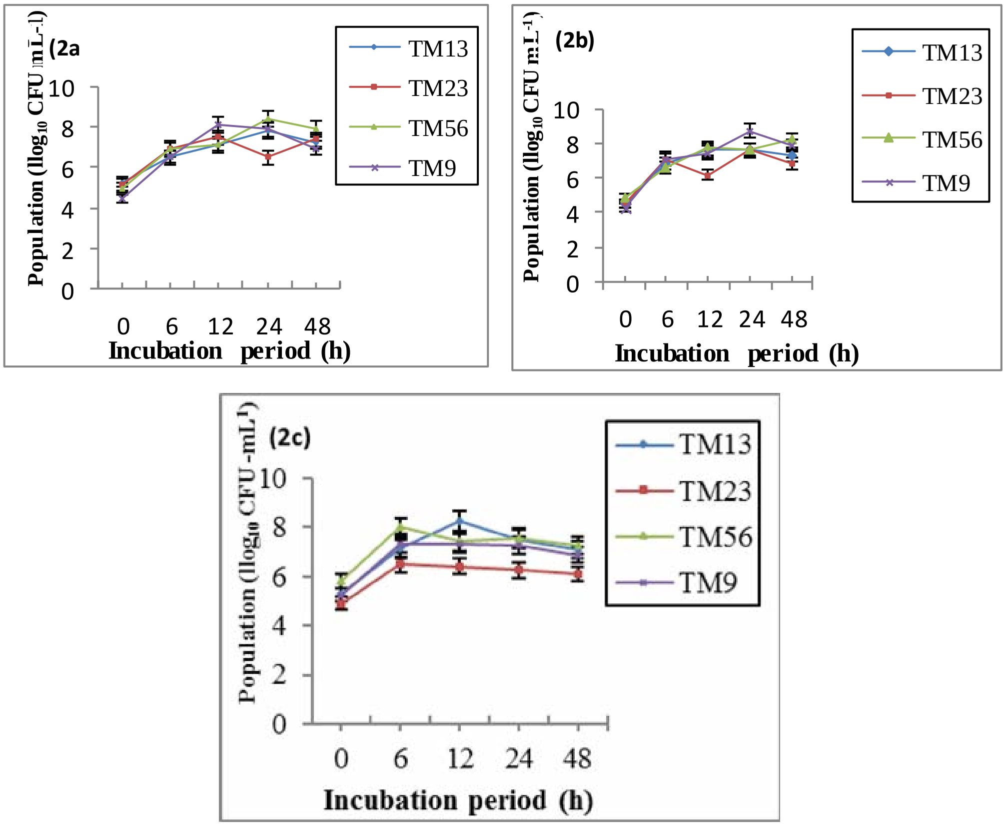

Fig. 2. Bacterial population in media supplemented with (2a) zinc oxide, (2b) zinc carbonate and (2c) zinc phosphate during 48 hrs incubation period.

Table 2. IAA production by ZSB and rice plants in growth culture at 21 days of growth period under gnotobiotic condition.

Treatment	IAA production (mg/l)
T1 = (Non-inoculated + without zinc)	4.2d
T2 = (TM56 + without zinc)	6.5bc
T3 = (TM9 + without zinc)	3.8e
T4 = (Non-inoculated + with zinc)	5.5cd
T5 = (TM56 + with zinc)	12.8a
T6 = (TM9 + with zinc)	6.5bc

TM56 = *Acinetobacter* sp., TM9 = *Serratia* sp. Means with same letters are not significantly different at $p > 0.05$ using Tukey's HSD.

IAA production among the isolates with or without zinc source in culture solution were significantly different (Table 2). TM56 in 0.2 mg/l zinc showed the highest IAA production (12.8 mg/l) followed by TM9 in 0.2 mg/l (6.5 mg/l) which is not significantly different with TM56 without zinc. This is because, isolates from rice endophytes have ability to produce IAA as plant growth hormone and also act as zinc solubilizer for plant uptake (Etesami *et al*. 2014). While, the presence of zinc source helped for complete nutrition of both plant and bacteria (Saravanan *et al*. 2003). These results indicated that the ZSB isolates had potential for promoting rice plant growth in presence of insoluble zinc.

The study showed that bacterial isolates from lowland rice soils were able to solubilize different forms of insoluble zinc. TM56 was found to be significantly better for zinc carbonate and zinc oxide solubilization, followed by TM23 for zinc phosphate. The pH decreased occurred in zinc oxide and zinc phosphate, while zinc carbonate is lowest. Growth of bacteria were also significantly affected by different insoluble zinc in the liquid salts media. TM56 of ZSB isolates and rice plants also produced significantly higher IAA for plant growth in presence of zinc source. In conclusion, ZSB proved its varied efficiency for *in vitro* zinc solubilization and also increased IAA production of rice plant which can be a better potential for plant zinc nutrition using microbes.

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