

Original Research Article

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Isolation, Screening and Characterisation of Plant Growth-Promoting Endophytic Bacteria from Weed Species

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ABSTRACT

With the intervention of green revolution many of the high yielding varieties, pesticides and fertilizers were introduced in agriculture which resulted in gradual replacement of traditional agricultural practices to chemical farming but, the chemical farming not only deteriorated the soil health. In order to overcome the effects of the chemical farming, the biological means of crop production is gaining lot of importance. Among the biological means, the endophytic microorganisms are a class of microbes that live in internal plant tissues of apparently healthy host plant parts like roots, stems, leaves and seeds which play an important role in growth and development of the crop by nutrient solubilization, biological control and production of phytohormones. So far, the crop endophytes have been exploited more but, the weed endophytic bacteria are not concentrated much in crop production. Hence, there is a need to give attention on the use of the weed endophytic bacteria in agriculture. In an attempt to isolate, screen and characterize the plant growth promoting endophytic bacteria from roots and stems of selected weed species which are grown in cultivated areas of Shivamogga district, Karnataka, India. Out of 5 weed species, as many as twenty seven endophytic bacterial isolates were obtained and assessed for their plant growth promotional activities viz., phosphorus, potassium, zinc and silicon solubilization. Among 27 endophytic bacterial isolates tested, four isolates viz., SENDO – 5, SENDO – 6, SENDO – 17 and SENDO – 27 were efficient in phosphorus, potassium, zinc and silicon solubilization ability. Further, based on morphological and biochemical characters the efficient endophytic bacterial isolates were tentatively identified as *Achromobacter* and *Citrobacter* species. Scaleup studies are required to develop and evaluate the different formulations of the efficient endophytic bacterial isolates on different crops for their sustainable production.

Keywords

Isolation, screening, characterization, endophytic bacteria, nutrient solubilization

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Introduction

Soils are living systems where organic and inorganic fractions interact to sustain diverse microbial communities and regulate nutrient availability. Many root-associated microorganisms mobilise nutrients locked in insoluble forms, thereby increasing plant growth and soil fertility (Ledin *et al.*, 1996).

The Green Revolution boosted crop yields but also introduced intensive chemical inputs. Over time, this has degraded soil health and allowed pesticide and fertiliser residues to accumulate in the food chain, weakening human immunity and contributing to chronic diseases. Consequently, biological approaches to crop production are receiving renewed attention.

Microorganisms act as soil engineers, supporting nutrient cycling and suppressing pests and diseases. Nitrogen fixers such as *Azotobacter*, *Rhizobium* and *Azospirillum* along with phosphate and potassium solubilising bacteria, are widely used as biofertilizers. However, endophytes from weeds are largely untapped reservoir remain neglected despite their potential resilience. This study aimed to isolate, screen and characterise plant growth promoting endophytic bacteria from weeds.

Material and Methods

Sample Collection

The roots and stems of five weed species *viz.*, *Parthenium hysterophorus*, *Chromolaena odorata*, *Celosia argentea*, *Lantana camara* and *Tephrosia purpurea* were collected aseptically from cultivated fields in Shivamogga district, India and the samples were brought to the laboratory under aseptic conditions in sterile polythene bags and stored at 4 °C for further isolation of endophytic bacteria.

Isolation of Endophytic Bacteria

The bacterial endophytes were isolated from root and stem of the healthy weed plants collected from different regions of Shivamogga district. The isolation was done from the plants immediately after collection by two methods.

First method involved cutting root and stem samples into 2 halves and each half was imprinted on nutrient agar

plates and incubated at room temperature for 24 hours.

In the second method, the root and shoot samples were macerated in 10 ml of distilled water using sterile pestle and mortar. For each macerated sample, that is root and stem serial dilutions were made, up to 10⁻⁵ dilutions. One hundred micro litres from each dilution of the respective sample were transferred to the sterilized petri plates labelled from 10⁻¹ to 10⁻⁵ and the cooled nutrient agar medium was poured and then plates were rotated in clockwise and anti-clockwise direction for uniform distribution of the suspension and allowed for solidification. Then, the plates were incubated at 28 °C for 24 to 72 hrs for isolation of bacterial endophytes. Further, the isolates were purified by streaking and maintained as Sahyadri Endophytic Bacterial (SENDO) isolates.

In vitro Screening of Endophytic Bacteria for their Plant Growth Promotional Activities

The pure cultures of isolated native endophytic bacterial isolates were screened for the plant growth promotional traits *viz.*, phosphorus, potassium, silicon and zinc solubilization using specific media like Sperber's, Aleksandrow's, Bunt and Rovira's with Mg₂Si₃O₈ and mineral salt media supplemented with 1 per cent zinc oxide respectively and the nutrient solubilization efficiency was calculated based on per cent solubilization index of P, K, Si and Zn using the formula as given by Pande *et al.*, 2017.

$$\text{Nutrient Solubilization Index} = \frac{\text{Halo zone diameter} + \text{Colony diameter}}{\text{Colony diameter}}$$

Selection and Characterisation of Efficient Isolates

The isolates showing superior performance in all the plant growth promotion traits were selected for further characterization studies. The colony morphology (size, shape, pigmentation, elevation) (Anon, 1957) and Gram staining (Rangaswami, 1975) and biochemical tests *viz.*, catalase, nitrate reduction, citrate utilisation, indole, Vogor Proskauer, methyl red, urease, casein and starch hydrolysis, gelatin liquefaction, acid/gas production, oxidase were done using standard protocols and the efficient isolates were identified tentatively up to genus level (Barthalomew and Mittewer, 1950).

Statistical Analysis

The statistical analysis of the data was carried out for completely randomized design (Panse and Sukhatme, 1985) as well as for Dunkun's multiple range test (Steel and Torrie, 1960).

Results and Discussion

Sample collection

A total of 50 root and stem samples were collected and was used for isolation of endophytic bacteria. The details of the locations and weed plant samples collected are furnished in Table 1.

Isolation of endophytes

The bacterial endophytes were isolated from root and stem of weed plants which were collected from cultivated areas of Shivamogga district by following two methods, root and stem imprinting and serial dilution pour plate method. As many as 27 endophytic bacterial isolates were obtained from surface sterilized roots and stem portions of the five weed species (Table 2 and Plate 1). Further for easy identification all the bacterial isolates were named tentatively as Sahyadri Endophytic bacteria (SENDO).

Weed plants, often overlooked in microbial ecology, have been reported as reservoirs of diverse microbial endophytes with functional traits relevant to agriculture (Strobel and Daisy, 2003; Hardoim *et al.*, 2015). The successful recovery of multiple isolates in this study confirms the earlier findings that weeds harbour diverse and metabolically active bacterial populations capable of colonizing host tissues (Kumar *et al.*, 2023).

Screening of endophytic bacteria for plant growth promotional traits

In the *In vitro* screening studies, out of the twenty-seven endophytic bacterial isolates tested for plant growth promotional activities revealed the patentability of isolated endophytes to solubilize nutrients such as phosphorous, potassium, zinc and silicon (Table 3).

Among the isolated endophytic bacteria, eleven strains exhibited the phosphorus solubilizing ability and recorded phosphorus solubilisation indices (PSI) ranging

from 1.31 to 3.45, and the efficient endophyte SENDO – 27 resulted the highest phosphorus solubilization index value of 3.45 (Table 4 and Plate 2). Similarly, twelve isolates showed potassium solubilization and the potassium solubilization index (KSI) ranged between 1.31 and 3.42, However, among the potassium solubilizing endophytic bacteria the isolate SENDO – 17 solubilized maximum of potassium on Aleksandrow's media containing mica (Table 5 and Plate 3). With respect to zinc solubilisation, fifteen isolates, showed with zinc solubilisation indices (ZnSI) ranging from 1.30 to 3.24 and again the SENDO – 27 resulted the highest zinc solubilizing ability of 3.24 ZnSI (Table 6 and Plate 4). On the other hand, only five isolates performed the silicon solubilization, with silicon solubilization indices ranging from 1.38 to 2.96, and the SENDO – 6 exhibited the highest silicon solubilization potentiality (Table 7 and Plate 5). Finally, based on the combined performance across all four nutrient solubilization ability, four isolates *i.e.*, SENDO – 5 and SENDO – 6 from *Parthenium hysterophorus*, SENDO – 17 from *Lantana camara* and SENDO – 27 from *Tephrosia purpurea* were selected for further characterization studies.

The findings are in line with earlier reports by Sharma *et al.* (2013), who highlighted that phosphate solubilizing bacteria (PSB) mobilize insoluble phosphate primarily through the secretion of low molecular weight organic acids, which lower the pH of the surrounding medium and chelate metal ions such as Ca, Fe and Al, thereby liberating soluble orthophosphate and the study thus authenticate previous research while emphasizing the ecological and agronomic significance of microbial diversity in phosphate solubilization. Harnessing efficient isolates such as SENDO – 27 may provide a sustainable alternative to chemical phosphorus fertilizers by improving phosphorus use efficiency and promoting crop productivity.

With respect to potassium solubilization, the study verifies the earlier findings, where potassium solubilizing bacteria (KSB) belonging to genera such as *Bacillus*, *Pseudomonas* and *Enterobacter* significantly enhanced K availability in soils and improved nutrient uptake in cereals (Liu *et al.*, 2012).

The superior performance of isolate SENDO – 17 highlights its potential as an efficient bioinoculant, capable of mobilizing otherwise unavailable soil potassium, thereby contributing to nutrient cycling and reducing dependence on chemical fertilizers.

In the present investigation, fifteen endophytic bacterial isolates showed the ability to solubilize zinc, and among which SENDO – 27 recorded the highest solubilization potential.

The findings are in agreement with earlier reports where endophytic bacteria were shown to enhance zinc bioavailability through the secretion of organic acids and siderophores that mobilize Zn^{2+} from insoluble complexes (Singh *et al.*, 2022).

The variability observed among isolates may be linked to differences in their metabolic capabilities to secrete organic acids, or chelating metabolites. Such zinc

solubilizing endophytes are particularly relevant in cereal crops, as they not only improve plant Zn uptake but also contribute to grain biofortification, which is crucial for addressing Zn malnutrition in human populations (Sultana *et al.*, 2021). In addition to zinc, the present study also revealed that only five isolates were capable of solubilizing silicon and among them SENDO – 6 showed the maximum solubilization index. The ability of endophytic bacteria to solubilize silicon has been reported previously, where microbial activity facilitated the release of plant available monosilicic acid, thereby strengthening plant defence responses and enhancing growth under stress prone conditions (Mehboob *et al.*, 2022).

Table.1 Collection of samples from cultivated areas of Shivamogga district

Sl. No.	Areas	Weeds
1.	Horenahalli	<i>Parthenium hysterophorus</i>
2.	Tumbri	<i>Tephrosia purpurea</i>
3.	Arodi	<i>Chromolaena odorata</i>
4.	Ammanghatta	<i>Celosia argentea</i>
5.	Koduru	<i>Lantana camara</i>
6.	Chamundipura	<i>Celosia argentea</i> and <i>Parthenium hysterophorus</i>
7.	Thirthahalli	<i>Chromolaena odorata</i> and <i>Tephrosia purpurea</i>
8.	Seebinekere	<i>Parthenium hysterophorus</i>
9.	Yoginarasipura	<i>Celosia argentea</i>
10.	Soppugudde	<i>Lantana camara</i>
11.	Mattur	<i>Tephrosia purpurea</i> and <i>Lantana camara</i>
12.	Talale	<i>Lantana camara</i>
13.	Hosmane	<i>Celosia argentea</i>
14.	Thimkapura	<i>Parthenium hysterophorus</i>
15.	Kudugalamane	<i>Tephrosia purpurea</i>
16.	Megaravalli	<i>Chromolaena odorata</i> and <i>Lantana camara</i>

Table.2 Endophytic bacterial isolates from weed plants

Sl. No.	Weed Plants	No. of Isolates	Isolates Code
1	<i>Parthenium hysterophorus</i>	10	SENDO 1 – SENDO 10
2	<i>Lantana camara</i>	7	SENDO 11 – SENDO 17
3	<i>Chromolaena odorata</i>	4	SENDO 18 – SENDO 21
4	<i>Celosia argentea</i>	3	SENDO 22 – SENDO 24
5	<i>Tephrosia purpurea</i>	3	SENDO 25 – SENDO 27

Note: SENDO – Sahyadri Endophytic Bacteria

Table.3 Plant growth promoting ability of isolated endophytic bacteria

Sl. No.	Isolates	Plant Growth Promotional activity			
		PS	KS	ZnS	SiS
1	SENDO – 1	-	-	-	-
2	SENDO – 2	-	+	-	+
3	SENDO – 3	-	-	+	+
4	SENDO – 4	-	-	+	-
5	SENDO – 5	+	+	-	-
6	SENDO – 6	+	+	-	+
7	SENDO – 7	+	+	+	-
8	SENDO – 8	-	-	+	-
9	SENDO – 9	-	-	-	-
10	SENDO – 10	+	-	+	-
11	SENDO – 11	-	-	+	-
12	SENDO – 12	-	-	+	-
13	SENDO – 13	-	-	+	-
14	SENDO – 14	+	+	+	-
15	SENDO – 15	+	+	+	-
16	SENDO – 16	-	-	-	-
17	SENDO – 17	+	+	-	+
18	SENDO – 18	-	-	+	+
19	SENDO – 19	-	-	+	-
20	SENDO – 20	-	-	+	-
21	SENDO – 21	+	+	-	-
22	SENDO – 22	-	-	-	-
23	SENDO – 23	-	+	-	-
24	SENDO – 24	-	+	-	-
25	SENDO – 25	+	+	-	-
26	SENDO – 26	+	-	+	-
27	SENDO – 27	+	+	+	+

Note:

+ = Positive, - = Negative

PS = Phosphorous solubilization, KS = Potassium solubilization, ZnS = Zinc solubilization, SiS = Silicon solubilization.

SENDO – Sahyadri Endophytic Bacteria

Table.4 Phosphorous solubilizing potentiality of endophytic bacterial isolates

Sl. No.	Isolates	PSI
1	SENDO – 5	2.73 ^c
2	SENDO – 6	2.92 ^b
3	SENDO – 7	1.62 ^g
4	SENDO – 10	1.48 ^h
5	SENDO – 14	1.31 ⁱ
6	SENDO – 15	1.91 ^e
7	SENDO – 17	2.15 ^d
8	SENDO – 21	1.24 ⁱ
9	SENDO – 25	1.68 ^{fg}
10	SENDO – 26	1.80 ^{ef}
11	SENDO – 27	3.45 ^a
	S. Em ±	0.042
	p	<0.01
	CV	3.630
	CD (1 %)	0.169

Note:

SENDO – Sahyadri Endophytic Bacteria

PSI – Phosphorous Solubilizing Index

Means followed by the same letter do not differ significantly

Table.5 Potassium solubilizing potentiality of endophytic bacterial isolates

Sl. No.	Isolates	KSI
1	SENDO – 2	1.80 ^f
2	SENDO – 5	2.80 ^d
3	SENDO – 6	2.88 ^c
4	SENDO – 7	1.87 ^e
5	SENDO – 14	1.42 ⁱ
6	SENDO – 15	1.67 ^g
7	SENDO – 17	3.42 ^a
8	SENDO – 21	1.70 ^g
9	SENDO – 23	1.34 ^j
10	SENDO – 24	1.31 ^j
11	SENDO – 25	1.51 ^h
12	SENDO – 27	2.99 ^b
	S. Em ±	0.012
	p	<0.01
	CV	0.981
	CD (1 %)	0.046

Note:

SENDO – Sahyadri Endophytic Bacteria

KSI – Potassium Solubilizing Index

Means followed by the same letter do not differ significantly

Table.6 Zinc solubilizing potentiality of endophytic bacterial isolates

Sl. No.	Isolates	ZnSI
1	SENDO – 3	1.96 ^{def}
2	SENDO – 4	1.52 ⁱ
3	SENDO – 7	1.85 ^{fg}
4	SENDO – 8	1.30 ^j
5	SENDO – 10	1.64 ^{hi}
6	SENDO – 11	1.73 ^{gh}
7	SENDO – 12	2.52 ^b
8	SENDO – 13	2.02 ^{de}
9	SENDO – 14	1.93 ^{ef}
10	SENDO – 15	2.42 ^b
11	SENDO – 18	2.21 ^c
12	SENDO – 19	2.08 ^d
13	SENDO – 20	2.29 ^c
14	SENDO – 26	1.56 ⁱ
15	SENDO – 27	3.24 ^a
	S. Em ±	0.043
	p	<0.01
	CV	3.668
	CD (1 %)	0.166

Note:

SENDO – Sahyadri Endophytic Bacteria

ZnSI – Zinc Solubilizing Index

Means followed by the same letter do not differ significantly

Table.7 Silicon solubilizing potentiality of endophytic bacterial isolates

Sl. No.	Isolates	SiSI
1	SENDO – 2	1.89 ^c
2	SENDO – 3	1.38 ^e
3	SENDO – 6	2.96 ^a
4	SENDO – 17	2.81 ^b
5	SENDO – 18	1.52 ^d
	S. Em ±	0.023
	p	<0.01
	CV	1.875
	CD (1 %)	0.102

Note:

SENDO – Sahyadri Endophytic Bacteria

SiSI – Silicon solubilizing index

Means followed by the same letter do not differ significantly

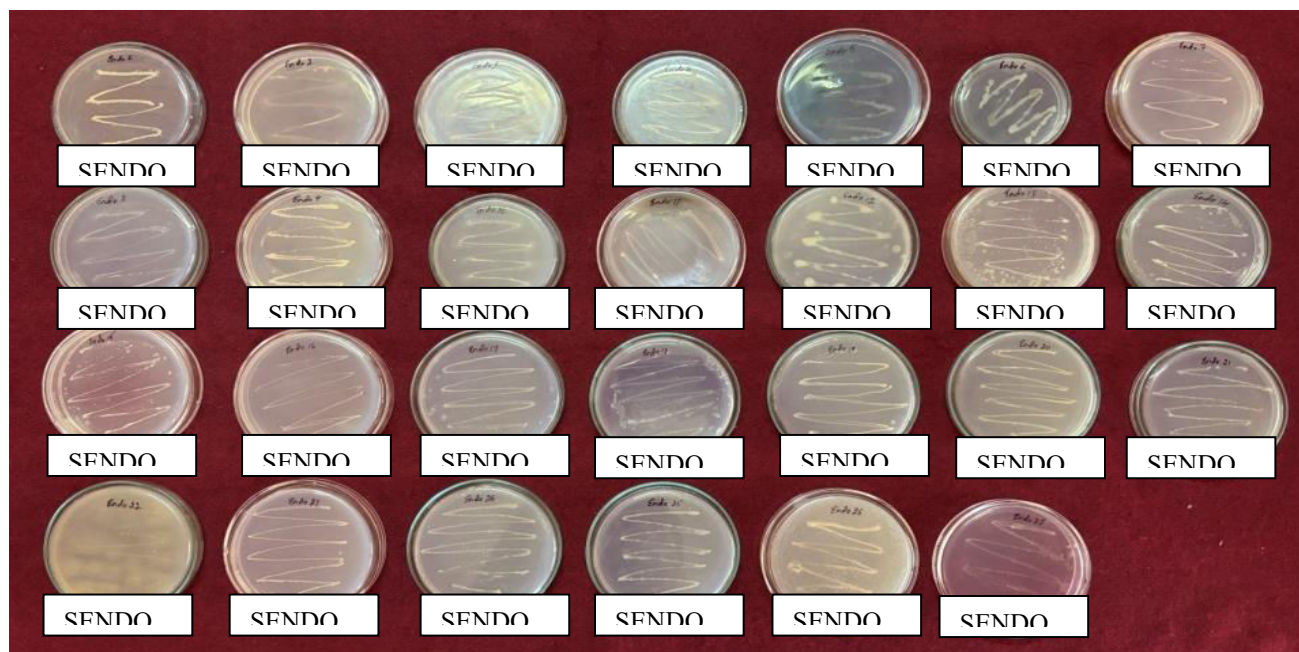
Table.8 Morphological and biochemical characterization of efficient endophytic bacterial isolates

Isolates	Morphological Test		Biochemical Test														Probable Genus
	Colony	Gram's Staining and Cell shape	CT	H ₂ S	IP	NR	MR	VP	CH	CU	UA	SH	GL	AP	GP	OT	
SENDO – 5	Medium circular, moist, smooth and convex	^{-ve} G Rod	+	+	-	+	+	-	-	+	+	-	+	+	+	-	<i>Citrobacter</i> sp.
SENDO – 6	Transparent creamy, smooth, opaque and convex	^{-ve} G Rod	+	-	-	+	-	-	+	+	-	-	+	-	-	+	<i>Achromobacter</i> sp.
SENDO – 17	Circular, moist, smooth and convex	^{-ve} G Rod	+	+	-	+	+	-	-	+	+	-	+	+	+	-	<i>Citrobacter</i> sp.
SENDO – 27	Circular, smooth and slightly raised	^{-ve} G Rod	+	-	-	+	-	-	+	+	-	-	+	-	-	+	<i>Achromobacter</i> sp.

Note:

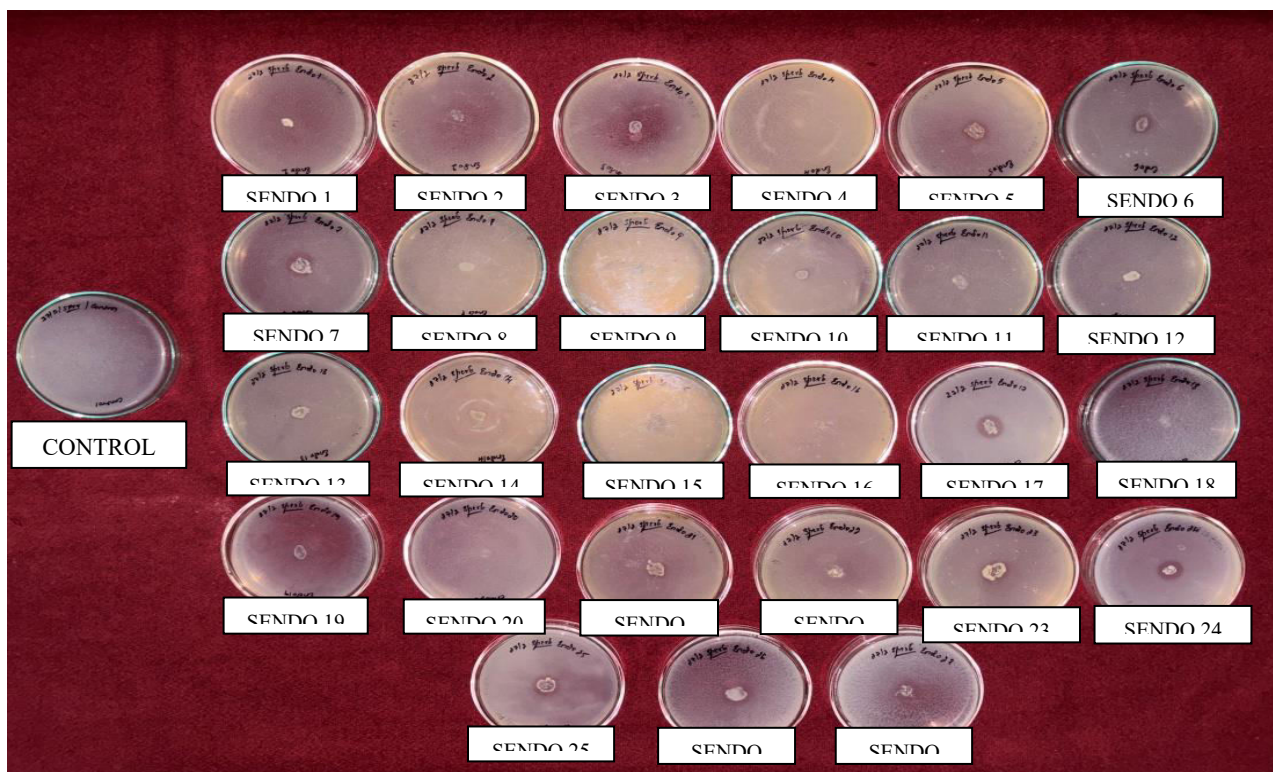
CT = Catalase test, H₂S = H₂S production, IP= Indole production, NR = Nitrate reduction, MR = Methyl red test, VP =Voges Proskauer's test, CH = Casein Hydrolysis, CU = Citrate utilization, UA = Urease activity, SH = Starch Hydrolysis, GL = Gelatin Liquefaction, AP = Acid production, GP = Gas Production, OT = Oxidase Test, SENDO – Sahyadri Endophytic Bacteria.

Plate.1 Endophytic bacterial isolates isolated from different weed plants collected from different crop ecosystems of Shivamogga district



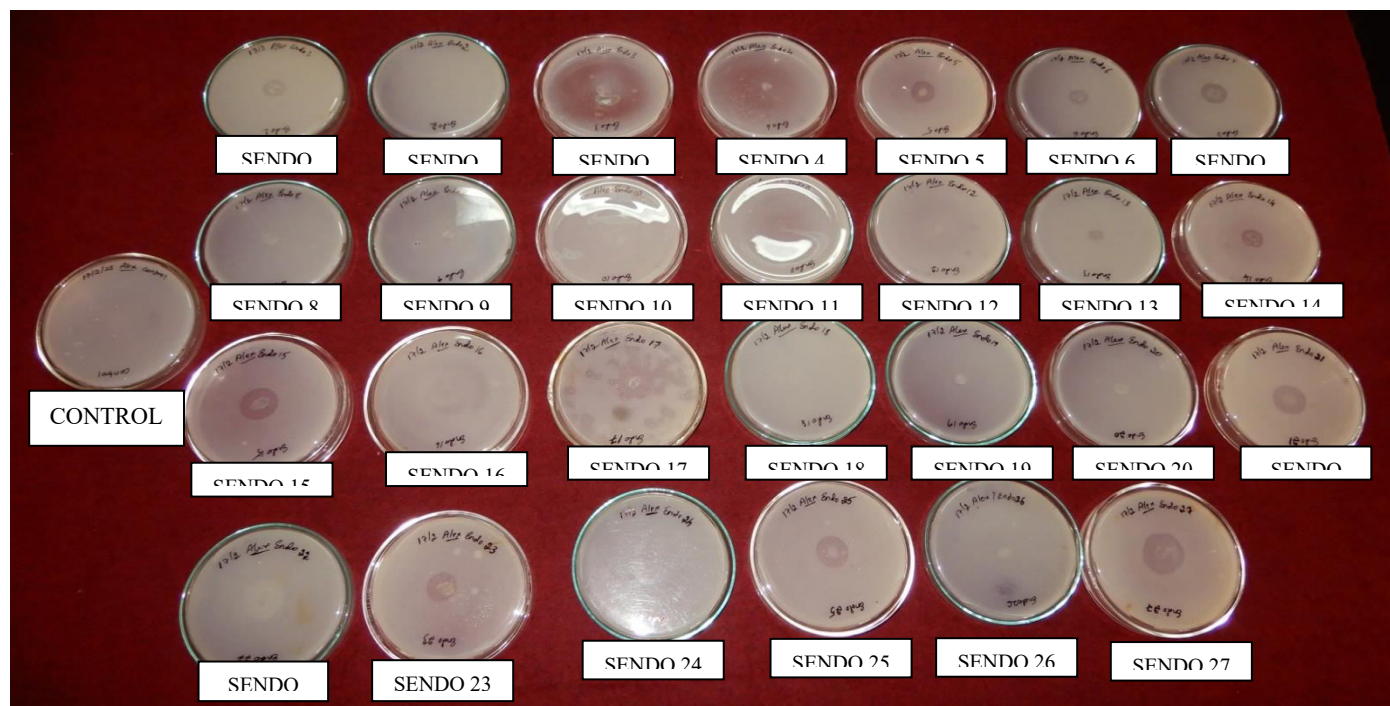
Note: SENDO – Sahyadri Endophytic Bacteria

Plate.2 Phosphorous solubilization ability of Sahyadri endophytic bacterial isolates



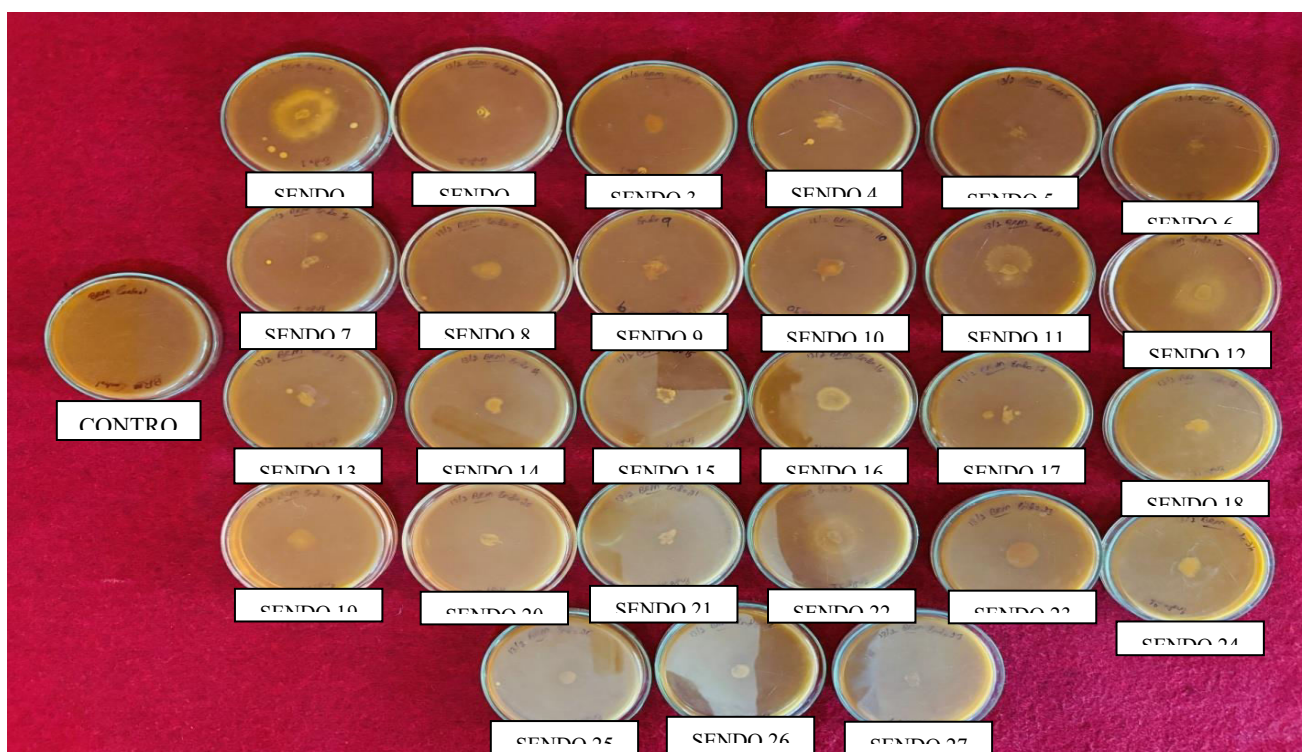
Note: Sahyadri Endophytic Bacteria

Plate.3 Potassium solubilization ability of Sahyadri endophytic bacterial isolates



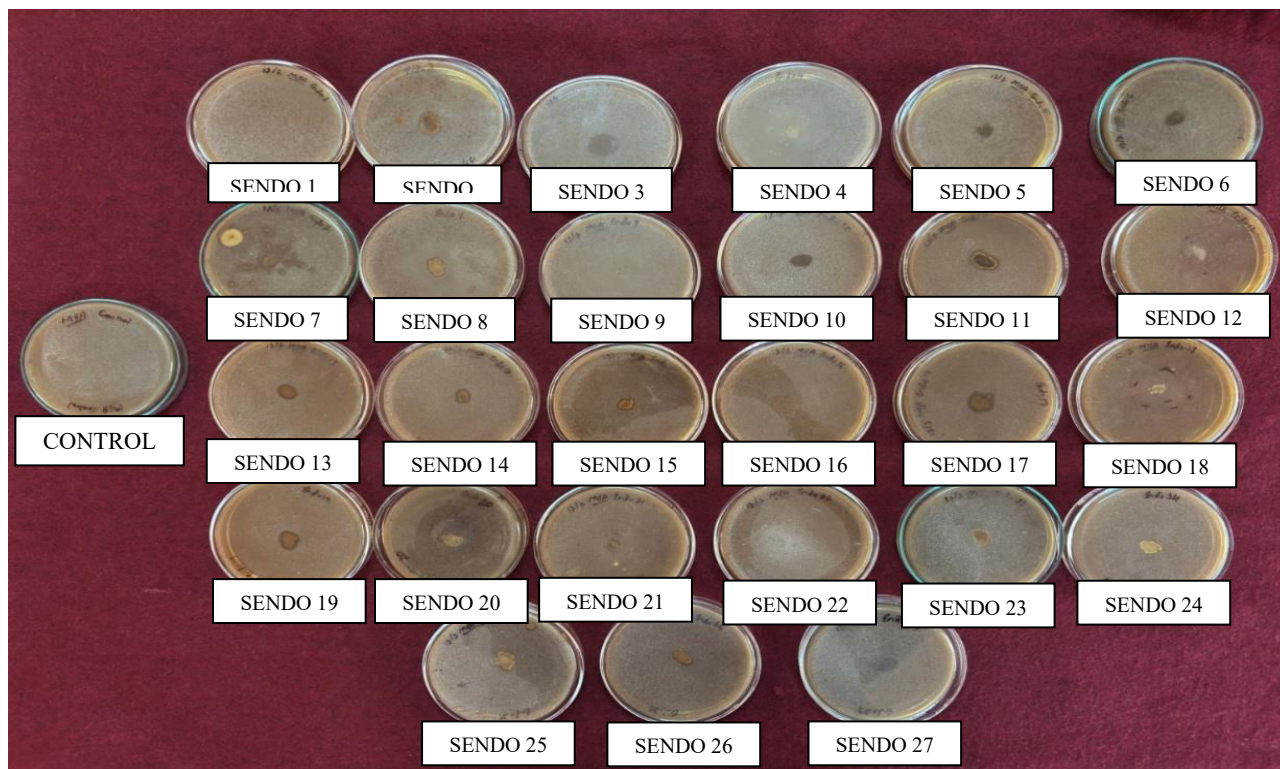
Note: SEND0 – Sahyadri Endophytic Bacteria

Plate.4 Zinc solubilizing potentiality of Sahyadri endophytic bacterial isolates



Note: SEND0 – Sahyadri Endophytic Bacteria

Plate.5 Silicon solubilizing potentiality of Sahyadri endophytic bacterial isolates



Note: SENDO – Sahyadri Endophytic Bacteria

Morphological and Biochemical Characterization

The four selected efficient isolates *i.e.*, SENDO – 5, SENDO – 6, SENDO – 17 and SENDO – 27 were identified as Gram-negative rods. However, with respect to the colony morphology the SENDO – 5 formed medium-sized, moist colonies, SENDO – 6 developed transparent, creamy colonies, SENDO – 17 produced moist, circular colonies, and SENDO – 27 appeared smooth colonies with a slightly raised elevation.

On the other hand, the biochemical characters revealed that all four isolates were positive for catalase activity, nitrate reduction, citrate utilisation and gelatin liquefaction. In addition, SENDO – 5 and SENDO – 17 tested positives for hydrogen sulphide production, methyl red reaction, urease activity and acid and gas production but were negative for casein hydrolysis and oxidase activity.

Conversely, SENDO – 6 and SENDO – 27 were positive for casein hydrolysis and oxidase activity but negative

for hydrogen sulphide production, methyl red reaction, urease activity and acid and gas production.

Based on their morphological and biochemical profiles, SENDO – 5 and SENDO – 17 were tentatively identified as *Citrobacter* species, whereas SENDO – 6 and SENDO – 27 were identified as *Achromobacter* species (Table 8). The findings are in line with the findings of Oteino *et al.*, 2015 who have previously characterized the plant growth promoting endophytic bacteria having the phosphate solubilization, hormone production, and stress tolerance. Similarly, the findings strongly support the findings of Fadji *et al.*, 2023; Hongrattipun *et al.*, 2014; Jha and Kumar, 2009 who isolated and identified endophytic bacteria from different plant parts and reported as *Achromobacter* and *Citrobacter*.

In conclusion, the results of the study will pave the way to create awareness about the native endophytic plant growth promoting bacterial usage among farmers of the region which results in transfer of technology from lab to land and also the use of native endophytic bacterial consortia containing SENDO – 5, SENDO – 6, SENDO – 17, and SENDO – 27 can be effectively used to

evaluate their performance in field conditions on the sustainable production different crops which will reduce the cost of fertilizers in agriculture and also helps in conserving soil health.

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Author Contributions

J. N. Ashish: Investigation, formal analysis, writing—original draft. M. S. Nandish: Validation, methodology, writing—reviewing. B. C. Dhananjaya:—Formal analysis, writing—review and editing. G. K. Girijesh: Resources, investigation writing—reviewing. M. Divya: Validation, formal analysis, writing—reviewing. C. K. Kavya: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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