Journal of Experimental Agriculture International

44(9): 132-143, 2022; Article no.JEAI.87983
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Screening of Zinc Solubilizing Plant Growth Promoting Rhizobacteria (PGPR) as Potential Tool for Biofortification in Rice

Md Shafiul Islam Rion [a,b], Atiqur Rahman [a], Mst. Julekha Khatun [a], H. M. Zakir [a], M. Harun Rashid [c] and Quazi Forhad Quadir [b*]

[a] Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[b] Division of Plant and Soil Sciences, West Virginia University, WV-26506, USA.
[c] Department of Agronomy, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2022/v44i930858

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/87983

Original Research Article

Received 25 March 2022
Accepted 08 June 2022
Published 14 June 2022

ABSTRACT

Aims: Plant growth-promoting rhizobacteria enhance growth by making plant nutrients available to plants under a variety of growing conditions. The study was designed to screen zinc (Zn) solubilizing rhizobacteria and test their colonization ability in the rice rhizosphere.
Place and Duration of Study: The experiments were conducted in the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh-2202, from January 2019 to July 2019.
Methodology: Initially, thirty-two previously isolated rhizobacteria were used for screening Zn solubilizing ability under a solid medium. Selected rhizobacteria from previous screening were used to quantify Zn solubilization in broth culture and evaluate their root colonization in rice using water agar media in a growth chamber. Early seedling growth was monitored for two weeks, and root-shoot lengths were recorded. Eleven of the tested rhizobacteria responded positively to ZnO-containing media.
Results: The Zn solubilizing index (ZSI) of the positive isolates ranged between 1.08-2.25 after 5 days of incubation. Isolate MQ1 solubilized the highest Zn both in solid medium (ZSI=2.25) and culture broth (solubilized 220.20 ppm Zn). The colonization of bacteria in the root zone was also

**Corresponding author: E-mail: qfq@bau.edu.bd;*

investigated via bio-primed rice seeds of Binadhan-20. Eight of 11 isolates (MQ1, MQ2, MQ3, MQ4, OSbr5, OSbr6, EC1, and MQL9) showed substantial colonization in the rhizosphere after two weeks. The germination percentage of bio-primed seeds was not increased over the control, however, in most cases, bio-priming boasted early seedling growth. The OSbr5, OSbr6, and MQ6 isolates were superior performers in case of root and shoot growth.

Conclusion: The study identified some Zn solubilizing isolates, revealed their root colonizing ability, and observed early plant growth promotion. These isolates could be used as a potential tool for the Zn biofortification approach in rice.

Keywords: Plant growth-promoting rhizobacteria; Zn solubilization; biofortification; root colonization.

1. INTRODUCTION

Zinc (Zn) deficiency is a well-known mineral malnutrition in human populations causing a number of health complications [1]. Globally, Zn and vitamin A deficiencies are two of the most serious nutrient deficiencies and are responsible for childhood mortality, especially, among those under five years of age [2]. Zinc insufficiency alone accounts for nearly 4% of young child illnesses and a loss of nearly 16 million global disability-adjusted life years [3].

In most cases, insufficient dietary intake of Zn and Fe is the reason behind Zn deficiency [4,5]. The worldwide prevalence of Zn deficiency in crops is due to low solubility of Zn, rather than low Zn content in soil [6,7] and poor plant uptake. Therefore, billions of people globally are suffering from micronutrient starvation [8,9]. To overcome this problem, improvement of Zn bioavailability in cultivated soils may enhance Zn contents in the staple food grains which would possibly mitigate the major health risks associated with Zn deficiency. Multifarious efforts are in place by plant scientists to enhance the absorption and bioavailability of Zn in grain crops via management practices, preferably fertilization, and plant breeding programs. Developing Zn-efficient crop varieties following breeding and molecular genetic protocols is a time-consuming process often taking several years to release a new variety. On the other hand, agronomic biofortification involves using different synthetic fertilizers, and overuse of these may lead to environmental pollution.

Plant growth-promoting rhizobacteria (PGPR) are gaining attention as a potential alternative to conventional agronomic biofortification since rhizobacteria have great potential to increase nutrient availability in the soil [10–15]. The PGPR seem successful in colonizing soil ecosystems due to their high adaptability in a wide variety of environments, faster growth rate and biochemical versatility to metabolize a wide range of natural and xenobiotic compounds as a nutrient source [16,17]. Once the PGPR establish themselves in the rhizosphere and surrounding soil if the ecosystem is favorable for their growth and development, they do not require a yearly application. PGPR promotes plant growth by increasing the availability and/or uptake of nutrients from the confined nutrient pool in the rhizosphere by many direct and indirect mechanisms. Zinc solubilizing PGPR helps to solubilize the fixed form of Zn and increases its uptake leading to Zn biofortification in grains [18–20]. The purpose of this study was to screen Zn-solubilizing rhizobacteria, quantification of their Zn-solubilizing potential and *In vitro* evaluation of root colonization and early seedling growth of a zinc-efficient rice variety (e.g., Binadhan-20).

2. MATERIALS AND METHODS

2.1 Sources of Rhizobacteria

Thirty-two bacteria were taken from the bacterial stock culture of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh, which were previously isolated from different plant rhizosphere (Table 1). The stock cultures were re-grown and maintained in a nutrient agar medium until further use.

2.2 Screening of Zn solubilizing Rhizobacteria

The screening of Zn solubilizing bacteria was done on modified Pikovskaya's (PKV) agar media containing ZnO [22]. The composition of the modified media was ZnO 0.1%, $(NH_4)_2SO_4$ 1.0 g/L, Sucrose 10.0 g/L, KH_2PO_4 0.1 g/L, KCl 0.2 g/L, $MnSO_4$ 0.2 g/L, Agar 1.5%. The pH of the media was adjusted to 7.0 with 0.1M KOH and 0.1M HCl before autoclaving. The bacteria were spot inoculated onto the solidified agar media using separate sterile toothpicks. After inoculating the bacteria, the petri dishes were

Table 1. Sources of rhizobacteria used in this study. A total of 32 rhizobacteria were used for screening Zn solubilizing bacteria [21]; The isolates were stocked at the Dept. of Agricultural Chemistry in low temperature and revived on nutrient agar media before use

Plant sources	Scientific name	Code Name	Rhizobacterial Isolates
Rice	*Oryza sativa*	OS	OSbr4, OSbr5, OSbr6, OSn7, OSn8, OS29(1), OS29(2), OS29(3)
Shama	*Echinochloa crusgalli*	EC	EC1, EC2, EC3, EC4, EC5, ECL1
Shusnishak	*Marsilea quadrifolia*	MQ	MQ1, MQ2, MQ3, MQ4, MQ5, MQ6, MQL7, MQL8, MQL9
Fern	*Pteris spp.*	Fr	Fr1, Fr2, Fr3, Fr4, Fr5, Fr6, Fr7
Soybean	*Glycine max*	GM	GM1, GM2

placed in the incubator (Neuve EN120, Turkey) at 28±1°C. Zinc solubilization was indicated by the formation of a clear halo zone surrounding the bacterial colony. The growth of the bacteria was monitored, and the colony diameter was recorded every 24 hours for five consecutive days. Based on their Zn-solubilizing index (ZSI) best performing 11 rhizobacteria were selected for further study. The ZSI was calculated using the following formula:

$$ZSI = \frac{Colony\ diameter + Halo\ zone\ diameter}{Colony\ diameter}$$

2.3 Quantification of Zn Solubilizing Capacity of the Rhizobacteria

Upon the quantification of Zn solubilizing potential of the selected rhizobacteria based on ZSI, they were inoculated in the modified PKV liquid broth poured on to 50 ml falcon tubes. The tubes containing the inoculated broth were placed on a rotary shaker (JSR JSOS-500, Korea) at 150 rpm for 2 days. Then the tubes were centrifuged in a centrifuge machine (Neuve NF800, Turkey) at 5000 rpm for 5 minutes. The culture supernatants were filtered by Whatman no. 42 filter paper and the volume of the filtrates were made up to 50 mL using distilled water. Finally, the filtrates were subjected to Zn analysis by AAS (Shimadzu AAS-7000, Japan) to measure the amount of Zn solubilized by the rhizobacteria.

2.4 Evaluation of Zn solubilizing PGPR on Rice (Binadhan-20) Growth Characteristics

Seeds of the rice variety Binadhan-20 were used as test crops to assess the plant growth performance of selected Zn solubilizing PGPR. For the germination test, 200 seeds were imbibed on overnight grown bacterial cultures in culture bottles for bio-priming the seeds. The culture bottles were in the shaker at 150 rpm for 24 hours. The overnight soaked seeds were placed on a paper towel in separate petri dishes for observing the germination of the seeds. The paper towel was soaked with sterile distilled water and each petri dish received fifteen seeds. The petri dishes were placed inside the plant growth chamber (JSR JSPC-960C2) with a day and night cycle of 14 and 10 hours, respectively. The petri dishes were observed for germination till the 9th day and data were recorded accordingly.

To observe the colonization of the bacteria in the rhizosphere of the rice (Binadhan-20), the seeds were grown in the growth chamber (JSR JSPC-960C2) keeping a 14/10 hours of day/night cycle, with a controlled day (28±1°C) and night (20.8±1°C) temperatures. Full strength Hoagland's solution along with 1% agar was used as substrate and the pH of the prepared Hoagland's solution was adjusted between the range (pH 5.8-6.3) by adding 0.1M KOH or 0.1M HCl before adding agar powder. Then 30 ml of the media were poured in 100 ml growth tubes and the tubes were then autoclaved. After cooling the tubes, bio-primed seeds (2 seeds per tube) were placed in each tube keeping them inside the Biohazard Safety Cabinet to minimize the chance of contamination. At last, all the tubes were placed inside the growth chamber, keeping them in a vertical position. Seeds were moistened every day using sterilized distilled water. The tubes were kept in the growth chamber for two weeks and then they were removed from the chamber for measuring their comparative root and shoot growth and to observe their colonization, if any, in the root zone.

The relative growth of each seedling in the tubes was measured for both their shoot and root length. Lengths were measured by ImageJ software (version 1.8.0) from the captured photos

taken with the help of a digital camera (Panasonic LX-2, Japan). Then seedling vigor index was calculated according to the formula mentioned in [23].

$$Vigor\ index\ (VI) = (Root\ length + Shoot\ length cm \times \%\ Germination$$

2.5 Statistical Analysis

All data collected were subjected to analyses of variance using Minitab 17.0 (Minitab, State College, PA, USA). When significant differences were found, the means were separated by Tukey's Pairwise Comparisons at a 95% confidence level.

3. RESULTS AND DISCUSSION

3.1 Screening of Rhizobacteria for Zn Solubilization

The colonization ability of the rhizobacteria in the rootzone is considered one of the most important criteria for the successful application of microbial biofertilizers in agriculture. In this study, we have investigated the Zn solubilization potential of some previously isolated PGPR, their ability to colonize in the rice root system, and their effect on early seedling growth. After culturing for 120 hours, eleven bacteria out of thirty-two showed putative Zn solubilization by forming a clear halo zone surrounding the colony in a solid medium containing ZnO as a source of Zn in varying intensity (Table 2). The ZSI of these eleven isolates was then evaluated and it ranged between 1.08 to 2.25 after 5 days of incubation (Table 3 and Plate 1). The isolate MQ1 showed the highest Zn solubilization in the medium (ZSI=2.25) followed by EC1 and OSbr5 (1.75 and 1.57, respectively). However, the rhizobacteria under investigation didn't have a significant effect on the change of in pH of the liquid medium (Supporting document 1). In our study, we have selected 11 isolates based on their ZSI to quantify the amount of Zn made available in broth culture. The MQ1 (220.20 ppm) solubilized the highest amount of Zn among the isolates. Such microbial solubilization of metal nutrients is done by several mechanisms, predominantly through the production of organic acids [24]. Other mechanisms include the release of anions chelators [25], siderophore production [26], proton, oxidoreductase systems of cell membranes, and chelated ligands [27,28] by the microbes to solubilize metal nutrients. Organic acid production results in the initial drop of pH of the culture medium, however, the bacterial isolates used in this study did not reduce the pH of the culture medium substantially (Supporting Document 1), which indicates that Zn solubilization by the investigated rhizobacteria might use mechanisms other than organic acid production. Some reports indicated that the Zn-compound used as a source of Zn (ZnO in the present study) might have intrinsic buffering potential to resist pH drop even though the microorganism produces organic acids [29]. [30] reported no decrease of pH when amended the medium with ZnO instead of $Zn_3(PO_4)_2$.

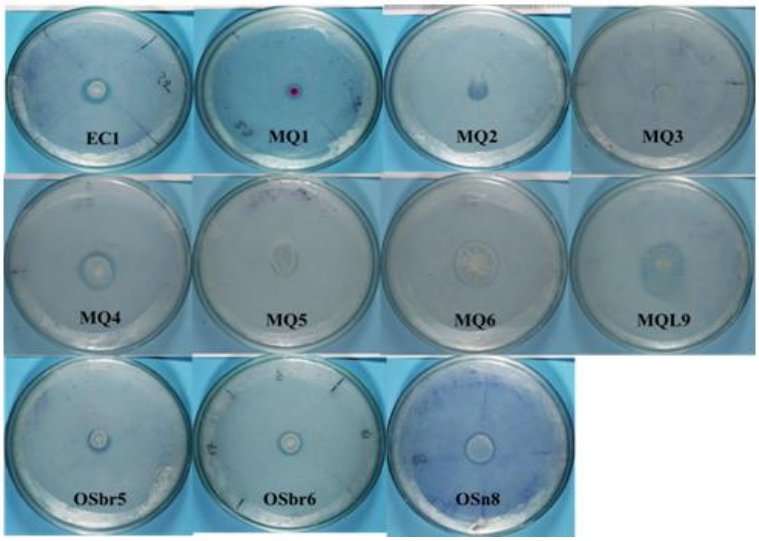

Plate 1. Solubilization of inorganic ZnO in vitro by eleven rhizobacterial isolates after 5 days of incubation at 28±1°C. The formation of halo zone around the bacterial colonies demonstrated the solubilization of zinc oxide

Table 2. Relative bacterial growth of 32 bacterial isolates in Zn media after 24 hours and 120 hours in case of colony size based on visualization (where "+" indicates positive response/growth in the medium, "-" indicates negative response/growth in the media, and darker colors indicates comparatively more Zn solubilization).

Rhizobacterial Isolates	Zinc solubilization ability	
	24 hours	120 hours
OS29(1), OS29(2), OS29(3), OSbr4, EC2, EC3, EC4, EC5, Fr1, Fr2, Fr3, Fr5, Fr6, GM2, ECL1	-	-
OSn7, Fr4, GM1	-	+
OSbr5, OSbr6, OSn8, EC1, Fr7, MQL7, MQL8, MQL9	+	+
MQ3, MQ5	+	++
MQ1	++	++
MQ2, MQ4	++	+++
MQ6	+++	+++

Table 3. Effect of rhizobacterial isolates to solubilize ZnO (in terms of ZSI) in culture media, germination, and seedling vigor of rice (Binadhan-20). Only 11 of the isolates responded positively to the Zn solubilization assay

Rhizobacterial treatment	ZSI	Germination Percent	Seedling vigor index
Control	-	93	512.18
EC1	1.75	87	1099.93
MQ1	2.25	93	946.67
MQ2	1.33	93	1228.15
MQ3	1.29	93	1130.89
MQ4	1.42	93	976.03
MQ5	1.30	87	803.79
MQ6	1.08	93	1451.16
MQL9	1.27	87	863.67
OSbr5	1.57	87	1451.62
OSbr6	1.38	93	1181.92
OSn8	1.27	100	524.79

3.2 Quantification of solubilized Zn by Rhizobacteria

Quantification of the amount of Zn solubilized by selected rhizobacteria was done in a broth medium containing ZnO as an inorganic source of Zn (Fig. 1). Among the 11 isolates, statistically (P<0.001) the highest amount of Zn was solubilized by MQ1 (220.20 ppm) followed by MQ2 (218.44 ppm), and MQ3 (207.67 ppm) which solubilized substantial amount of insoluble ZnO in the liquid media. The isolate OSbr5 was the lowest Zn solubilizer. In general, isolates collected from shusnishak (*Marsilea quadrifolia*) solubilized more Zn compared to the isolates from other plants. The variations in quantities of Zn solubilized by the selected rhizobacteria were observed in the present study. This might be due to the origin of the rhizobacteria. In general, it was found that the isolates from *M. quadrifolia* performed better over other isolates. The *M. quadrifolia* is a weed that can thrive harsh growing conditions and so does their associated rhizobacteria. The growth-promoting traits of the selected rhizobacteria were reported in an earlier study [21]. The isolate MQ1 outperformed all other isolates in terms of Zn solubilization, and was also a good phosphate solubilizer and IAA producer (Table 4).

3.3 Effect of Rhizobacteria on Seed Germination and their Root Colonization

The number of seeds germinated in each petri dish and the percentage of their seed germination after 9 days are presented in Plate 2 and Table 3. All the seeds treated with OSn8 isolate germinated, while 87% of seeds germinated for the OSbr5, EC1, MQ5, and MQL9 isolates. Meanwhile, OSbr6, MQ1, MQ2, MQ3, MQ4, and MQ6 isolates have the same germination percentage as the control (93%). Though there were no major variations in

germination rate among bio-primed and control seeds, the seedlings from primed seeds showed better early growth promotion.

The *in vitro* root colonization in rice (Binadhan-20) root system was observed to investigate the rice root colonization ability of selected rhizobacteria from different sources. The isolates which colonized the root system showed a whitish growth around the roots. Colonization of the rhizobacteria in the roots of rice (Binadhan-20) was not the same for all the selected isolates investigated (Plate 3). The isolates MQ2, MQ3, MQ4, and MQ6 colonized in the rice roots quite significantly while OSbr5, OSbr6, MQ1, EC1, and MQL9 colonized moderately. However, OSn8 and MQ5 did not show colonization as indicated in Plate 3. Root colonization by the candidate PGPR is an important criterion to be considered as a plant growth promoter. The water agar test tube method [31] was employed to visualize the root colonization ability of the selected PGPR. Significant root colonization of the rice root system was observed in the test tube as indicated by the umbrella-like hazy and turbid growth on the immediate surroundings of the root. [32] also used a similar method to visualize root colonizing bacteria and preselect them as a potential PGPR. Like our study, they also observed variations in bacterial colonizing ability among the isolates. While the isolate MQ1 colonized visibly better than other isolates by forming an umbrella-like structure, EC1, OSn8, and MQ5 isolates colonized to a lesser extent. Various factors could be involved in root colonization by bacteria. [33] found that root biofilm can be induced by factors like nutrient availability, temperature, and relative humidity, whereas root exudates also trigger root colonization of the introduced rhizobacteria [34].

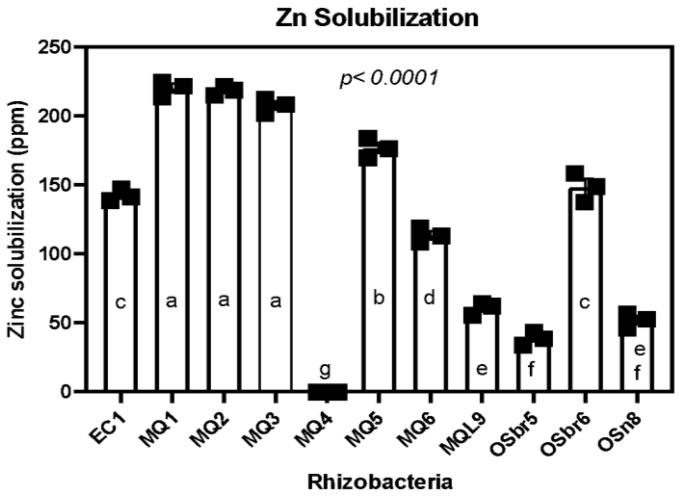

Fig. 1. Efficacy of rhizobacterial isolates to solubilize ZnO under laboratory conditions. Bars indicate the standard error of means. Columns with different letters are significantly different according to Tukey's Pairwise Comparisons at a 95% confidence level

Table 4. Biofunctionalities of the eleven selected PGPR used for Zn solubilization and in vitro root colonization assay [21] Here, "+" indicates positive response/growth and "-" indicates negative response/growth in the media

Isolate Code	PSB	IAA	Nitrogen fixation
EC1	-	+	+++
MQ1	++	++	++
MQ2	+++	-	+++
MQ3	+	-	+++
MQ4	++	-	++
MQ5	+	+++	++
MQ6	-	-	+
MQL9	+	-	+++
OSbr5	-	-	+
OSbr6	-	+++	++
OSn8	++	+++	++

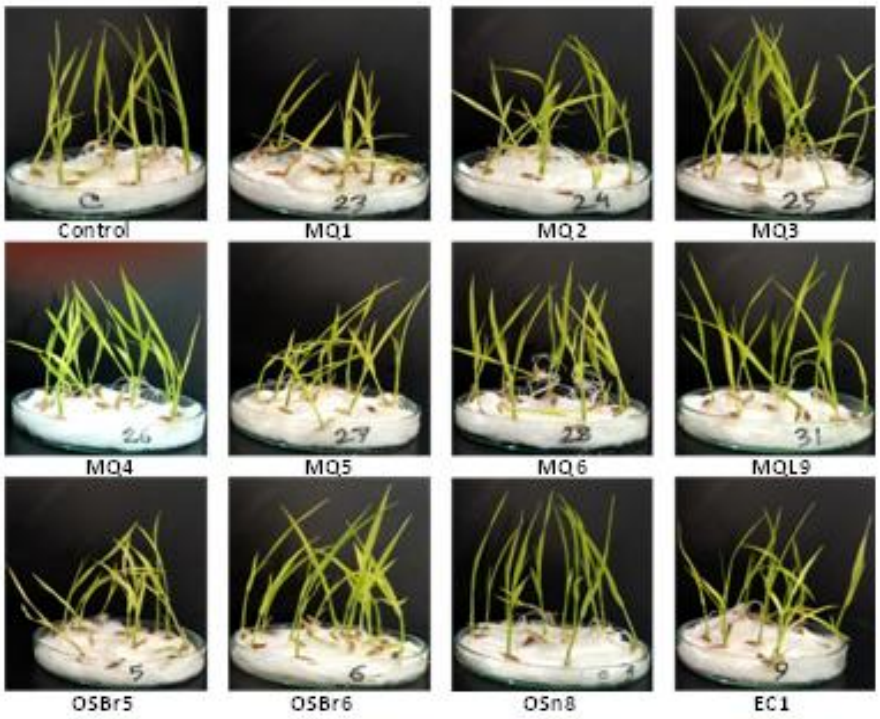

Plate 2. Effect of eleven Zn solubilizing rhizobacteria on the germination of rice seedlings. The germination rate didn't vary significantly in bio-primed seeds compared to control. Some rhizobacteria inoculated seeds resulted in enhanced seedling vigor in germination assay, maximum in Osn8 and minimum in MQ1

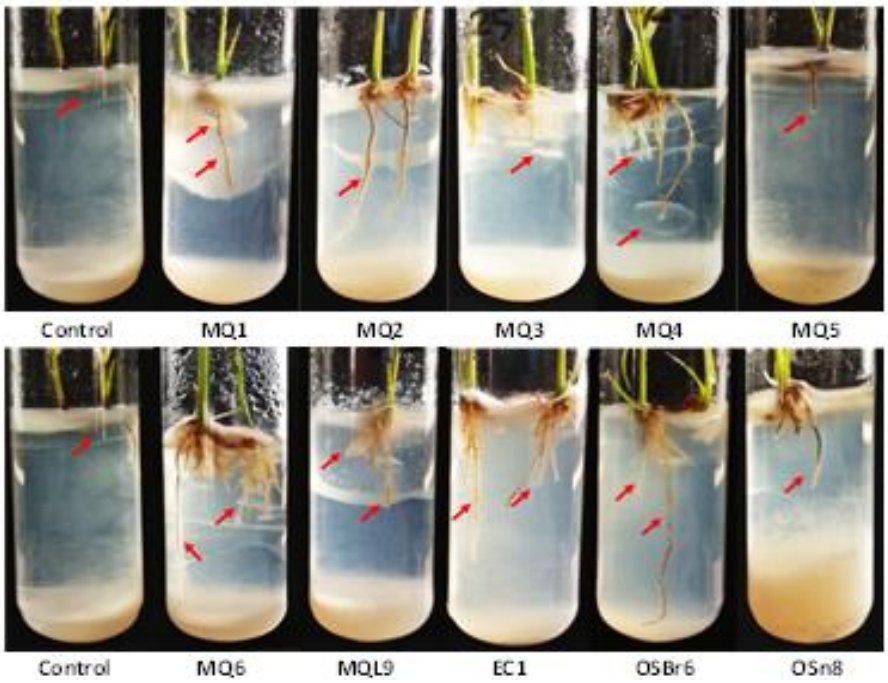

Plate 3. Depiction of root colonization of Zn solubilizing rhizobacteria in rice (Binadhan-20) underwater agar culture tubes. The rhizobacteria colonized surrounding the root of bio-primed rice seedlings as indicated by the red arrows. The arrows in the control tube indicate a drastic reduction in root growth and root colonization

3.4 Early Seedling Growth and Vigor Index

There was a significant difference (*P<0.0001*) in root and shoot growth between control and bio-primed seedlings with rhizobacteria (Fig. 2 and Plate 4). The seeds primed with OSbr5 produced the highest shoot growth (124.22 mm) which was followed by MQ6 (120.30 mm). The lowest shoot growth was observed for control seeds (non-inoculated seeds). Considering the root growth, statistically, significant differences between bio-primed and control seedlings were evident. The highest root length was obtained from MQ6 bio-primed seedlings (35.74 mm) followed by OSbr6 (35.44 mm), MQ2 (28.43 mm), and EC1 (23.43 mm). The lowest root lengths were observed in plants grown from seeds primed with OSn8 (9.03 mm) and MQ5 (8.99 mm) isolates. The rhizobacteria used in this study promoted both root and shoot growth, and induced root branching compared to the control except for one isolate (MQ5). The production of IAA [35], gibberellins [36], and cytokinins [37] from root-associated bacteria have a reasonable influence on plant growth. [21] tested the isolates previously for functionalities like phosphorus solubilization, IAA production, and nitrogen-fixing capability. All the 11 isolates can fix nitrogen whereas some of them are good IAA producer, and some can solubilize phosphorus (Table 4). Some of the bacteria were not able to solubilize significant quantities of Zn in solid and liquid medium but provided enhanced growth of rice seedlings in water agar culture tubes. This enhanced root and shoot growth might be attributed to their ability to solubilize mineral phosphate and the production of IAA. Though isolates like MQ6, and OSbr5 had no IAA producing capability, they returned the higher seedling growth and vigor index among the isolates. On the other hand, OSn8 having higher IAA producing ability performed poorly in the case of seedling growth. Root colonization is a precondition for the biofunctional traits to be effective for plant growth promotion. Poor root colonization of isolates like OSn8 may be responsible for inferior seedling growth in rice.

Despite the lack of growth-promoting traits like PSB or IAA production, OSbr5 showed higher shoot growth of bio-primed rice seedlings. Non-IAA-producing isolates may possibly adopt mechanisms other than plant hormones to facilitate seedling growth, as root colonizing rhizobacteria can also promote plant growth through K and Zn solubilization, siderophore production [10,26,38], and by minimizing other biotic and abiotic stressors. However, the plant growth promotion by rhizobacteria under controlled conditions should only be used as a preliminary selection criterion for rhizobacteria as a potential PGPR candidate. Under field conditions, an array of microclimatic factors other than temperature, humidity, and light play an important role in the successful colonization and promotion of plant growth.

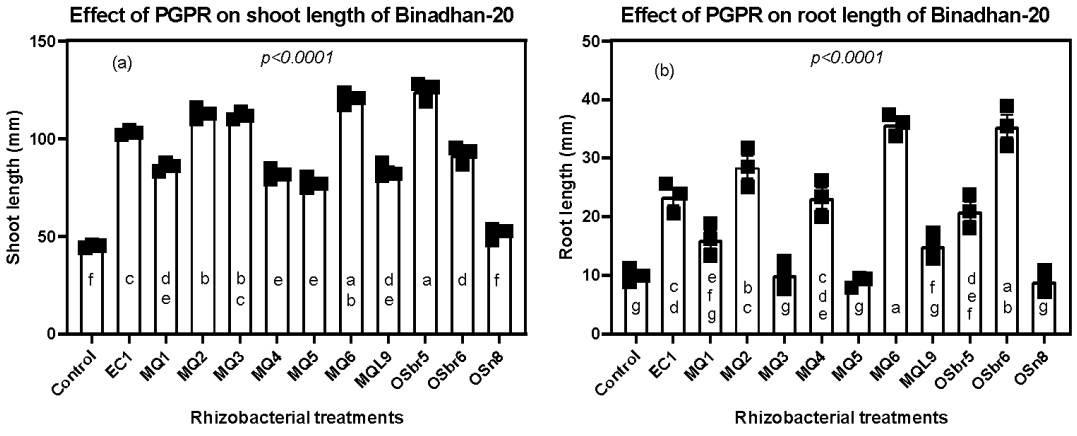

Fig. 2. Effect of Zn solubilizing rhizobacteria on the shoot (a) and root (b) growth of rice (Binadhan-20) seedlings. Bars indicate the standard error of means. Columns with different letters are significantly different according to Tukey's Pairwise Comparisons at a 95% confidence level. Except for Osn8, all other seedlings from bio-primed seeds showed a significant increase in shoot growth compared to the control (a). The root growth of rice seedlings was also stimulated by the rhizobacteria compared to the control except for three isolates, MQ3, MQ5, and Osn8 (b)

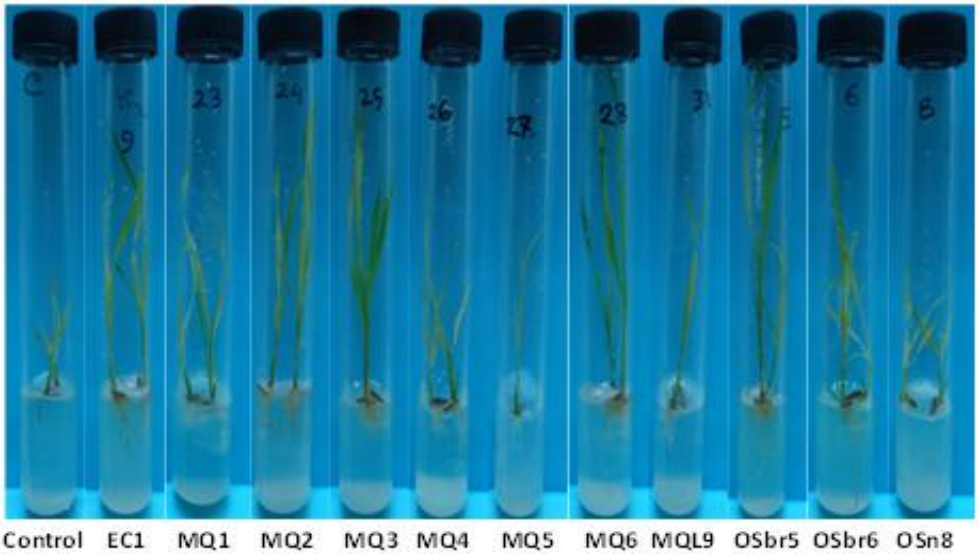

Plate 4. Effect of Zn solubilizing rhizobacteria on rice (Binadhan-20) growth under axenic conditions in water agar culture tubes. The plants were grown for two weeks and root and shoot lengths were recorded. The plants from bio-primed seeds showed significant variation in shoot growth compared to uninoculated control plants

4. CONCLUSION

This study revealed the Zn solubilizing potential of some growth-promoting bacteria along with their ability to colonize the rice root system. The expression of Zn solubilization by the candidate PGPR is very much crop-specific and requires good root colonization. This study identified Zn solubilizers as candidate PGPR that could be used for bioformulation and to evaluate the possibility of Zn biofortification in a sustainable and eco-friendly approach.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Bangladesh Agricultural University Research System (BAURES) funded research project entitled 'Bio-formulation with multifunctional rhizobacteria for crop improvement' (Project # RPP-2018-127) and Ministry of Science and Technology for NST Fellowship.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Krebs NF. Update on zinc deficiency and excess in clinical pediatric practice. ANM. 2013;62(Suppl. 1):19–29.
2. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, et al. Maternal and child undernutrition: Global and regional exposures and health consequences. The Lancet. 2008; 371(9608):243–60.
3. Gibson RS. Zinc deficiency and human health: Etiology, health consequences, and future solutions. Plant Soil. 2012;361(1): 291–9.
4. Cakmak I, Pfeiffer WH, McClafferty B. Review: Biofortification of durum wheat with zinc and iron. Cereal Chemistry. 2010;87(1):10–20.
5. Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany. 2004;55(396):353–64.

6. Gontia-Mishra I, Sapre S, Sharma A, Tiwari S. Alleviation of Mercury Toxicity in Wheat by the Interaction of Mercury-Tolerant Plant Growth-Promoting Rhizobacteria. J Plant Growth Regul. 2016;35(4):1000–12.
7. Iqbal U, Jamil N, Ali I, Hasnain S. Effect of zinc-phosphate-solubilizing bacterial isolates on growth of Vigna radiata. Ann Microbiol. 2010;60(2):243–8.
8. Cakmak I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? Plant Soil. 2008;302(1):1–17.
9. White PJ, Broadley MR. Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytologist. 2009;182(1):49–84.
10. Asha MN, Rahman A, Quadir QF, Islam MS. Isolation and screening of multifunctional rhizobacteria from the selected sites of Madhupur, Narshingdi and Mymensingh, Bangladesh. Research in Agriculture Livestock and Fisheries. 2015;2(1):1–8.
11. Choudhary DK, Sharma KP, Gaur RK. Biotechnological perspectives of microbes in agro-ecosystems. Biotechnol Lett. 2011;33(10):1905–10.
12. Islam MT, Deora A, Hashidoko Y, Rahman A, Ito T, Tahara S. Isolation and Identification of potential phosphate solubilizing bacteria from the rhizoplane of *Oryza sativa* l. cv. BR29 of Bangladesh. Zeitschrift für Naturforschung C. 2007;62(1–2):103–10.
13. Meena VS, Maurya BR, Bahadur I. Potassium solubilization by bacterial strain in waste mica. Bangladesh Journal of Botany. 2014;43(2):235–7.
14. Meena VS, Verma JP, Meena SK. Towards the current scenario of nutrient use efficiency in crop species. Journal of Cleaner Production. 2015;102:556–7.
15. Rahman A, Talukder NM, Islam MT. Screening phosphate solubilizing bacteria from rhizoplane of tomato. Bangladesh Journal of Progressive Science and Technology. 2005;4:1–6.
16. Rahman MS, Quadir QF, Rahman A, Asha MN, Chowdhury MAK. Screening and characterization of phosphorus solubilizing bacteria and their effect on rice seedlings. Research in Agriculture Livestock and Fisheries. 2014;1(1):27–35.
17. Taher AM, Quadir QF, Zakir HM, Nazneen T, Rahman A. Screening and isolation of arsenic tolerant Rhizobacteria from arsenic contaminated areas of Bangladesh. Progressive Agriculture. 2019;30:17–25.
18. Rana A, Joshi M, Prasanna R, Shivay YS, Nain L. Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. European Journal of Soil Biology. 2012;50:118–26.
19. Barbagelata PA, Mallarino AP. Field correlation of potassium soil test methods based on dried and field-moist soil samples for corn and soybean. Soil Science Society of America Journal. 2013;77(1):318–27.
20. Gontia-Mishra I, Sapre S, Tiwari S. Zinc solubilizing bacteria from the rhizosphere of rice as prospective modulator of zinc biofortification in rice. Rhizosphere. 2017;3:185–90.
21. Khatun MJ, Rahman A, Quadir QF, Rion MSI, Zakir HM. Isolation and characterization of plant associated rhizobacteria for plant growth promoting traits. Fundamental and Applied Agriculture. 2021;6(1):95–106.
22. Pikovskaya RI. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species – ScienceOpen. Microbiologia. 1948;17:362–70.
23. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria1. Crop Science. 1973;13(6): cropsci1973.0011183X001300060013x.
24. Alexander M. Introduction to soil microbiology. New York: Wiley; 1961.
25. Jones DL, Darrah PR. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant Soil. 1994;166(2):247–57.
26. Saravanan VS, Kumar MR, Sa TM. Microbial Zinc Solubilization and Their Role on Plants. In: Maheshwari DK, editor. Bacteria in Agrobiology: Plant Nutrient Management [Internet]. Berlin, Heidelberg: Springer. 2011 [cited 2021 Sep 4];47–63. Available:https://doi.org/10.1007/978-3-642-21061-7_3
27. Chang HB, Lin CW, Huang HJ. Zinc-induced cell death in rice (*Oryza Sativa* L.) roots. Plant Growth Regul. 2005;46(3):261–6.
28. Wakatsuki T. Metal oxidoreduction by microbial cells. Journal of Industrial Microbiology. 1995;14(2):169–77.
29. Franz A, Burgstaller W, Schinner F. Leaching with *Penicillium simplicissimum*:

Influence of metals and buffers on proton extrusion and citric acid production. Applied and Environmental Microbiology. 1991;57(3):769–74.

30. Saravanan VS, Madhaiyan M, Thangaraju M. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. Chemosphere. 2007;66(9): 1794–8.
31. Silva HSA, Romeiro R da S, Mounteer A. Development of a root colonization bioassay for rapid screening of rhizobacteria for potential biocontrol agents. Journal of Phytopathology. 2003; 151(1):42–6.
32. Mafia RG, Alfenas AC, Ferreira EM, Binoti DHB, Mafia GMV, Mounteer AH. Root colonization and interaction among growth promoting rhizobacteria isolates and eucalypts species. Rev Árvore. 2009;33:1–9.
33. Stanley NR, Lazazzera BA. Environmental signals and regulatory pathways that influence biofilm formation. Molecular Microbiology. 2004;52(4):917–24.
34. Lugtenberg BJJ, Kravchenko LV, Simons M. Tomato seed and root exudate sugars: composition, utilization by *Pseudomonas* biocontrol strains and role in rhizosphere colonization. Environmental Microbiology. 1999;1(5):439–46.
35. Patten CL, Glick BR. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Applied and Environmental Microbiology. 2002;68(8):3795–801.
36. Brown ME. Plant growth substances produced by micro-organisms of soil and rhizosphere. Journal of Applied Bacteriology. 1972;35(3):443–51.
37. Hussain A, Krischke M, Roitsch T, Hasnain S. Rapid determination of cytokinins and auxin in cyanobacteria. Curr Microbiol. 2010;61(5):361–9.
38. Rahman A, Sitepu IR, Tanf SY, Hashidoko Y. Salkowski's reagent test as a primary screening index for functionalities of rhizobacteria isolated from wild dipterocarp saplings growing naturally on medium-strongly acidic tropical peat soil. Bioscience, Biotechnology, and Biochemistry. 2010;74(11):2202–8.

APPENDIX

Supporting Document 1. Changes of pH of the liquid media after inoculating PGPRs into them after 48 hours after inoculation of bacteria in the media

Bacteria	pH	Fluctuation (unit pH)
Control	7.53	
MQL9	7.53	0
MQ6	7.54	0.01
MQ5	7.53	0
MQ4	7.53	0
MQ3	7.54	0.01
MQ2	7.54	0.01
MQ1	7.53	0
EC1	7.55	0.02
OSn8	7.53	0
OSbr6	7.54	0.01
OSbr5	7.61	0.07

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/87983