ORIGINAL RESEARCH PAPER

Microbiology

ISOLATION AND SCREENING OF ZINC MOBILIZING MICROORGANISMS FROM RHIZOSPHERIC SOIL

KEY WORDS: Zinc Mobilizing bacteria, Solubilization, Zinc oxide, Zinc carbonate.

Komal Rudani Department of Microbiology, Shri Maneklal M. Patel Institute of Sciences and Research, Kadi(North Gujarat),Gujarat, India

Kalavati Prajapati* Department of Microbiology, HVHP Institute of Post Graduate Studies and Research, Kadi(North Gujarat),Gujarat, India *Corresponding Author

ABSTRACT

Interaction between soil microbes and minerals play a major role in environmental cycling-processes, which leads to the mobilization of nutrient from soil components into available forms for biological uptake which enhances growth and yield. In the present study zinc mobilizing bacteria (ZnMB) and zinc mobilizing fungi (ZnMF) were isolated from garden soil and rhizosphere soil collected from kadi region. The colonially and morphologically diverse bacterial and fungal cultures showing solubilization zone for zinc oxide (ZnO) and zinc carbonate (ZnCO3) were isolated. The isolated bacterial (ZMB) and fungal (ZMF) isolates were screened for solubilization of insoluble zinc sources i.e. Zinc oxide (ZnO) and zinc carbonate (ZnCO3). These isolates were then characterized on the basis of morphological, cultural characteristics, enzyme spectrum and sugar utilization. Among the primary screened bacterial and fungal isolates which show maximum zone of Insoluble zinc solubilization were selected for further study.

Introduction:

Zinc (Zn) is an essential nutrient required in some fertilizer programs for crop production. While some soils are capable of supplying adequate amounts for crop production, addition of zinc fertilizers is needed for others. Both quantitative and qualitative yield of the plants are strongly dependent on this micronutrient. In India about 50% of the soils are deficient in zinc and this remains the most important nutritional disorder affecting majority of the crop production (K.Sunithakumari and et al.2016). Zn plays an essential role in the biosynthesis of IAA through the formation of tryptophan the precursor of IAA. The application of Zn is converted to $ZnCO_3$ in calcium rich alkali soil, and to Zn $(PO_3)_4$ in near neutral to alkali soils of high P application.(Beulah Jerlin and et al.2017). Zn is an important component of different enzymes catalyzing many metabolic reaction in plants. The essential process of life in plants are influenced by Zn, such as (A) nitrogen metabolism i.e. nitrogen and protein uptake quality; (B) photosynthesis i.e. synthesis of chlorophyll and carbon anhydrase activity (C) resistance against biotic and abiotic stresses i.e. .resistance against oxidative damage. Zinc also plays a significant role in plant resistance against disease, photosynthesis, cell membrane, integrity, protein synthesis, pollen formation and enhances the level of antioxidant enzymes and chlorophyll within plant tissues. . (AzharHussain and et al. 2015)

Inorganic fertilizers are recommended as good source of Zn but they are quickly fixed on soil medium, causind poor availability to plants. Microbes are potential alternate that by could cater plant zinc requirement by solubilizing the insoluble source of Zinc (ZnO) and complex zinc in soil.(AzharHussain and et al. 2015).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.

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 (Bhagwan Singh Dhaked and et al. 2017).A diverse range of bacteria including species of Rhizobium, Psudomonas, Azospirillum, Bacillus, Azotobacter, Klebsiella, Enterobacter and many others have been shown to promote plant growth. These plant growth promoting rhizobacteria (PGPR) can be used as bio-control agents (Irumnaz and et al. 2016).

Materials and Methods:

Samplecollection

Samples were collected from the various insoluble Zinc mineral containing soils from areas like Costal saline area of Gujarat (Mandvi) and Soil samples by Kadi region were collected.

Adaptation and Enrichment

Collected soil samples were mixed with insoluble Zinc Oxide (ZnO) and Zinc Carbonate ($ZnCO_3$) incubated for 1 week at room temperature. After adaptation 1 gm of soil was inoculated in 100 ml liquid medium containing 1% glucose, 0.05%yeast extract and 0.5% Zinc Oxide (ZnO) incubated at 37^{0} C on 120 rpm for 1 week.

Isolation and Screening of Zinc Mobilizing microbes

Enriched samples were inoculated in liquid mineral salt agar medium constituted glucose 1%, $(NH_4)_2$ $SO_4$0.1% , KCl 0.02%, $K_2HPO_4$0.01%, $MgSO_4$ 0.02%, 1% ZnO and agar 3% pH-7 and incubated at 37^{0} C for 1 week. For isolation of fungi same medium supplemented and pH-4.0-4.5 incubated at 30^{0} C. Colonies exhibiting clear zone of Zinc solubilization were selected as Zinc solubilizers. Secondary screening was carried out from the different isolates by studying their ability of higher Zinc solubilization by Khandeparkar's selection ratio.

Ratio = D/d = Diameter of zone of clearance/Diameter of growth

Selected fungal and bacterial isolates were also inoculated on the same Liquid minerals salt agar medium with pH indicator dye (0.025% bromothymol blue). Fungal isolates were characterized from their colonical and morphological characteristics. The higher zinc solubilizing strain was identified using standard cultural, morphological methodology and the isolates which shows higher zone of ZnO solubilization were selected.

Characterization of Zinc Mobilizing Bacterial Isolates

Bacterial isolates were characterized using different cultural, microscopical and biochemical characteristics. In addition to other studies including various enzymes production, and sugar utilization.

Enzyme spectrum

Selected bacterial isolates were studied for the production of various enzymes i.e. Amylase, Protease, Lipase, Geletinase, Catalase etc. The Amylase, Protease, Lipase, Geletinase were studied by plate assay method using Starch Agar, Casein Agar, Trybutyrene Agar, Geletin Agar respectively. Urease and catalase were studied by inoculating into urea broth and nutrient broth.

Sugar fermentation test

Various Sugar i.e. Glucose, Galactose, Fructose, Maltose, Mannitol, Xylose and sucrose fermentation tests were studied. Nutrient sugar broth with Durham's vial and Andrade's Indicator were inoculated and incubated at 37^{0} C for 48 to72 hours.

RESULTS AND DISCUSSION:

Isolation and screening

Colonies exhibiting zone of clearance indicating Zinc Mobilizing were selected. The colonies were selected which were morphologically distinct. Total 10 bacterial and 3 fungal isolates were isolated as Zinc solubilizers and named as ZnMB1 to ZnMB10 and ZnMF1 to ZnMF3.

Table-1: Zinc solubilization values of bacterial isolates by Khandeparkar's selection ratio.

Isolates	Diameter of Zn Clearance Zone (D) mm	Diameter of microbial growth (d) mm	D/d ratio
	Bacteria		
ZnMB-1	**1.8**	**0.5**	**3.6**
ZnMB-2	**1.7**	**0.5**	**3.4**
ZnMB-3	1.7	0.7	2.4
ZnMB-4	1.9	0.8	2.3
ZnMB-5	1.5	0.6	2.5
ZnMB-6	**1.3**	**0.3**	**4.3**
ZnMB-7	**1.6**	**0.4**	**4.0**
ZnMB-8	**1.7**	**0.3**	**5.6**
ZnMB-9	1.4	0.5	2.8
ZnMB-10	1.2	0.4	3.0
	Fungal		
ZnMB-1	1.6	0.5	3.2
ZnMB-2	**1.4**	**0.4**	**3.5**
ZnMB-3	1.5	0.6	2.5

From that isolates five bacterial ZnMB-8, ZnMB-6, ZnMB-7, ZnMB-1 and ZnMB-2 (Fig.1) one fungal ZnMF-1 isolates exhibiting higher ratio of clear zone of Zinc Mobilization by Khandeparkar's selection ratio were selected. All the isolates shows solubilization mechanism through acid production when tested by Bromothymol Blue containing agar medium(Fig.2).

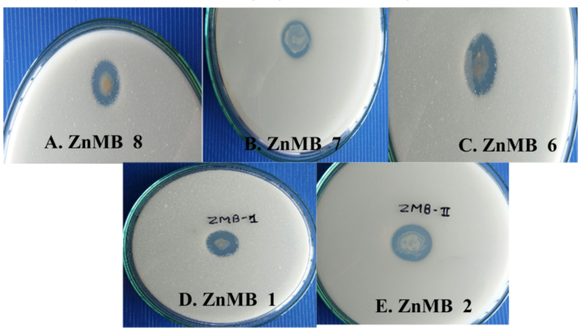

Fig.1 Zone of ZnO Soulubilization by bacterial isolates on Mineral Salt Agar Medium (A. ZnMB 8, B. ZnMB 7, C. ZnMB 6 D.ZnMB 1 E. ZnMB 2)

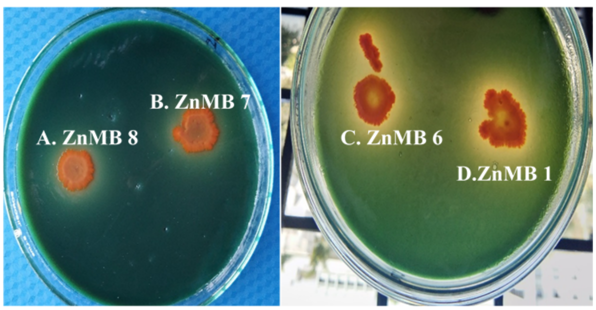

Fig. 2. Zone of ZnO Soulubilization by bacterial isolates on Mineral Salt Agar +Bromothymol Blue Medium (A. ZnMB 8, B. ZnMB 7, C. ZnMB 6 D.ZnMB 1)

Cultural, Microscopical and Biochemical Characteristics

The morphological and the Biochemical characteristics of the 5 good Zinc solubilizers are presented in Tables 2. Most of these isolates are aerobic,2 of them are Gram positive while 3 are gram negative. The biochemical characters are studied for the five isolates of Zinc solubilizing bacteria presented in (Table-2).

Table-2: Colonical, Morphological and biochemical characteristics of best Zinc mobilizing bacterial isolates.

Isolates	Colony characters	Morphological characters Gram reaction & cell shape	H_2S Production
ZnMB-1	Big Yellowish, Opaque, moist, Smooth	Gram Negative, Short rod Shaped	-ve
ZnMB-2	Medium Greenish, Translucent, Smooth, Raised	Gram Negative Short rod Shaped	-ve
ZnMB-6	Small white, Translucent, Smooth mucoid, Viscous	Gram Positive Thick rod Shaped	-ve
ZnMB-7	Small White, Opaque, Rough, Raised	Gram Negative Short rod Shaped	-ve
ZnMB-8	Small White, Transperant, Smooth, Raised	Gram Positive Thick rod Shaped	-ve

Out of five selected bacterial strains three are gram negative while two are gram positive bacteria. All the five bacterial strains do not produce H2S.

Studied the zinc solubilizing ability of Bacillus spp. and Pseudomonas spp. by using ZnO, zinc sulphide and zinc corbonate in both plate and broth assay. Bacillus spp. showed the highest solubilization in the presence of zinc sulphide and Pseudomonas spp. showed more solubilizing ability in the presence of both ZnO and ZnCO3. (Saravanan et al. 2003)

Total nine different enzymes were checked for all the five bacterial strains.

Table-3: Enzymatic Activity by Zinc mobilizers.

Isolates	1	2	3	4	5	6	7	8	9
ZnMB-1	+++ve	++ve	++ve	+++ve	-ve	-ve	+ve	+ve	-ve
ZnMB-2	+++ve	++ve	++ve	+++ve	-ve	-ve	+ve	+ve	-ve
ZnMB-6	+++ve	++ve	++ve	++ve	-ve	+ve	+ve	-ve	-ve
ZnMB-7	++ve	++ve	+ve	++ve	-ve	-ve	++ve	+ve	+ve
ZnMB-8	+++ve	++ve	++ve	++ve	-ve	-ve	+ve	-ve	+ve

1-Amylase, 2-Geletinase, 3-Lipase, 4-Protease, 5-Urease, 6-Decarboxylase, 7-Nitrate reductase, 8-Dehydrogenase, 9-Deaminase.

Table-4: Various carbohydrates utilization by Zinc Mobilizers.

Isolates	1	2	3	4	5	6	7
ZnMB-1	+++ve	++ve	++ve	+ve	++ve	++ve	++ve
ZnMB-2	++ve	+++ve	+ve	++ve	+++ve	++ve	++ve
ZnMB-6	+ve	++ve	++ve	+ve	+ve	++ve	+ve
ZnMB-7	++ve	++ve	-ve	++ve	++ve	+++ve	+ve
ZnMB-8	++ve	+ve	++ve	+ve	++ve	++ve	+ve

1-Glucose, 2-Galactose, 3-Sucrose, 4-Maltose, 5-Mannitol, 6-Fructose, 7-Xylose.

DISSCUSION

Numerous microorganisms particularly those associated with roots have the ability to increase plant growth and productivity (Cheng et al., 1986). However, certain groups of microorganisms can directly or indirectly transform rocks and minerals in quantities large enough to influence the geological distributions. These transformations include enzymatic oxidation, reduction reactions, formation of chelates and complexes with protein, amino-acids, organic acids etc. (Henderson and Duff, 1963).

The chances of getting microbial isolates for solubilization of insoluble mineral nutrients is more in the rhizosphere soils of many crops (Altamare, 1999). The results are in agreement with the findings of who also isolated Azotobacter, Azospirullum, Bacillus and Pseudomonas strains from diverse crop production system as mineral zinc solubilizers. (Desai et al. 2012)

In support of also isolated bacterial strains capable of solubilizing different form of insoluble zinc compounds like ZnO, ZnCO3 and Zn3 (PO4)2. (Saravanan and Raj (2004), Madhaiyan et al. (2004), Silva et al. 2010)

Out of ten isolates, five ZnMB strains were further screened for their ability to solubilize insoluble zinc compounds like ZnO in agar and broth medium. The zone of solubilization by all the five strains at 72 hours after incubation (HAI). Such observation were made earlier that among the insoluble zinc compounds ZnO, ZnCO3 and Zn3 (PO4)2 found to solubilize readily (Saravanan et al., 2003, Saravanan et al., 2006, Saravanan et al., 2007 and Sarathambal et al., 2010).

In the present study, the results of all the experiments concludes that Pseudomonas and Bacillus sp. was potential in solubilizing the zinc mineral in this study. The results are in agreement with the isolated Pseudomonas and Bacillus sp. from various sources. (Saravanan and Raj (2004), Long Xinxian et al. (2010), Desai et al. (2012), Sushil et al. (2012) and Mukherjee et al. 2011)

Based on colony morphological and biochemical characters the organisms were tentatively identified as Pseudomonas sp. then the organisms were inoculated to solid and broth medium containing insoluble zinc sources. Similarly also isolated and characterized bacteria (Pseudomonas aeruginosa (CMG 823) and Pseudomonas fluorescencs respectively) capable of solubilizing two insoluble zinc compounds like zinc oxide and zinc phosphate. (Fasim et al. (2002) and Di Simine et al. 1998)

The morphological characterization revealed that all the zinc solubilizing bacteria were gram negative, short to long rods but differed in their physiology and nutrition. Similarly reported Pseudomonas sp. solubilized insoluble zinc compounds. (Mukherjee et al. (2011), Saravanan and Raj (2004) and Desai et al. 2012)

CONCLUSION:

After secondary screening five bacterial isolates were select out of 10 isolates. Among them ZnMB1, ZnMB2 and ZnMB7 are gram negative, short rods while ZnMB3and ZnMB8 are Gram positive bacillus. All the bacterial strains having various enzymatic activity as well as different Biochemical characterization. Zinc solubilizing all bacterial isolates utilize variety of the carbohydrate sources. Among 5 bacterial strains ZnMB1, ZnMB2 and ZnMB7, Gram negative are wide range of the only ZnMB7 capable producing deaminase enzyme and two bacterial strain ZnMB6 and ZnMB8, Gram Positive are ZnMB 6 only able to catalyze decarboxylase enzyme. So these all characteristics make these bacterial strains to solubilize the Zinc. These isolates can use as a good zinc mobilizers for sustainable solution to improve the zinc availability for plant nutrition and production.

ACKNOWLEDGEMENT:

The authors are thankful to the Department of Microbiology and Management of Kadi Sarva Vishwavidyalaya (KSV), Kadi, for providing the facilities for this research.

REFERENCES

1. Sunithakumari K., Padma devi S. N. AND Vasandha S. (2016) "Zinc solubilizing bacterial isolates, from the agricultural field of Coimbatore Tamil Nadu, India" Current sci. vol.110(2).
2. Beulah Jerlin, Sharmila S., Kathiresan K. and Kayalvizh K. (2017) "Zinc solubilizing Bacteria from Rhizospheric soil of Mangroves" Intrnational Journal of microbiology and Biotechnology 2(3) 148-155.
3. AzharHussain, Muhammad Arshad, Zahir Ahmad Zahir and Muhammad Asghar (2015) "Prospects of Zinc Solubilizing Bacteria for Enhancing Growth of Maize" Pak. Journal agri. Sci. vol. 52(4) 915-922.
4. IrumNaz, Habib Ahmad, ShahidaNasreenKhokhar, Khalid Khan and AzaharHussain Shah (2016) "Impact of Zinc Solubilizing Bacteria on Zinc Contents of Wheat" American-Eurasian J. Agric.& Environ.Sci. vol.16(3) 449-454.
5. Bhagwan Singh Dhaked, S. Triveni, R. Subhash Reddy and G. Padmaja (2017)" Isolation and Screening of Potassium and Zinc Solubilizing Bacteria from Different Rhizosphere Soil" Int.J.Curr.Microbiol.App.Scivol.6(8):1271-1281
6. Saravanan, V. S., Subramoniam,S. R., and Anthoni Raj, S. (2003), "Assesing in-vitro solubilisation potential of different zinc solubilisisng bacterial isolates." Brazilian.J. Microbiol., 34:121125.
7. Brian J. Alloway (2008) "Zinc in Soils and Crop Nutrition".
8. Cheng, Y.C., Barker, R., Kleifeld, O. and Chet, I., (1986) "Increased growth of plants in the presence of biological control agents Trichoderma harzianum". Plant Dis., 70: 145-148.
9. Henderson, M.E.K. and Duff, R.B., (1963), "The release of metallic and silicate ions from minerals and soils by fungal activity". J. Soil Sci., 14: 236-246
10. Altamare, C., Norvell, W.A., Bjorkman, T. and Harman, G.E., (1999) "Solubilization of phosphates and micronutrients by the plant growth promoting and bacterial fungus Trichoderma harzianum Rifai", App. Environ. Microbiol., 65: 2926-2933.
11. Desai, S., Praveen Kumar, G., Sultana, U., Pinishetty, S., Ahmed, M. H., Amalraj, D. L. and Gopal Reddy., (2012), "Potential microbial candidate strain for management of nutrient require of crops". African J. Microbiol. Res., 6(17): 3924-3921.
12. Saravanan, V. S. and Raj. S. A., (2004), "Isolation and characterization of zinc solubalising bacteria from paddy soil". Biofer. technol., 12: 379-385.
13. Silva, D. and Ferreira, J., (2010), "Isolation and characterization of bacterial strain associated with sugarcane with features for promoting plant growth". M.Sc., Universidad Estadual do Norte Fluminense Darcy Ribeiro.
14. Madhaiyana, M., Saravanan, V. S., Jovi, S. S. B. D., Lee, H., Thenmozhi, R., K. Harie, K. and Tongmin Sa, T., (2004), "Occurrence of Gluconacetobacter diazotrophicus in tropical and subtropical plants of Western Ghats". Microbiol. Res., 159:233–243
15. Sharma,K. S., Mahaveer, P., Sharma, Aketi, Ramesh, Joshi, P. O., (2012), "Characterization of zincsolubilizing Bacillus isolates and their potential to influence zinc assimilation in soybean seeds". J. Microbiol. Biotechnol., 22(3): 352~359
16. Di Simine, C. D., Sayer, J. A. and Gadd, G. M., (1998), "Solubilization of zinc phosphate by a strain of Pseudomonas fluorescens isolated from forest soil". Biol. Fertile. soils., 28: 87-94.
17. Fasim, F., Ahmed, N., Pasons, R. and Gadd, G. M., (2002), "Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery". FEMS Microbiol. Lett., 213: 1-6.
18. Saravanan, V. S., Subramoniam,S. R., and Anthoni Raj, S., (2003), "Assesing in-vitro solubilisation potential of different zinc solubilisisng bacterial isolates". Brazilian.J. Microbiol., 34: 121125
19. Saravanan, V. S., Kalaiarasan, P., Madhaiyan, M. and Thangaraju, M., (2006), "Solubilization of insoluble zinc compounds by Gluconacetobacter diazotrophicus and the detrimental action of zinc ion (Zn2+) and zinc chelates on root knot nematode Meloidogyne incognita". Lett. Appl. Microbial., 44(3):235-41.
20. Saravanan, V. S., Madhaiyan, M. and Thangaraju, M., (2007), "Solubilisation of zinc compounds by the diazotrophic, plant growth promoting bacterium Gluconacetobacter diazotrophicus". Chemosphere. 66:1794-1798
21. Sarathambal, C. and Thangaraju, M., et al. (2010), "Assessing the zinc solubilization ability of Gluconacetobacter diazotrophicus in maize rhizosphere using labelled (65) Zn compounds". Indian J. Microbiol., 50:103-9.