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Research Article



Selenorhizobacteria mediated selenium biofortification in *Vigna radiata* under selenium deficient region – a sustainable food approach

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Abstract

Biofortification is receiving more devotion to enhance the Phyto-availability of micronutrients such as Fe, Zn, and Se, in the major food crops. Isolation of selenorhizobacteria was done on a nutrient agar plate supplemented with Na₂SeO₃. Among them, the best strain MPJ12 was chosen due to its Plant Growth Promoting Rhizobacteria (PGPR) characteristics, including its capacity to produce siderophore, phosphate, ammonia, etc., Selenorhizobacteria were further applied for *Vigna radiata* pot study with selenium-deficient soil. MPJ12 strain was identified as *Bacillus mycoides* by 16S rRNA sequencing method. The strain was submitted to NCBI under accession no. MG694542. Pot study indicates there was an increase in the vegetative parameters like root and shoot length. *Bacillus mycoides* are capable to enhance selenium content by 0.89 ppm in *Vigna radiata* as well as in leaves, roots, and shoots of *Vigna radiata*. The use of selenorhizobacterial microbes that endorse *Vigna radiata* growth is becoming an effective sustainable agricultural approach to substitute synthetic fertilizers, pesticides, and supplements in selenium-deficient regions.

Keywords: Biofortification, Selenium, Selenorhizobacteria, PGPR, *Vigna radiata*

Introduction

Biofortification involves enhancing the nutritional quality of food crops through agronomic practices (Patel et al., 2018a; Patel et al., 2020a). The new area of research involves the use of crop-microbe interactions to promote selenium biofortification. Selenorhizobacteria is beneficial plant growth promoting rhizobacteria having the ability to enhance selenium content in food crops. Various Plant Growth Promoting mechanisms revealed by rhizobacteria could promote the growth of plants (Patel et al., 2019). Selenium deficit in humans may cause immune system disorders such as cardiovascular diseases, diabetes, and several cancers, etc. which are related to health risks, which is why it is necessary to improve Se content in the human diet (Sarwar et al., 2020).

Se Biofortification in crops is one of the well-developed approaches to fight against existence of Se deficiency in humans (Sarwar et al., 2020). Plants are the major source of Se for consumers (Zhou et al., 2020). That is why; there is considerable attention in understanding the factors that responsible for the accumulation and distribution of Se in the edible portion of plants, especially with an understanding toward improving Se biofortification (Zhou et al., 2020).

The main source of Se for consumers is plants, which accumulate Se (Schiavon et al., 2020). Some plants, in the existence of higher inorganic Se, can take up and accumulate Se in the form of organic derivatives (Amato et al., 2020). Whereas in case of plants present in Se deficient region, selenorhizobacteria could apply and they biologically take up

and accumulate Se. During seedling growth, young leaves comprise comparatively higher content of Se (Hossain et al., 2021). The bioaccumulation of Se generally happens in the vacuoles of the plant cells, and via sulfate transporters, Se may be effluxed from the vacuole (Hossain et al., 2021). When bioaccumulation occurs in edible portions of crop tissues, this process permits the production of Se-supplemented foods that have been used as potential nutraceutical products for humans and animals (Amato et al., 2020).

Soil's geographic distribution of Se is extremely diverse and approx. ranges from 0.01 to 2 mg/kg (Patel et al., 2018). Selenium present in soils is influenced by the type of soil, texture, geological history, mineralogy, amount of organic matter, rainfalls, and Se deposition (Schiavon et al., 2020). Several factors manage the mobility and solubility of Se in soils like pH, redox potential, and organic and inorganic compounds in soils (Schiavon et al., 2020). The use of Se-enriched fertilizers in soil may involve in the improvement of Se bioavailability to plant edible products. Applications of selenorhizobacteria in soil or the application of PGP-selenorhizobacteria to plants might enhance the biofortification of Se plants (Patel et al., 2020b).

Materials and Method

Isolation of Selenorhizobacteria

A soil sample was taken from the rhizosphere of a leguminous plant in Ahmedabad. Isolation of selenorhizobacteria was done on a nutrient agar plate supplemented with 1mM Na_2SeO_3 as selenium source and incubated at 37°C for 48h (Patel et al., 2020b; Acuna et al., 2013). Quantitative analysis was done on the Se content found in isolated selenorhizobacteria. 10 ml of tri-acid mixture of $\text{HNO}_3:\text{HClO}_4:\text{H}_2\text{SO}_4$ were used to digest 1 ml of bacterial culture (Duran et al., 2013). Followed by atomic absorption spectrophotometer was used to measure the Se content (HG-AAS).

PGPR efficacy of Selenorhizobacteria

The ability of selenorhizobacteria to produce siderophores using the CAS-shuttle siderophores test techniques (Patel et al., 2018b), production of phosphate by Pikovskaya's broth medium, Ammonia production by peptone water broth, IAA production by Salkowski's reagent, was performed to check their potential as PGPR (Patel et al., 2020b; Patel et al., 2018c).

Identification of Selenorhizobacteria

Isolates were identified using 16s rRNA at chromas biotech Pvt. Ltd., Bangalore. Sequin software was used to align the sequence data using BLAST analysis before it was submitted to NCBI. Their phylogenetic tree analysis was done by MEGA 4.0 software was used to analyse their phylogenetic tree (Tamura et al., 2007).

Application of Selenorhizobacteria on *Vigna radiata*

On the basis of PGPR efficacy best three isolates MPJ2, MPJ12, and MPJ18 were further individually applied for *Vigna radiata* pot study having selenium-deficient soil. To coat the seeds with cells, sterilized seeds were immersed for 60 min in the grown culture of selenorhizobacteria (Yasin et al., 2015). Without applying culture treatment, the control was prepared. Na_2SeO_3 as chemical fertilizer was also applied for comparative study. After harvesting at 60 DAS, Se content enhanced in treated *Vigna radiata* was analyzed by HG-AAS as same as selenorhizobacteria mentioned above (Duran et al., 2013). And also note down their vegetative characteristics including the seed germination rate, vigour index, shoot, and root length, and total fresh weight (Patel et al., 2020b; Jha and Saraf, 2012).

Statistical Data Analysis

One-way analysis of variance (ANOVA) was used to statistically analyse the data. Each experiment was run three times, and the results were shown as means \pm standard errors (P value \leq 0.05).

Results and Discussion

Three different morphological red colour colonies were isolated on nutrient agar medium supplemented with Na_2SeO_3 as selenorhizobacteria. It suggested they possessed the ability to convert selenium from inorganic Na_2SeO_3 to elemental Se (Se^0). Followed by labeled MPJ2, MPJ12, and MPJ18; selected for further study (Fig. 1).

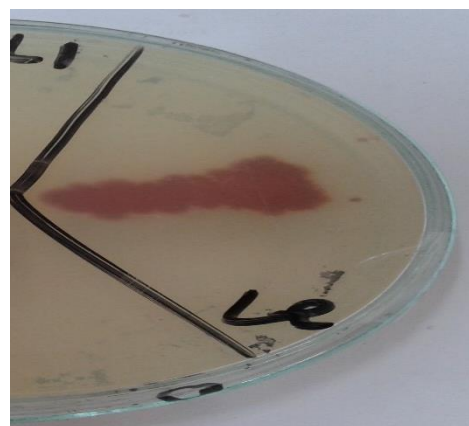


Fig. 1 MPJ12 selenorhizobacteria on Nutrient agar plate supplemented with 1mM Na_2SeO_3

The MPJ12 isolate was able to accumulate 84.4% of the Se present in selenorhizobacteria, which suggests that it has a stronger ability to convert Na_2SeO_3 to elemental selenium. As well as, MPJ2 isolate was capable to accumulate 70.1% Se followed by MPJ18 isolate was showed 63.5% Se accumulation capability (Fig. 2). Researchers reported that

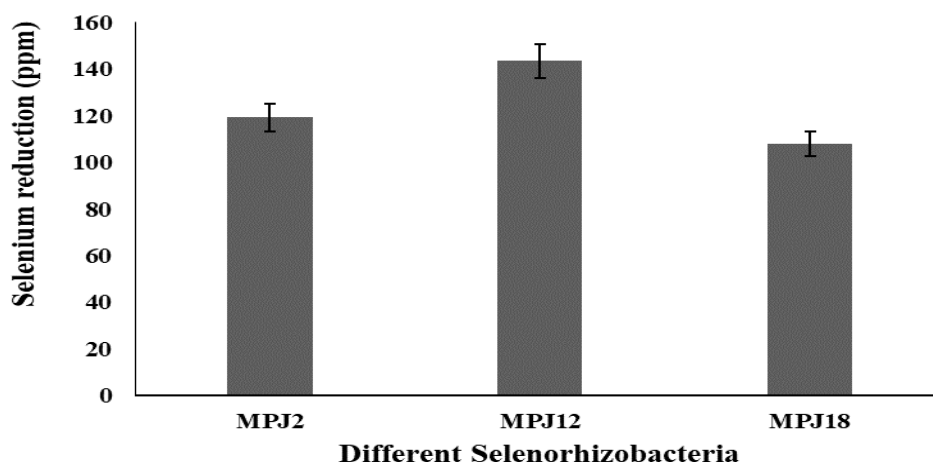


Fig. 2 Selenium Content present in different Selenorhizobacteria

Bacillus sp. have capacity to metabolize the selenium in various organic forms (Duran et al., 2015).

All three isolates were characterized for their PGP efficacy to check their potential as PGPR. All the isolates were found to be solubilised an insoluble mineral tri-calcium phosphate in liquid Pikovskaya’s broth. After 7 days, Maximum liquid phosphate solubilization was found by isolate MPJ12 as 52µg/ml, whereas MPJ2 and MPJ8 solubilized 23µg/ml and 18µg/ml, respectively, after 7 days (Table 1). There are different types of siderophore such as catecholates, hydroxamate, etc. were produced by isolates it indicates it have a high affinity towards iron. According to the results, all three isolates produced siderophores at a rate ranging from 20% to 75%. After 48 h of incubation, the best isolate MPJ12 yielding 75% siderophore was identified (Table 1).

IAA plays a leading role in the elongation of plant roots. Production of IAA was recorded in between the range of 66µg/ml to 89µg/ml at 96 h of incubation in dark. MPJ12 recorded maximum IAA production of 89µg/ml, followed by MPJ2 and MPJ18 isolates were 74µg/ml and 66µg/ml, respectively (Table 1). After incubation, the production of ammonia by isolates was recorded for 13 days and estimated by Nessler’s reagent. The ammonia production was confirmed by the development of brown to yellow color. The maximum ammonia production 34µg/ml was found by the MPJ12 isolate whereas, MPJ2 and MPJ18 were reported to produce 21µg/ml and 19µg/ml, respectively at 11 days (Table 1). Results specify that isolates exposed to the most significant attributes of PGPR were helpful for the vegetative growth of the plant.

Table 1. Plant growth promoting attributes of selected selenorhizobacteria

	Siderophore production (% at 48h)	Phosphate solubilization (µg/ml at 7days)	Ammonia production (µg/ml at 11days)	IAA production (µg/ml at 96h)
MPJ2 isolate	32	23	21	74
MPJ12 isolate	75	52	34	89
MPJ18 isolate	20	18	19	66

Phylogenetic tree analysis was constructed on the basis of 16S rRNA gene sequences and the data was aligned with already available data to National Centre for Biotechnology Information (NCBI) library. A *Bacillus mycooides* strain called MPJ12 was identified by 16S rRNA analysis to have a 98% match to the reported gene sequence. It showed

similarity with *Bacillus mycooides* strain LZH with GenBank accession No. KY049893.1 (Fig. 3). The sequence of MPJ12 isolate was deposited with accession number MG694542 at NCBI GenBank (Table 2). Our study reported for the first time *Bacillus mycooides* reported as Se bio-fortifier in *Vigna radiata*.

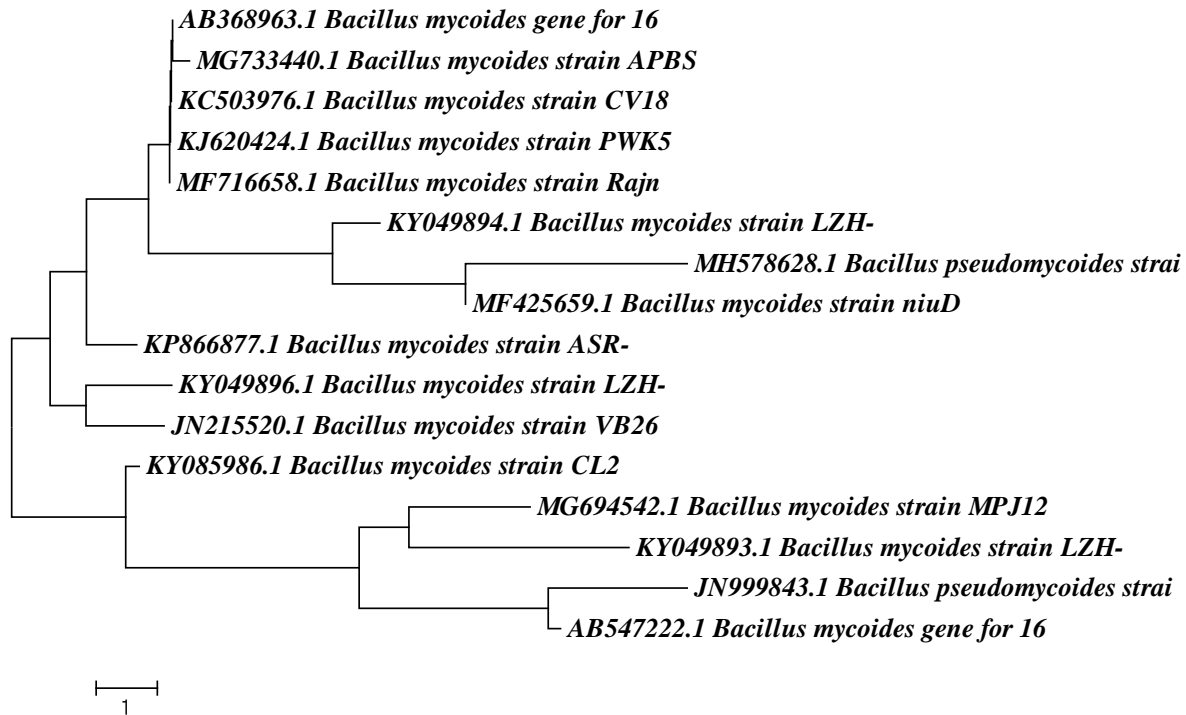


Fig. 3 Phylogenetic tree analysis of MPJ12 isolate

Table 2. 16S rRNA sequencing based phylogenetic similarity of isolates

Isolate	MPJ12
Genera	<i>Bacillus</i> sp.
Strain	<i>Bacillus mycooides</i>
Similarity (%)	98
Accession No.	MG694542

Seed germination percentage showed that higher seed germination 73.3% was observed by MPJ12 isolate followed by 70% was obtained by MPJ2 and MPJ18 isolates, whereas in control *Vigna radiata* and 1mM Na₂SeO₃ treated *Vigna radiata* 60% and 66.6% germination of seed was found (Table 3). Maximum seed vigour index of 3855.6 was recorded by MPJ12 isolate treated seed followed by, 2905 by MPJ2 isolate treated seed and 2863 by MPJ8 isolate treated seed which was highly appreciable than the control (1932) and 1 mM Na₂SeO₃ (2524.8) treated seed (Table 3). Moreover, it revealed the seedling fitness. Results demonstrated that MPJ12 isolate treated *Vigna radiata* generated higher quality seed and better seedlings than control *Vigna radiata*.

Vegetative characteristics showed the longest possible shoots and roots (43cm and 9.6cm) were recorded by MPJ2 isolate treated plant followed by, MPJ18 isolate treated plant (33.3cm and 8.2cm), MPJ8 isolate treated plant (32.9cm and 8cm). It was reported to be higher than the control plant (25.2cm and 7cm) and 1mM Na₂SeO₃ treated plant (30.01cm and 7.9cm) respectively (Table 3).

Results found a positive increase in total fresh weight in between the range of 1.3g to 7.2g. MPJ12 isolate treated plant showed a higher fresh weight of 7.2g (Table 3), which indicates a 45.3% increase in fresh weight than the fresh weight of the control plant. Followed by MPJ2 (3.3%) and MPJ18 (4.1%) showed an increase as compared to the control plant. As well as it also increases fresh weight by 15.7% than the 1mM Na₂SeO₃ treated plant. According to the findings, MPJ12 was able to produce a lot more biomass than the control and 1mM Na₂SeO₃ treated plant.

The result revealed overall maximum vegetative growth was recorded by the MPJ12 isolate. It clearly shows that MPJ12 could promote the maximum vegetative parameters of plant growth when compared to the 1mM Na₂SeO₃ treated plant a positive control and the control plant a negative control.

Table 3. Effect of various selenorhizobacteria and Na₂SeO₃ on the vegetative characteristics of inoculated *Vigna radiata*

	Seed Germination (%)	Seed Vigour Index	Shoot Length (cm)	Root Length (cm)	Total Fresh Weight (g)
Control plant	60	1932	25.2±0.12	7±0.12	1.3±0.12
1mM Na₂SeO₃ treated plant	66.6	2524.8	30.01±0.23	7.9±0.12	2.8±0.32
MPJ2 treated plant	70	2905	33.3±0.10	8.2±0.12	5.4±0.09
MPJ12 treated plant	73.3	3855.6	43±0.04	9.6±0.06	7.2±0.06
MPJ18 treated plant	70	2863	32.9±0.28	8±0.15	5.1±0.12

* Mean ± standard error (n=3), significant difference P≤0.05

Pot experiments showed at the time of harvesting Se content improvement in treated *Vigna radiata* was recorded in between range of 0.31ppm to 0.89ppm (Fig. 4). At 60DAS, maximum presence of Se content was found in MPJ12 isolate

treated *Vigna radiata* (0.89 ppm), followed by MPJ2 isolate treated *Vigna radiata* (0.72ppm) and MPJ18 isolate treated *Vigna radiata* (0.64ppm); whereas 1mM Na₂SeO₃ treated *Vigna radiata* (0.49ppm) and Untreated control *Vigna radiata* (0.31ppm) (Fig. 4).

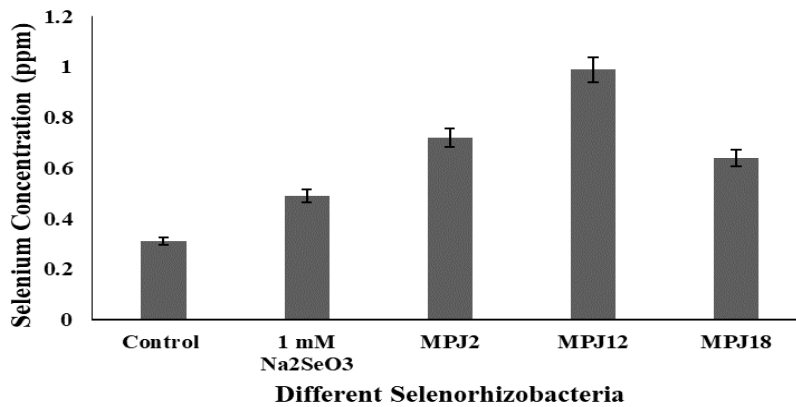


Fig. 4 Selenium content enhanced in *Vigna radiata* at 60 DAS

After 60DAS, at the harvesting time of *Vigna radiata*, In comparison to the control and 1mM Na₂SeO₃ treated *Vigna radiata*, the MPJ12 isolate treated *Vigna radiata* showed 2.19-fold and 1.02-fold higher Se content, respectively (Figure 4), which significantly indicates MPJ12 isolate had best potential to improve *Vigna radiata* Se content. Researchers reported the Zn content improvement in *Vigna radiata* (L.) (Haider et al., 2021) as well as Se content increase in *Glycine Max* by *Paraburkholderia megapolitana* as selenobacteria (Trivedi et al., 2019). Whereas certain researchers recorded enhancement in Se content in fruits of *Phaseolus vulgaris* leguminous plant by *Rhizobium selenitireducens* selenorizobacteria (Patel et al., 2020b). Moreover, biofortification of *Vigna radiata* by siderophore producing bacteria also reported by some researchers but it was in term of iron biofortification of *Vigna radiata* (Patel et al., 2020a). Our study revealed for the first time increase in Se content (2.19-fold) of *Vigna radiata*.

According to the present study, selenorhizobacteria could be biofortified food to improve their nutritional quality. The status of Se for life, its human health significances and economic probabilities, suitable biofortification policies may oblige healthier to eliminate its hidden hunger instead of its artificial supplementation.

Conclusion

MPJ12 has the best potential to produce siderophore, Ammonia, and IAA as well as siderophore solubilization. MPJ12 could enhance 0.89 ppm (2.19-fold) selenium content in *Vigna radiata* which indicates a significant 2.19-fold selenium content increase in *Vigna radiata* as compared to control plant *Vigna radiata*. Among all *Bacillus mycoides* (MPJ12) strains used as the best bio-fortifier against treated other selenorhizobacteria and chemical fertilizer under selenium-deficient soil. Using selenorhizobacterial microbes that endorse *Vigna radiata* growth is becoming an effective

sustainable agricultural approach to substitute synthetic fertilizers, pesticides, and supplements under selenium-deficient regions.

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Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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