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Harnessing Endophytic Bacteria from Finger Millet Landraces for Biocontrol of Foot Rot Disease

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Abstract

Finger millet (*Eleusine coracana* (L) Gaertn. subsp. *coracana*), a staple crop in arid and semi-arid regions of Africa and Asia, is praised for its drought resistance and high nutritional value. However, it faces significant yield losses due to foot rot caused by the soil-borne pathogen *Sclerotium rolfsii*. Chemical fungicides, while commonly used, pose environmental risks and contribute to fungal resistance. In search of solution, the best alternative for the chemical management is biological management. Thus, this study investigates the potential of bacterial endophytes isolated from finger millet landraces for biological control of foot rot. Totally sixty-nine endophytic bacteria were isolated from seeds, roots and leaves of finger millet landraces, by following surface sterilization and spread plating method for seeds, tissue imprinting for leaves and roots. These endophytes were screened for their antagonistic activity against *S. rolfsii* using a dual culture assay. Among sixty nine isolates, eight endophytic bacteria (AES, BMEL1, GDEL1, GES, HKES, KEL1, KER1 and KER2) exhibited maximum antagonistic activity with inhibition rates ranging from 44.39 per cent to 85.57 per cent were selected. Further these isolates were characterized morphologically (Gram staining) and biochemically (catalase activity, siderophore and hydrogen cyanide production). All the isolates were able to exhibit catalase activity, isolate AES able to produce highest siderophore and BMEL1, KEL1, KER1 and KER2 showed positive for hydrogen cyanide production. Thus, the result ensured the use of endophytes from landraces for sustainable management of foot rot in finger millet.

Keywords : Endophytes, Biocontrol, Finger millet landraces, *Sclerotium rolfsii*

Finger millet (*Eleusine coracana* (L) Gaertn. sub sp. *coracana*), renowned for its moderate drought resistance, is widely cultivated across the arid and semi-arid regions of Africa and Asia, serving as a staple for millions of people (Tadele, 2016). In India finger millet was cultivated in 8.91 lakh ha, where Karnataka alone accounts for over 5.27 lakh hectares, contributing more than 60 per cent of India's total production (Vennila and Murthy, 2021). Esteemed for its exceptional nutritional profile, the United States National Academies have designated finger millet as a 'super cereal'. It is rich in minerals and boasts a high micronutrient density (Kumar *et al.,* 2016). Nutritionally comparable to rice in terms of protein (6-8%) and fat (1-2%) content, finger millet surpasses both rice and wheat in mineral and micronutrient levels and also provides notable antioxidant and antibacterial properties (Verma & Patel, 2013 and Chandra *et al.,* 2016).

Finger millet is highly valued by local farmers for its resilience to stress conditions and resistance to a

variety of pathogens (Goron and Raizada, 2015). Nevertheless, it remains susceptible to several diseases, with foot rot caused by *Sclerotium rolfsii* emerging as a significant threat, particularly under irrigated and high-rainfall conditions. This disease has been documented to cause yield losses exceeding 50 per cent (Nagaraja and Reddy, 2009). According to the All India Coordinated Research Project on Small Millets, foot rot is a primary cause of yield loss in Mandya and Jagdalpur regions, accounting for 15 per cent of the overall finger millet losses across India (Millets Pathology - 32nd AGM - 2021 - IIMR). To mitigate this significant issue, several fungicides have been in use, including propiconazole, hexaconazole, mycobutanil, thiophanate, tebuconazole and carbendazim, to control diseases caused by *Sclerotium rolfsii* (Das *et al.,* 2014). However, the intensive application of these chemicals poses significant environmental risks, raising serious concerns about the long-term sustainability of modern agricultural practices and the development of fungal resistance (Moss, 2008 and Cardoso *et al.,* 2010). Given that *S. rolfsii* is a soil-borne pathogen, fungicide application is often not economical, as it requires drenching the entire field and resistance development remains a common issue (Manu *et al.,* 2016). As a result, there is a growing interest in alternative method for managing plant diseases, one such approach is biological agents.

Biological agents in plant disease management have already been implemented in agricultural crop production. In recent decades, novel research on biological control of plant diseases has focused on the role of a particular class of microbes that colonize the internal tissues of host plants, known as endophytes (Mousa and Raizada, 2013). Consequently, endophytes have garnered significant attention as a potential source of natural solutions for agricultural challenges. These endophytes were present in each and every plant and possess numerous benefits (Srikanth *et al*., 2023). Thus endophytes are hypothesized to confer resistance to host plants against fungal pathogens (Mousa and Raizada, 2013). The promising role of endophytes in enhancing plant resilience naturally complements the adaptability seen in local landraces.

Local landraces, characterized by their dynamic populations and association with traditional farming systems, demonstrate remarkable adaptability to harsh and unpredictable environmental conditions. Disease incidence in these landraces is notably less than 25 per cent compared to commercial varieties, even without the application of fungicides (Villa *et al.,* 2005; Vom Brocke *et al.,* 2014; Sanchez-Martín *et al.,* 2017). This study posits that cross-inoculation of endophytes from landraces to susceptible varieties which could enhance pathogen resistance, leading to increased yield in an environmentally sustainable manner. Consequently, this research focused on isolating bacterial endophytes from finger millet landraces and assessing their effectiveness against foot rot caused by *Sclerotium rolfsii*.

Material and Methods

Isolation of Endophytes from Landraces of Finger Millet

Endophytes were isolated from various plant parts of landraces of finger millet, including roots, leaves and seeds. Seeds of landraces were procured from the All India Coordinated Research Programme on Millets (AICRP) at the Zonal Agricultural Research Station (ZARS), V. C. Farm, Mandya, Karnataka. The seeds were grown in the pots for the isolation of endophytes from the plant parts. The plant samples were initially washed and cut into 2-3 cm pieces. These pieces were surface-sterilized by immersing them in 70 per cent ethanol for one minute, followed by treatment with 1.5 per cent sodium hypochlorite for 2 mins. Subsequently, the samples were rinsed thoroughly with distilled water to eliminate any residual sodium hypochlorite, with the washing process repeated 5-6 times using sterile distilled water. The surface-sterilized samples were then blot-dried with sterile filter paper, cut into two halves and each half was placed on nutrient agar plates in triplicate. Spread plating was carried out from the final wash of surface sterilisation, serving as the control. The plates were incubated at room temperature for 24-48 hours (Jabborova *et al.,* 2020).

Screening for Biocontrol Activity of Endophytic Bacteria Against Foot Rot Pathogen

Dual Culture Assay

Endophytic isolates were assessed for their antagonistic activity against the foot rot pathogen, *Sclerotium rolfsii*, which was obtained from AICRP, ZARS, V. C. Farm, Mandya. The evaluation followed the dual culture plate assay method as described by Dennis and Webster (1971). In this method, both the endophytic bacterial isolate and the pathogen were inoculated on a single potato dextrose agar (PDA) plate. A 5 mm diameter disc of the pathogen was placed at the center of the PDA plate and a 24-hour-old culture of endophytic bacteria (at a concentration of 10^8 cfu/ml) was streaked at the edge of the plate. The plates were then incubated at 27 ºC for four to eight days, with each test conducted in triplicate. Observations were made once the pathogen had fully grown on the control plate. The percentage inhibition of the pathogen's growth was calculated using the formula suggested by Vincent (1927).

$$I = \frac{(C-T)}{C} \times 100$$

Where,

I = Per cent inhibition,

C = Growth of fungal plant pathogens in control (mm),

T = Growth of fungal plant pathogens in dual culture plate (mm)

The efficient isolates that inhibited the higher per cent growth of pathogen in the dual culture method were selected for further characterization.

Morphological Characterisation of Bacterial Endophytes

The following morphological tests *viz*., cell shape, Gram reaction were carried out to tentatively characterize the identified endophytes. The purified cultures, at log phase were observed microscopically for the cell morphological characteristics. Gram staining was carried out using the procedure given by Harrigan and McCance (2014). The slides were viewed with the compound microscope at 100 x under oil-immersion.

Biochemical Characterization of Efficient Endophytic Isolates

Siderophore Production

The bacterial isolates were spotted onto CAS (Chrome Azurol Sulfonate) plates and incubated at 28 ± 2 ºC for 24 and 72 hours to allow for bacterial growth. The plates were then examined for the presence of a yellow halo surrounding the bacterial colonies, which indicates positive siderophore production (Maheshwari *et al.,* 2019).

Catalase Hydrolysis

The catalase activity in the isolates was checked by flooding 3 per cent H_2O_2 over the bacterial colony. Formation of efferve scence was confirmed as positive, while the absence of bubbles or a few scattered bubbles was confirmed as negative (Shah *et al.,* 2021).

Hydrogen Cyanide

Hydrogen cyanide production was assessed following the method outlined by Bakker and Schippers (1987). Petri dishes containing 10 per cent Trypticase Soy Agar, supplemented with 4.4 g/L of glycine, were inoculated with the bacterial endophytes. The dishes were then inverted and covered with a lid that had been impregnated with a filter paper containing 0.5 per cent picric acid and 2 per cent sodium carbonate. The plates were incubated at 28 °C for three to five days. Cyanide production was indicated by a color change on the filter paper from yellow to orange-brown.

Statistical Analysis

The data was statistically analysed using OPSTAT statistical tool and the means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Isolation of Endophytic Bacteria from Landraces of Finger Millet

A total of sixty-nine bacterial endophytes were isolated from twenty-four landraces of finger millet. The majority of these endophytes were obtained from seeds (34 isolates), followed by roots (18 isolates) and leaves (17 isolates) (Plate 1). This result is also validated by the results of Bhagyashree *et al*., (2023), were more number of endophytes were isolated from the seed of finger millet landraces. The abundance of seed-associated endophytes suggests that seeds might serve as important reservoirs for microbial diversity in finger millet landraces, potentially influencing seed germination and early seedling growth in adverse environmental conditions. As the result of elimination of seed-endophytic bacteria from these landraces reduced germination at the unfavourable environment (Arellano-Wattenbarger *et al.,* (2023). Endophytes in seed positively influence root and shoot development, contribute to root hair formation as these endophytes are in direct contact with root and shoot and enhance the chlorophyll content of seedlings (Shearin *et al.,* 2018). The isolates were subsequently purified and categorized based on their associated crop varieties. Among these, only those exhibiting distinct morphological traits, such as variations in colony shape, color and texture, wereselected for further characterization.

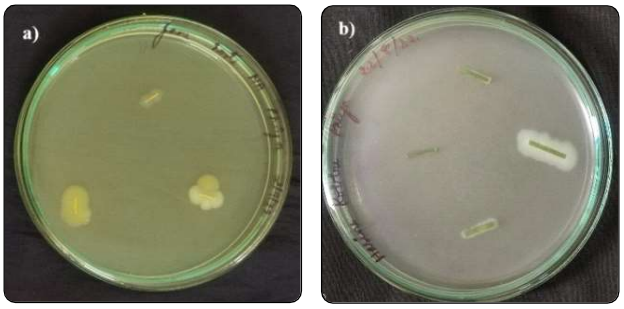

Plate 1 : Isolation of endophytic bacteria from the different parts of landraces of finger millet

Note : a) Isolation of endophyte from roots;
b) Isolation of endophyte from leaves

Table 1

Endophytic bacteria isolated from landraces of finger millet

Land races	Isolates
Ayyan	AES
Benne mundaga	BMES1, BMES2, BMEL1, BMEL2, BMEL3, BMER1, BMER2
Bilikkadi	BES, BEL1, BEL2
Dodda	DES1, DES2
Gana	GES
Gidda	GDES1, GDES2, GDES3, GDEL1, GDEL2
Guli	GUES1, GUES2
Guppe	GPES1, GPES2
Gutteginthalu	GGES
Haalu	HES
Haaluguli	HGES1, HGES2, HGEL, HGER1, HGER2, HGER3
Hasina kombu	HKES, HKEL1, HKEL2, HKER1, HKER2, HKER3
Hasirumundaga	HMER1, HMER2
Jenu	JES, JEL1, JEL2, JER1, JER2, JER3
Kapputhene	KTES
Keenya	KES, KEL1, KEL2, KEL3, KER1, KER2
Kolimotte	KMES
Kuntukulu	KKES1, KKES2, KKES3
Mallige	MES
Mundaga	MUES, MUEL, MUER1, MUER2
Nepal	NES, NEL, NER
Shakthi	SES1, SES2
Thenemundaga	TES
Zimbabae	ZES1, ZES2

Note : E- Endophyte, S- Seed, L- Leaf and R- Root

Screening for Biocontrol Activity of Endophytic Bacteria against Foot Rot Pathogen

The antagonistic potential of sixty nine purified bacterial endophytes against *Sclerotium rolfsii* was assessed using a dual culture technique. Inhibition zones were measured to calculate the percentage of inhibition, which ranged from 85.57 per cent (exhibited by HKES) to 44.39 per cent (shown by KMES). Based on these results, eight isolates - AES,

BMEL-1, GDEL-1, GES, HKES, KEL-1, KER-1 and KER-2which significantly differed (P= < 0.005) from other isolates, were selected for further characterization basedon their superior inhibitory activity (Plate 2) (Fig. 1). This effectiveness may be due to the production of secondary metabolites and volatile organic compounds by the bacterial endophytes, which inhibit fungal growth and sclerotium germination and indirectly by inducing systemic resistance in plants (Li *et al.,* 2023 and Rajani *et al*., 2019). This evidently suggests that endophytes from landraces contribute to higher disease resistance compared to modern varieties, since landraces have been shown to be highly adaptive under multi-site evaluation. (Sanchez-Martín *et al.,* 2017).

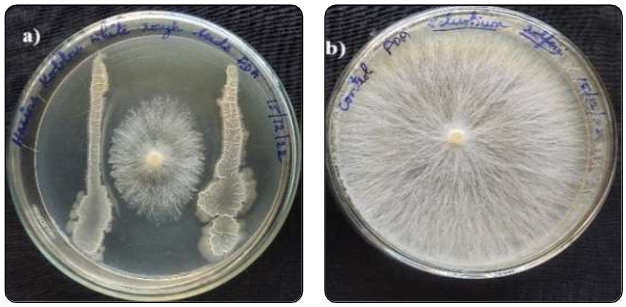

Plate 2 : Screening the biocontrol activity by dual culture technique

Note : a) biocontrol activity of HKES isolate
b) control (*Sclerotium rolfsii*)

Characterisation of the Endophytes

Under gram staining and microscopic observation, among the 8 isolates, 6 were gram positive and 2 were gram negative. The morphological characters of the endophytic isolates are mentioned in the Table 2.

Biochemical Characterization of Efficient Bacterial Endophytic Isolates

The eight selected bacterial endophytes subjected to a series of biochemical assays to assess their potential for characterization and identification. These assays included tests for siderophore production, catalase activity and hydrogen cyanide (HCN) production. In the siderophore production test, the AES isolate, sourced from the seeds of the Aayan landrace, exhibited the largest yellow halo. This indicates a high level of siderophore production, which is essential for iron uptake and competitive inhibition of pathogenic microbes (Table 3) (Plate 3b). These results are consistent with those reported by Longoria-Espinoza *et al*. (2024), where endophytes isolated from potato plants also demonstrated significant side rophore production, as evidenced by the formation of a yellow halo around the bacterial colonies.

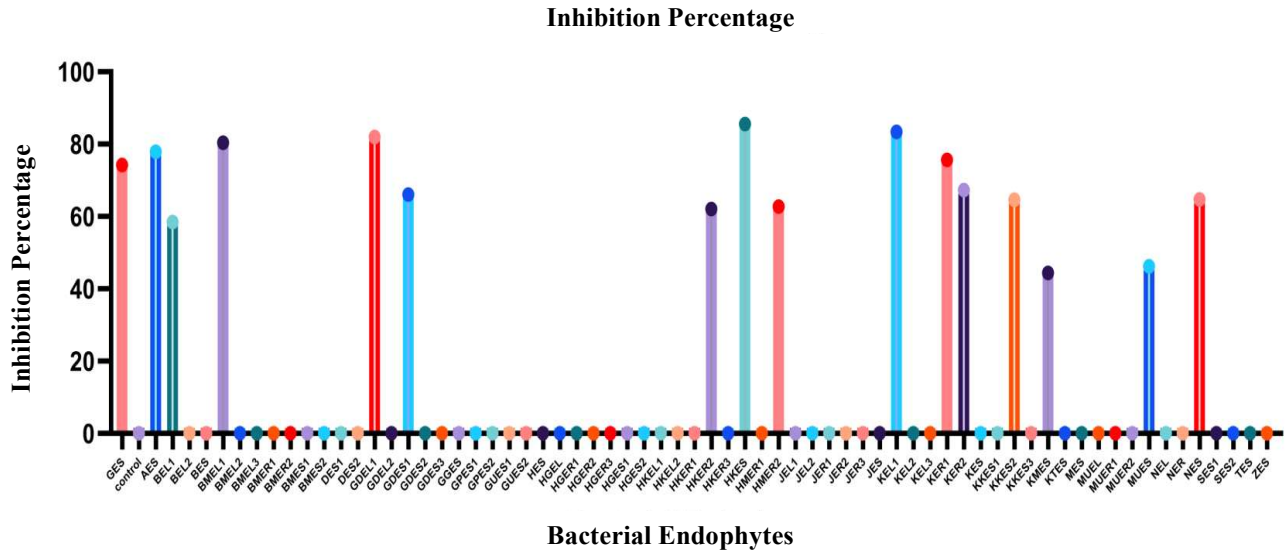

Fig. 1 : Antagonistic potential of endophytic bacterial isolates in dual culture technique

TABLE 2

Morphological characteristics of the endophytic bacteria isolated from landraces of finger millet

Isolates	Gram Staining	Shape	Colony Colour
GES	Negative	Cocci	White
HKES	Positive	Cocci	White
GDEL1	Positive	short rods	White
KEL1	Positive	short rods	White
BMEL1	Positive	short rods	White
KER2	positive	short rods	White
AES	Positive	Cocci	White
KER1	negative	Cocci	White

TABLE 3

Biochemical Characterization of Efficient Bacterial Endophytic Isolates

Isolates	HCN Production	Siderophore Production	Catalase
GES	Negative	Cocci	White
AES	-	+++	+
BMEL1	+	++	+
GDEL1	-	++	+
GES	-	++	+
HKES	-	+	+
KEL1	+	+	+
KER1	+	+	+
KER2	+	++	+

Note : (-) -Negative, (+) -good, (++) -very good, (+++) -excellent

All endophyticisolates were tested positive for catalase activity, as indicated by the effervescence production when 3 per cent hydrogen peroxide was applied to the culture plates (Table 3) (Plate 3c). This result aligns with findings from Fariska *et al*. (2024), where endophytes isolated from cassava leaves also showed positive catalase activity, which is a key enzyme involved in the breakdown of hydrogen peroxide and protection of cells from oxidative damage.

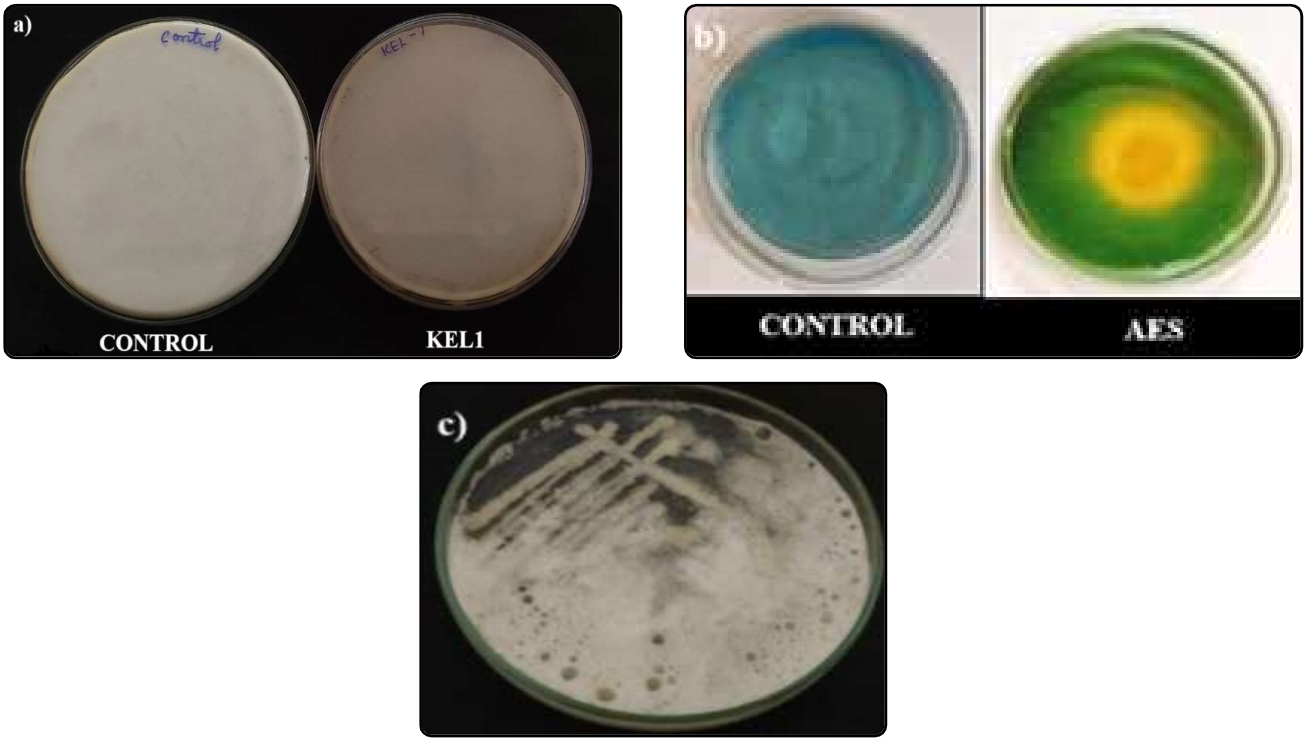

Plate 3 : (A) HCN Production of KEL1; (B) Siderophore production of AES; (C) Catalase activity of GDEL1

In the hydrogen cyanide production test, isolates BMEL1, KEL1, KER1 and KER2 exhibited positive results by changing the filter paper color from yellow to brown. This change indicates the production of hydrogen cyanide, a volatile compound known for its antifungal properties (Table 3) (Plate 3a). Shreshtha *et al*. (2024) also observed similar results in their study, where 66 per cent of 90 endophytes isolated from cucumber plants were capable of producing hydrogen cyanide, further supporting the efficacy of these compounds in microbial antagonism.

A total of sixty nine bacterial endophytes were initially screened for their biocontrol potential against *Sclerotium rolfsii*, the pathogen responsible for foot rot in finger millet. The dual culture assay identified eight isolates with significant antagonistic activity. The biocontrol efficacy of these isolates is likely due to the production of secondary metabolites, volatile organic compounds and allelochemicals that inhibit pathogen growth. Among these, isolates HKES-1, GDEL-1 and KEL-1 exhibited the highest levels of inhibition against *Sclerotium rolfsii*. These findings suggest that the endophytes from landraces not only offer potential as biological control agents but also possess the ability to with stand and thrive under varied environmental conditions. The promising results highlight the potential for utilizing these endophytes in sustainable agricultural practices to manage foot rot effectively while minimizing environmental impact.

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