

Plant Archives

Journal homepage: http://www.plantarchives.org DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.258

CHARACTERIZATION OF ENDOPHYTIC BACTERIA OF FINGER MILLET [ELEUSINE CORACANA (L.)] FROM SOUTHERN STATES OF INDIA

S. Akshitha^{1*}, G. Padmaja², G. Rajesha³ and S.N.C.V.L. Pushpavalli⁴

¹Department of Plant Pathology, College of Agriculture, P.J.T.S.A.U., Rajendranagar, Hyderabad - 500 030, Telangana, India.

²Regional Agricultural Research Station, P.J.T.S.A.U., Warangal, Telangana, India.

³I.C.A.R.-Indian Institute of Millet Research, Rajendranagar, Hyderabad, Telangana, India.

⁴Institute of Biotechnology, P.J.T.S.A.U., Hyderabad, Telangana, India.

*Corresponding author E-mail : singitham.akshitha@gmail.com (Date of Receiving-23-06-2024; Date of Acceptance-28-08-2024)

Endophytic bacteria residing inside the plants, influence the host fitness by disease suppression, and plant growth promotion and have the ability to act as biocontrol agent. The present study aimed to isolate and characterize endophytic bacterial endophytes of finger millet [Eleusine coracana (L.)]. A total of thirty bacterial endophytes were isolated from healthy finger millet plants collected from the growing districts of Telangana, Andhra Pradesh and Karnataka. Among the 30 endophytic bacterial isolates, 17 isolates were from root, 10 isolates were from stem and 3 isolates were from leaves were isolated. The highest number of bacterial endophytes about 70% were found in roots followed by above ground parts. All the isolates of endophytes have been characterized based on morphology and colony characteristics. The characteristics of colony morphology were used to evaluate the diversity of endophytic bacteria which showed variation with respect to colony size, shape, colour, texture, optical property, elevation and staining. An equal number ABSTRACT of Gram positive and Gram-negativebacterial endophytes were found among the 30 isolates most of the bacterial cells being rod shaped. The bacterial colonies showed circular and irregular form with even or undulate colony marginhaving flat to raised elevations. The pigmentation of the isolated coloniesvaried with varying locations. The dendrogram constructed from colonymorphological characteristics grouped into three broad groups (Cluster I, cluster II and Cluster III) with jaccard's similarity coefficient of 33%. The cluster pattern showed separation of FSEB-4G from other isolates due to its dissimilarities in colony characters compared to other isolates. The isolates FREB-4H and FSEB-3T had similar colony characters even though the isolated parts and region were different.

Key words : Finger millet, Endophytes, Biocontrol agent, Plant growth promotion, Phenotypic characterization.

Introduction

Finger millet [*Eleusine coracana* (L.) Gaertn] is one of India's most significant millet crop commonly referred as the "Nutri-millet" and "poor man's food" due to its superior nutritional value compared to many cereals. It contains 65-75% carbohydrates, 5-8% protein, 15-20% dietary fiber and 2.5-3.5% minerals (Chetan and Malleshi, 2007). The Annual global output of finger millet is estimated at around 3.7 million tonnes from a total area of about 2.1 million hectares with a productivity of 600 kg ha⁻¹ (FAOSTAT, 2020 and Gairhe *et al.*, 2021). India is the world's foremost producer of finger millet, with a

total production area of 1 million hectares with an average productivity of 1747 kg ha⁻¹ (Gebreyohannes *et al.*, 2021 and Indiastat, 2020), accounting for 46% of the global share.

The production and productivity of finger millet is being decreased due to varying climatic conditions which influence the biotic and abiotic stresses. Among the biotic stresses fungal and bacterial disease are prominent which affect the yield. Among these diseases, foot rot is one of the important emerging diseases of finger millet especially under irrigated and high rainfall situations (Nagaraja and Reddy, 2009) it is causing yield loss of upto 50 per cent (Batsa and Tamang, 1983).

Foot rot of finger millet disease caused by *Sclerotium rolfsii* Sacc. is a soil borne pathogen, first reported by Coleman (1920) in India from the princely state of Mysore. Use of fungicides may not be economical and also leads to development of resistance to the fungicides. Owing to its broad host range and ability to infect over 500 crops, cultural measures such as crop rotation were likewise ineffective against *Sclerotium rolfsii*. Hence, management of this disease using endophytic biocontrol agents is considered as one of the best alternatives to chemical control.

Endophytes are one of the potential microorganisms investigated for suppressing of pathogens. Endophytic bacteria are those that remain in plant tissues and do not cause significant harm or gaining benefit other than residency (Kobayashi and Palumbo, 2000). Plants engage in continuous interactions with a diverse array of microorganisms and are protected from microbial competition and environmental stress by their host plants.

Although aerial plant parts like flowers, stems, and cotyledons can be a point of entry, though the root zone is the main route by which endophytes penetrate plant tissue (Kobayashi and Palumbo, 2000). Moreover, Kobayashi and Palumbo (2000) reported the isolation of many distinct bacterial species from a single plant. Bacterial endophytes are abundant and diverse in various crops such as rice (Stoltzfus *et al.* 1997), maize (Fisher *et al.*, 1992), cotton (McInroy and Kloepper, 1995), cucumber (Mahafee and Kloepper, 1997) and potato (Garbeva *et al.*, 2001).

Endophytes are also reported to act as antagonistic by suppressing the pathogen growth by various plant growth promoting activities like phosphate solubilization, production of ammonia, siderophores, HCN *etc.*, Thus, the present investigation was taken up to isolate and characterize endophytic bacteria from finger millet [*Eleusine coracana* (L.)] from different growing locations of Southern India to carry out further research activities.

Materials and Methods

Collection of finger millet samples

The finger millet plant samples were randomly collected from g rowing field for isolation of bacterial endophytes during flowering stage from Southern states of India *viz.*, Telangana, Andhra Pradesh and Karnataka states during *kharif* 2023. Healthy plant samples were collected from all the location and brought to the lab for isolation.

Isolation of endophytic bacteria

Bacterial endophytes were isolated from collected plant parts (root, stem and leaf tissues) of healthy finger millet plants. The samples were rinsed thrice with tap water followed by sterile distilled water twice and these sterile plant parts were cut to small pieces with a sterile scalpel. The cut pieces are then rinsed in 70% ethanol for 30 seconds and then sterilized with 1% NaClO for 1 minutes. Finally, roots were thoroughly washed 6 times with sterile distilled water (Gagne et al., 1987) and the final wash was spread on nutrient agar (NA) plates as control check. Surface-disinfected tissue was aseptically macerated with homogenizers and serial dilution (10⁻¹ to 10^{-6}) was made (Plate 1). Hundred microliters of 10^{-6} dilution was inoculated into NA plates by following pour plate method and incubated at 28±2°C for 24-48 hours. After 48 hours of incubation period bacterial colonies were picked up from culture plate and streaked into separate plate to obtain the pure culture and stored for further studies.

Cultural and morphological characterization

The pure culture of each bacterial endophyte was streaked on NA plates to record the cultural and morphological features. After 24-48 hours of incubation, the shape, margin, elevation, size, texture, appearance and pigmentation of the bacterial colonies were noted as per the colony morphology parameters as outlined by Smibert and Krieg (1994).

The cultural and morphological characters of all the bacterial endophytic isolates were used to generate binary matrix. The dendrogram was based on the proximity matrix obtained from the jaccord coefficient and Sequential Agglomerative Hierarchiel Non-overlapping (SAHN) method and clustering was done using the Unweighted Pair Group Method with Arthimetic Average (UPGMA) Method (Sneath and Sokal, 1973).

Results and Discussion

Collection of finger millet samples

The healthy finger millet plant samples were collected from Telangana, Andhra Pradesh and Karnataka districts of South India. Samples of root, shoot and leaves were collected from two locations from each district. The detailed list on collection of various plant samples is presented in Table 1.

Isolation of endophytic bacteria

A total of 30 bacterial isolates have been isolated from six different locations by using different plant parts of root, shoot and leaves. Out of these 30 endophytic bacteria, the plates with root sap (17) showed high number

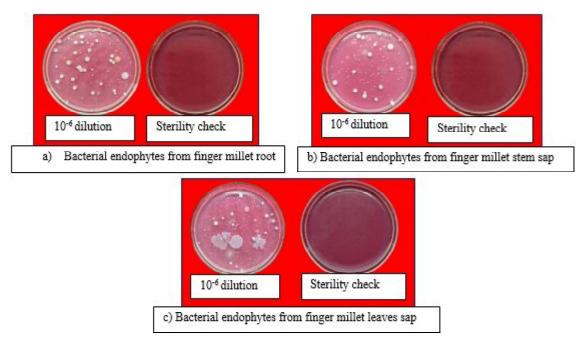


Plate 1: Isolation of bacterial endophytes from a) roots b) stems c) leaves of finger millet.

of colonies followed by stem (10) and leaves (3). Among these different parts of plant, the root region had a greater number of endophytic bacterial colonies compared to other parts like stem and leaf (Plate 1).

Our results are consistent with the findings of Gupta *et al.* (2015), who found that *Prosopis cineraria* roots had a higher population density and variety of endophytes than stems and leaves. Similarly, Liu *et al.* (2017) reported that although while endophyte is found in every part of the plant, the roots that have the closest contact with the soil may serve as the initial point of entry for endophyte bacteria.

Morphological and cultural characterization of bacterial endophytes

Based on the culture and morphological characteristics, the characterization of the 30 endophytic bacterial isolates was done (Table 2). It was found that out of 30 endophytic bacterial isolates from root, stem and leaf parts an equal number of isolates showed Gram positive (15 isolates) and Gram negative (15 isolates). Similar results were reported by Zinniel *et al.* (2002) and Ebrahimi *et al.* (2010) that the equal number of Gram positive and Gram-negative endophytic bacteria can be present in the plant parts. However, the contractor results were reported by Stoltzfus *et al.* (1997), Elbeltagy *et al.* (2000) that Gram negative bacteria predominated in the tissues of various plants.

Most of the endophytic bacterial isolates from the root sap were regular in shape. About 13 isolates of root and 7 isolates from stem sap were found to be circular. The endophytic bacterial isolates FREB-3M, FREB-4M, FREB-5M in case of root and FSEB-3S, FSEB-4G and FSEB-2B from stem were irregular in shape though most of the isolates showed circular in shape. It was also observed that all of the endophytic bacterial isolates isolated from the leaves were irregular in shape (Table 2).

Based on the colony margin, bacterial isolates were divided into even and undulate. Among the 30 isolates most of the isolates showed even margin except FLEB-5H, FREB-1S, FSEB-3S, FSEB-5S, FREB-2G, FSEB-3G, FSEB-4G, FLEB-5G, FREB-3M, FREB-2G, FSEB-5M, FREB-2B, which showed undulate.

Majority of the isolates from root and stem were found to have slightly raised colonies. Most of the colonies isolated from the leaf were flat except the isolate FLEB-5T. Raised colonies were also found in the isolates FREB-2H, FSEB-4S, FSEB-5S, FREB-2T, FSEB-4T, FLEB-5T, FSEB-4G, FREB-1M, FREB-2B.

The size of the colonies varied from pin point to large size colonies. Most of the colonies were small size *viz.*, FREB-2H, FREB-3H, FSEB-4S, FREB-1T, FREB-2T, FSEB-4T, FREB-1G, FSEB-3G, FREB-1M, FREB-2B, FSEB-3B, FSEB-5B and pinpoint size viz., FREB-1H, FREB-4H, FREB-1S, FREB-2S, FSEB-3T, FSEB-4G, FREB-2M, FREB-1B, FSEB-4B. Few isolates *viz.*, FLEB-5H, FSEB-3S, FLEB-5T, FREB-2G, FLEB-5G, FREB-5M produced medium size colonies. 3 Isolates FSEB-5S, FREB-3M, FREB-4M from Mandya and Sangareddy produced large colonies.

S. no.	Isolate name	State	District	Place of collection	Latitude N ⁰	Longitude E ⁰	Isolated part
1	FREB-1H	Telangana	Hyderabad	Rajendranagar	17.3242	78.3939	Root
2	FREB-2H	Telangana	Hyderabad	Rajendranagar	17.3242	78.3939	Root
3	FREB-3H	Telangana	Hyderabad	Rajendranagar	17.3242	78.3939	Root
4	FREB-4H	Telangana	Hyderabad	Rajendranagar	17.3242	78.3939	Root
5	FLEB-5H	Telangana	Hyderabad	Rajendranagar	17.3242	78.3939	Leaves
6	FREB-1S	Telangana	Sangareddy	Zaheerabad	17.4244	77.3644	Root
7	FREB-2S	Telangana	Sangareddy	Zaheerabad	17.4244	77.3644	Root
8	FSEB-3S	Telangana	Sangareddy	Zaheerabad	17.4244	77.3644	Stem
9	FSEB-4S	Telangana	Sangareddy	Zaheerabad	17.4244	77.3644	Stem
10	FSEB-5S	Telangana	Sangareddy	Zaheerabad	17.4244	77.3644	Stem
11	FREB-1T	Andhra Pradesh	Tirupati	Perumallapalli	13.6259	79.3719	Root
12	FREB-2T	Andhra Pradesh	Tirupati	Perumallapalli	13.6259	79.3719	Root
13	FSEB-3T	Andhra Pradesh	Tirupati	Perumallapalli	13.6259	79.3719	Stem
14	FSEB-4T	Andhra Pradesh	Tirupati	Perumallapalli	13.6259	79.3719	Stem
15	FLEB-5T	Andhra Pradesh	Tirupati	Perumallapalli	13.6259	79.3719	Leaves
16	FREB-1G	Andhra Pradesh	Guntur	Maddiboinavaripalem	15.8999	80.4764	Root
17	FREB-2G	Andhra Pradesh	Guntur	Maddiboinavaripalem	15.8999	80.4764	Root
18	FSEB-3G	Andhra Pradesh	Guntur	Maddiboinavaripalem	15.8999	80.4764	Stem
19	FSEB-4G	Andhra Pradesh	Guntur	Maddiboinavaripalem	15.8999	80.4764	Stem
20	FLEB-5G	Andhra Pradesh	Guntur	Maddiboinavaripalem	15.8999	80.4764	Leaves
21	FREB-1M	Karnataka	Mandya	Gandalu	12.5702	76.8271	Root
22	FREB-2M	Karnataka	Mandya	Gandalu	12.5702	76.8271	Root
23	FREB-3M	Karnataka	Mandya	Gandalu	12.5702	76.8271	Root
24	FREB-4M	Karnataka	Mandya	Gandalu	12.5702	76.8271	Root
25	FREB-5M	Karnataka	Mandya	Gandalu	12.5702	76.8271	Root
26	FREB-1B	Karnataka	Bangalore	Mavallipura	13.1201	77.5293	Root
27	FREB-2B	Karnataka	Bangalore	Mavallipura	13.1201	77.5293	Root
28	FSEB-3B	Karnataka	Bangalore	Mavallipura	13.1201	77.5293	Stem
29	FSEB-4B	Karnataka	Bangalore	Mavallipura	13.1201	77.5293	Stem
30	FSEB-5B	Karnataka	Bangalore	Mavallipura	13.1201	77.5293	Stem

Table 1 : Collection of healthy plant samples of finger millet from different places to isolate endophytes.

Colony colour variation has been observed among the 30 bacterial endophytes viz., white, cream, creamy white, greenish yellow, light brown, brown. The colony colour of the isolates varied from place to place *i.e.*, isolates FREB-1H, FREB-2H, FREB-3H, FLEB-5H, FLEB-5G isolated from Hyderabad and Guntur produced white colour colonies. Most of the bacterial endophytic colonies viz., FREB-4H, FREB-2S, FSEB-3S, FREB-2T, FSEB-3T, FSEB-4T, FSEB-3G, FREB-4M, FREB-5M, FREB-2B were observed as cream colour and the isolates FREB-1S, FSEB-4S, FSEB-5S, FREB-3M, FREB-1B, FSEB-3B were shown as creamy white colour. Greenish yellow pigmentation was seen in the isolates of Andhra Pradesh and Karnataka viz., FREB-1T, FLEB-5T, FREB-2G, FREB-2M, FSEB-5B. Light brown to brown colony colour was seen in the isolates of Andhra Pradesh and Karnataka *viz.*, FREB-1G, FSEB-4G, FREB-1M and FSEB-4B.

The isolates predominantlyhad mucoid type of texture but fewer isolates found to have butyrous texture *viz.*, FREB-1S, FSEB-4S, FSEB-5S, FREB-1G, FREB-2G, FREB-4M, FREB-2B, FSEB-3B from the districts of Sangareddy, Guntur, Mandya and Bangalore. About 3 isolates FLEB-5H, FSEB-3G, FREB-2M from Hyderabad, Guntur and Mandyawere found to have dry texture.

The endophytic bacterial isolates appeared in 3 different types- smooth, rough and veined. Majority of isolates were found to be smooth in appearance though few isolates FLEB-5H, FSEB-3S, FSEB-4S, FSEB-5S, FSEB-3G, FREB-2M FREB-5M, FREB-2B were

S. no.	Cultural characteristics	Types	No. of isolates	Name of the isolates	
1.	Colony shape	Circular	21	FREB-1H, FREB-2H, FREB-3H, FREB-4H, FREB-1S, FREB-2S, FSEB-4S, FSEB-5S, FREB-1T, FREB-2T, FSEB-3T, FSEB-4T, FREB-1G, FREB-2G,	
				FSEB-3G, FREB-1M, FREB-2M, FREB-1B, FSEB-3B, FSEB-4B, FSEB-5B	
		Irregular	9	FLEB-5H, FSEB-3S, FLEB-5T, FSEB-4G, FLEB-5G, FREB-3M, FREB-4M, FREB-5M, FREB-2B	
2. Margin		Even	18	FREB-1H, FREB-2H, FREB-3H, FREB-4H, FREB-2S, FSEB-4S, FREB-1T, FREB-2T, FSEB-3T, FSEB-4T, FLEB-5T, FEBR-1G, FREB-1M, FREB-2M, FSEB-1B, FSEB-3B, FSEB-4B, FSEB-5B	
		Undulate	12	FLEB-5H, FREB-1S, FSEB-3S, FSEB-5S, FREB-2G, FSEB-3G, FSEB-4G, FLEB-5G, FREB-3M, FREB-4M, FREB-5M, FREB-2B	
3.	Elevation	Flat	5	FLEB-5H, FREB-1S, FREB-2S, FLEB-5G, FREB-5M	
		Slightly raised	16	FREB-1H, FREB-3H, FREB-4H, FSEB-3S, FREB-1T, FSEB-3T, FREB-1G, FREB-2G, FSEB-3G, FREB-2M, FREB-3M, FREB-4M, FREB-1B, FSEB-3B, FSEB-4B, FSEB-5B	
		Raised	9	FREB-2H, FSEB-4S, FSEB-5S, FREB-2T, FSEB-4T, FLEB-5T, FSEB-4G, FREB-1M, FREB-2B	
4.	Size	Pinpoint	9	FREB-1H, FREB-4H, FREB-1S, FREB-2S, FSEB-3T, FSEB-4G, FREB-2M, FREB-1B, FSEB-4B	
		Small	12	FREB-2H, FREB-3H, FSEB-4S, FREB-1T, FREB-2T, FSEB-4T, FREB-1G, FSEB-3G, FREB-1M, FREB-2B, FSEB-3B, FSEB-5B	
		Medium	6	FLEB-5H, FSEB-3S, FLEB-5T, FREB-2G, FLEB-5G, FREB-5M	
		Large	3	FSEB-5S, FREB-3M, FREB-4M	
5.	Texture	Mucoid	19	FREB-1H, FREB-2H, FREB-3H, FREB-4H, FREB-2S, FSEB-3S, FREB-1T, FREB-2T, FSEB-3T, FSEB-4T, FLEB-5T, FSEB-4G, FLEB-5G, FREB-1M, FREB-3M, FREB-5M, FREB-1B, FSEB-4B, FSEB-5B	
		Butyrous	8	FREB-1S, FSEB-4S, FSEB-5S, FREB-1G, FREB-2G, FREB-4M, FREB-2B, FSEB-3B	
		Dry	3	FLEB-5H, FSEB-3G, FREB-2M	
6.	Appearance	Smooth	21	FREB-1H, FREB-2H, FREB-3H, FREB-4H, FREB-1S, FREB-2S, FREB-1T, FREB-2T, FSEB-3T, FSEB-4T, FLEB-5T, FREB-1S, FREB-2S, FSEB-4G, FREB-1M, FREB-3M, FREB-4M, FEBR-1B, FSEB-3B, FSEB-4B, FSEB-5B	
		Rough	8	FLEB-5H, FSEB-3S, FSEB-4S, FSEB-5S, FSEB-3G, FREB-2M, FREB-5M, FREB-2B	
		Veined	1	FLEB-5G	
7.	Pigmentation	White	5	FREB-1H, FREB-2H, FREB-3H, FLEB-5H, FLEB-5G	
		Cream	10	FREB-4H, FREB-2S, FSEB-3S, FREB-2T, FSEB-3T, FSEB-4T, FSEB-3G, FREB-4M, FREB-5M, FREB-2B	
		Creamy white	6	FREB-1S, FSEB-4S, FSEB-5S, FREB-3M, FREB-1B, FSEB-3B	
		Greenish yellow	5	FREB-1T, FLEB-5T, FREB-2G, FREB-2M, FSEB-5B	
		Light brown	3	FREB-1G, FSEB-4G, FSEB-4B	
		Brown	1	FREB-1M	

Table 2 continued...

Table 2 : Morphological characteristics of endophytic bacteria isolates.

8.	Optical	Opaque	28	FREB-1H, FREB-2H, FREB-3H, FREB-4H, FLEB-5H, FREB-1S, FREB-2S,
	property			FSEB-3S, FSEB-4S, FSEB-5S, FREB-1T, FSEB-3T, FSEB-4T, FLEB-5T,
				FREB-1G, FREB-2G, FSEB-3G, FSEB-4G, FLEB-5G, FREB-2M, FREB-3M,
				FREB-4M, FREB-5M, FREB-1B, FREB-2B, FSEB-3B, FSEB-4B, FSEB-5B
		Transparent	2	FREB-2T, FREB-1M
Ν	Morphological Types		No. of	Name of the isolates
cł	naracteristics		isolates	
9.	Gram staining	Gram+	15	FREB-3H, FLEB-5H, FREB-2S, FSEB-3S, FREB-1T, FSEB-4T, FLEB-5T,
				FREB-1G, FREB-2G, FREB-1M, FREB-3M, FREB-5M, FREB-1B, FSEB-3B,
				FSEB-4B
		Gram-	15	FREB-1H, FREB-2H, FREB-4H, FREB-1S, FSEB-4S, FSEB-5S, FREB-2T,
				FSEB-3T, FSEB-3G, FSEB-4G, FLEB-5G, FREB-2M, FREB-2B, FSEB-5B
10.	Cell shape	Coccus	12	FREB-1H, FREB-1S, FSEB-4S, FSEB-5S, FSEB-4T, FSEB-3G, FSEB-4G,
				FREB-2M, FREB-4M, FSEB-3B, FSEB-4B, FSEB-5B
		Rod	17	FREB-2H, FREB-3H, FREB-4H, FLEB-5H, FREB-2S, FSEB-3S, FREB-1T,
				FREB-2T, FSEB-3T, FLEB-5T, FREB-1G, FREB-2G, FLEB-5G, FREB-1M,
				FREB-5M, FREB-1B, FREB-2B,
		Spirillum	1	FREB-3M

Table 2 continued...

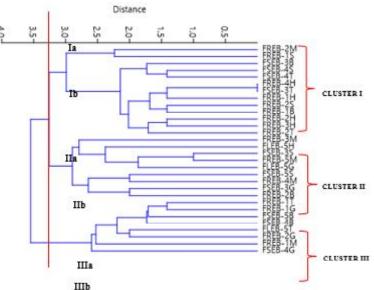


Fig. 1: Hierarchial non overlapping algorithm and clustering using Unwighted Pair Group Method with Arthmetic Average and leaf isolates from different places of Southern (UPGMA) of endophytic bacterial isolates collected from India, which were closely related to each other on Southern states of India.

butyrous and FLEB-5G were found to have veined appearance.

Based on the optical property the endophytic bacterial colonies were categorized into opaque and transparent. Out of 30 endophytic bacterial isolates, only two isolates viz., FREB-2T and FREB-1M from Tirupathi and Mandya were observed to have transparent type of colonies on the nutrient agar plate where the remaining isolates were observed to have opaque nature.

Singh et al. (2013) found similar results after isolating seven endophytic bacteria from sugarcane and observed that the majority of the isolates had spherical colonies with smooth and wavy edges, convex elevation, and white to creamy coloration. Similarly, three endophytes were identified by Gupta et al. (2015) from the roots of Prosopis cineraria plants. These endophytes were characterized as round in form, even, smooth, flat and yellowish to orange in color with an opaque nature.

The Hierarchial non overlapping algorithm and clustering using Unweighted Pair Group Method with Arthmetic Average (UPGMA) grouped these 30 endophytic bacterial isolates into 3 clusters (Cluster I, Cluster II and Cluster III) at the similarity co-efficient of 33% (Fig. 1).

Cluster I accommodated only the root, stem

the basis of colony and cell characteristics. The Cluster I comprised of the 13 isolates which were further sub divided into 2 subgroups (Cluster Ia and Cluster Ib). Subgroup cluster Ia comprised 2 isolates FREB-2M, FREB-1S from the districts Sangareddy and Mandya were distinct from each other in cultural characteristics. Subgroup Ib comprised of 11 isolates from Hyderabad, Sangareddy, Tirupathi, Bangalore *i.e.*, isolates FSEB-3B, FSEB-4S, FSEB-4T, FREB-4H, FSEB-3T, FREB-1H, FREB-2S, FREB-1B, FREB-2H, FREB-3H, FREB-2T. Among these 11 isolates, isolates FSEB-3T and FREB-

1H showed no variation between the cultural characteristics which were isolated from root and stem parts of plant collected from Hyderabad and Tirupathi.

Cluster II accommodated 9 isolates which is further sub divided into 2 groups *i.e.*, cluster IIa consisting of FREB-3M, FLEB-5H, FSEB-3S, FREB-5M, FLEB-5G and cluster IIb consisting of FSEB-5S, FREB-4M, FSEB-3G, FREB-3B.

Cluster III accommodated 8 isolates which was sub grouped into 2 *viz.*, cluster IIIa and cluster IIIb. One sub group Cluster IIIa comprised of the isolates FREB-1T, FREB-1G, FSEB-5B, FSEB-4B, FLEB-5T, FREB-2G, FREB-1M and other sub group Cluster IIIb comprised of only one isolate FSEB-4G from Guntur district.

According to Salaki *et al.* (2010), every microbial strain is categorized into a homogenous taxon group using the numeric-phenetic classification approach.Based on a variety of phenotypic information, including macro morphology, colony morphology, use of carbon sources, enzyme reducers, the capacity to break down macromolecules and several physiological characteristics, those taxon species have been identified.

Conclusion

The diversity of thirty endophytic bacteria isolates collected from Southern states of India were isolated from different tissues of the hosts. The assessment for colony morphology gave an indication of the variation among the endophytic isolates. The isolates studied were chosen for their dominance as well as uniqueness and differences with other in colony morphology. Interestingly, Gram positive and Gram negative isolates were equally distributed among the isolates from different places. Most of the isolated bacterial colonies were circular with even margin and smooth appearance. Majority of the colonies were small in size with slight elevation. Mucoid type of texture was predominantly seen among the isolated colonies from different locations. However, the pigmentation of the colonies varied from place to place. Dendrogram based on cultural characterization pattern grouped these 30 endophytic bacterial isolates into three broad groups (Cluster I, cluster II and Cluster III) with jaccard's similarity coefficient of 33%. This phenotypic variation may indicate the ability of an organism to survive, adapt and acclimatize in diverse climatic condition.

Acknowledgement

Authors are grateful to the ICAR- Indian Institute of Millet Research and Department of Plant Pathology, Professor Jayashankar Telangana State Agricultural University, Rajendranagar. Hyderabad - 500 030, India.

References

- Batsa, B.K. and Tamang D.B. (1983). Preliminary report on the study of millet diseases in Nepal. In: *Maize and Finger millet*. 10th Summer workshop 23-28 Jan., 1983, Rampur, Chitwan, Mysore.
- Chethan, S. and Malleshi N.G (2007). Finger millet polyphenols: Characterization and their nutraceutical potential. *Amer. J. Food Technol.*, **2**, 582-592.
- Coleman, L.C. (1920). The cultivation of ragi in Mysore. *Bull. Dep. Agric. Gen. Ser*, **11**.
- Ebrahimi, A., Asgharian S. and Habibian S. (2010). Antimicrobial activities of isolated endophytes from some Iranian native medicinal plants. 217-222
- Elbeltagy, A., Nishioka K., Suzuki H., Sato T., Sato Y.I. and Morisaki H. (2000). Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *Soil Sci. Plant Nutr.*, **46**, 617-629.
- FAOSTAT (Food and Agriculture Organization of the United Nations STAT) (2020). FAOSTAT. Available online:<u>https://www.fao.org/_faostat/en/#data</u>
- Fisher, P.J., Petrini O. and Scott H.L. (1992). The distribution of some fungal and bacterial endophytes in maize (*Zea mays L.*). *New Phytologist*, **122**, 299-305.
- Gagne, S., Richard C., Roussean H. and Antoun H. (1987). Xylem-residing bacteria in alfalfa roots. *Candian J. Microbiol.*, 33, 996-1000.
- Gairhe, S., Gauchan D. and Timsina K.P. (2021). Prospect and potentiality of finger millet in Nepal: nutritional security and trade perspective. J. Agricult. Nat. Resour., 4, 63–74
- Garbeva, P., Van Overbeek L.S., Van Vuurde J.W.L. and Van Elsas J.D. (2001). Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microbial Ecology*, **41**, 369-383.
- Gebreyohannes, A., Shimelis M., Laing, Mathew I., Odeny D.A. and Ojulong H. (2021). Finger millet production in Ethiopia: Opportunities, problem diagnosis, key challenges and recommendations for breeding. Sustainability, 13, 13463.
- Gupta, R.M., Kale P.D., Rathi M.L. and Jadhav N.N. (2015). Isolation, characterization and identification of endophytic bacteria by 16S rRNA partial sequencing technique from roots and leaves of *Prosopis cineraria* plant. *Asian J. Plant Sci. Res.*, **5**, 36-43.
- Indiastat (2020). Socio-economic statistical data and facts about India. <u>https://www.indiastat.com/</u>
- Kobayashi, D.Y. and Palumbo J. D. (2000). Bacterial endophytes and their effects on plants and uses in agriculture. *Microbial Endophytes*, CRC Press: 213-250.
- Liu, H., Carvalhais L.C., Crawford M., Singh E., Dennis P.G., Pieterse C.M. and Schenk P.M. (2017). Inner plant values: diversity, colonization and benefits from Endophytic bacteria. *Front. Microbiol.*, 8, 2552.
- Mahaffee, W.F. and Kloepper J.W. (1997). Bacterial

communities of the rhizosphere and endorhiza associated with field-grown cucumber plants inoculated with a plant growth-promoting rhizobacterium or its genetically modified derivative. *Canadian J. Microbiol.*, **43**, 344-353.

- McInroy, J.A. and Kloepper J.W. (1995). Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant and Soil*, **173**, 337-342.
- Nagaraja, A. and Anjaneya Reddy B. (2009). Foot rot of finger millet - an increasing disease problem in Karnataka. *Crop Res.*, **38**, 224-225.
- Salaki, C.L., Situmorang J., Sembiring L. and Handayani N. (2010). Analysis of the diversity of pathogenic *Bacillus thuringiensis* isolates against cabbage pest insects (*Crocidolomia binotalis*) with a numerical systematic approach. *Biota*, 15, 469-476.
- Singh, D., Sharma A. and Saini GK. (2013). Biochemical and molecular characterisation of the bacterial endophytes

from native sugarcane varieties of Himalayan region. *Biotechnology*, **3**, 205-212

- Smibert, R.M., Krieg N.R., Gerhardt P., Murray R. and Wood W. (1994). Methods for general and molecular bacteriology. *Amer. Soc. Microbiol.*, Washington, DC, 607-654.
- Sneath, P. (1973). The principles and practice of numerical classification. *Numerical Taxonomy*, 573.
- Stoltzfus, J.R., So R.M.P.P., Malarvithi P.P., Ladha J.K. and De Bruijn F.J. (1997). Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *Plant and Soil*, **194**, 25-36.
- Zinniel, D.K., Lambrecht P., Harris N.B., Feng Z., Kuczmarski S and Higley P. (2002). Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Appl. Environ. Microbiol.*, 68, 2198-2208.