Advances in Bioresearch
Adv. Biores., Vol 13 (1) January 2022: 01-08

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.13.1.108

Advances in Bioresearch

ORIGINAL ARTICLE

Bacterial assisted improved Zn consignment in root and shoot of rice plant by zinc solubilizing *Serratia marcescence* bearing plant probiotic traits

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ABSTRACT
The current study was carried out to evaluate the plant probiotic traits of zinc solubilizing bacterial strain (FMAR105) isolated from Finger millet rhizosphere, and to determine bacterium role in substantial uptake of zinc in shoot and root of rice plant. The strain was identified as Serratia marcescens on the basis of 16S rDNA sequencing. The PGP traits of bacterial strain were determined on the basis of solubilization of phosphate, production of siderophore, indole acetic acid, ammonia, and EPS. The plant probiotic attributes of the bacterium inoculant was evaluated under a short term pot trial, and the different parameters such as shoot and root height and biomass of plant (fresh and dry weight of plant) were measured at 21 and 42 days of sowing. At both intervals, the increased biomass was reported for the strain (FMAR105) in combination with the Zn supplement (ZnO). After harvesting (at 42 day), the zinc content in root and shoot was also determined where bacterial inoculants with ZnO supplement increased Zn level in shoot and root in comparison with uninoculated control and single bacterial inoculant. Moreover, available Zn in soil was highest in bacterial treated soil with added Zn supplement (ZnO). The overall study revealed that ZSB inoculant (FMAR105) in combination with Zn supplement can enhance the plant growth and improve the Zn content in plant parts.
Key words: *Zinc solubilizing bacteria (ZSB), Plant probiotic, Zinc, Rice, Serratia marcescens*

Received 08.06.2021 Revised 16.09.2021 Accepted 15.11.2021

How to cite this article:
V K Upadhayay, A Khan, J Singh and A V Singh. Bacterial assisted improved Zn consignment in root and shoot of rice plant by zinc solubilizing *Serratia marcescence* bearing plant probiotic traits. Adv. Biores. Vol 13 [1] January 2022. 01-08

INTRODUCTION

The Agricultural sector has a promising place in the world economy and contributes its fabulous role in feeding the enormously growing world population. But the food intake by people should be plentiful with the required amount of micronutrients such as iron (Fe) and zinc (Zn). Zn is an important for the plants and as well as for the human being and animals in terms of keeping several metabolic functions viable [1]. The shortage of this imperative micronutrient commences the consequence of zinc malnutrition [2]. The outbreak of ailments occurs in response to Zn malnutrition include 'retarded growth', 'deferred wound healing', 'diarrhea', 'skeletal abnormalities', 'increased risk of abortion' [3], 'impairments of physical growth', 'greater risk of various infections' [4], 'erectile dysfunction', 'hyperammonemia', 'severe immune dysfunction', 'alopecia', 'glossitis', 'neurosensory disorders' and 'cancer' [5, 6, 7, 8]. Therefore, there must be an incorporation of a firm solution to counter the effect of Zn related malnutrition. The health supplements are a good option but perhaps away from the reach of economically deprived people. So, improving Zn content in the edible parts of crops may offer the required amount of zinc to the consumer, as this strategy well knowingly ascribed as 'biofortification' which fruitfully challenges the risk of malnutrition [9].

A large section of the world population relies on rice as a staple food crop, but several native agro-climatic and soil conditions affect the uptake of zinc by plants [10]. Therefore, agronomic methods, plant breeding, and transgenics are the imperative biofortification approaches provided a sustainable solution for enormous uptake of micronutrients from soil to plants [11, 12]. These approaches are prolific but not

economically feasible, and then there is always a need to seek novel technology in such field. Using zinc solubilizing bacteria (ZSB) is a new and environment-friendly approach and may provide a sustainable solution for augmenting zinc in plant parts [9]. These are especially a rhizospheric bacteria colonize the plant roots and producing organic acids or employing other strategies to solubilize the nonsoluble form of zinc in surrounding soil. Zinc solubilizing bacteria were also studied to have multiple traits of plant growth promotion including phosphate solubilization, production of siderophore, IAA, and EPS [13, 14]. They were also reported to show several plant probiotic effects in terms of promoting plant growth (plant height, dry weight, yield, etc) [1].

The present study was done to illustrate the role of zinc solubilizing bacterial strain bearing plant probiotic traits on paddy growth and their parallel impact on improved zinc uptake in the shoot and root portion of the plant as well as on availability of Zn in soil.

MATERIAL AND METHODS

Soil and Microorganism:

The Finger millet rhizospheric soil from agricultural field in Almora, Uttarakhand, India was selected for the isolation of bacteria, and brought to the lab immediately. One gram soil sample was serially diluted (up to 10^6) in a normal saline solution. 100µl suspension from the diluted soil sample was uniformly spreaded over the nutrient agar plates. Afterward, the plates were kept for the incubation at 28±2ºC for 24h. Bacterial colonies showing distinct morphological features were selected.

Isolation and selection of zinc solubilizing bacteria

The isolated bacterial strains were assessed for their Zn solubilization behavior by adapting the method of Ramesh *et al* [15]. The mineral salt medium separately amended with 0.1% of three different insoluble Zn sources i.e., zinc oxide, zinc carbonate, and zinc phosphate were prepared and poured into petri plates. The freshly grown cultures were inoculated with help of a toothpick at the center of medium containing plates. The plates were incubated at 28±2ºC for seven days and observed for the appearance of the halo zone around the bacterial colony. This halo zone was indicative of the positive result of Zn solubilization. The isolate showed better solubilization potential and solubilization efficiency (%) was selected for further study.

Biochemical and molecular characterization

Genomic DNA of bacterial isolate FMAR105 was isolated as per the method of Bazzicalupo and Fani [16] and 16Sr DNA amplification was done using universal primers set [16S Forward Primer: 5'–CAGGCCTAACACATGCAAGTC–3' and 16S Reverse Primer: 5'– GGCGGATGTGTACAAGGC – 3']. Amplification was carried out on the thermal cycler 'PTC-200 thermal cycler' (M.J. Research) and amplified product of the isolate was sequenced by Chromus biotech (Bangalore, India). The identification of ZSB isolate FMAR105 was performed on the basis of 16S rRNA gene sequence homology by MEGA5 software through a neighbor-joining method with 1000 bootstrap replicates [17].

Plant growth promoting assays

The test bacterial strain was assessed for PGP (plant growth promotion) abilities assays. Various plant growth-promoting tests (P solubilization, siderophore production, IAA production, ammonia production, HCN production, and EPS production) were done to assess the PGP ability of selected zinc solubilizing bacterial isolate. The methods of Edi-Premono *et al* [18] and Nautiyal [19] were followed to evaluate the phosphate solubilization ability in both qualitative and quantitative manner, respectively. Siderophore production efficacy of test ZSB isolate (both qualitative and quantitative) was performed by the method of Schwyn and Neilands [20]. IAA (Indole-3-acetic acid) production efficiency of selected ZSB strain was evaluated as per Patten and Glick [21]. The production of volatile compound 'HCN' was determined through the method of Miller and Higgins [22]. The tests of ammonia and EPS production were performed by methods illustrated by Cappuccino and Sherman [23] and Siddikee *et al* [24], respectively.

Short term pot trial

The plant probiotic traits of selected isolate (FMAR105) were determined on rice under a short term pot trial. The trial was performed in net house at Department of Microbiology, College of Basic Sciences and Humanities, GBPUA&T, Pantnagar, India. The 3kg capacity of plastic pots was filled with 2kg sterile soil. The trial was laid out in CRD with three replications. This trial included five treatments, viz., T1 (Control), T2 (ZSB-inoculant 'FMAR105'), T3 (ZSB-inoculant 'FMAR105'+ZnO as Zn supplement @ 60kg/hectare). The seeds of paddy (variety- Pant Dhan 1) were procured from BSPC (Breeder Seed Production Centre), Pantnagar, Uttarakhand, India. The seeds of the paddy were sterilized sequentially, first dipping seeds in 2.5% sodium hypochlorite solution (for the 1 minutes) following washed with sterilized distilled water; secondly, seeds were treated with 70% ethanol (for 2 minute) followed by sterilization with 0.1 % $HgCl_2$ solution (for 5 minutes) and thoroughly rinsing with sterile distilled water 8-10 times. Before sowing, the

seeds intended for treatments (T2 and T3) were primed with test bacterial inoculum through dipping of seeds in test bacterial suspension for four hours. In treatment T1,the seeds were sown without priming with bacterial suspension and maintained as uninoculated control. The ten paddy seeds were sown in each pot. The plant probiotic traits such as shoot and root length, fresh and dry weight of plant were determined at two successive intervals viz., 21, and 42 days. At 42 days the plants were uprooted and Zn content was determined in root and shoot part of rice plant through AAS using acid digestion method illustrated by Estefan *et al* [25]. Moreover, the available Zn content in soils of each treatment soils was also analyzed through DTPA extraction method [26].

RESULT AND DISCUSSION

Isolation and determination of zinc mobilizing activity of bacterial isolate

Indeed, the importance of Zn is widely known for crop productivity, but it is also required for conquering the issue of Zn malnutrition in the human population of developing countries [27]. Therefore, it becomes imperative to augment dietary Zn uptake [15, 28]. Using rhizospheric bacteria possessing Zn solubilizing trait as potential bioinculants can be a sustainable option to curve Zn malnutrition as they mobilize the rate of Zn from soil to plants, and also increase the growth and yield of plants [1]. Rhizospheric soil is an important hub for rhizospheric bacterial communities, and it provides a good source for the isolation of numerous culturable bacteria. Rhizospheric bacteria assist plant growth by depicting wide ranges of plant growth-enhancing features and as biostimulants make a roadmap for sustainable agriculture production [29]. The current study was begun with isolating 16 bacterial isolates from rhizospheric soil of Finger millet. One potential isolate, FMAR105, showed better results for zinc solubilization, was selected for the study. This isolate formed halo zone of different sizes viz., 1.63cm, 1.50cm, and 1.2cm on ZnO, $ZnCO_3$ and $ZnPO_4$ containing mineral salt medium (Fig. 1). The solubilization of Zn might be due to the secretion of organic acids of bacterial origin as reported by Fasim *et al* [30], and the involvement of other mechanisms such as proton secretion [11, 12, 31], and siderophore production [32, 33].

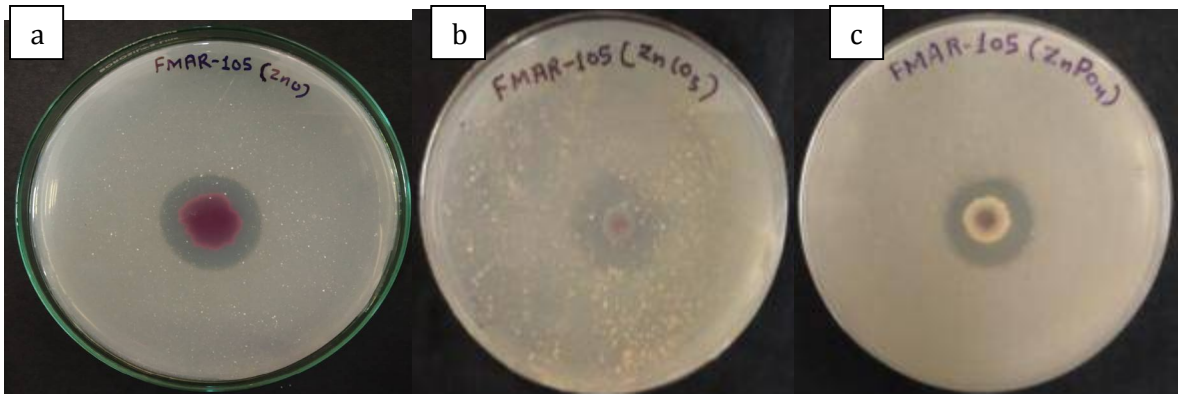

Figure 1: Zn solubilization traits of FMAR105 bacterial strain on ZnO (a), $ZnCO_3$(b) and $ZnPO_4$(c) containing mineral media

Characterization of isolate

The bacterial isolate FMAR105 was a gram-negative, small rod, catalase-positive, producing reddish, spherical, and smooth colony on nutrient agar medium. It was indole positive, methyl red negative, Voges-Proskauer negative, and Simmon citrate positive characteristics. Bacterium FMAR105 had positive results for the gelatinase and caseinase test, while it showed negative results for the amylase and cellulose test. The consequences of the BLAST search of the 16S rRNA gene sequences depicted FMAR105 isolate was closely related to *Serratia marcescens*. On the basis of the phylogenetic tree constructed with the 16S rDNA similarity (%), it was acknowledged as *S. marcescens* (accession number MW843567), and the utmost similarity was found with *Serratia marcescens strain* 1602 chromosome and the next closest homolog was found to be *Serratia marcescens* subsp. *Sakuensis* strain WRK17 (Figure 2).

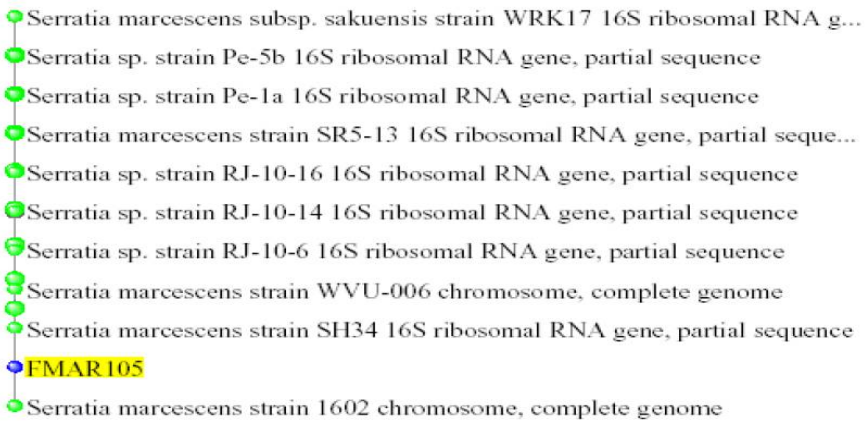

Fig 2: Phylogenetic tree of isolate FMAR105

Plant growth promoting assays:

Positive results for functional plant growth-promoting tests revealed the important PGP ability of FMAR105 (Table 1). The qualitative P solubilization test was assumed positive on the basis of halo zone formation around the bacterial colony (Fig 3). Similarly, a considerable value of solubilized phosphate i.e., 218.3 µg/ml was calculated in the response to the bacterial strain on 7th day of incubation.

Figure 3. Qualitative phosphate solubilization determination of isolate FMAR105

The qualitative test for siderophore production by isolate FMAR105 was determined as positive on basis of the yellowish zone around the bacteria colony. The value of 58.16 % siderophore unit was estimated in a quantitative test, which illustrated the siderophore production in liquid medium by the bacterium. Test isolate was found positive for the IAA production and produced 13.27 µg/ml IAA in tryptophan amended broth. The isolate was also found positive for the production of ammonia and exopolysaccharide (EPS). The value for EPS was determined as 2.14mg/ml when it measured quantitatively. The multiple plant growth-promoting traits of this strain defined it as a 'prolific plant probiotic'. In past, ZSB potentially screened for the plant growth-promoting attributes such as the production of phytohormones, siderophores, ACC deaminase, ammonia, EPS, HCN, and solubilization of phosphate [13, 14, 15, 32, 34, 35].

Table 1: Plant growth promoting (PGP) abilities of ZSB FMAR105

Plant growth promoting traits	*Qualitative*	Quantitative
Phospahte solubilization	+	218.33 (µg/ml)
Siderophore production	+	58.16 (% siderophore unit)
IAA production	+	13.27 (µg/ml)
Ammonia production	+	-
EPS Production	+	4.42 (mg/ml)

'+' indicates positive result for qualitative tests; the data are average of triplicate experimental values

Influence of bacterial strain on rice plant under short term pot trial

Plant probiotic traits of isolate FMAR105 is depicted in Table 2. Results illustrate that shoot length increased for FMAR105 inoculated plants in comparison to control. The best results for increment in shoot length at 21 days (22.77cm) and 42 days (36.75) were recorded for the treatment T2 containing

only a single bacterial inoculant. On the contrary, the augmented root length was recorded at 21 days (9.97cm) and 42 day (18.77) for FMAR105+ZnO treatment (T3).

Table 2: The effect of ZSB strain FMAR105 on plant probiotic traits (shoot length, root length, wet weight and dry weight) of rice

Treatments	21 days				42 days			
	Shoot length (cm)	Root length (cm)	Fresh weight per plant(g)	Dry weight per plant (g)	Shoot length (cm)	Root length (cm)	Fresh weight per plant (g)	Dry weight per plant (g)
T1 (Uninoculated Control)	19.28±1.84	6.13±0.29	0.202± 0.02	0.011±0.002	29.78±0.74	11.50±0.43	0.528±0.05	0.042±0.008
T2 (ZSB inoculant FMAR105)	22.77±0.69 (18.10)	8.27±0.15 (34.91)	0.235±0.007 (16.33)	0.018±0.002 (63.63)	36.75±0.50 (23.40)	15.90±0.75 (38.26)	0.561±0.05 (6.25)	0.048±0.034 (14.28)
T3 (ZSB inoculant FMAR105 + ZnO Supplement)	22.28±0.40 (15.56)	9.97±1.00 (62.64)	0.247±0.01 (22.27)	0.019±0.001 (72.72)	35.15±0.62 (18.03)	18.77±0.75 (63.21)	0.771±0.02 (46.02)	0.051±0.012 (21.42)

The data are average of triplicate experimental value;Values in parenthesis indicate percent increase; '±' indicates standard deviation

Similarly, the data pertaining to fresh weight and dry weight of plant indicated that bacterial treated plants showed higher values over control treatment. The FMAR105 + ZnO treatment showed values of percent increase for fresh weight (22.27% and 46.02% at 21 days and 42 days, respectively) and dry weight (72.72% and 21.42% at 21 days and 42 days, respectively) in comparison with control and single bacterial inoculants. An augmentation in root and shoot length could be accredited to the competence of isolate FMAR105 to mobilize Zn from native resource; hence the Zn solubilized by this bacterial strain is enough for rice plant growth. The zinc solubilizing bacterial strains (*Pseudomonas aeruginosa, Ralstonia pickettii, Burkholderia cepacia, Klebsiella pneumonia*) were also suggested to be better bio- inoculants for increasing root and shoot length and dry mass of rice plant [35]. Likewise, if this Zn mobilizing competence of FMAR105 is compared with treatment T3 where combined treatment of this strain and ZnO was given, there is a considerable enhancement in root length (Table 2). It has been studied that inoculation of Zn solubilizing bacteria and rate of Zn source influence plant growth [36].

Table 3: Response of ZSB strain on Zn content of root and shoot of plant, and on available Zn of soil.

Treatments	Zinc in shoot (mg/kg)	Zinc in root (mg/kg)	Available zinc in soil (mg/kg)
T1	10.21±0.70	16.01±1.33	1.43±0.04
T2	11.93±1.26	22.80±1.95	1.78±0.15
T3	16.97±0.17	28.01±4.04	2.50±0.09

The data are average of triplicate experimental values; '±' indicates standard deviation

The data showing Zn content in shoot and root part of the plant and available Zn in the soil under the influence of bacterial treatment after 42 days were illustrated in Table 3. The FMAR105+ZnO treatment (T3) exhibited the highest value for Zn content in both shoot (16.97mg/kg) and root (28.01mg/kg). These values were more than control and single bacterial inoculated treatment. However, the single bacterial inoculant also significantly improved the Zn content in the shoot and root part in comparison to control. Moreover, the soils from each pot were taken after harvesting of the plant (at 42 days) and determined to

illustrate the effect of treatments on available Zn in soil. The higher availability of Zn was determined in the soil of the combined treatment of bacterial inoculant and ZnO (FMAR105+ZnO). The soil of single bacterial treatment without added ZnO was also showed an enhanced value of available Zn in comparison with uninoculated control. The enhancement in Zn uptake might be attributable to the Zn solubilizing action of isolate FMAR05. As this strain was found to solubilize three different Zn source, it might have solubilized the non-soluble Zn source in soil resulting in the improved soluble fraction of Zn, consequently improving shoot and root Zn contents of rice. The study of Sharma *et al* [37] reported the increased percentage of Zn in root and shoot part in response to Zn solubilizing bacterial isolate. The increment in DTPA extractable Zn in the soil in T3 treatment might be due to the production of organic acids by bacteria and added source of Zn supplement as ZnO. It is well documented that Zn solubilizing bacteria reduce the rhizospheric pH and help in Zn solubilization, and thus eventually increased the uptake of Zn by the plant [1, 38, 39]. Similarly, turn down in the rhizospheric pH with the inoculation of bacterial strain (*Bacillus cereus* W9) was earlier reported by Yu *et al* [40].

CONCLUSION

The present study demonstrated the prolific contribution of zinc solubilizing bacterial on rice plant growth. Bacterial strain *S. marcescens*FMAR105 isolated from the finger millet rhizosphere was determined as a proficient plant probiotic strain on the ground of numerous plant growth-promoting traits. It exhibited plant growth in terms of increased shoot and root length and increased rice plant biomass. Moreover, the effect of bacterium *S. marcescens* with the ZnO as a zinc supplement, it improved the Zn contents in the shoot and root parts and depicted its nature as a potential 'natural biofortifying agent'. The augmentation in available zinc in the soil further illustrated the efficacy of this strain as a potential soil bioinoculant. The study suggests the inoculation of this zinc solubilizing strain might be an auxiliary factor for increasing zinc in food parts of crops in the future study if it could be applied as 'biofertilizer' under field conditions.

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