GOLDEN RESEARCH THOUGHTS

ISSN: 2231-5063
IMPACT FACTOR : 4.6052 (UIF)
VOLUME - 10 | ISSUE - 12 | JUNE – 2021

# "STUDIES ON ISOLATION OF ENTOPHYTIC BACTERIA AGAINST FUNGAL PLANT PATHOGEN FROM WHEAT"

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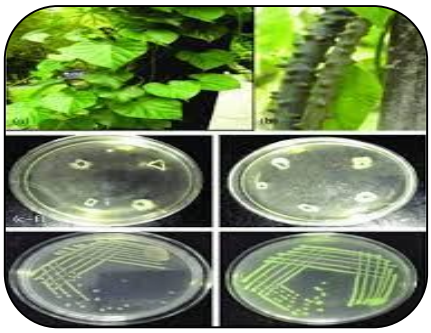
**ABSTRACT :-**



Endophytes are known to enhance plant nitrogen fixation or secrete plant hormone-like substances to promote growth and also to produce versatile secondary metabolites.This study aims to discover the antagonistic effects of some endophytic bacteria isolated from wheat. In present investigation a total 5 strains of an endophytic bacteria were isolated from wheat. Further on the basis of morphological, cultural and biochemical characteristics of endophytic bacterial isolates it was observed that total (4) genera viz; EBI-1 & EBI-5 belong to Bacillus, EBI-2 belongs to Klebsiella sp., EBI-3 belongs to Staphylococci, EBI-4belongs to Pseudomonas. Hence, the results indicated that the isolated bacterial endophytes EBI 1, EBI 4, and EBI 5 may be the possible candidates and can be used as bio control agent for the management of the Aspergillus Niger infections in wheat. Hence, these endophytic isolates were further considered for interaction studies against Aspergillus Niger.

**KEYWORDS:** Endophytes, Wheat, Plant pathogen, Aspergillus Niger, Klebsiella.

## INTRODUCTION:

Endophytes are known to enhance plant nitrogen fixation or secrete plant hormone-like substances to promotegrowth (Tan et al.,2003;Sevillaet al., 2001),and also to produce versatile secondary metabolites, which have medical and industrial applications (Hardoimet al., 2015; Ryan et al., 2008).

In addition, plant disease control by endophytes is one of the useful properties because endophytes can support sustainable agriculture and they do not cause environmental pollution or harmful toxic effects unlike chemical pesticides.

Seed endophytes may come from different plant organs, being transferred to seeds via vascular connections or through gametes, resulting in colonisation of embryo and endosperm; and reproductive meristems may also be the source (Malfanovaet al., 2013).Vertical transition from one plant generation to the following may then occur, as suggested by several authors (Gagne-Bourgueet al., 2013).

The role of seed endophytes has not been unraveled yet. It has been demonstrated that some of them can increase plant growth due to the production of plant hormones or to their contribution in plant nutrients acquisition, especially nitrogen and phosphorus (Gagne-Bourgueet al., 2013; Xuet al., 2009).

A worldwide fungal plant pathogen impacting severely on cereals production and quality, as this microorganism is a source of mycotoxins, which affect human and animal health. The purpose of this work was to survey the presence of bacterial endophytes in the seeds of a commercial wheat cultivar widely shown. May be identify the most abundant genera and to screen these isolates for some features which are involved in direct or indirect mechanisms of plant growth promotion.

## Materials and Methods

### Isolation and Identification of Endophytic bacteria in wheat

### Sample Collection

For current study, from3 different locations of Washim district each sub-location 3-5 healthy plants were selected randomly. The plants were uprooted, sealed into plastic bags and well labelled. All samples were processed immediately after collection.

### Surface Sterilization

The collected plants were washed under tap water to remove soil and further separated into plant parts viz., root, stem and leaf.All root, stem and leaf samples were washed twice in distilled water then surface sterilized as described by Hung and Annapurna, (2004).

### Isolation and identification of Endophytic Bacteria

The surface disinfected samples were macerated (Hung and Annapurna, 2004). The slurry was filtered through sterile musclin cloth. The filtrate was serially diluted upto 10-5 dilution and 1 mL aliquot of 10-5 dilution was spreaded onto nutrient medium. For isolation of bacteria nutrient agar supplemented with nistatin (200g/mL).Bacterial isolates were identified on the basis of conventional cultural morphological and biochemical characteristics according to Bergey's Manual of Determinative Bacteriology(Holt et al., 1994).

### Isolation and Identification of region specific fungal pathogens in wheat

### Screening and Collection of Diseased Plants

The diseased plants of wheat with characteristics symptomswere screened from each field during the cultivation period.The diseased plants were uprooted and sealed into sterilized plastic bags and labelled. (Goswamiet al., 2010)

### Isolation of fungal pathogens

The plant materials were cleaned properly and the infected plant parts (roots, stem, leaves etc.) were separated. Tissue isolation method was used for isolation (Chen et al., 2006). The infected plant tissue was cut into 5 mm segments. The segments were surface disinfected in 1 % sodium hypochlorite solution for upto 3-5 min. The segments were separately dried in between sheets of sterile filter paper.The segments were plated (3 pieces per plate) on fresh potato dextrose agar (PDA) medium supplemented with streptomycin (@0.2 g L-1) and incubated for 7 days at 25 ± 2°C (Yaqub and Shahzad, 2005).Isolation of fungal pathogens from soil was carried out adopting serial dilution technique (Aneja, 2006).

## Identification of isolated fungal pathogens

The identification of isolates was done on the basis of morphological and cultural characteristics followed by comparing with the available standard literature (Gilman, 2001).

## Pathogenicity Test

Pathogenicity of the fungal isolates was tested in vivo individually by the standard procedure as described by Mahesha, (2006) with slight modifications.

## Analysis of Antagonistic Activity

## Screening of Endophytic bacteria for Antagonistic Activity against isolated fungal pathogens of wheat

- The isolated endophytic bacterium was screened for their in vitro antagonistic activity against the isolated pathogens of wheat.(Vanitha&Ramjegathesh, 2014).

## In vitro Assay for Antagonist Activity of Endophytic bacteria against isolated Fungal Pathogens.

The assay for antagonism was performed on PDA by inoculating the test pathogen adopting dual culture method as suggested by Zivkovicet. al., (2010).

## Determination of Percent Growth Inhibition (PGI)

The Percent growth inhibition (PGI) was calculated using the formula;

$PGI = (C-T)/C \times 100$

## Result and Discussion

## Isolation and identification of endophytic bacteria associated in wheat:

The results on isolation and identification are presented in (Table:1) in present investigation a total 5 strains of an endophytic bacteria were isolated from wheat. Further on the basis of morphological, cultural and biochemical characteristics of endophytic bacterial isolates it was observed that total (4) genera viz; EBI-1 & EBI-5 belong to Bacillus, EBI-2 belongs to Klebsiella sp., EBI-3 belongs to Staphylococci, EBI-4belongs to Pseudomonas

**Table1: Morphological, cultural and biochemical characteristics of Endophytic Bacterial Isolates (EBI)**

| Characters | EBI-1 | EBI-2 | EBI-3 | EBI-4 | EBI-5 |
|---|---|---|---|---|---|
| Shape | Irregular | Round | Circular | Round | Round |
| Size | 2-5mm | 4-5mm | 1-4mm | 2-3mm | 4-10mm |
| Elevation | Flat | Flat | Convex | Slimy | Flat |
| Surface | Rough | Smooth | Smooth | Flat | - |
| Margin | Entire | Entire | Entire | Regular | Irregular |
| Color | Whitish | Grayish | Yellow | Cream | White |
| Pigmentation | Yellowish | - | Golden yellow | Yellowish orange | - |
| **Morphological Characters** | | | | | |
| Gram Reaction | + | - | + | - | + |
| Cell Shape | Rod | Rod | Cocci | Rod | Rod |
| **Biochemical Characters** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Glucose | + | + | + | - | + |
| Sucrose | - | - | + | - | + |
| Lactose | + | + | + | - | - |
| Maltose | + | + | + | - | + |
| Indole | - | - | - | - | - |
| Methyl Red | - | - | + | - | - |
| VogesPrausker | + | + | + | - | + |
| Citrate | + | + | + | + | + |
| Catalase | + | - | + | + | + |
| Oxidase | - | - | - | + | + |
| Nitrate | + | - | + | + | + |
| Starch Hydrolysis | + | - | + | + | + |
| Gelatin Liquefaction | + | - | + | + | + |
| Casein Hydrolysis | + | - | + | - | + |
| $H_2S$ | - | - | - | + | - |
| Possible Species | *Bacillus species.* | *Klebsiella species* | *S. aureus species* | *Pseudomonas species* | *Bacillus species* |

## Isolation and Identification of region specific fungal pathogens in wheat

The cultural and morphological characters of fungal isolates were presented in **(Table-2)** from the observed character, the identified fungal isolates were detected to be **FusariumOxysporiumand Aspergillus Niger** hence**,** and these pathogens were considered for further studies. It was also observed that after 3rd day of incubation, total 10 isolates were recovered and found to be slow growing which ranges between 15-17mm of colony diameter. Among which 06 - isolates were identified as **FusariumOxysporium, 04** – isolates were identified as **Aspergillus Niger**. The result also indicates predominance of **FusariumOxysporium and Aspergillus Niger** infection to wheat, hence this fungal isolate was considered as test pathogen for further study.

**Table 2: Cultural and morphological characters of isolated fungal pathogens of wheat**

| Characters | IFP-1 | IFP-2 |
|---|---|---|
| Growth (Colony Diameter) | 15mm | 17mm |
| Shape | Circular | Globular |
| Margins | Smooth | Smooth |
| Color | White | Greenish black |
| Texture | Thick | Wrinkled |
| Zonation | Absent | Absent |
| Mycelium | Separate | Separate |
| Possible species | *FusariumOxysporium* | *Aspergillus sp.* |
| No. of isolated identified on 3rd day | 06 | 04 |

\+ Slow – Mean colony diameter of 15-45mm on 3rd day
++ Moderate – Mean colony diameter of 45-65mm on 3rd day
+++ Good – Mean colony diameter of 65-85mm on 3rd day
++++ Excellent – Mean colony diameter of ▪ 85mm on 3rd day

## Analysis of Antagonistic Activity

### Screening of Endophytic bacteria for Antagonistic Activity against isolated fungal pathogens of wheat

The isolated endophytic bacteria of wheat were analysed for antagonistic activity against isolated test fugal pathogen viz;FusariumOxysporium and Aspergillus Niger as to screen the potential candidates for diseases control.

### In vitro Assay for Antagonist Activity of Endophytic bacteria against Aspergillus Niger

The data in Table 3 represents the efficacy of endophytic isolates to control the mycelial growth of Aspergillus Niger from the results. It was observed that endophytic bacterial isolates exhibited mycelial growth inhabitation of Aspergillus Niger. Among the tested isolates EBI 1, EBI4, and EBI 5 were found to be display the inhibition of mycelial growth Aspergillus Niger. The maximum inhibition was observed in treatment of EBI5 with 57.22 as Percent growth inhibition, followed by EBI 1 with as 47.77and EBI 4 with as 42.77, respectively as its PGI .whereas the isolates EBI 2 and EBI 3 does not showed the inhibition of Aspergillus Niger. Hence, the results indicated that the isolated bacterial endophytes EBI 1, EBI 4, and EBI 5 may be the possible candidates and can be used as bio control agent for the management of the Aspergillus Niger infections in wheat. Hence, these endophytic isolates were further considered for interaction studies against Aspergillus Niger.

### Table 3: In vitro Assay for Antagonist Activity of Endophytic bacteria against Aspergillus Niger

Endophytic Isolates Mean Mycelia Growth (mm) Growth InhibitionPercent Growth Inhibition (%)

| Endophytic Isolates | Mean Mycelia Growth (mm) | Growth Inhibition | Percent Growth Inhibition (%) |
|---|---|---|---|
| EBI 1 | 9.4 | I+ | 47.77 |
| EBI 2 | 18.1 | NI | NI |
| EBI 3 | 16.3 | NI | NI |
| EBI 4 | 10.3 | I+ | 42.77 |
| EBI 5 | 7.7 | I+ | 57.22 |
| Control | 14.3 | NI | NI |

**I : Inhibition, NI: No Inhibition.**

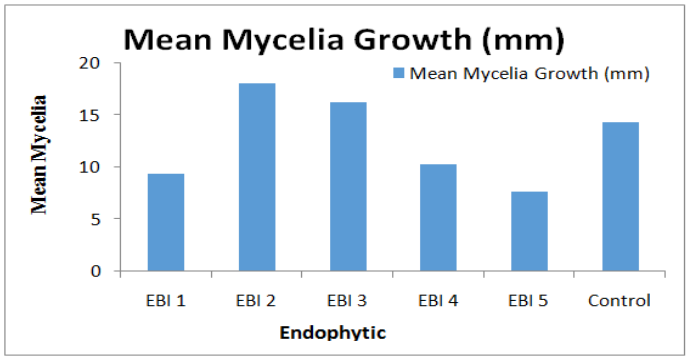



**Fig 1: Mean Mycelia Growth (Mm) of Bacterial Endophytic Against Aspergillus Niger**

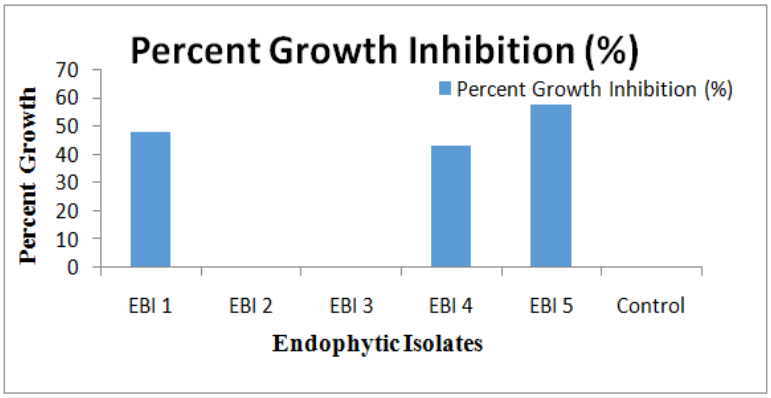



**Fig 2: Percent Growth Inhibition (%) of Bacterial Endophytic Against Aspergillus Niger**

## In vitro Assay for Antagonist Activity of Endophytic bacteria against FusariumOxysporium

The data in Table 4 represents the efficacy of endophytic isolates to control the mycelial growth of FusariumOxysporiumfrom the results. It was observed that endophytic bacterial isolates exhibited mycelial growth inhabitation of FusariumOxysporium. Among the tested isolates EBI 1, EBI 4,and EBI 5 were found to be display the inhibition of mycelial growth FusariumOxysporium. The maximum inhibition was observed in treatment of EBI 1 with 53.88 as percent growth inhibition, followed by EBI 5 with as 50.55and EBI 4 with as 41.11, respectively as its PGI .whereas the isolates EBI 2 and EBI 3 does not showed the inhibition of FusariumOxysporium. Hence, the results indicated that the isolated bacterial endophytes EBI 1, EBI 4, and EBI 5 may be the possible candidates and can be used as bio control agent for the management of the FusariumOxysporium infections in wheat. Hence, these endophytic isolates were further considered for interaction studies against FusariumOxysporium.

**Table 4: In vitro Assay for Antagonist Activity of Endophytic bacteria against FusariumOxysporium**

| Endophytic Isolates | Mean Mycelia Growth (mm) | Growth Inhibition | Percent Growth Inhibition (%) |
|---|---|---|---|
| EBI 1 | 8.3 | I+ | 53.88 |
| EBI 2 | 18.5 | NI | NI |
| EBI 3 | 19.2 | NI | NI |
| EBI 4 | 10.6 | I+ | 41.11 |
| EBI 5 | 8.9 | I+ | 50.55 |
| Control | 18.1 | NI | NI |

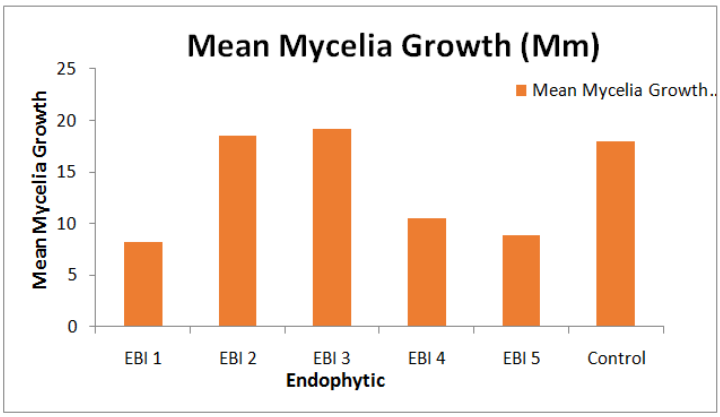



**Fig 3: Mean Mycelia Growth (Mm)of Bacterial Endophytic Against FusariumOxysporium**

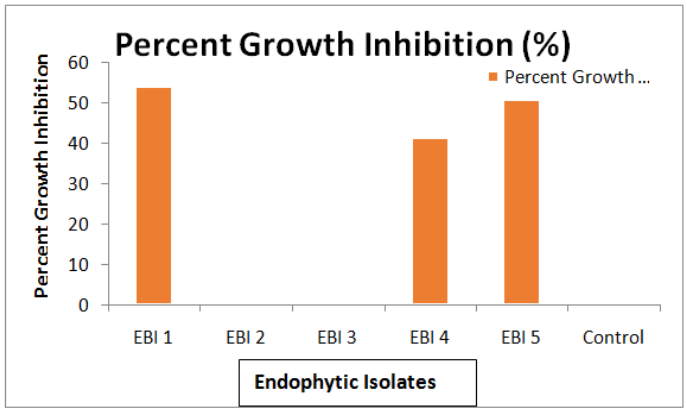



**Fig 4: Percent Growth Inhibition (%) of Bacterial Endophytic Against FusariumOxysporium.**

## CONCLUSION

The studies on resident endophytic bacteria of wheat for control of fungal pathogens was carried out as a model study to exploit the bacterial endophytes for its application in agriculture for plant diseases control in present study the fungal plant pathogen were isolated from diseased plant of wheat. The high proportion of fungal pathogen was screened on the other hand the bacterial endophytes were isolated from healthy plants of wheat and screened for its antagonistic activity against fungal pathogen.

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