The Pharma Innovation Journal 2022; SP-11(11): 111-113

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 111-113

www.thepharmajournal.com
Received: 19-09-2022
Accepted: 22-10-2022

Eramma
PhD Scholor, Department of Agricultural Microbiology, UAS, Raichur, Karnataka, India

Dr. Mahadeva Swamy
Professor and Head, Department of Agricultural Microbiology, University of Agricultural Sciences, Raichur Karnataka, India

Dr. Nagaraj M Naik
Assistant Professor, Department of Agricultural Microbiology, University of Agricultural Sciences, Raichur, Karnataka, India

Dr. Yallappa M
Assistant Professor, Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka, India

Dr. Ramesh YM
Assistant Professor, Department of Agronomy, Agriculture Research Station, University of Agricultural Sciences, Raichur, Karnataka, India

Dr. Yenjerappa ST
Professor and Head, Department of Plant Pathology, University of Agricultural Sciences, Raichur, Karnataka, India

Corresponding Author:
Eramma
PhD Scholor, Department of Agricultural Microbiology, UAS, Raichur, Karnataka, India

Bio control potentiality of bacterial endophytes against maize diseases

Eramma, Mahadeva Swamy, Dr. Nagaraj M Naik, Dr. Yallappa M, Dr. Ramesh YM and Dr. Yenjerappa ST

Abstract
Plant growth promoting endophytic bacteria were isolated from maize (*Zea mays* L.) from different maize growing regions of Kalyana Karnataka. Totally 92 endophytic bacterial isolates were isolated from different parts of maize. Further based on the PGP traits exhibited by the endophytes, six efficient were selected and identified. The isolates were screened for antagonistic activity against *Rhizoctonia solani* and *Fusarium moniliforme,* causing banded leaf and sheath blight and stalk root and ear rot diseases in maize following dual culture method. Among the six efficient endophytic isolates, EKSB-8 showed highest inhibition of 42.00% against *Rhizoctonia solani* and EPSB-6 produced highest inhibition of 43.33% against *Fusarium moniliforme* respectively. The study confirmed the potential of plant growth promoting endophytes EKSB-8 (*Pantoea dispersa*) and EPSB-6 (*Pseudomonas libanensis*) as bio control agents against *R. solani* and *Fusarium moniliforme* pathogens of maize].

Keywords: Biocontrol, antagonistic, *Rhizoctonia solani,* and *Fusarium moniliforme,* endophytes

Introduction
Among all the cereal crops in the world, maize is of greater importance because it is a highly consuming staple food around the world and its wide range of adaptability to varied agro-climatic regions (Mahajan *et al.,* 2015) [11]. *Rhizoctonia solani* is a soil borne fungus, causes Banded leaf and sheath blight represents significant economic yield loss in maize cultivation Singh *et al*., 2014) [15]. In the last two decades its severity has been increasing, causing the yield loss upto 40-70% which can often be managed with chemical fungicides *viz*., carbendazim, propiconazole, hexaconazole, tebuconazole (Sharma *et al*., 2002) [14].
Fusarium moniliforme is a facultative fungal endophyte stalk root and ear rot disease in maize. In both biotrophic and saprophytic growth *F. moniliforme* produces fumonisins and reduce the commercial value of maize. It transmits to the subsequent generations through clonal infection of seeds and plant debris via both vertical and horizontal transmission.
It is important to adapt environmentally friendly disease control strategies like biological control in near future since use of chemical fungicides causes various human health problems and also pathogens develop resistance against fungicides. It is of public concern to use chemical fungicides as it causes numerous human health problems and also pathogens resist fungicides (Hallmann *et al*., 2009) [7].
Plant growth promoting rhizobacteria and endophytes can be used as substitute for management of fungal diseases. The presence of endophytes has been found in all types of plants. Endophytes colonize the internal tissues of the host plant without causing any damage to those plants. A large number of endophytic bacteria are common rhizospheric bacteria. In presence of pathogens endophytes produce antibiotics, secondary metabolites, hydrolytic enzymes and volatile compounds through mechanisms similar to PGPR to counteract their negative effects (Lodewyckx *et al*., 2002; Schulz *et al.*, 2006) [10, 13]. As endophytic bacteria inhabit internal living tissues of host plant and their close association with pathogens, they are used as bio control agents against most plant pathogens (Berg *et al*., 2005) [2]. Present investigation was conducted to profile the endophytic bacteria associated with maize and to evaluate their antagonistic activities, bio control potential *in vitro* and *in vivo* conditions against and *R. solani and F. moniliforme* pathogens which cause banded leaf and sheath blight and stalk root and ear rot diseases in maize respectively.

Material and Methods

Maize plant samples were collected from maize growing regions of Kalyan Karnataka region. Totally 92 endophytic bacteria were isolated from root, stem and leaf of maize plant samples. Among all isolated endophytes, 40 were phosphate solubilizers, 30 were zinc solubilizers and 22 were potassium solubilizers.

Isolation of endophytic bacteria

The isolation of endophytes was conducted by following the standard procedures given by Bacon *et al*. (2002) [1]. In order to remove soil particles, maize plant samples were washed thoroughly under running tap water. During surface sterilization 2cm pieces of roots and stems were cut and sequentially washed for one minute in 70 % ethanol, followed by 5% sodium hypochlorite for 5 minutes in young plants whereas, 10 minutes in older plants and finally in 70 % ethanol for one min. Lastly they were rinsed four times with sterile distilled water and samples were dried aseptically.

One gram of plant sample was macerated aseptically using a sterilized mortar and pestle in 9 ml of sterile distilled water. From this, 1.5 ml of aliquot was transferred to a sterile microfuge tube and centrifugation was done at 1,300 rpm at 40 °C for 10 min. Serial dilution was performed for I ml of supernatant upto 10^{-8} dilutions. A fter this, 0.1ml of each dilution was spread onto the plates containing specific media and incubated for 4 to 5 days at 27-30 °C. Specific media namely sperber media (Sperber, 1958) [16], aleksandrow media (Hu *et al.*, 2006) [8] and TRIS minimal media (Di *et al.*, 1998) [6], were used for the isolation of phosphate, potassium and zinc solubilizing bacteria respectively. Morphologically distinct endophytic bacterial colonies were picked and pure cultures were preserved in 40% (v/v) glycerol solution at -20 °C.

Pathogens: Virulent isolates of *R. solani* and *F. moniliforme* isolated from naturally infected maize samples were obtained. To the new PDA plates, actively growing hyphae were successively transferred and the cultures were maintained on slants and preserved at 4 °C.

Bio control efficiency of endophytic isolates against fungal pathogens

Dual culture technique was used to measure the effect of isolated endophytic bacteria on two maize pathogens *Rhizoctonia solani* (Banded leaf and sheath blight) and *Fusarium moniliforme* (stalk root and ear rot). In a petri plate containing PDA medium, endophytic bacterial isolate was streaked on one side and disc of mycelium from 7 days old fungal pathogen was placed in the middle and incubated at 28 ± 2 ºC for seven days.

Isolates which were positive for antagonistic activity, inhibited growth of the fungus when it grew towards the endophytic bacterial colony on potato dextrose agar. The per cent inhibition was measured using the formula (Mugiastuti *et al.*, 2020) [12].

$$I\ \% = \{(C - T) / T\}\ 100$$

I = Percent inhibition of pathogen
C- Growth of mycelium in control plate (cm)
T - Growth of mycelium in treatment plate (cm)

Result and Discussion

A total of 92 endophytic bacteria were isolated from different maize growing regions of Kalyana Karnataka. Among those, 40 were phosphate solubilizers, 30 were zinc solubilizers and 22 were potassium solubilizers and two efficient nitrogen fixing were collected from Department of Agricultural Microbiology. Based on PGP traits six efficient strains were selected and further evaluated for antagonistic activity against maize pathogens *Rhizoctonia solani* and *Fusarium moniliforme* following dual culture technique (Table 1).

Table 1: Bio control potentiality of endophytic bacterial isolates against fungal pathogens

Endophytic isolate	*Rhizoctonia solani*		*Fusarium moniliforme*	
	Mycelium growth (cm)	Inhibition of pathogen (%)	Mycelium growth (cm)	Inhibition of pathogen (%)
EPSB-6	5.44	40.00%	5.12	43.33%
EPSB-16	-	-	5.91	34.33%
EZSB-6	6.34	29.55%	-	-
EKSB-8	5.22	42.00%	5.18	42.44%
ENFB-5	5.65	37.22%	5.27	41.44%
ENFB-21	6.01	33.22%	6.60	26.66%
Control	9.00	100%	9.00	100%
S.Em.±		0.27		0.25
CD		1.03		0.92

Mean values are average of three replication

In case of *Rhizoctonia solani*, among the four efficient endophytic isolates, EKSB-8 (*Pantoea dispersa*) showed highest inhibition of 42.00% with radial growth of pathogen as 5.22 cm followed by EPSB-6 (*Pseudomonas libanensis*), recorded inhibition percent of 40.00% with mycelium growth of pathogen as 5.44 cm, while EZSB-6 (*Microbacterium neimengense*) recorded the lowest inhibition of 29.55% with mycelium diameter of 6.34 cm. No inhibition was recorded by an isolate EPSB-16 (*Acinetobacter pittii*) (Plate 1).

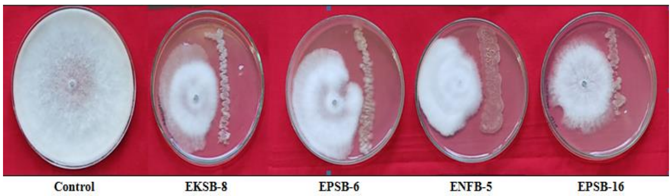

Plate 1: Bio control potentiality of endohytic bacterial isolates against maize fungal pathogen *Rhizoctonia solani*

In case of *Fusarium moniliforme*, among the four efficient endophytic isolates, EPSB-6 produced highest inhibition of 43.33% with radial growth of pathogen as 5.12 cm followed by EKSB-8, which recorded inhibition percent of 42.44% with radial growth of pathogen as 5.18 cm while the lowest inhibition of 26.66% was recorded by ENFB-21 with mycelium diameter of 6.60 cm, EZSB-6 failed to inhibit the pathogen (Plate 2).

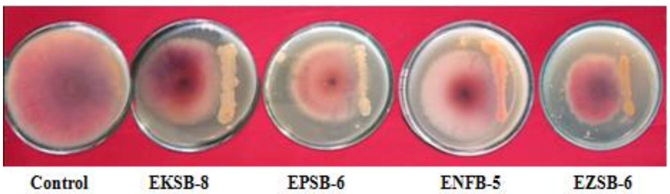

Plate 2: Bio control potentiality of endohytic bacterial isolates against maize fungal pathogen *Fusarium moniliforme*

Endophytes inhibit the pathogen growth by secreting some of the secondary metabolites antimicrobial compounds that cause alteration in structural architect and lysis of mycelia, also due to the mutual exchange of genetic materials between endophytes and host plant by horizontal transfer. Similar outcomes were obtained by, Summi *et al*., (2013) [13], who isolated 52 endophytic bacteria from the maize roots and carried out *in vitro* studies against maize pathogens such as *Curvularia lunata* and *Exserohilum turcicum*, which exhibited maximum inhibition of pathogen 82% and 80% by endophytic bacteria EB23 and EB22.
Similarly, using agar diffusion method, Dawut *et al*., (2016) [5] screened two endophtic *Bacillus substilis* XJAS-AB-11 and XJAS-AB-13 strains for their ability to inhibit the maize pathogens responsible for spot in maize *i.e*., *Bipolaris maydis* and *Exserohilum turcicum*. Inhibitory activity of XJAS-AB-11 and XJAS-AB-13 broth against *E. turcicum and B. maydis* were 45.0%, 63.33% and 58.34%, 23.33% respectively under *in vivo*. Similarly, Bolivar *et al*., (2021) [3] isolated endophytic bacteria *Bacillus subtilis* from *Zea mays*. Upon characterization, various strains of *Bacillus subtilis* showed growth inhibition towards *B. cinerea* under in vitro cultures. Similarly, Chauhan *et al*., (2016) [4] reported bio control potentiality of *Bacillus cereus* against *Fusarium solani* causing rhizome rot in turmeric. Similarly the Antifungal activity was exhibited by bacterial endophytes, *B. cereus*, *B. thuringiensis*, *B. pumilis*, *P. putida* and *Clavibacter michiganensis*, against *F. solani*, *A. pullulans*, *Alternaria alternata* and *B. fulva* pathogens in turmeric (Kumar *et al*., 2016) [9].

Conclusion
The present study revealed the importance of endophytic bacteria for bio control traits. In this study, based on *in vitro* experiments, two strains of endophyte *viz*., *Pantoea dispersa* and *Pseudomonas libanensis* exhibited significantly higher antagonistic activity, which confirms the potential of endophytes as bio control for sustainable maize cultivation. For the best of our knowledge, this is the first report on the strains of endophytes *viz*., *Pantoea dispersa* and *Pseudomoas libanensis* as bio control agents against maize pathogens *R. solani* and *F. moiliformae*.

References

1. Bacon CW, Glenn AE, Hinton DM. Isolation, in planta detection and culture of endophytic bacteria and fungi. Journal of Food Science and Technology. 2002;17:136-138.
2. Berg G, Krechel A, Ditz M, Faupel A, Sikora RA, Ulrich A, *et al*. Endophytic and ectophytic potato associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiology Ecology. 2005;51:215-229.
3. Bolivar AHJ, Rodriguez VE, Cantoral JM, Sanchez D, Collado IG, *et al*. Endophytic bacteria *Bacillus subtilis*, isolated from *Zea mays*, as potential biocontrol agent against *Botrytis cinerea*. Biology. 2021;10:492.
4. Chauhan AK, Maheshwari DK, Kim K, Bajpai VK. Termitarium-inhabiting *Bacillus endophyticus* TSH42 and *Bacillus cereus* TSH77 colonizing *Curcuma longa* L.: isolation, characterization, and evaluation of their biocontrol and plant-growth-promoting activities. Canadian Journal of Microbiology. 2016;62:880-892.
5. Dawut M, Abliz A, Ghopur W, Behti P, Obulkhasim B, Mijit G. Biocontrol potentiality of active ingredients from endophytic *Bacillus subtilis* isolated from *Alhagi pseudalhagi* Desv on maize spot diseases. Journal of Plant Pathology and Microbiology. 2016;7:11-18.
6. Di SCD, Sayer JA, Gadd GM. Solubilization of zinc phosphate by a strain of *Pseudomonas fluorescens* isolated from a forest soil. Biology and Fertility of Soils. 1998;28(1):87-94.
7. Hallmann J, Davies KG, Sikora R. Biological control using microbial pathogens, endophytes and antagonists. Root-Knot Nematodes; c2009. p. 380-411.
8. Hu X, Chen J, Guo J. Two phosphate and potassium solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World Journal of Microbiology and Biotechnology. 2006;22:983-99.
9. Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. 3 Biotech. 2016;6:1-8.
10. Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi S, Mezgeay M, *et al*. Endophytic bacteria and their potential applications. CRC Crit. Rev. Pl. Sci. 2002;21:583-606.
11. Mahajan V, Yadav OP, Kumar P, Kumar B, Chikkappa GK, Kaul J, *et al*. Annual Progress Report Kharif Maize All India Coordinated Research Project on Maize. Eds. Indian Institute of Maize Research, Pusa Campus, New Delhi 110-012, India; c2015. p. 999.
12. Mugiastuti E, Suprayogi, Prihatiningsih N, Soesanto L. Isolation and characterization of the endophytic bacteria, and their potential as maize diseases control. Biodiversity Journal. 2020;21(5):1809-1815.
13. Schulz BJE, Boyle CJC, Sieber TN. Microbial root endophytes, Springer; c2006. p. 1-13.
14. Sharma RC, Srinivas P, Batsa BK. Banded leaf and sheath blight of maize its epidemiology and management. In N. P. Rajbhandari, J. K. Ransom, K. Adhikari, and A. F. E. Palmer (Eds.), Proc. of a maize symposium, NARC and CIMMYT: Kathmandu; 2001. p. 108-112.
15. Singh V, Kumar S, Lal M, Hooda KS. Cultural and morphological variability among *Rhizoctonia solani* isolates from trans-gangetic plains of India. Research on Crops. 2014;15:644-650.
16. Sperber JI. The incidence of apatite solubilizing organisms in the rhizosphere and soil. Australian Journal of Agricultural Research. 1958;9:778-781.
17. Summi D, Vendan K, Santhosh GP, Mahadevaswamy, Aswathnarayana D. Endophytic bacteria and fungi as biocontrol agents against foliar diseases of Maize. Mysore Journal of Agricultural Sciences. 2013;47:523-531.