Chapter-4

Results

4.1. Isolation of bacterial endophytes from finger millet

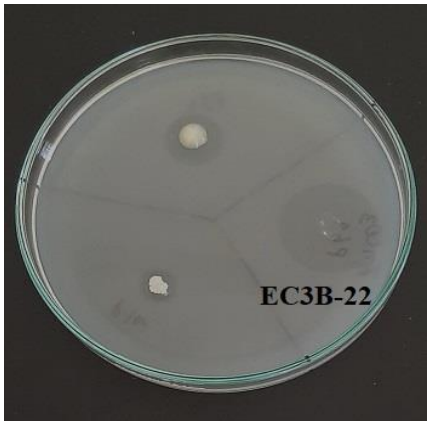
All three varieties of the finger millet plant, including the roots, shoots, leaves, and seeds (Figure 4.1), yielded a total of 112 endophytic bacteria. These isolates were selected on the basis of various morphological types for further characterization and identification, and they were purified and given names in accordance with the crop variety names.

Figure 4.1: Finger millet seeds (VL-348, VL-352, and PRM-1), and plants for the isolation of Endophytes.

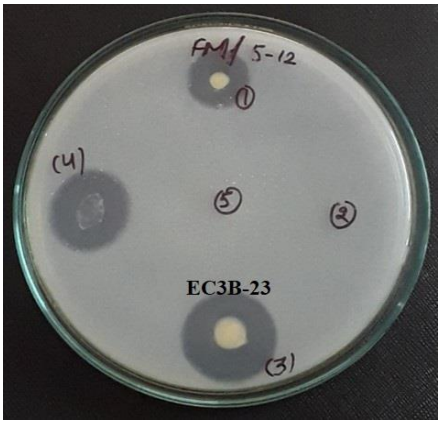
4.2. Screening of efficient iron and zinc-solubilizing bacterial endophytes

By measuring the diameter of the zone of clearance, it was possible to ascertain the endophytic isolates' capacity to bind iron and zinc. Six bacterial isolates were selected during the preliminary screening among the 112 endophytic bacterial isolates based on zone development on modified basal medium and Tris minimum medium agar plates with iron and zinc salts, respectively i.e., EC1B-24, EC3B-12, EC3B-22, EC3B-23, EC3B-1, and EC2B-21. All the six isolates solubilized all three zinc salts and exhibited solubilization index in varying amount. Maximum solubilization index was showed by EC3B-23 for zinc oxide. For zinc carbonate again EC3B-23 showed highest zone and gave the maximum solubilization index (4.8 mm), followed by EC3B-22 (4.3 mm); EC1B-24, 3.5 mm; EC3B-12, 2.8 mm; EC2B-21, 2.2 mm, and EC3B-1, 1.9 mm. zinc oxide was solubilized by all the isolates maximum solubilization index was showed by EC3B-23 followed by EC3B-22, EC1B-24, EC3B-12, EC2B-21, and least solubilization was showed by EC3B-1 which was 5.6, 3.4, 3.2, 3.0, 3.0, and 2.1 mm respectively. Similarly, all bacteria endophytic isolates were able to utilize zinc phosphate with maximum solubilization index displayed by EC3B-1 (3.6 mm), EC2B-21, and EC3B-12 shared similar solubilization index (3.1 mm) followed by EC1B-24 (3.0 mm), EC3B-23 (2.3 mm), and EC3B-22 (2.1 mm). For iron solubilization ferric phosphate is used as a source of iron, bacterial endophytes solubilized ferric phosphate. EC3B-22 showed maximum solubilization index (4.2 mm), followed by EC3B-23

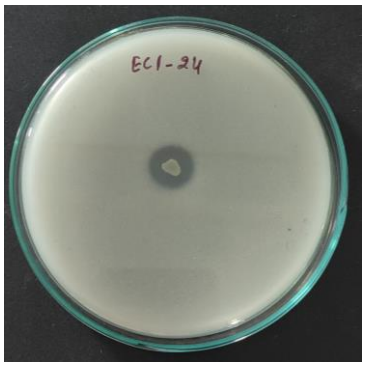
(3.1), EC3B-1 (3.0), EC1B-24 (2.9), EC2B-21 (2.8), and EC3B-12 (2.8 mm). Table 4.1 displays the zinc and iron solubilization indices of the chosen endophytic bacterial isolates, while figure 4.2 showing the solubilization zone (zinc and iron) formed by endophytic bacteria.



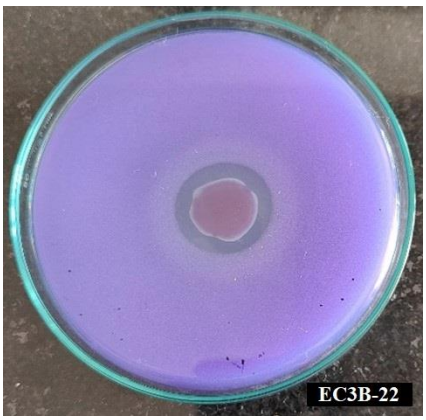
a. Zinc phosphate solubilization

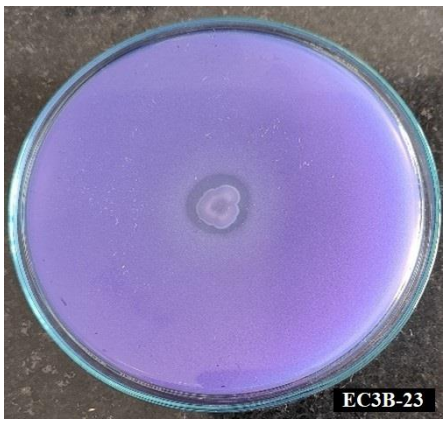


b. Zinc carbonate solubilization



c. Zinc oxide solubilization





d. Iron solubilization

Figure 4.2: figure depicting the zinc phosphate, zinc carbonate, zinc oxide (a, b, and c respectively) solubilization on Tris minimum medium, and ferric phosphate solubilization (d) on modified basal medium.

Table 4.1: Qualitative zinc and iron solubilization by bacterial endophytes using three different insoluble zinc salts (ZnO, $ZnCO_3$, and $ZnPO_4$), and iron salt ($FePO_4$) respectively.

Endophytic Isolates	Zn solubilization			Fe solubilization
	$ZnCO_3$	ZnO	$ZnPO_4$	$FePO_4$
	SI (mm)			
EC1B-24	3.5±0.51	3.2±0.59	3.0±0.51	2.9±0.51
EC3B-12	2.8±0.50	3.0±0.55	3.1±0.54	2.8±0.52
EC3B-22	4.3±0.58	3.4±0.71	2.1±0.35	4.2±0.67
EC3B-23	4.8±0.61	5.6±0.82	2.3±0.49	3.1±0.48
EC3B-1	1.9±0.63	2.1±0.62	3.6±0.53	3.0±0.51
EC2B-21	2.2±0.52	3.0±0.61	3.1±0.54	2.8±0.52

4.3. Plant growth endorsing attributes of iron and zinc solubilizing bacterial

4.3.1. Synthesis of IAA

All of the iron and zinc solubilizing isolates displayed changes in colour that varied from a lightest pink to deep red with the addition of Salkowski's mixture for IAA qualitative testing, signifying hormone synthesis. The findings showed that all isolated bacterial endophytes produced IAA differently. In the presence of tryptophan, among all six bacterial isolates EC3B-22 synthesized IAA in highest amount (117.2 µg/ml) (Figure 4.3), followed by EC3B-23 synthesized

IAA production of 105.5 µg/ml, EC3B-12 (104.4 µg/ml), EC2B-21, EC1B-24, and EC3B-1 with 87.4 µg/ml, 75.4 and 65.4 µg/ml, respectively. (Table 2) (Figure 4.4).

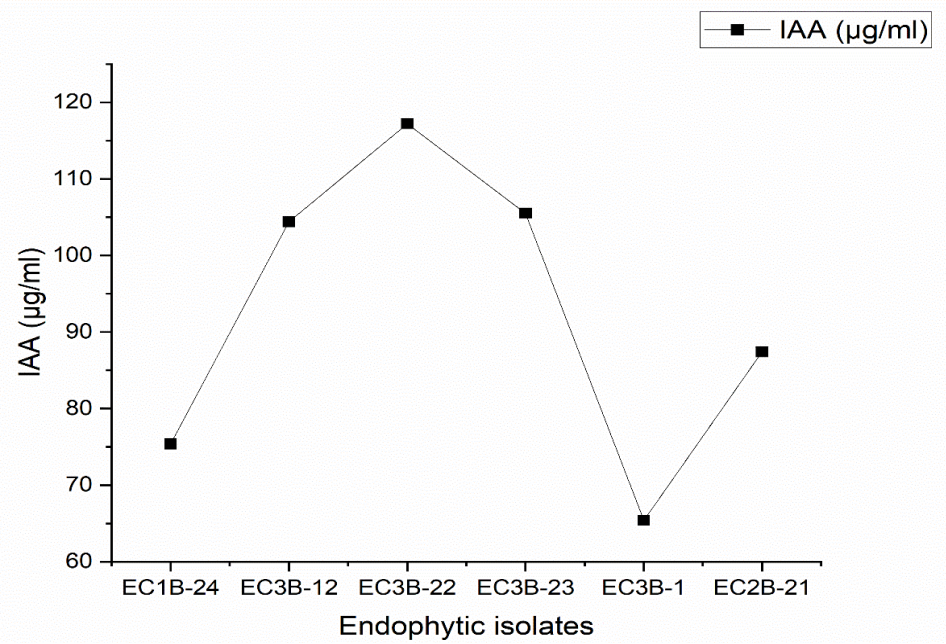


Figure 4.3: Graphical representation for IAA production by bacterial endophytes

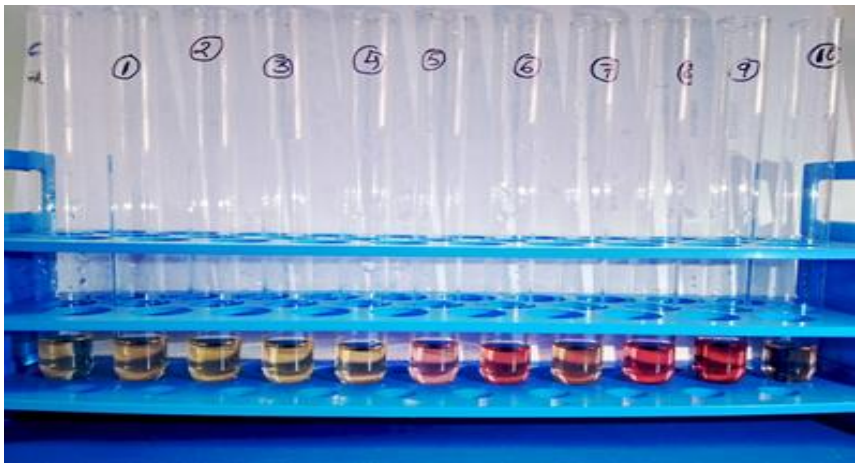


Figure 4.4: Image depicting production of IAA by endophytes.

4.3.2. Synthesis of HCN

All of the bacterial endophytes, with the exception of EC2B-21, were capable of synthesizing hydrogen cyanide in various amounts. Qualitatively, EC1B-24 and EC3B-22 displayed strong reaction (+++), but EC3B-12 displayed a moderate response (++), and EC3B-23, EC3B-1 showed weak positive (+), and negative result was shown by EC2B-21. The bacterial endophytes were additionally assayed spectrophotometrically to quantify the HCN production. It was observed that isolate EC3B-22 synthesized HCN in significant amount and exhibited the maximum absorbance value i.e., 0.043, followed by EC1B-24, EC3B-12, EC3B-23, EC3B-1, and EC2B-21 with a value of 0.041, 0.036, 0.029, 0.016, and 0.003, respectively (Table 4.2; Figure 4.5).

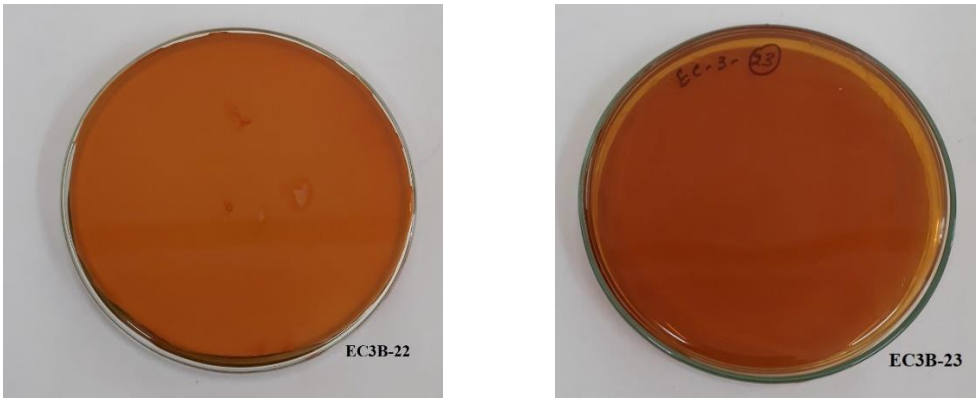


Figure 4.5: HCN production by bacterial endophytes

4.3.3. Synthesis of Ammonia

Selected bacterial endophytes were tested for ammonia synthesis, only the endophytic isolates EC3B-23 and EC2B-21 exhibited the ammonia synthesis

when Nessler's reagent was added. Other endophytic bacteria did not demonstrate ammonia production. (Table 4.2; Figure 4.6).

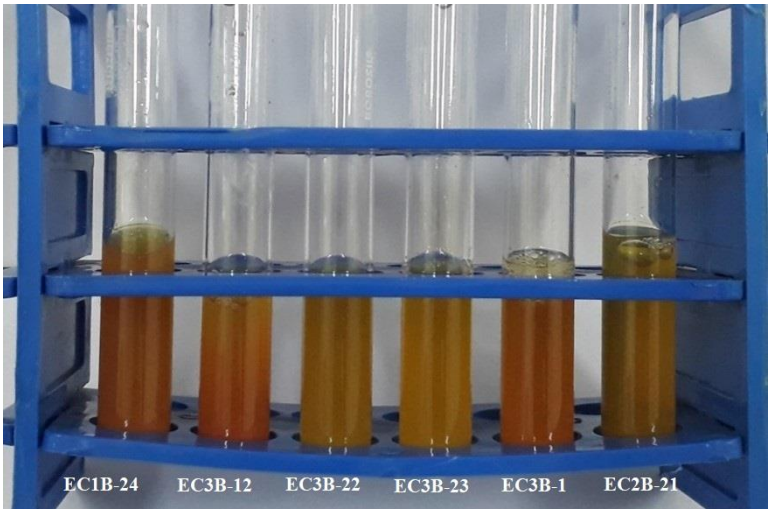
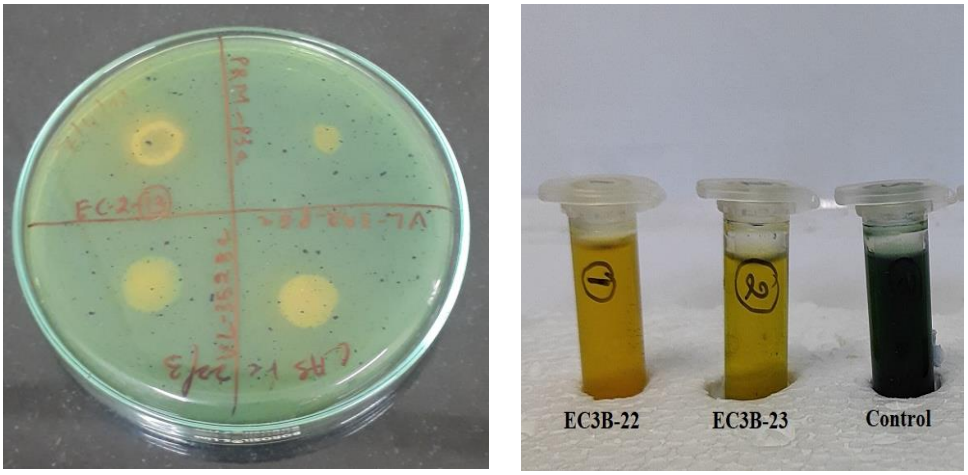


Figure 4.6: Ammonia production by endophytes

4.3.4. Synthesis of siderophore

An orange colour ring that formed around the colony developed using the CAS-agar approach under restricting iron circumstances served as a visual indicator of an effective siderophore production. All of the bacterial endophytes produced siderophores and had an orange zone surrounding the colony except EC1B-24. Quantitative assessment displayed that the bacterial endophytes, exhibited siderophore in varying amount and maximum percent siderophore unit (psu) was showed by EC3B-22 (71.8 psu) followed by EC3B-1 (66.09 psu), EC3B-12 (61.4 psu), EC2B-21 (51.4 psu), EC3B-22 (23.74 psu), and EC1B-24 (13.4 psu). (Table 4.2; Figure 4.7).



a. Qualitative b. Quantitaive

Figure 4.7: figure (a) depicting qualitative estimation of siderophore production on modified CAS agar medium, (b) quantitative siderophore estimation, by bacterial endophytes.

4.3.5. Solubilization of Phosphate

Using the agar plate technique, the phosphorus solubilization capability of iron- and zinc-solubilizing endophytes was assessed. Phosphate solubilizing potential of endophytic isolates was assessed by examining halo zones surrounding the colonies on NBRI-BPB medium. Bacterial isolate EC2B-21showed maximum solubilization index (3.6 mm), while EC3B-22 resulted in least solubilization index (2.4 mm). Similarly, endophytic bacteria EC3B-1 also solubilized phosphate with solubilization index (3.3 mm) followed by EC1B-24 (3.2 mm), EC3B-12 (2.6 mm), and EC3B-23 (2.5 mm) (Table 4.2; Figure 4.8).

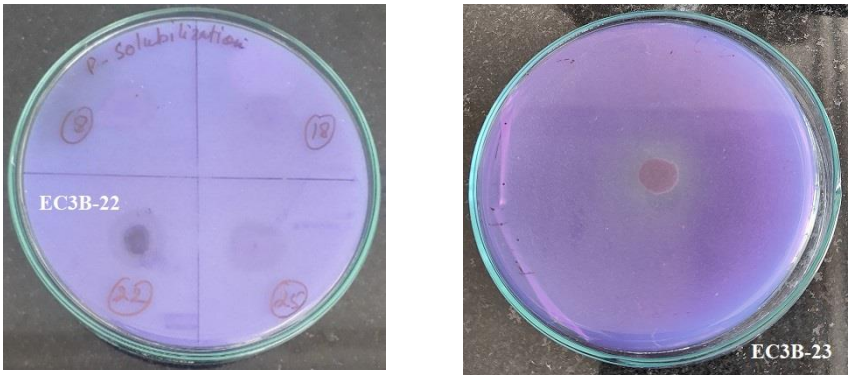


Figure 4.8: qualitative phosphate solubilization of zinc and iron solubilizing endophytes

Table 4.2: Indole acetic acid, Phosphate solubilization, ammonia, hydrogen cyanide, and organic acid synthesis, by selected endophytic bacterial isolates. Mentioned pH values are at the period of organic acid synthesis. The values are the average of three replicates; and the values after ± signify the standard deviation.

Endophytic isolates	Phosphate Solubilization	Siderophore		Indole acetic acid (µg/ml)	Hydrogen cyanide		Ammonia	Organic Acid	pH
	SI (mm)	Qualitative	PSU		Qualitative	Quantitative			
EC1B-24	3.2±0.63	-	13.4±0.54	75.4±0.48	+++	0.041±0.001	-	+	4.96±0.11
EC3B-12	2.6±0.66	+	61.4±0.44	104.4±0.64	++	0.036±0.002	-	+	5.14±0.16
EC3B-22	2.4±0.63	+	23.74±0.60	117.2±0.67	+++	0.043±0.002	-	+	5.16±0.12
EC3B-23	2.5±0.56	+	71.8±0.63	105.5±0.59	++	0.029±0.001	+	+	5.04±0.16
EC3B-1	3.3±0.64	+	66.09±0.54	65.4±0.46	+	0.016±0.001	-	-	4.84±0.13
EC2B-21	3.6±0.68	+	51.4±0.48	87.4±0.74	-	0.003±0.002	+	+	5.14±0.12

4.3.6. Production of Organic acid

The endophytic isolates that may generate organic acids by plate test are listed in Table 2. Yellow colour formation around the colonies was considered affirmative for organic acids production. All bacterial isolates showed formation of yellow zone round the colonies which shows that they were capable to synthesize organic acids except isolate EC3B-1. pH of the broth medium decreased as a result of the production of organic acid, and bacterial endophytes EC3B-22, EC3B-12, EC2B-21, EC3B-23, EC1B-24, and EC3B-1 lessened the pH to 5.1, 5.1, 5.1, 5.0, 4.9, and 4.8 respectively (Table 4.2; Figure 4.9).

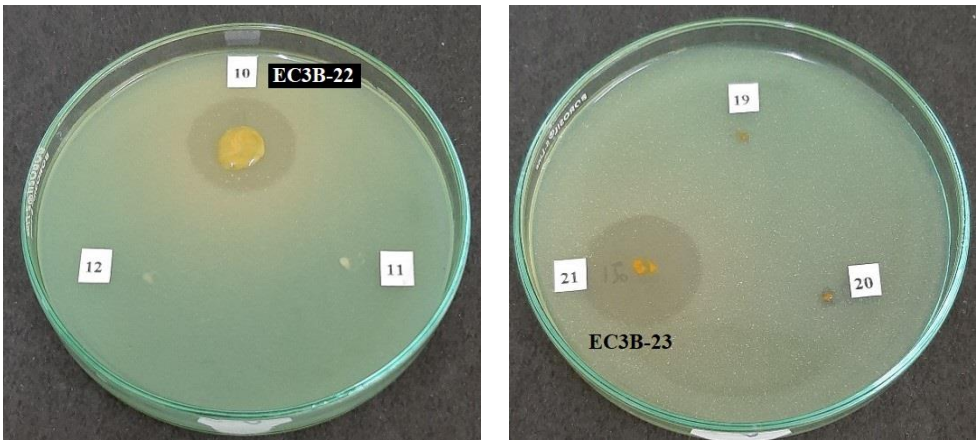


Figure 4.9: Organic acid production by bacterial endophytes

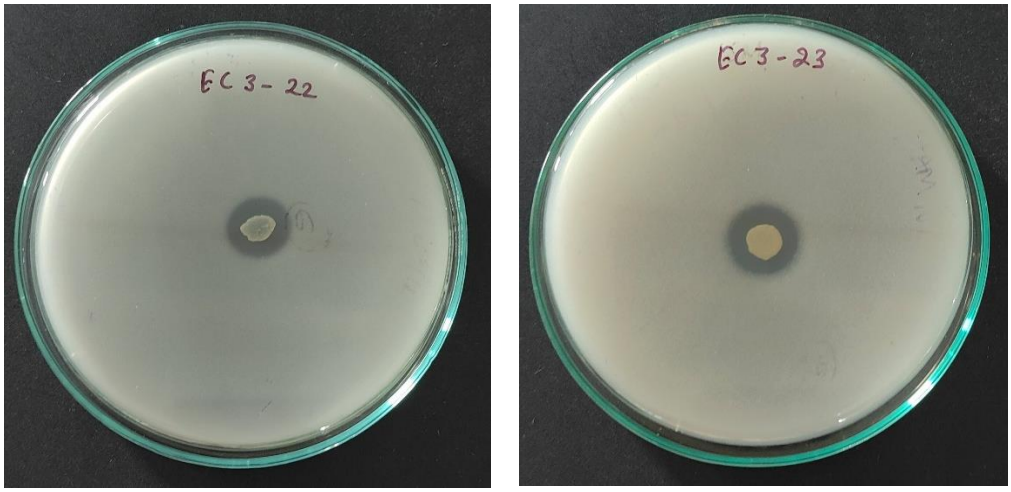
4.4. Extra cellular enzyme production

All of the endophytic isolates displayed a variety of enzymatic activity. Bacterial isolates EC3B-23, EC3B-1, and EC2B-21 exhibited lipase and amylase activity; EC1B-24 was also displayed production of for lipase. EC3B-23 exhibited urease production. All the selected isolates were unable to synthesize tannase. Furthermore, endophytic isolates EC3B-1, EC3B-24, EC1B-1, and were positive for gelatinase. Similarly, EC3B-

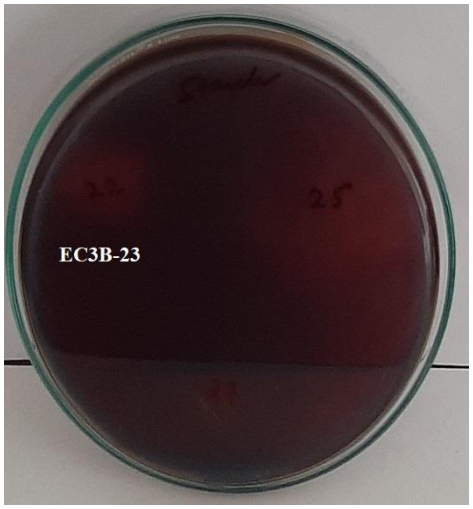
22, EC1B-24, and EC2B-21 were positive for protease, while All of the tested isolates produced phytase (Table 4.3; Figure 4.10).

Table 4.3: Extra cellular enzyme production by bacterial endophytes

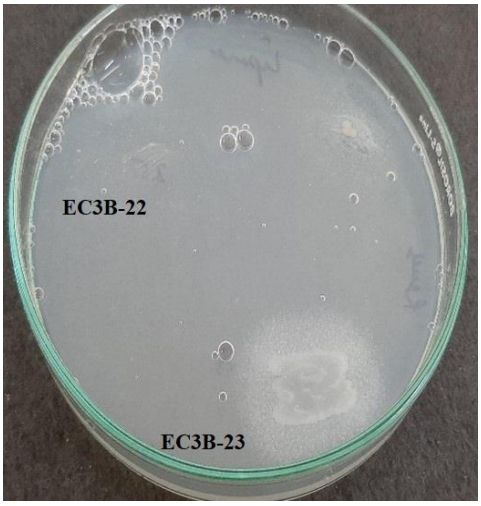
S.no.	Name of bacterial isolate	Name of enzymes						
		Amylase	Lipase	Urease	Tannase	Gelatinase	Protease	Phytase
1.	**EC1B-24**	-	+	-	-	+	+	+
2.	**EC3B-12**	-	-	-	-	-	-	+
3.	**EC3B-22**	-	-	-	-	+	+	+
4.	**EC3B-23**	+	+	+	-	-	-	+
5.	**EC3B-1**	+	+	-	-	+	-	+
6.	**EC2B-21**	+	+	-	-	-	+	+



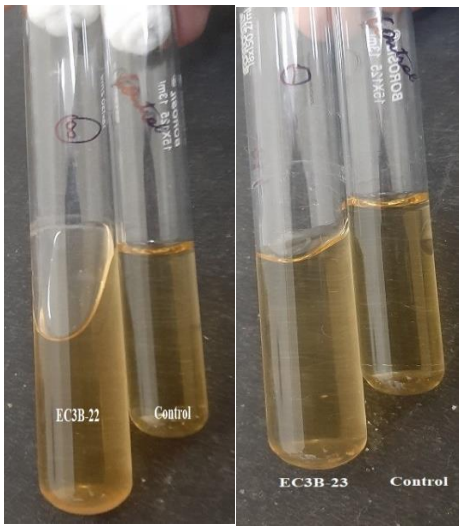
a. Phytase synthesis



b.Amylase synthesis

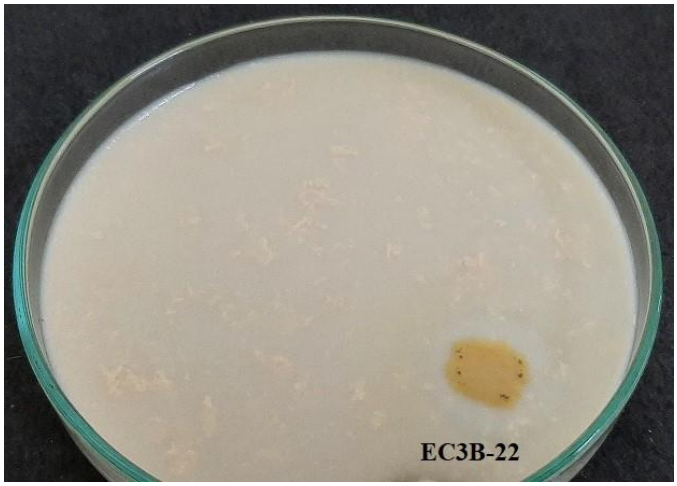


c.Lipase synthesis



d.Gelatin hydrolysis

e.Urease synthesis



f. Protease synthesis

Figure 4.10: Extra cellular enzyme production by iron and zinc solubilizing bacterial endophytes

4.5. Stress tolerance potential of selected bacterial endophytes

The adaptability of each finger millet endophytic bacterial isolate was evaluated at various pH levels, temperatures, and NaCl concentrations, and their optimal growth was documented. All of the isolates were found to grow well in the pH range of 5.0 to 9.0, however they favoured a pH range of 6.0 to 8.0 for optimal growth. Even at pH 10.0, little growth was seen. On the other hand, with regard to temperature, both bacterial isolates successfully grown at temperatures amid 15°C to 35°C. The endophyte EC3B-23 was additionally survived at 40°C and 50°C. At 40°C, EC3B- 22, EC3B-1, and EC2B-21 displayed constrained growth. NaCl concentrations between 0.5% and 3% are ideal for all of the bacterial isolates. Isolate EC2B-21 showed good growth on NaCl concentration 8.0% and restricted growth was recorded on concentration 10.0%. (Table 4.4).

Table 4.4: Table depicted the stability of bacterial endophytes to Diverse abiotic stress

Bacterial isolate		EC1B-24	EC3B-12	EC3B-22	EC3B-23	EC3B-1	EC2B-21
Growth on different pH range	4.0	-	-	++	++	+	++
	5.0	+	+	++	++	++	+++
	6.0	+++	++	+++	+++	+++	+++
	7.0	+++	++	+++	+++	+++	+++
	8.0	+	+	+++	++	++	+++
	9.0	+	+	+	+	++	+++
	10.0	-	+	-	-	+	+++
Growth at temperature (°C)	5	-	-	-	-	-	-
	15	++	++	+++	+++	++	+++
	25	+++	+++	+++	+++	++	++
	30	+++	+++	+++	+++	+++	+++
	35	+++	++	++	+++	+++	+++
	40	+	+	+	++	++	++

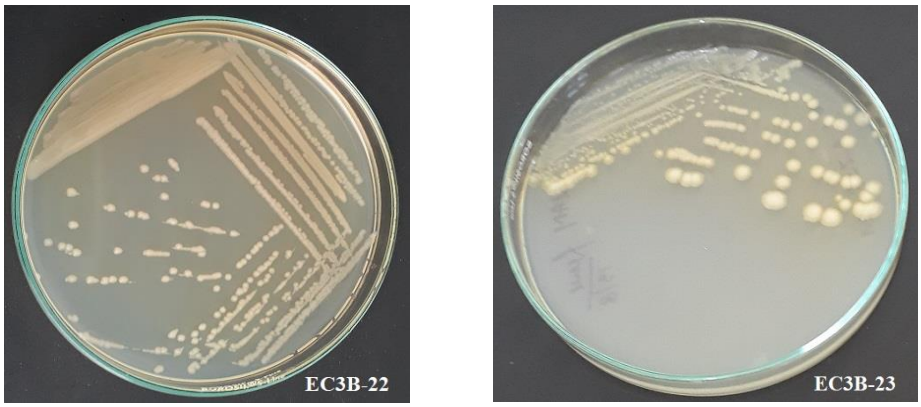
	50	-	-	-	++	-	++
Growth on different NaCl concentration (%)	0.50	+++	+++	+++	+++	+++	+++
	1	+++	+++	+++	+++	+++	+++
	2	+++	++	+++	+++	++	+++
	3	++	+	+++	+++	-	+++
	4	-	-	-	-	+	+++
	5	-	-	-	-	-	+++
	6	-	-	-	-	-	+++
	7	-	-	-	-	-	+++
	8	-	-	-	-	-	+++
	9	-	-	-	-	-	++
	10	-	-	-	-	-	+
	11	-	-	-	-	-	-
	12	-	-	-	-	-	-

4.6. Identification of selected iron and zinc solubilizers:

4.6.1. Morphological and biochemical characterization of the endophytic isolates

On the basis of iron and zinc solubilization index two most prominent endophytic bacteria (EC3B-22, and EC3B-23) were selected for further examination. Endophytic isolates were identified using colony morphology as well as biochemical and molecular characterization. EC3B-22 is Gram-negative with circular cells and a smooth, convex elevation, while EC3B-23 is Gram-positive with similar cellular features. Both strains exhibit motility and possess a yellowish colour, but EC3B-22 displays a remarkable yellow-blue fluorescent pigmentation. Biochemically, both strains are positive for oxidase and catalase enzymes, indicating their ability to perform aerobic respiration. However, they differ in H2S production, with EC3B-22 being negative and EC3B-23 positive. Similarly, the MR and VP tests reveal contrasting results, with EC3B-23 demonstrating methyl red and citrate utilization, while EC3B-22 does not. Furthermore, EC3B-23 is positive for the nitrate, urease, and gelatin tests, indicating its capacity to reduce nitrate, hydrolyse urea, and liquefy gelatin, respectively, whereas EC3B-22 is

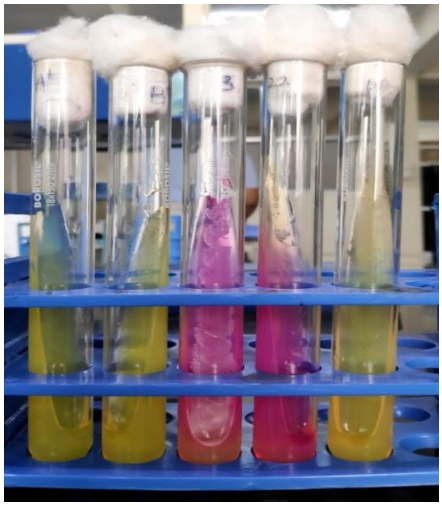
negative in these tests. Both strains exhibit fermentative capabilities for various sugars like glucose, sucrose, mannitol, and maltose (Table 4.5; Figure 4.11).



a. Bacterial endophytes on NA culture plates

b. Catalase production

c. MR test



d. Uresae test

e. Indole test

Figure 4.11: Figure depicting biochemical characterization of bacterial endophytes

Table 4.5: Morphological and biochemical characteristics of bacterial endophytes

Characteristics	EC3B-22	EC3B-23
Gram's reaction	Negative	Positive
Motility	Motile	Motile
Cell shape	Circular	Circular
Colour	Yellowish	Yellowish cream
Pigmentation	Yellow-blue fluorescent	-
Elevation	Convex	Convex
Texture	Smooth	Smooth
Oxidase	+	+
Catalase	+	+
H_2S	-	+
Indole	-	-
MR	-	+
VP	-	-
Citrate	+	+
Nitrate	-	+
Starch	-	+
Urease	-	+
Gelatin	+	+
Glucose	+	+
Sucrose	+	+
Mannitol	+	+
Maltose	+	+

4.6.2. Molecular characterization of promising isolates

EC3B-22 and EC3B-23, two potential bacterial endophytes, were selected for additional research based on their iron and zinc solubilization index. From both isolates, DNA was extracted. On a 1.0% agarose gel used to assess its quality, a single band of high-molecular weight DNA was observed (Figure 4.12). 16S rRNA forward and reverse primers were used to amplify a 16S rRNA gene fragment. On an agarose gel, a single discrete 1500 bp PCR amplicon band was observed (Figure 4.13). The genome sequences had been uploaded to NCBI Genbank and assigned accession numbers. (Table 4.6). The endophytes were characterized using the 16S rRNA gene sequence analysis. The endophytic isolates EC3B - 22, and EC3B - 23 displayed a pairwise

sequence alignment (EZbiocloud database) that revealed sequence similarity to *Pseudomonas bijieensis* L22-9^{T} and *Priestia megaterium* NBRC 15308^{T} of 99.85% and 99.51%, respectively. Because isolate EC3B-22 does not group with other *Pseudomonas* species according to the phylogenetic analysis (Figure 4.14), it is possible that EC3B-22 isolates represent a novel species. In contrast, *Priestia megaterium* NBRC 15308^{T} and isolate EC3B-23 are closely connected in the evolutionary tree (Figure 4.15).

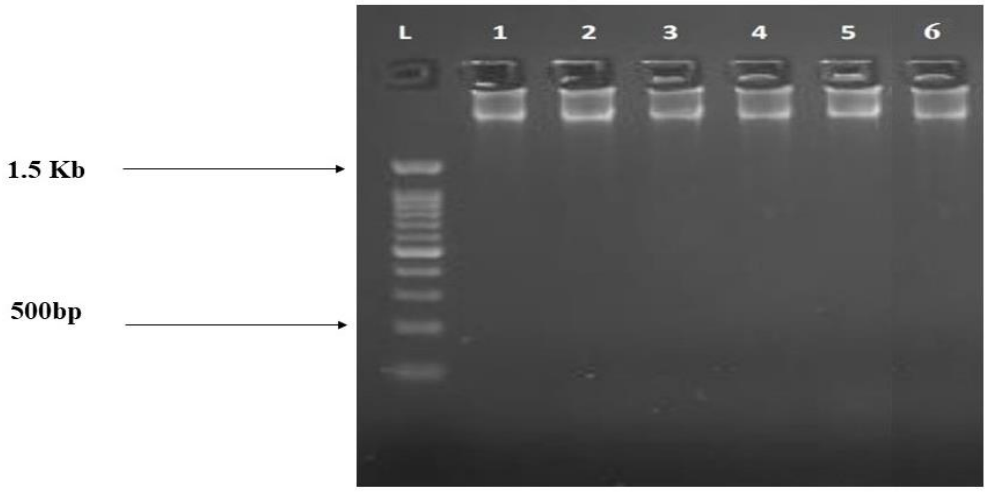


Figure 4.12: gDNA loaded on 1% Agarose gel

Lane Description:

L: DNA Marker; 1. EC1B-24; 2. EC3B-12; 3. EC3B-22; 4. EC3B-23; 5. EC3B-1; 6. EC2B-21

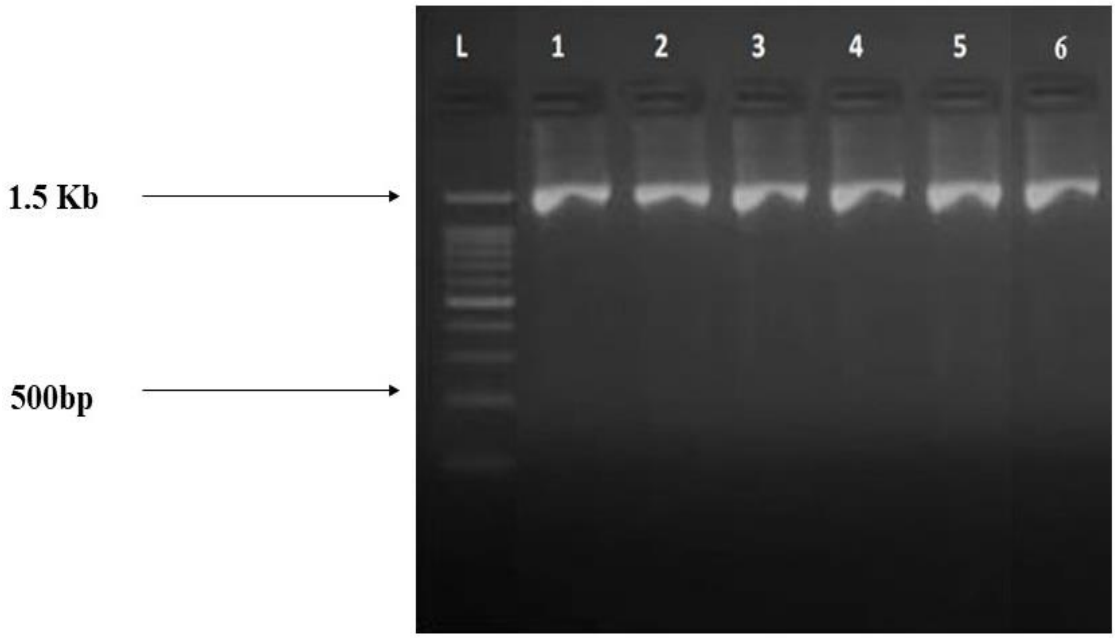


Figure 4.13: PCR products loaded on 1% Agarose gel

Lane Description:

L: DNA Marker; 1. EC1B-24; 2. EC3B-12; 3. EC3B-22; 4. EC3B-23; 5. EC3B-1; 6. EC2B-21

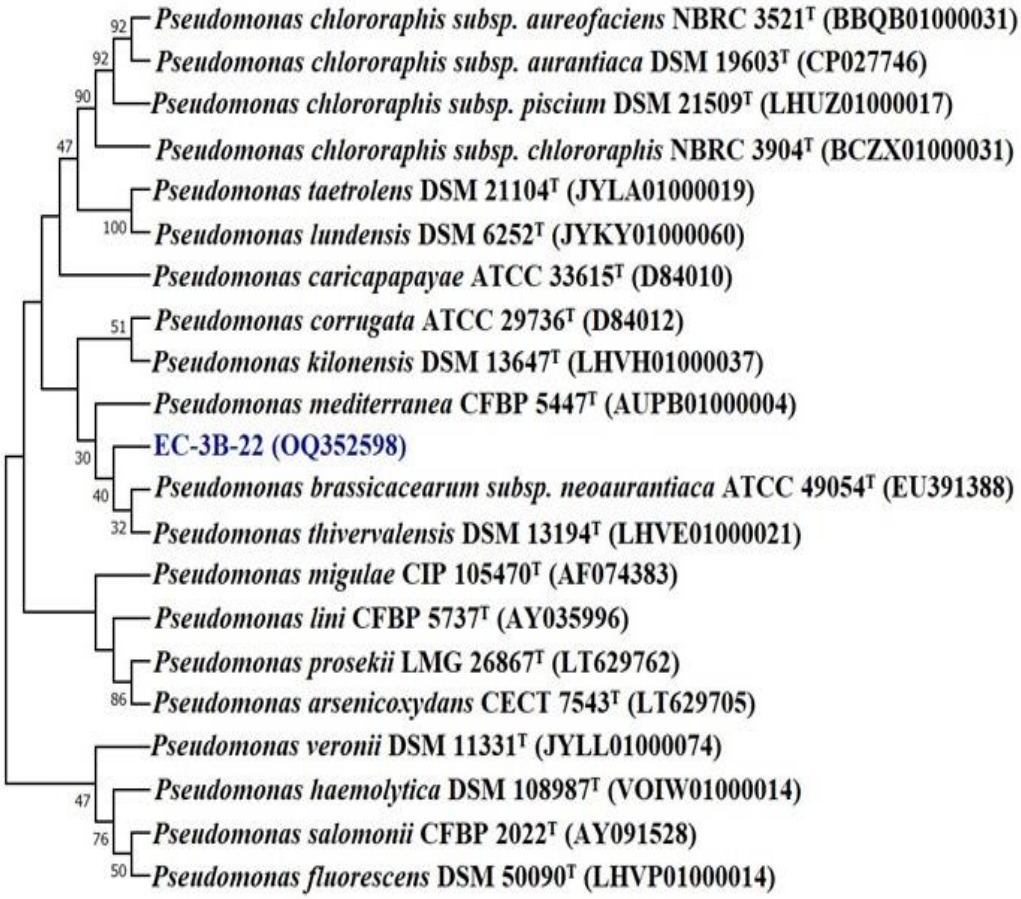


Figure 4.14: Maximum likelihood approach used to reconstruct the phylogenetic tree of the isolate EC3B-22, *Pseudomonas sp*. using the Kimura 2-parameter model.

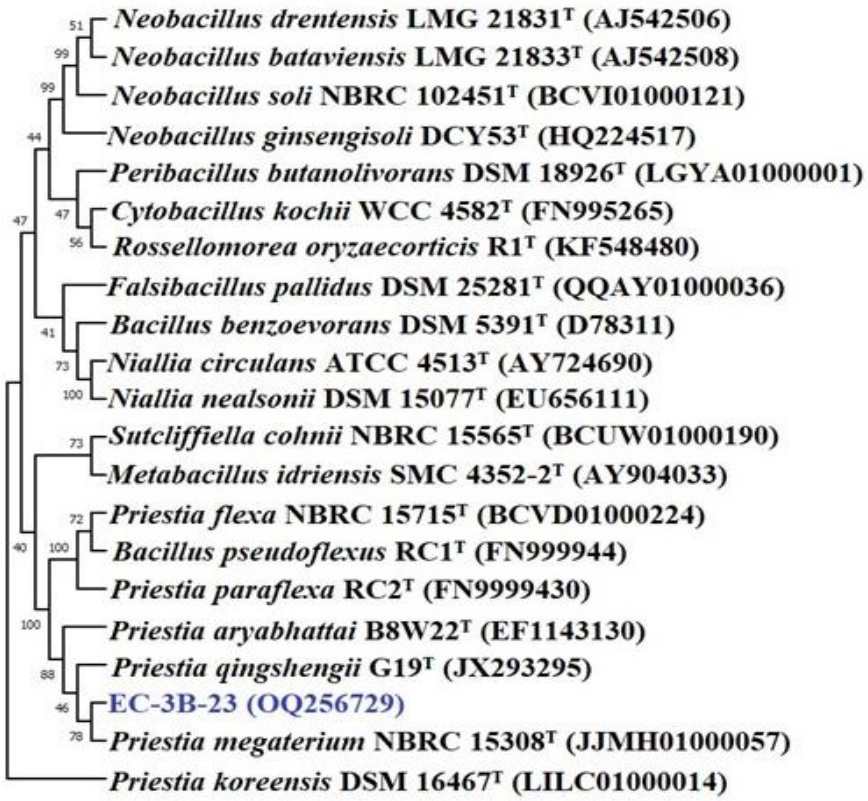


Figure 4.15: Maximum likelihood approach used to reconstruct the phylogenetic tree of the isolate EC3B-23, *Priestia* sp. using the Kimura 2-parameter model.

Table 4.6: The bacterial isolates' accession numbers and identities of sequences (differences/total nucleotides) reveal their closest association with specific species.

S.No.	Bacterial Isolates	Accession numbers	Identities of sequences (Diff./total nt)	Most closely associated with
1.	EC3B-22	OQ352598	99.85% (2/1318)	*Pseudomonas bijieensis L22-9^T*
2.	EC3B-23	OQ256729	99.51% (7/1440)	*Priestia megaterium NBRC 15308^T*

Additionally, the influence of diverse sources of nitrogen and carbohydrates on the development and growth of bacterial endophytes were assessed. In terms of nitrogen sources, both EC3B-22 and EC3B-23 demonstrate good utilization of peptone, yeast extract, beef extract, ammonium bromide, ammonium persulphate, ammonium

bicarbonate, ammonium molybdate, ammonium dichromate, and ammonium ferrous sulphate. However, EC3B-22 shows limited utilization of ammonium acetate, while EC3B-23 demonstrates moderate utilization. Both isolates also do not utilize ammonium thiocyanate and ammonium vanadate. Regarding carbon sources, both isolates exhibit good utilization of glucose, fructose, inositol, malt extract, sorbitol, and mannitol. EC3B-22 shows limited utilization of lactose, maltose, galactose, arabinose, rhamnose, sucrose, mannose, and dextrin. On the other hand, EC3B-23 shows moderate utilization of lactose and maltose but does not utilize galactose, arabinose, rhamnose, ribose, trehalose, sucrose, mannose, and dextrin. (Table 4.7)

Table 4.7: Utilization and growth of endophytic bacteria on varied carbon, and nitrogen sources

Different nitrogen and carbon sources	Bacterial isolate	
	EC3B-22	EC3B-23
Nitrogen sources		
Peptone	+++	+++
Yeast Extract	+++	++
Beef Extract	+++	+++
Ammonium Bromide	+++	+++
Ammonium Thiocyanate	-	-
Ammonium Persulphate	+++	+++

Ammonium Vanadate	**-**	**-**
Ammonium Bicarbonate	**+++**	**+++**
Ammonium Acetate	**++**	**+++**
Ammonium Molybdate	**+++**	**+++**
Ammonium Dichromate	**+++**	**+++**
Ammonium Ferrous Sulphate	**+++**	**+++**
Carbon sources		
Glucose	**+++**	**+++**
Fructose	**+++**	**+++**
Lactose	**+**	**+++**
Inositol	**+++**	**+++**
Maltose	**+**	**+++**
Malt extract	**+++**	**+++**
Sorbitol	**+++**	**+++**
Mannitol	**+++**	**+++**
Galactose	**+++**	**-**
Arabinose	**+++**	**-**
Rhamnose	**+**	**-**
Ribose	**-**	**-**
Trehalose	**-**	**-**
Sucrose	**+++**	**-**
Mannose	**+++**	**-**
Dextrin	**+**	**-**

4.7. Seed germination

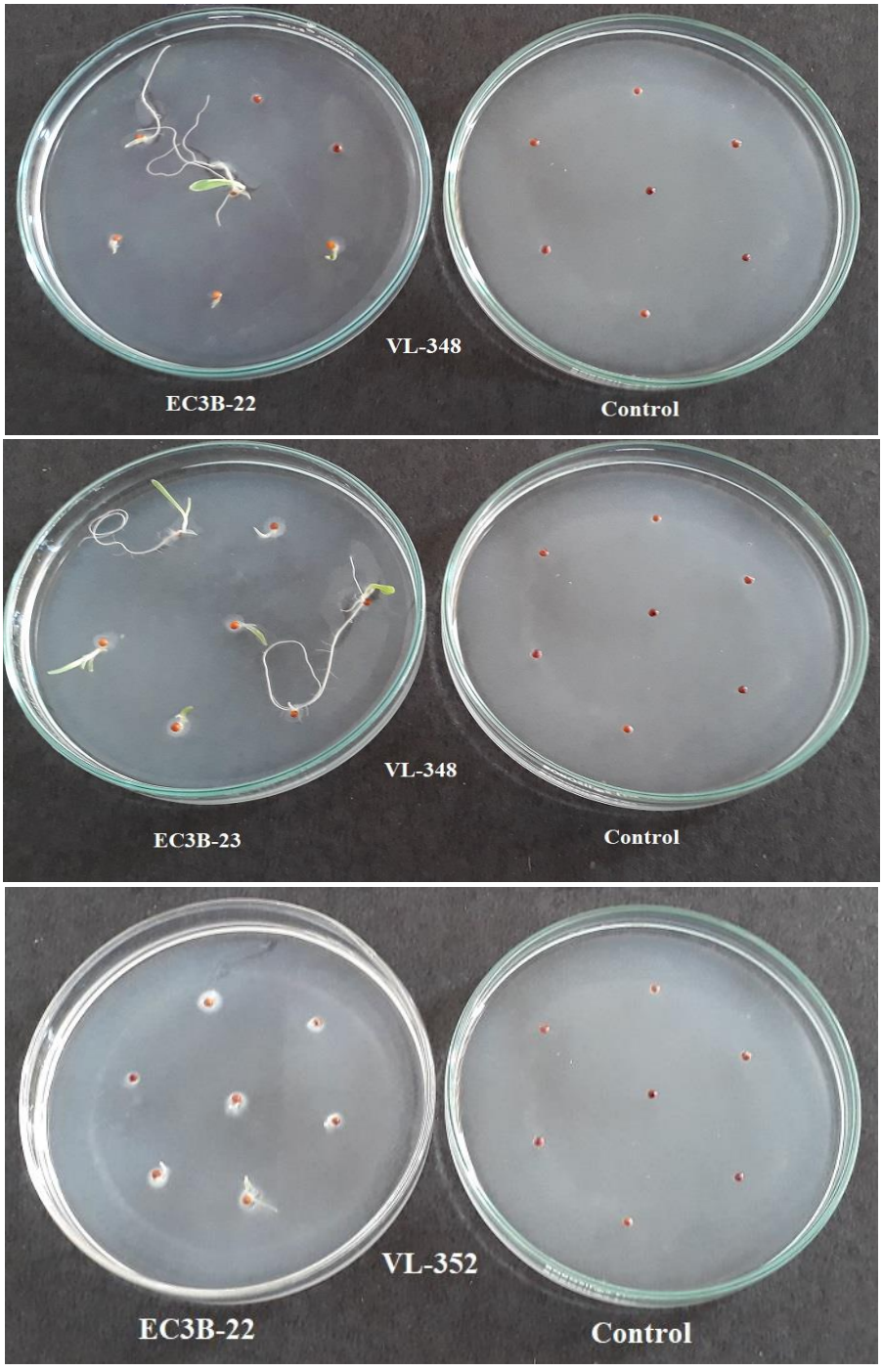
To study the effect of selected bacterial endophytes on seed germination under laboratory conditions was observed by inoculating the sterilized finger millet seeds with bacterial cells (10^{-9}/ml). The control set was hydro primed for 50 mins with distilled sterile water. After inoculation, the Petri plates were incubated in plant growth chamber for 4 days, percent germination and length of radical and plumule was observed. The treated seed of variety VL-348 with treatment B2 showed the highest radical growth (10.3 cm) and plumule length (5.1 cm). Similarly in variety, VL-352 maximum radical and plumule length was observed in treatment B2 i.e., 6.8 and 3.2 cm respectively. Similar pattern was observed in variety PRM-1. Maximum germination was observed

in variety VL-348 (100%) with treatment B2, and in variety VL-352 (100%) with treatment B1. Germination percentage, radical, and plumule length in showed in table 4.8.

Even after 4 days the hydro primed seeds did not show germination. The results obviously indicated that biopriming of finger millet seeds with selected endophytes resulted in early germination as compared to the hydro primed control treatment (Figure 4.16).

Table 4.8: Effect of endophytes on seed germination percentage, plumule and radial length

Cultivar	**PRM-1**		**VL-348**		**VL-352**	
Treatment	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**
Germination percentage	57	71	86	100	100	86
Plumule length (cm)	1.1	1.7	2.5	5.1	2.0	3.2
Radical length (cm)	2.6	2.8	8.7	10.3	3.5	6.8


VL-348
EC3B-22
Control
VL-348
EC3B-23
Control
VL-352
EC3B-22
Control

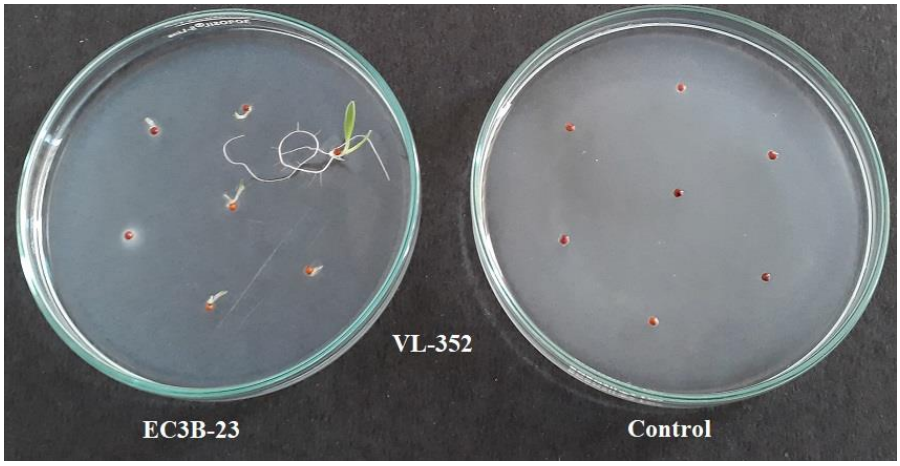


Figure 4.16: Effect of iron and zinc solubilizing endophytes on finger millet seed germination (PRM-1, VL-352, VL-348)

4.8. Pot experiment for Plant growth and micronutrient (Fe/Zn) solubilization and accumulation

Two bacterial endophytes were selected for a plant growth and micronutrient (Fe and Zn) absorption experiment based on their capacity to solubilize iron and zinc. Selected bacterial endophytes exhibited the ability to synthesize a range of extracellular enzymes, phosphate solubilization, phytohormones, and siderophores. The finger millet plants that had been treated with bacterial endophytes under pot experiment conditions were harvested 90 days after being inoculated.

4.8.1. Plant growth parameters

Inoculation of bacterial endophytes influenced the length of the shoot and roots. Results of a pot trial in a plant growth experiment displayed that all endophytic bacterial isolates ominously augmented the shoot and root and constraints (dry biomass, and length of shoot-root) as compared to uninoculated plants. After inoculation with the endophytic bacterial strain EC3B-23 (*Priestia megaterium*), a substantial increase in plant height and root length was seen in cultivar VL-352. The maximum shoot and root dry biomass, respectively, were obtained by finger millet cultivars VL-348 and VL-352 treated with the EC3B-23. With regards to plant height and root length, all FM cultivars of exhibited increased length of shoot and root as compared to control. In the PRM1 variety, the bacterial isolate EC3B-23 resulted in maximum plant length i.e., 66.8 cm which is higher than EC3B-22 i.e., 65.1 cm. Similarly, the application of both bacterial endophytes enhanced root length more favourably than the control treatments (20.1, and 19.8 cm, respectively). The same pattern was recorded in variety VL-348, where EC3B-23 showed the highest plant height (67.2 cm), then by EC3B-22 (64.8 cm), which was greater than the control treatment (54.4 cm) and zinc carbonate alone treatment (54.9 cm). The maximum root length exhibited by EC3B - 23 and EC3B - 22 bacterial inoculated treatments i.e., 21.1 cm and 19.5 cm, that was greater than the uninoculated

treatments (13.4 cm) and zinc carbonate treatment (17.3 cm). Stimulatingly, cultivar VL-352 also showed similar trend, with the maximum plant heights, 68.3 and 66.7 cm, respectively, being displayed by endophytes EC3B-23 and EC3B-22. Contrasted with the control (53.3 cm), bacterial inoculated VL-352 plants exhibited higher plant height. Similar to this maximum root length was showed by endophytic isolate EC3B-23 (22.3 cm) followed by EC3B-22 (20.4 cm), both were more than control (14.1cm) and zinc carbonate treatment (18.2cm). (Figure 4.17). In contrast to the uninoculated control, a considerable enhancement in plant height and root length was seen after the inoculation of endophytic bacteria EC3B-23.

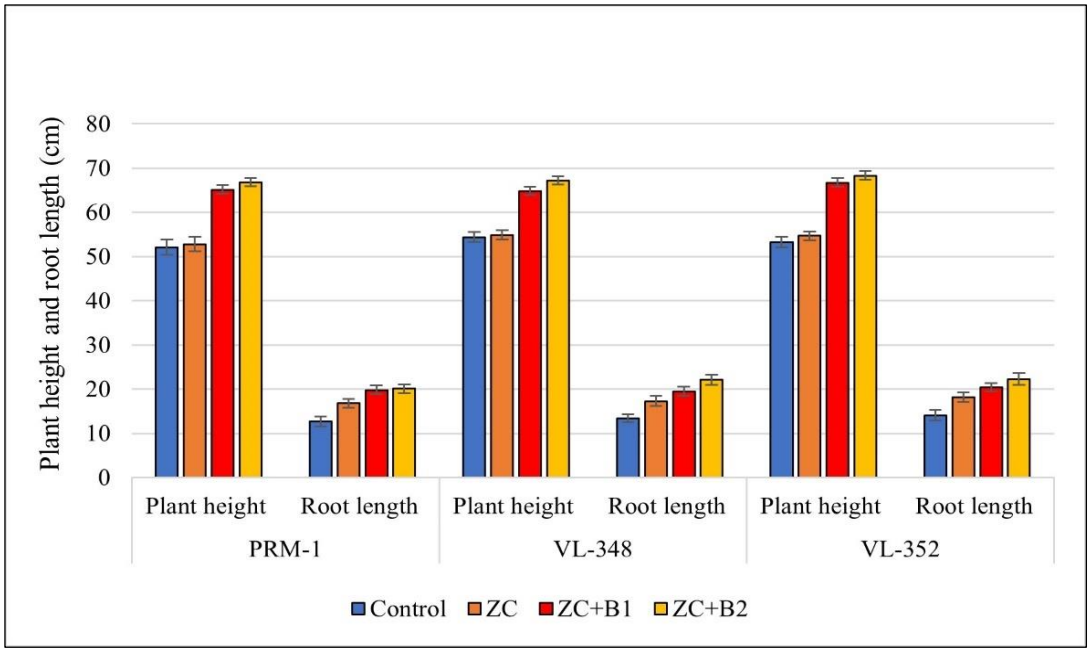


Figure 4.17: Impact of endophytic inoculation on plant height and root length of FM cultivars (VL-352, PRM-1, and VL-348), error bars denote the mean value ± SD of the experiment conducted in triplicates (control- uninoculated seeds, ZC- zinc carbonate, B1- EC3B-22; *Pseudomonas bijieensis*, B2- EC3B-23; *Priestia megaterium*)

Regarding the dry weight of the plant and the root, in finger millet cultivar PRM-1 the maximum plant dry weights was observed after the inoculation of EC3B-23 (*Priestia megaterium*) followed by EC3B-22 (*Pseudomonas bijieensis*) treatments, respectively, of 21.2 g and 20.7 g, which was more than control and zinc carbonate treatment (15.9 g and 16.5 g). In terms of root dry weight, the similar pattern was seen, with EC3B-23 exhibiting the maximum root dry mass (6.31 g) followed by EC3B-22 (5.29 g). Similar findings were seen with the bacterial treatments EC3B-23 and EC3B-22 in cultivars VL-348 and VL-352, as compared to the uninoculated plants. (Figure 4.18).

Compared to the uninoculated control treatment, the finger millet plants that had received endophytic inoculation showed considerably better growth traits.

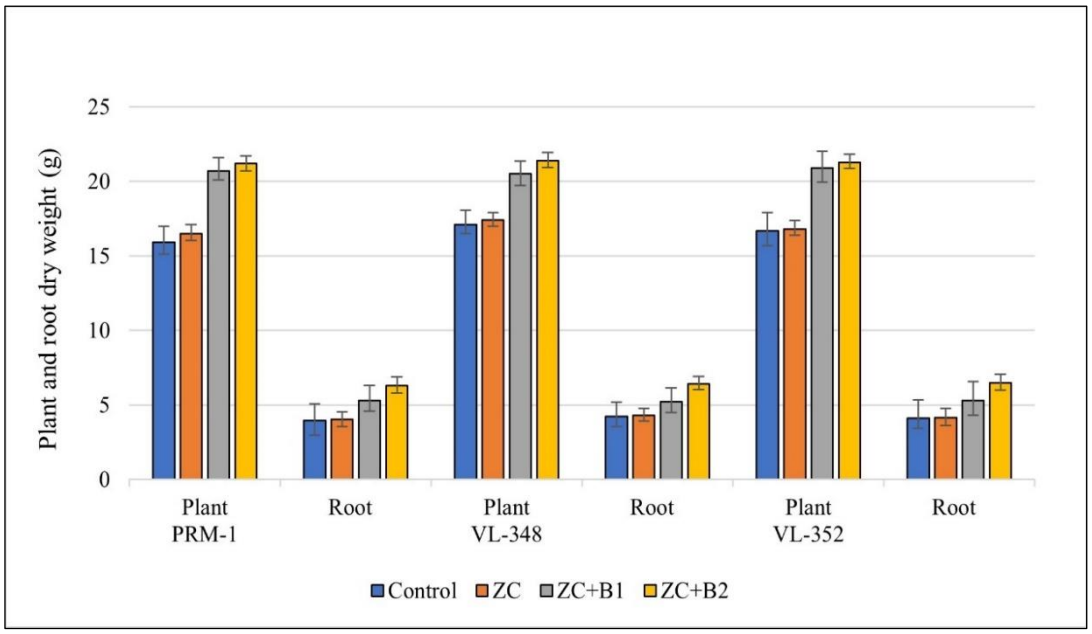


Figure 4.18: Impact of endophytic inoculation on plant and root dry weight of FM cultivars (VL-352, PRM-1, and VL-348), error bars denote the mean value ± SD of the experiment conducted in triplicates (control- uninoculated seeds, ZC- zinc carbonate, B1- EC3B-22; *Pseudomonas bijieensis,* B2- EC3B-23; *Priestia megaterium*).

4.8.2. Zinc content in finger millet grains

Endophytic inoculation also showed an influence on the zinc concentration of grains as compared to control treatment. The bacterial endophytes primed treatments showed maximum zinc content. Treatment EC3B-23 in cultivar PRM-1 exhibited highest zinc (2.73 mg) content, and it was 18.8% higher than the control. On the other hand, Zn content of 2.66 mg in PRM-1 was observed when treated with endophyte EC3B-22, which was 15.15% greater than control. The highest Zn content in variety VL-348 was found in the EC3B-23 treatment, which was 15.35% more than control. The next-highest grain zinc content was found at 2.69 mg in the EC3B-22 inoculated treatment, which was 11.61% more than the control. In finger millet cultivar VL-352, bacteria EC3B-22 showed the highest zinc concentration i.e., 2.75 mg, compared to the control, which was 16.03% higher. The remaining microbial treatment revealed grains with zinc contents that were 13.5% more than the uninoculated treatment (Figure 4.19). Comparing the uninoculated treatments, among all three varieties VL-348 and VL-352 showed considerably higher Zn concentration in grains.

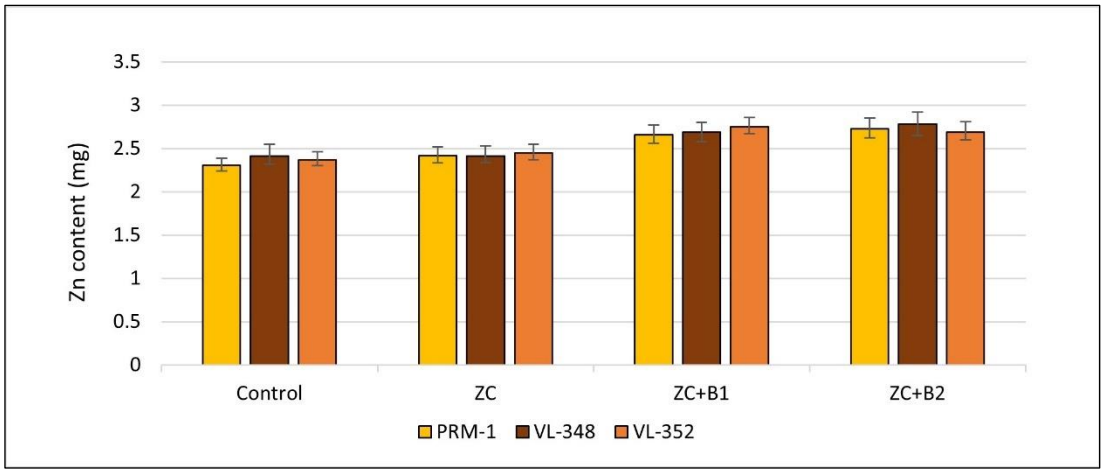


Figure 4.19: Impact of endophytic inoculation on zinc content of FM grains (VL-348, VL-352, and PRM-1,), error bars indicate the mean value ± SD of the experiment conducted in triplicates (control- uninoculated seeds, ZC- zinc carbonate, B1- EC3B-22; *Pseudomonas bijieensis*, B2- EC3B-23; *Priestia megaterium*)

4.8.3. Zinc content in finger millet shoot and root

Endophytic inoculation had an impact on the zinc content of the root and shoot as well; contrary to uninoculated group, endophytic treated plants exhibited higher zinc content. In cultivar PRM-1, the treatment EC3B-23 showed the highest zinc content in shoot and root i.e., 2.51 mg and 2.42 mg respectively, which is 16.7 % and 15.7 % higher as compared to control. Shoot and root zinc content increased to 2.45 mg and 2.37 respectively, after the endophytic bacteria EC3B-22 were inoculated, which was 13.9% and 13.3% higher in comparison to uninoculated control and zinc carbonate treatment. Similar to this, in variety VL-348 bacterial isolate EC3B-23 showed maximum zinc content (16.5%; shoot, and 17.06%; root) as compared to control followed by treatment EC3B-22 (11.4%; shoot, and 11.8%; root). In variety VL-352 higher content was

observed by the inoculation of EC3B-22 i.e., for shoot 16.2 % and for root 17.3 % as compared to control (Figure 4.20).

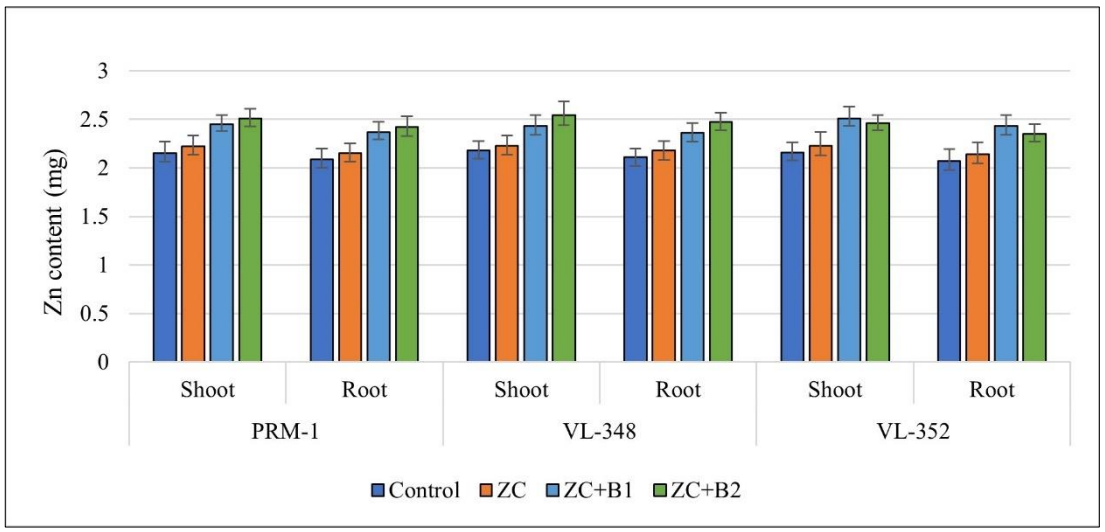


Figure 4.20: Impact of endophytic inoculation on zinc content of finger millet shoot and root (VL-348, VL-352, and PRM-1,), error bars indicate the mean value ± SD of the experiment conducted in triplicates (control- uninoculated seeds, ZC- zinc carbonate, B1, EC3B-22; *Pseudomonas bijieensis*, B2, EC3B-23; *Priestia megaterium*)

Analysis of the Zn concentration in finger-millet showed that plants absorbed Zn in its available forms through their roots, and that biopriming of plants with endophytes having potential of Zn/Fe mobilization and possess attributes to stimulate plant growth can help to expediate the transportation of zinc from the roots to the shoots. The colonisation of the plants by these effective endophytes is crucial for the mobilisation of zinc from the root to the grain via the shoot.

4.8.4. Iron content in finger millet grains

Endophyte inoculations also affected the iron concentration in finger millet. Among all varieties, maximum iron content was recorded in VL-348 after the inoculation of EC3B-23 with (4.5 %) followed by PRM-1 (6.1 %), and VL-352 (6.4%) more as compared to

control. In variety PRM-1, bacterial endophyte EC3B-23 showed maximum iron content (3.63 mg; 6.14%), followed by EC3B-22 (3.59 mg; 4.97%), as compared to control treatment (3.42 mg). Similarly, in variety VL-348, bacterial isolate EC3B-23 showed iron enhancement i.e., 3.65 mg followed by EC3B-22 with iron concentration 3.62 mg which was higher than control and ferric phosphate treatments (3.49 mg and 3.48 mg respectively) Similar pattern was observed in variety VL-352, among both bacterial isolates, EC3B-23 showed maximum enhanced iron concentration (3.62 mg; 6.47%), as compared to control followed by EC3B-22 (3.59 mg) (Figure 4.21).

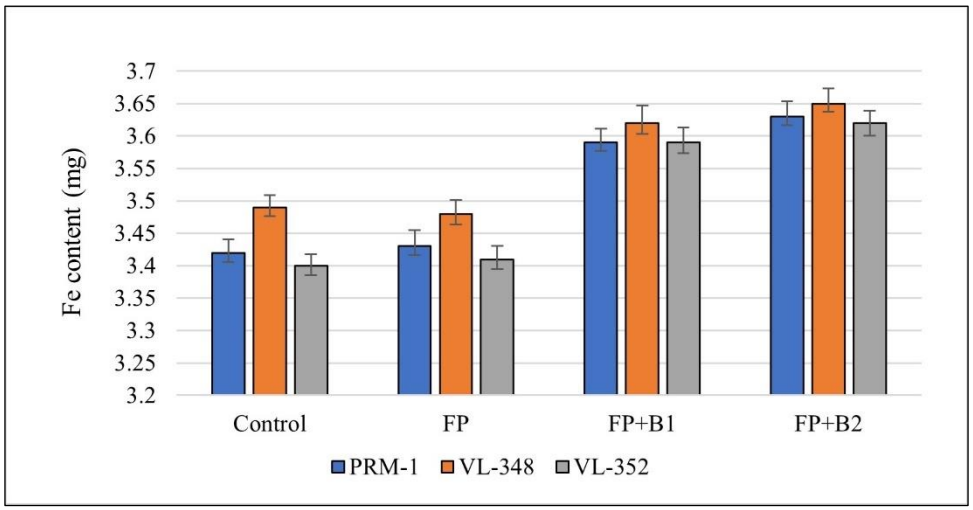


Figure 4.21: Impact of endophytic inoculation on iron content of FM grains (VL-348, VL-352, and PRM-1), error bars indicate the mean value ± SD of the experiment conducted in triplicates (control- uninoculated seeds, FP- ferric phosphate, B1- EC3B-22; *Pseudomonas bijieensis,* B2- EC3B-23; *Priestia megaterium*)

4.8.5. Iron content in finger millet shoot and root

According to our findings, endophyte inoculation enhanced the Fe content of shoots, and roots in all three finger millet varieties. In case of PRM-1 bacterial isolate EC3B-23 showed maximum iron content in shoot i.e., 2.43 mg. Other isolate (EC3B-22; 2.39 mg) were also found to be effective inoculant in the enhancement of iron content in shoot as compared to control treatment (2.22 mg). Along with shoots, endophytes also increased the iron content in the roots., and maximum iron content was recorded was 2.35 mg (EC3B-23). Similar to this, in variety VL-348 bacterial isolate EC3B-23 resulted in enhanced and maximum increased shoot and root iron content (2.42 mg and 2.39 mg); EC3B-22 was also helpful in increased shoot and root iron content (2.39 mg and 2.31 mg) in comparison to control (2.2 mg; shoot and 2.12 mg; root) and ferric phosphate alone treatment (2.21 mg; shoot and 2.13 mg; root). Similar pattern was observed in variety VL-352, bacterial endophyte EC3B-23 (shoot; 2.45 mg and root; 2.4 mg) contributed in maximum iron content enhancement, which is higher among all three variety and all sets of treatments (Figure 4.22).

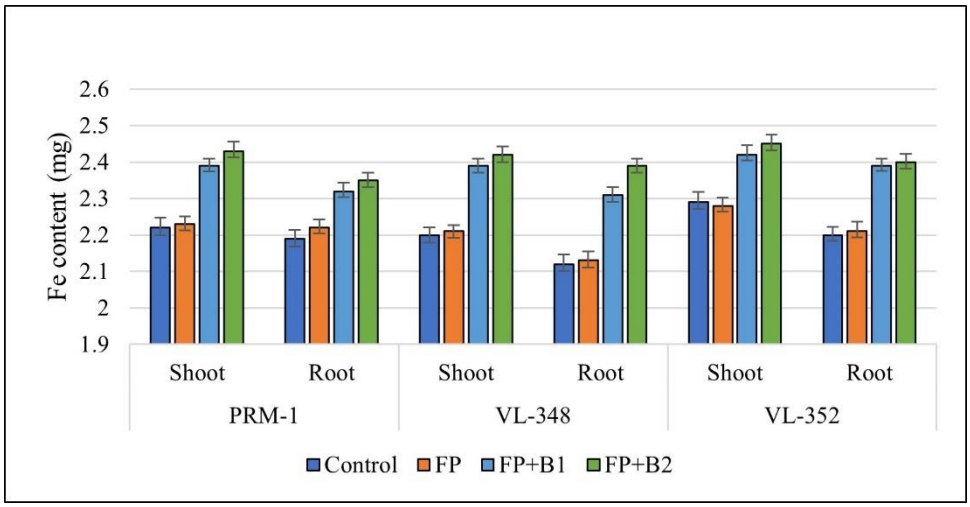


Figure 4.22: Impact of endophytic inoculation on iron content of finger millet shoot and root (VL-348, VL-352, and PRM-1), error bars indicate the mean value ± SD of the experiment conducted in triplicates (control- uninoculated seeds, FP- ferric

phosphate, B1-EC3B-22; *Pseudomonas bijieensis,* B2-EC3B-23; *Priestia megaterium.*

4.8.6. NPK content in finger millet grains

Endophytic inoculation substantially influenced the NPK contents in grains when compared to the control treatment; the inoculated plants showed increased content in all three finger millet cultivars. Maximum NPK content was showed in cultivar VL-348, in treatment ZC+B2 (EC3B-23; (*Priestia megaterium*) i.e., 1.36, 0.251, 0.642 mg; higher than the ZC+B1 (EC3B-22; *Pseudomonas bijieensis*), ZC, and uninoculated control treatment. Endophytic isolates; also enhanced the NPK content in other finger millet cultivars; PRM-1, and VL-352 (Figure 4.23).

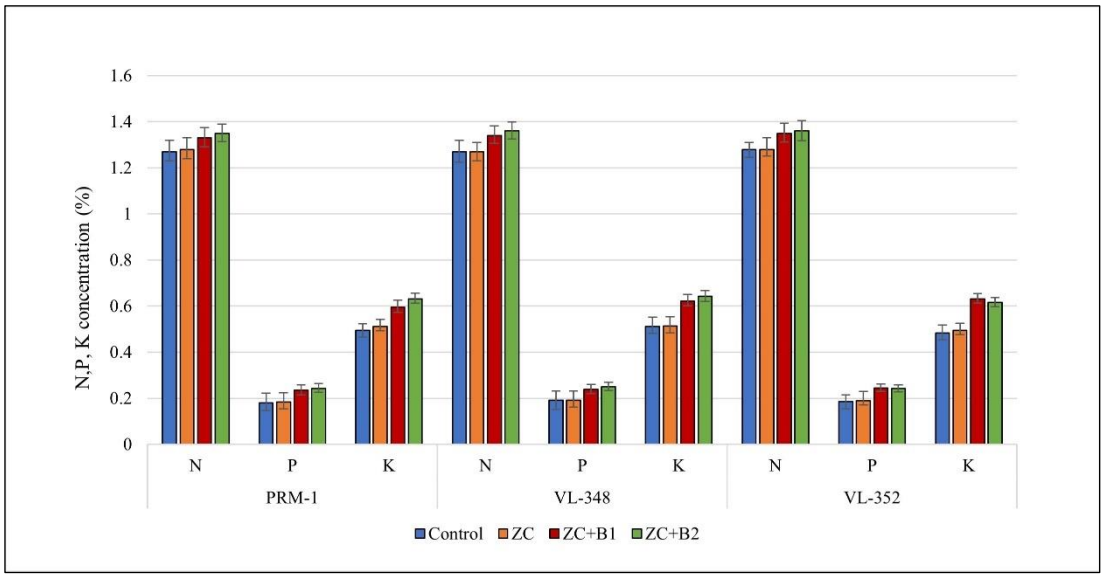


Figure 4.23: Impact of endophytic inoculation on NPK content of FM grains (VL-348, VL-352, and PRM-1), error bars represent the mean value ± SD of the experiment conducted in triplicates (control- uninoculated seeds, ZC- zinc carbonate, B1- EC3B-22; *Pseudomonas bijieensis,* B2- EC3B-23; *Priestia megaterium*).