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# STUDY OF METABOLIC POTENTIAL OF ENDOPHYTIC BACTERIA IN FINGER MILLET

**Shikha Shirsh*** JRF, Department Of Biotechnology, Lalit Narayan Mithila University, Darbhanga-846004, India *Corresponding Author

**Jaykar Jha** Retd. Professor And H.O.D, Department Of Biotechnology, Lalit Narayan Mithila University, Darbhanga-846004, India

**ABSTRACT** Present study was carried out in the fields of Darbhanga to study the metabolic potential of endophytic bacteria inhabiting the roots, stems and leaves of finger millet.The plant samples were collected from the fields and were analysed for the metabolic potential endophytic bacteria by first isolating them and then further studying their ability to produce IAA,cellulase and protease and to solubilise phosphorous and zinc qualitatively.The endophytic bacteria found positive for these traits can be considered to be a potent plant growth promoting bacterial endophyte(PGPBE) which can be further used to increase the area of cultivation and productivity of finger millet.This will further encourage the farmers to grow finger millet and will ensure food security and sustainable agriculture.

**KEYWORDS :** Finger Millet.endophytic Bacteria,metabolic Potential

## INTRODUCTION

Finger millet (Eleusine coracana) is a staple food for drought prone areas of the world.It is popularly known as ragi,madua,nagli and kapai in vernacular languages in different parts of the country.It is considered to be a crucial crop for food and nutritional security.In India, it is not only an important crop amongst the small millets but also third in its importance among millets after sorghum and pearl millet.

Moreover,it is cultivated mostly as a rain-fed crop under different environmental conditions in India.The crop is grown in an area of 1.6 million hectare and production of 2.1 million tonne.

## FINGER MILLET AS A CROP IN DARBHANGA

The total area of the district is 241443.2 hectare which includes 198415 hectare of cultivable land, 19617 hectare of upland,37660 hectare of medium land and 38017 of low land.Moreover,there is 29706 hectare of waterlogged or chaur area.

Major crops grown in Darbhanga include paddy,wheat, maize, pulses, oil seeds,sugarcane and ragi.According to the Directorate of Statistics and Evaluation,Bihar area,production and yield of ragi during year 2017-2018 can be given as follows:Out of 198415 hectare of total cultivable land,5891 hectare of land is under ragi cultivation and the total production is 4963 metric tonne per annum and 1186 kg/hectare.

Out of 18 blocks of Darbhanga,ragi is grown mostly in Gaura Bauram,Biraul,Benipur,Kusheshwar sthan(West), Ghanshyampur, Alinagar ,Kiratrpur and Baheri.

## ROLE OF FINGER MILLET IN FOOD SECURITY AND SUSTAINABLE AGRICULTURE

Finger millets are really important in the overall development of agriculture in the country.As the bulk production of this is consumed at the village level,the real value of this crop is not appreciated. Moreo ver,their role is also not recognised in imparting food security to a large farming community which is basically a vulnerable group in different parts of the country.

Millets are eco-friendly and thus,are suitable for fragile and vulnerable ecosystems and should be preferred for sustainable and green agriculture.Thus,promotion of millets can lead to a much efficient natural resource management and finally to a more holistic approach in sustaining agro-biodiversity.

## ROLE OF ENDOPHYTIC BACTERIA IN PLANT GROWTH AND DEVELOPMENT

Plants constantly interact with a wide range of bacteria. Plant associated bacteria generally colonize the rhizosphere (rhizobacteria), phyllosphere (epiphytes) and inside the plant tissues (endophytes). Endophytes are the microorganisms that visibly do not harm the plants and are able to live inside the tissues to enhance plant growth and provide nutrition to host (Gaiero et al 2013). Endophytic bacteria are ubiquitous i.e. distributed in almost all the plant species either through their active colonization or as latent residents in plant tissues. Endo phytes initially enter the plant endosphere, adapt to the new enviro nment and eventually promote plant growth, yield and plant defence responses. Endophytic bacterial diversity within plants depends on the ability of bacteria to colonize plant tissues and establish population both inter and intracellularly.

Cellulase and protease secreted by the bacteria act as key enzymes for the invasion and colonization of plant roots (Susilowati et al 2015). Plant root colonization by the endophytic bacteria is actually the primary step towards successful initiation of plant microbe interaction (Nelson 2004; Bhattacharjee et al 2012). After initial colonization, some endophytes move to other parts of plant by entering the vascular tissues and spread within the plant systematically (Gaiero et al 2013).

Plant growth promoting bacterial endophytes (PGPBE's) facilitate plant growth via two different mechanisms i.e. direct and indirect mechanisms. In direct mechanism, promotion of plant growth occurs by the production of phytohormones like indole acetic acid (IAA), cytokinin, Zinc (Zn), Phosphorous (P) solubilization and increase in assimilable nitrogen availability to host plant through biological nitrogen fixation (BNF). On the other hand, in indirect mechanism, bacterial endophytes produce secondary metabolites i.e. siderophore, antibiotics, hydrogen cyanide (HCN) and enzyme like 1-Aminocyclopropane-1carboxylate (ACC) deaminase, cellulase and protease which play a crucial role in conferring tolerance to biotic and abiotic stresses (Grover et al 2011; Souza et al 2015).

## ENDOPHYTIC BACTERIA WITH REFERENCE TO FINGER MILLETS

Recently studies have been made about association of endophytic bacteria with various crops.These studies have shown that the bacterial communities associated with various plants favour plant growth in different known and unknown ways. The performance of finger millet as rain-fed crops in low nutrient soil and without much use of fertilisers hint that possibly this crop harbours various endophytes in its roots which help in better absorption and assimilation of the nutrients present in the soil.Though there have been several reports on composition and ecological roles of symbiotic bacterial communities associated with various terrestrial and fresh water plants but the role of endophytes with reference to the coarse grains has not been studied so far.It seems to be really interesting to explore the presence and relevance of endophytic bacterial communities in finger millet as this area of research is completely unexplored and untouched.There is a need to find out potential endophytic isolates of this promising as well as nutritious crop with plant growth promoting (PGP) traits to boost its production and to increase its area of production .

## MATERIALS AND METHODS

### COLLECTION OF SAMPLES

For the isolation endophytic bacteria,healthy leaves,stems and roots of *Eleusine coracana* were collected from randomly selected healthy wild and cultivated plants from the fields of Kusheshwar sthan(W), Biraul and Alinagar blocks of Darbhanga.

## SURFACE STERILISATION

The healthy plants after being washed under slow running tap water were separated into stems,roots and leaves.Stems and roots were cut into sections 2-3 cm long.Sub-samples were prepared from each sample for isolation of endophytes.The samples were disinfected by immersing the samples in 70% ethanol for 1-3 minutes and 4% aqueous solution of sodium hypochlorite for 1.5 minute,again rinsed with 70% ethanol and finally rinsed with sterile distilled water(SDW) 4-5 times in the laminar air flow cabinet.

## MEDIA FOR ISOLATING ENDOPHYTIC BACTERIA

Endophytic bacteria were isolated and cultured in Nutrient Agar culture media containing 3 g beef extract,5 g peptone,5 g NaCl,15 g agar and 1000 ml SDW with pH 7.0-7.2 and in Pseudomonas Agar (For Fluorescein) which contained Casein enzyme hydrolysate 10.0 g/ L,Protease peptone10.0 g/ L,K2HPO4 1.5 g/ L, MgSO4.7H2O 1.5 g/ L, Agar 15.0 g/ L and SDW 1000 mL with pH 7.0-7.2.

## ISOLATION,PURIFICATION AND SUBCULTURE OF ENDOPHYTIC MEDIA

For inoculation 0.1 ml of the aliquot was spread on the NA medium .The inoculations were done in triplicates separately for each sample of root,stem and leaf extract.The plates were then sealed using parafilm tape and were incubated 28°C for 48-72 hours in order to recover the maximum possible colonies of bacterial endophytes.Observations were taken after 48-72 hours.The total number of colonies was counted after the colonies appearedand expressed as cfu(colony forming unit).

**Table 1: Isolates of endophytic bacteria from different tissues of finger millet**

| Sources | Tissue | Endophytic bacteria |
|---|---|---|
| Biraul | Root(7) | BIRR1,BIRR2,BIRR3,BIRR4,BIRR5,BIRR6,BIRR7 |
| | Stem(4) | BIRS1,BIRS2,BIRS3,BIRS4 |
| | Leaf(5) | BIRL1,BIRL2,BIRL3,BIRL4,BIRL5 |
| | Total=16 | |
| Kusheshwar Sthan | Root(6) | KUSR1,KUSR2,KUSR3,KUSR4,KUSR5,KUSR6 |
| | Stem(5) | KUSS1,KUSS2,KUSS3,KUSS4,KUSS5 |
| | Leaf(4) | KUSL1,KUSL2,KUSL3,KUSL4 |
| | Total=15 | |
| Alinagar | Root(6) | ALIR1,ALIR2,ALIR3,ALIR4,ALIR5,ALIR6 |
| | Stem(4) | ALIS1,ALIS2,ALIS3,ALIS4 |
| | Leaf(5) | ALIL1,ALIL2,ALIL3,ALIL4,ALIL5 |
| | Total=15 | |
| | Grand total=46 | |

BIRR:Biraul root KUSR:Kusheshwar sthan root ALIR:Alinagar root
BIRS:Biraul stem KUSS:Kusheshwar sthan stem ALIS:Alinagar stem
BIRL:Biraul leaf KUSL:Kusheshwar sthan leaf ALIL:Alinagar leaf

## CHARACTERIZATION OF ENDOPHYTIC BACTERIA ISOLATES FOR PLANT GROWTH PROMOTIONAL (PGP) TRAITS

A) Quantitative analysis of Indole acetic acid (IAA) production: The production of IAA in the isolates of endophytes was detected according to Gordon and Weber (1951).This was done by inoculating bacterial suspension in 10 ml Luria Bertani broth containing tryptophan 0.01% (L-Trp) and incubating the tubes at 28°C for 3-6 days. Appearance of pink colour confirmed the production of IAA. The amount of IAA was determined quantitatively by adding about 2 ml of Salkowski's reagent (1 ml of 0.5ml $FeCl_3$ in 50 ml of 35% $HClO_4$) to 1 ml of culture supernatant.On the other hand, using uninoculated broth with Salkowski's reagent as a reference. Absorbance of pink colour was measured spectroscopically in the spectrophotometer at 535 nm after 20 min and the IAA concentration was quantified by standard curve.

B) Assay for Phosphate (P) solubilization

Qualitative estimation of phosphate solubilisation: The ability of endophytic bacteria to solubilise phosphate was determined qualitatively by streaking strains on National Botanical Research Institute's Phosphate growth medium (NBRIP) (Arora 2007). Presence of yellow clear zone around bacterial growth after 5-7 days of incubation period at 28 ° C indicated positive phosphorous solubi li sation.

Here, phosphate solubilization index (PSI) was calculated by using the given formula:

PSI Index = A/B

where A= Total diameter (colony + halo zone)

B= Diameter of colony

C) Zn solubilisation: The isolates were taken to solubilise zinc on tris-minimal medium supplemented with zinc oxide (ZnO) and zinc phosphate( $Zn_3(PO_4)_2$) separately at a concentration 0.1% Zn. After spot inoculation of endophytic bacteria on plates,the plates were incubated in dark at 28°C and then observed for formation of clear zone around bacterial growth after 7 days.

## CELL WALL DEGRADING ENZYME PRODUCTION

**A)** Protease production: Protease activity or casein degradation was determined by the appearance of clear zone on skimmed milk agar by endophytic bacterial isolates. Skimmed milk agar plates were prepared and spot inoculated with the endophytic bacterial isolates and incubated at 28°C for 2-5 days. Appearance of clear zone around the colony was considered as the positive test for protease production (Chaiharn et al 2008).

**B)** Cellulase production: The bacterial isolates were screened for cellulase activity by plating on Carboxy Methyl Cellulose (CMC) agar according to Ariffin et al (2006).Carboxy methyl cellulose (CMC) agar plates were prepared and spot inoculated with bacterial isolates and incubated at 28ºC for 5 days for the secretion of cellulase. Appearance of halo zone around the colony was considered as the positive test for cellulase production.

After incubation, the plates were flooded with the aqueous solution of congo red (1% w/v) for 15 minutes. Then congo red solution was poured off and plates were further flooded with 1M NaCl for 15 minutes.The formation of a clear zone of hydrolysis around the colony indicated cellulose activity and cellulose degradation. High cellulose activity was decided by measuring the diameter of clear zone.

## RESULTS AND DISCUSSION

## SCREENING OF ENDOPHYTIC BACTERIAL ISOLATES OF FINGER MILLET FOR MULTIFARIOUS PGP TRAITS

A) Indole acetic acid (IAA) production

Total 46 endophytic bacterial isolates were tested for their qualitative IAA production. The bacterial isolates indicated their ability to produce IAA by producing pink colour with Salkowasi's reagent. All the endophytic bacterial isolates of finger millet were cultured in Luria broth supplemented with 0.01% tryptophan for their quantitative analysis.The amount of IAA produced by the bacterial isolates ranged from 2.0 – 6.5 $\mu gml^{-1}$ in the absence of tryptophan and 7.6 – 19.8$\mu gml^{-1}$ in the presence of tryptophan after the incubation of 3 days (Table 4).

Out of 46 endophytic bacterial isolates,11isolates namely BIRR3,BIRR5,BIRL3,KUSR2,KUSR5,KUSS1,KUSS3,KUSL2,ALIR4,ALIS2 and ALIL3 produced high amount of IAA which ranged between 15 - 20 $\mu gml^{-1}$ at 3rd day of incubation in the presence of tryptophan. Of 46 endophytic bacterial isolates, 22% produced low amount of IAA, 54% produced medium amount of IAA and 24% produced high amount of IAA(Fig 2).

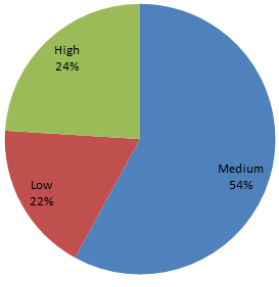


**Fig 1: Percent of IAA production by endophytic bacterial isolates of finger millet in the presence of tryptophan**

A) Phosphate solubilization

To analyse the phosphate solubilising activity of the bacteria,all the 46 endophytic bacterial isolates were grown on NBRIP medium.The media was modified by adding 0.5% tri-calcium phosphate as inorganic source of phosphorous. Out of 46 endophytic bacterial isolates, 23.91% (11) showed a very clear yellow halo zone around colony on NBRIP medium.This indicated the positive test for

phosphorous solubilization. Phosphate solubilization index (PSI) ranged from 1.06 to 1.33 (Table 5). Highest phosphorous solubilization index i.e. 1.31 was shown by KUSS1which was isolated from the sample collected from Kusheshwar sthan which was followed by BIRR3 which was isolated the sample collected from Biraul which showed the PSI of 1.17.On the other hand, lowest PSI was recorded for KUSR2 i.e.1.06 which was isolated from the sample collected from Kusheshwar sthan.

**Table 2: Phosphate Solubilization of endophytic bacterial isolates of finger millets shown by diameter of halo zone**

| Endophytic bacterial isolates | Diameter of Halo Zone (cm) | P-solubilization index (PSI) |
|---|---|---|
| BIRR3 | 0.3 | 1.17 |
| BIRR5 | 0.4 | 1.09 |
| BIRL3 | 0.1 | 1.07 |
| KUSR2 | 0.6 | 1.06 |
| KUSR5 | 0.2 | 1.08 |
| KUSS1 | 0.2 | 1.33 |
| KUSS3 | 0.3 | 1.12 |
| KUSL2 | 0.1 | 1.08 |
| ALIR4 | 0.5 | 1.07 |
| ALIS2 | 0.1 | 1.11 |
| ALIL3 | 0.2 | 1.09 |

C) Zinc solubilisation

All the bacterial endophytes from finger millet were subjected to measure Zinc solubilization efficiency on Tris-minimal medium.The media was amended by adding zinc oxide and zinc phosphate as inorganic source of zinc. Out of total 46 endophytic bacteria, three isolates (BIRR5, KUSL2 and ALIL3) were able to solubilize zinc phosphate which was incorporated in Tris- minimal medium. These three isolates produced a clear halo zone around the colony.Maximum diameter of halo zone was shown by ALIL3 (0.5) which was isolated from the sample taken from Alinagar which was followed by both BIRR5 and KUSL2(0.4)which were isolated from the sample collected from Biraul and Kusheshwar sthan respectively (Table ).On the other hand, all the endophytic bacterial isolates failed to solubilize zinc oxide incorporated in Tris-minimal medium.

**Table 3: Zinc solubilization efficiency of endophytic bacterial isolates of finger millet**

| Endophytic bacterial isolates | Halo zone diameter (cm) | | Zn solubilization efficiency (%) |
|---|---|---|---|
| | $Zn_3PO_4$ | ZnO | $Zn_3PO_4$ |
| BIRR5 | 0.4 | - | 16.5 |
| KUSL2 | 0.4 | - | 16.5 |
| ALIL3 | 0.5 | - | 21.5 |

## CELL WALL DEGRADING ENZYME

All the 46 isolates of endophytic bacteria were observed for qualitative production of protease and cellulase enzymes.This was done on skim milk and CMC agar media respectively. Of 46 isolates, 30.43% (14) were able to produce protease enzyme which was shown by the appearance of clear zone on the skimmed milk agar medium. Moreover, 17.39% (8) were able to produce cellulase enzyme which was shown by the appearance of halo zone on CMC agar medium. Highest protease production was shown by KUSR3 (2.1 cm) which was isolated from the root of the sample taken from Kusheshwar sthan followed by ALIR6 (2.0cm) which was isolated from the sample collected from Alinagar.On the other hand,in cellulase production highest halo zone was shown by BIRL5(1.5cm) isolated from the leaf of the sample collected from Biraul followed by KUSR3(1.4cm) isolated from the root of the sample taken from Kusheshwar sthan.

## CONCLUSION

Endophytic bacteria are believed to promote the growth of their host plant and also fitness through different direct and indirect mechanism (Vessey, 2003). Bacterial endophytes may strongly influence the performance, growth and stress tolerance of the plants in which they inhabit.There is need to select potential endophytic isolates with plant growth promoting traits (PGP) and colonization from root,stem and leaf tissues of coarse grains like finger millet.

During the present study,bacterial endophytes having potential plant growth promoting traits have been found which promote plant growth by producing IAA,phosphorous solubilisation and zinc solubilisation. These endophytic bacteria enter the plant by producing cell wall degrading enzymes i.e. cellulase and protease.This study primarily focuses on the qualitative production of IAA,cellulose and protease and solubilisation of phosphorous and zinc.More such studies need to be carried out in this field to encourage the cultivation of eco-friendly nutritious rain-fed crops like finger millet at the local level.

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