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Isolation, Characterization and Identification of Endophytic Bacteria from Roots of *Hordeum Vulgare* Plants

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Abstract: ***Endophytic bacteria, known for their ability to colonize plant tissues, play a crucial role in plant health and development. In this study, bacterial endophytes were isolated from the root tissues of Hordeum vulgare (barley) and characterized using morphological, biochemical, and genetic analysis. The isolates were identified as Bacillus subtilis and Pseudomonas spp., both of which demonstrated significant enzymatic activity and varying antibiotic resistance. Bacillus subtilis exhibited high amylolytic, catalase, and urease activity, whereas Pseudomonas spp. showed only catalase activity. Antibiotic sensitivity testing revealed that Bacillus subtilis was resistant to all tested antibiotics, while Pseudomonas spp. displayed sensitivity to ampicillin at higher concentrations. These findings highlight the potential of endophytic bacteria in improving plant resilience, nutrient uptake, and defense mechanisms. Given the increasing scientific interest in plant-microbe interactions, further research is warranted to explore the metabolic pathways and functional applications of these bacterial endophytes in sustainable agriculture and bioremediation.***

Keywords: endophytic bacteria, *Hordeum vulgare*, *Bacillus subtilis*, *Pseudomonas spp.*, plant-microbe interaction

1. Introduction

Endophytes are group of bacteria having the ability to enter inside the plant hosts colonizing the intercellular spaces. They exist in a range of tissues types within a broad range of plants, colonizing the plant systemically with bacterial colonies. They are ubiquitous, colonize most of the plants, and have been isolated from almost all plants examined till date. Endophytes are to live symbiotically within the plants. This type of association of endophytes with internal tissues of host plant has increasingly gained them scientific and commercial interest due to their potential to improve plant quality and growth (Carroll, 2001: Schulz et al 2000). They exhibit complex interactions with their hosts which involves mutualism and antagonism.

Their association can be obligate or facultative. Plants strictly limit the growth of endophytes, and these endophytes use many mechanisms to gradually adapt to their living environments. In order to maintain stable symbiosis, endophytes produce several compounds that promote growth of plants and help them adapt better to the environment. Some of the endophytes are known to protect their host from being attacked by fungi, insect and mammals by producing secondary metabolites (Zhang, 2007). Among them, endophytic bacteria are thought to interact closely with their host plants, and therefore could be used as biological control agents in sustainable crop production potentially (Sturz and Nowak, 2000; Taechowisan et al., 2003; Zhang et al., 2008). Endophytes are known to supply nutrients to plant by fixing atmospheric nitrogen and solubilizing ion (Marx 2004; Porras - soriaano et al 2009). This ultimately leads to increase in plant immune system as well as protects plant from infection by plant pathogens. studies have also shown role of endophytes in removal of soil contaminants (Barac et. al 2004; Doty et al 2009). Studies on endophytes their significance and role in plant metabolism is an important area to explore.

The present studies was therefore undertaken to investigate and identify endophytic bacteria present in the tissue of roots barley plant. The technique of 16s rRNA partial sequencing is also used for identification of the bacterial species. Studies were also performed on biochemical characterization of the endophytes and their ability to interact with different antibiotic.

2. Materials and Methods

Collection of the sample: Three months old plants of Barley were taken from a plant farm near Darbhanga, Bihar. These plants were maintained under controlled environmental conditions in well plastic pots in the green house of Department of Biotechnology, L. N. Mithila University, Darbhanga Bihar, India.

Isolation of endophytic bacteria from Barley roots

Surface sterilization: The root sample of the *Barley* plant were taken and processed separately. Healthy and undamaged root were collected from the plant grown under controlled environmental conditions. These were than washed under running water and dried followed by surface sterilization. Surface sterilization was done first washing in 1% savlon for 5 - 6 minutes. Subsequently these pieces of roots were treated with 0.1 % mercuric chloride for surface sterilization.

Isolation of endophytic Bacteria: The samples of roots were cut into small pieces and macerated separately in phosphate buffer of pH 7.2 with a sterile pestle and mortar. Tissue extract were then prepared for tenfold dilution in sterile saline. Serial dilutions were prepared from this extract. For inoculations 0.1ml of the aliquot was used on Nutrient Agar medium. The inoculations were done in triplicates separately for both roots tissue extract. These plates were then incubated at 37^0C. Observations were taken after 48 to 72 hrs. Bacterial colonies were differentiated on the basis of morphological colony characters. Bacterial isolates were picked from plates and Purified by streaking techniques and incubated at 37^0C. The isolation process repeated till monocultures were obtained for further experimentations. The media used for isolation was Yeast Manitol Agar, Picovasky's medium.

Characterization of endophytic bacteria

a) **Morphological:** Morphological characterization was done by Gram staining and motility test.
b) **Physiological Characterization**

Isolation, Characterization and Identification of Endophytic Bacteria

Effect of pH on growth of isolates: Optimization and standardization of growth of the isolates on range of pH was studied by inoculating the isolates on Nutrient broth media (Hi - media) having pH range from 1 to 14. These were then incubated at 37^0C. for 48 hours. The growth was determined by taking optical density at 520 nm.

Effect of temperature on growth of isolates: Effect of temperature on growth of isolates was determined by inoculating the isolates in nutrient broth and incubated at different temperature that is at 4^0C, 28^0C, 37^0C, 50^0Cfor 48 hours. Optical density at 520 nm was measured.

Biochemical Characterization

a) **Catalase Test**: Catalase activity of the isolates was estimated by using H_2O_2 solution onto the microscopic slides containing the culture of the isolates separately.
b) **Amylolytic activity:** Amylolytic activity was observed after inoculating the isolates in nutrient agar with 1% starch of pH 6.06. Culture plates were treated with iodine after incubation period. Clear zone was observed around colonies confirming the activity.
c) **Urease activity:** Isolates were grown on the medium containing urea agar. After incubation slant were observed for change in its colour from redish pink indicating positive urease activity.
d) **Antibiotic Sensitivity test:** The isolates to be screened for their sensitivity for antibiotics were cultured on to the nutrient agar plate. Antibiotic Kanamycin of varying concentration (5µg/ml, 50µg/ml, and 100µg/ml) used for treating the culture by using well technique. The protocol was repeated for Ampicillin and Amoxicillin antibiotic with concentration – 5µg/ml, 50µg/ml, and 100µg/ml on separate plates. Cultures inoculated for 24 hrs. at 37^0C the growth of the bacterial colonies was observed and zone of inhibition measured.

3. Results and Discussion

Three bacterial colonies were isolated from root tissue extract. Two bacterial colonies isolate II& III were found to be of same species.

Table 1: Morphological characterization of endophytes

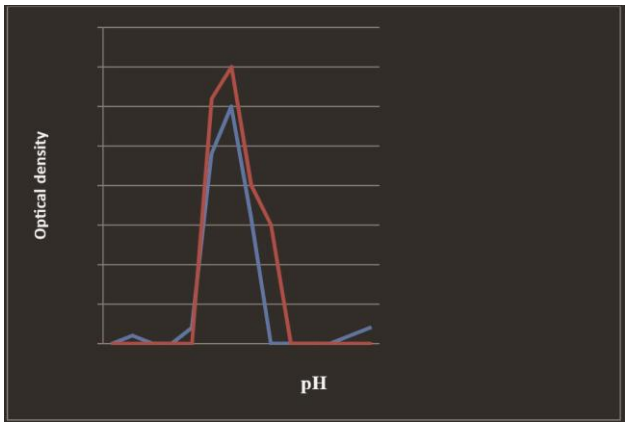
Colony characters	Isolate I/ root	Isolate II/ root	Isolate III/ root
Size	4mm	3mm	3.7 mm
Shape	Circular	Circular	Circular
Colour	Orange	Yellowish	Yellowish
Margine	Regular	Regular	Regular
Colony characters	Isolate I/ root	Isolate II/ root	Isolate III/ root
Opacity	Opaque	Opaque	Opaque
Elevation	Flat	Flat	Flat
Consistency	Smooth	Smooth	Smooth
Gram characters	Gram positive	Gram negative	Gram negative
Motility	Motile	Motile	Motile

Table 2: Screening of the isolates for enzyme activity

Organism	*Amylolytic activity*	*Urease activity*	*Catalase activity*
Bacillus subtilis	+	+	+
Pseudomonas spp.	-	-	+

+ = Positive, - = Negative.

Graph: Effect of pH on growth of isolates
Media: Nutrient Brothincubation: temp 37 ^{0}C time: 2 Days



Results and Discussion

Hordeum vulgare is used in many industries since many years. However, new discoveries in this plant research leads to development and opening of many more areas to explore. The plant is now gaining importance to develop some more new search for the future development by understanding the gene level studies. Therefore, considering its huge uses, there is an ample scope for future research and hence further pharmacological investigations are warranted. Endophytic bacteria are reported to enhance the growth of plant and may also increase the supply of nutrients to plants in turn helping in building the defence mechanism of the plant.

Isolation, Characterization and Identification of Endophytic Bacteria

The present investigations were undertaken to find out the presence of bacterial endophytes in root of this plant. By using nutrient agar and selective medium (YMA and Picovasky's medium) two endophytes were isolated from roots tissue of the plant. Hence ultimately two endophyte were present in root tissue of the barley plant and further characterization and sequencing were performed in these two isolate.

On the basis of the morphological and biochemical characterization of the endophytes they are identified as *Bacillus subtilis* and *Pseudomonas spp.* The isolate I shows 99% identity to *Bacillus subtilis* strain. The isolate 2 shows 99% identity to *Pseudomonas spp*. Strain. The sequences are submitted to NCBI GENE BANK and they are under process.

During present studies the endophytic bacteria *Bacillus subtilis* isolated from roots showed high amylolytic, catalase and urease activity though only catalase activity observed in *Pseudomonas* spp. (Table2). Sensitivity of Three different antibiotics at varying concentration was also tested for these two endophytes. *Bacillus subtilis* was Observed to be resistant to all three antibiotics kanamycin, ampicillin &amoxicillin as no zone of inhibition was observed whereas *Pseudomonas spp.* showed sensitivity to ampicillin at higher concentrations.

To conclude, two bacterial endophytes are isolated from root tissue of *Hordeum vulgare* plant. Both the Isolates *Bacillus subtilis* & *Pseudomonas spp.* were present in root tissue. Further research work is required to explore more about the role of these bacterial endophytes in the metabolism of the plant.

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