"Isolation, characterization and identification of endophytic bacteria of Hordeum vulgare by molecular sequencing technique"

Aisha parween, Shikha shirish
University Department of Biotechnology Lalit Narayan Mithila University Kameshwaranagar, Darbhanga, Bihar

Abstract

Endophytic bacteria have been found in virtually every plant studied where they colonize the internal tissues of their host plant and can form a range of different relationships including symbiotic, mutualistic, commensalism and trophobiotic. These bacteria colonize the rhizosphere and phyllo sphere and the inside plant tissue.

Isolation endophytic bacteria their characterization and also their identification by 16s rRNA sequencing technique have been discussed. Most endophytes appear to originate from the Rhizophora however some may be transmitted through the seed. These bacteria can promote plant growth and yield can act as biocontrol agent. Barley **(*Hordeum vulgare*),** a major crop grown in the temperate regions globally is known for being one of the oldest crops being cultivated. This paper summarizes currently available knowledge about endophytic bacteria in various plant species and specifically discusses the molecular identification of barley plant by different endophytes.

KEYWORDS: Bacterial endophytes, *Hordeum vulgare*, 16s rRNA.

INTRODUCTION

Our earth is a place of variety of living organisms. Among all living organism's micro-organism occupied almost all the habitat of our earth. In fact, plants and animals are associated with diverse micro-organisms. All bacteria that colonize the interior of plants including active and latent pathogens can be considered to be endophytes, (Misaghi and Donndelinger, 1990), (James and Olivares, 1997). The bacteria that establish endosymbiosis with the plant, where by the plant receives an ecological benefit from the presence of symbiont (Quispel, 1992). Most endophytes appear to originate from the Rhizophora however some may be transmitted through the seed. Endophytic bacteria can promote plant growth and yield can act as biocontrol agent.

BACTERIAL ENDOPHYTES:

Bacterial endophytes i.e. bacteria that are present with in plants have been known for more than 120years endophytic growth was recognized as a particular stage in the life of bacteria when it was described as an advance stage of infection and a close relation with mutualistic symbiosis. Endophytes have been defined as microorganism that could be isolated from surface sterilized plant organ (Hennie and villforth,1940).

Endophytic bacteria classified as obligate and facultative Obligate endophytes are strictly dependent on the host plant for their growth and survival, and transmission to other plants occurs vertically or via vectors. Facultative endophytes have a stage in their life cycle in which they exist outside host plants. In the extreme view, bacterial phytopathogens might be included as (facultative or obligate) endophytes, as they often occur in avirulent forms in plants. For example, Ralstonia solanacearum biovar 2, which can survive in water systems, can occur as an endophyte, in an apparently avirulent form, inside tomato plants (van Overbake et al., 2004). Moreover, Xylella fastidiosa is apparently extremely adapted to life within plant tissue, persisting for a long time without causing harm to the plant (Araujo et al., 2002). Given their transmission to other plants via insect vectors, and their apparent recalcitrance to growth outside the plant, organisms such as X. fastidiosa might be considered to represent obligate endophytes. Avirulent forms of plant pathogens should thus be regarded as endophytes, whereas virulent forms of these organisms should not be included.

The endophytic niche offers protection from the environment for those bacteria that can colonize the intercellular spaces, and they have been isolated from all plant compartments including seeds (Posada and Vega, 2005). A very comprehensive list of bacterial endophytes isolated from a broad range of plants to isolate and characterize endophytic species is provided by Rosenblueth and Martinez Romero (2006) and Bery and Hallmann (2006). Molecular approaches for the isolation and characterization of bacterial endophytes and plant associated bacteria and communities inhabiting roots of plant were analyzed by 16s rRNA gene-based techniques such as terminal restriction fragment length polymorphism, analysis, denaturing gradient gel electrophoresis as well as 16s rRNA gene cloning and sequencing. Endophytic bacteria have been isolated from large diversity of plant on earth.

Many endophytes such as Pseudomonas, Barkholderia and Bacillus (Lodewyekx et.al.) are members of common soil bacterial genera. These genera are well known for their diverse range of secondary metabolic products including antibiotics, anticancer compounds, antifungal, antiviral, insecticidal and immunosuppressant agents. A wide range of biologically active compounds have been isolated from endophytic organisms. For example, Pseudomonas Viridiana which has been isolated from grass species, Produces Ecomycine, active against human pathogens *Candida albicans* and *Cryptococcus neoformans.* Genomic analysis indicates that many species of bacteria have the potential to produce bioplastics (Kalia et.al., 2003).

Many endophytes having ability to promote plant growth. They increase plant growth through the improved cycling of nutrients and minerals such as nitrogen, phosphate and other nutrients. Endophytic organisms can also supply essential vitamins to plants.

Barley belongs to the genus *Hordeum* in the Poaceae also known as Gramineae family. In old world agriculture barley is one of the founder crops. It is also a model of experimental system because of its short life cycle and morphological and genetic characteristics. Barley is short

seasoned, early maturing grain found in environment globally. In Australia barley is the second largest field crop (after wheat). Barley is one of the cereals which tolerates rather well abiotic stress conditions. It was the staple food of people in many countries. It has gained importance in recent time because of its nutritional value. Its consumptions lead to lowering of low-density lipoprotein (LDL), cholesterol that reduces the risk of heart diseases and regulates blood sugar level in diabetics. Barley is rich source of fibers, which makes it a comfortable diet. It is also used in brewing industry so it is also known as industrial crop. It is a crop of cool climate. In India, it is basically a winter crop. Barley is popularly known as "JAU" in Hindi. It is one of the most important cereal grain crops after rice, wheat and maize. In India this crop is cultivated as a summer crop in temperate regions and as a winter crop in tropical regions. Coarse cereals are made out of this along with other cereals crops like oats, finger millet, corn etc. Barley is cultivated as a rabi season crop in India and sowing is undertaken from Oct. to Dec. and harvesting from Mar. to May. In India the major production states of barley are Rajasthan, Uttar Pradesh, West Bengal, Jammu &Kashmir, some regions in Bihar, Uttarakhand and Himachal Pradesh.

MATERIALS AND METHODS

The flowing objective can be achieved undergoing flowing steps.

1. **Collection of the sample:** Samples were collected from different sites of Darbhanga. All the samples were collected in a sterile plastic bag, transferred to laboratory and processed immediately.
2. **Isolation and purification of bacterial endophytes:** The collected samples were washed out with tap water to remove adhering soil, then they were superficially sterilized with 70% ethanol and air dried under laminar air flow hood. The outer tissue was removed from sample and inner tissue were cut into small pieces and macerated in sterile pestle and mortar.
3. **Culture of endophytic bacteria**: The isolated samples will be serially diluted and inoculation will be done on nutrient agar plate at 37°C in thermostat incubator. After incubation observation will be taken after 48 to 72hrs. Bacterial colonies will be differentiated on the basis of morphological colony characters.

Figure: streak plate purification of endophytic bacterial isolates.

4. **Characterization of endophytic bacteria:**

a) Morphological Characterization: Morphological characterization were done by using Gram staining technique**.** The microscopic examination performed with the oil immersion objective of the bright field microscope. A loopful bacterial culture taken for microscopic examination of motility of the bacteria isolated for identification and characterization. Slides were prepared by standard technique.
Morphological study was done on the basis of shape, elevation, texture, margin, colour, size, odour and pigmentation etc of colonies of endophytic bacteria grown on specific medium and incubated at 28±2°C for 2-3 days. Cell motility and shape of single colony was observed under light microscope.

Table. Morphological characteristics of endophytic bacterial isolates of Barley.

Characteristics	Morphology of endophytic bacterial isolates		
Cell shape	Rods	Rods	Rods
Colony shape	Round	Round	Polymorphic
Elevation	Flat	Raised	Umbonate
Texture	Mucoid	Mucoid	Mucoid
Margin	Smooth	Entire	Irregular
Colour	Light yellow	Cream	Cream
Size	Medium	Small	Medium
Pigmentation	Few isolates produced fluorescent green pigment	None	none

b) **Gram's staining**
Gram's reaction was done to differentiate endophytic bacteria Gram positive and negative on the basis of standard staining protocols (Bartholomew 1962).

c) Physiological characterization: Optimization of effect of pH and temp. of the isolates were studied. Effect of pH will be studied by inoculating the isolates on nutrient broth media having pH range 1 to 14. Effect of temp. on isolates will be determined by inoculating the isolates in nutrient broth and incubate at differing temps. i.e. at 4°C,28°C,37°C for 48hours.

d) Biochemical characterization: Antibiotic sensitivity test, Urease, and different enzymes test was done.
Catalase Test: A drop of 3% H2O2 was taken on glass slide. Small amount of endophytic bacterial culture was mixed with inoculating needle. Rapid and sustained production of gas bubbles or effervescence constituted positive test.
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.
Urease activity: isolates were grown on the medium containing urea agar. After incubation slant were observed for change in its clour from reddish pink indicating positive ureases activity.

5. **Molecular identification of bacteria:** Identification of endophytic bacteria isolated from barley would be done by using 16s rRNA gene from DNA of pure bacterial culture. 16s rRNA is a significant target to the molecular level identification. The upstream region of the 16s rRNA is known to be highly conserved in species to species. So, this region could

also be used for the verification of the thermodynamic stability on the basis of conserved secondary structure of RNA.

RESULTS AND DISCUSSION

The present investigation was undertaken to find out the presences of bacterial endophytes in root of barley plant. By using nutrient agar and selective medium different endophytic bacteria were isolated from root barely plant.

Various investigators reported endophytic microbes from various plant exists in different ecosystems. It is not worthy that of the nearly 3,00,000 plant species that exists on earth each individual plant is host to one or more endophytes. successful isolation of entophytic bacteria from barley plant was done from different location of Darbhanga Bihar. approx. 30 endophytic bacterial strains were isolated from root of barley plant.

Only a few of these plants have ever been completely studied relative to their endophytic biology. In this present study Total 30 bacterial strains were recovered from all the collected samples from selected plants. All the bacterial strains are markable different from terrestrial bacterial isolates. So far these are numerous. In India also countable number of reports showed on diversity of endophytic bacteria, fungi in medicinal plants but from available literature there is no report on endophytic bacteria from Barley root in Darbhanga, Bihar. In general, endophytic bacteria occur as lower population densities than rhizospheric bacteria or bacterial pathogens. Endophytes are the chemical synthesizers with in plants. Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against pathogens and some of these compounds have been proved for useful drug discovery. Up to now most of the natural products from endophytes are antibiotics, anticancer agents, biological control agents' antivirals, antidiabetic agents and other bioactive compounds by their different functional roles. Further these endophytic isolates will be a potential source for extracellular enzymes. Endophytic bacteria reside within plant tissues have been reported to promote plant growth. They promote plant growth directly or indirectly.

REFERENCES:

1) **Rekha M. Gupta, Prathamesh S. Kale, Madhuri L. Rathi and Nikhil N. Yadav. 2015.** isolation, characterization and identification of endophytic bacteria by 16s rRNA partial sequencing technique from roots and leaves of *Prosopis cineraria* plant, Maharashtra India
2) **Nawed Anjum, Ramesh Chandra 2015.** Endophytic bacteria: Optimization of isolation procedure from various medicinal plant and their preliminary characterization, BIT Ranchi.

3) **Polpass Arul Jose, ilangovanshanamugasundari, kunjukrishnan, kamalakshiSivkala& Solomon Robinson DabidJebakumar. 2013.** Molecular phylogeny and plant growth promoting traits of endophytic bacteria isolated from roots of seagrass *Cymodoceaserrulata*, Madurai.
4) **Robert P. Ryan, Kieran Germaine.2007.** Bacterial endophytic: Recent developments and application.
5) **Cindy lodewyckx, jacoVangronsveld, Fiona Porteous. 2002.** Endophytic bacteria and their potential application.
6) **Elvia Perez-Rosales, Lilia Alcaraz-Melendez, M.E. Puente, R.V. Juarez.2017.** Isolation and characterization of endophytic bacteria associated with roots of jojoba.
7) **V.Miao, J. Davies.2010.** Actinobacteria: The good, the bad and the ugly Anton leeuwenhoek,98, pp.143-150.
8) **Agarwal and Sheden.1987:** Tetrazolium reducing microorganism inside the roots of *Brassica species.* Curr.sci 56 :187-188.
9) **Chen, C., E.M. Bauke, G. Musson, R. Rodriguez Kabana and J.W. Kloepper.1995.** Biological control of fusarium wilt on cotton by use of endophytic bacteria. Biol. Control.5:83-91.
10) **Compeau, G., B.J. Al-Achi, E. Platsouka , and S.B Levy.1988.** \Survival of rifampin-rifampin-resistant mutants of pseudomonas fluorescens and pseudomonas putida in soil system. Appl.Environ. Micorodial, 54:2432-2438.
11) **Khandelwal Preeti, Sharma Ram Avatar, Agarwal Mala:** Journal of plant science (2015):3(1-1):33-39.
12) **Kobayashi D.Y.& Palumbo J.D.2000.** Bacterial endophytes and their effects on plants and uses in agriculture, in microbial Endophytes, edited by C.W. Bacon&J.F.Jr. White, Marcel Dekker, New York.
13) **Bensalim, S.J. Nowak and S.K. Asiedu. 1998.A** plant growth promoting rhizobacterium and temperature effects on performance of 18 clones of potato.Am.J. Potato res.75:145-152.
14) **Gayatri S, Saravanan D, Radhakrishnan M, Balagurunthan R and K.** endophytic bacteria from leaves of mangrove and salt marsh plant species, Indian journal of *biotechnology*, 9, 210, 397-402
15) **Arunachalam C and Gayathri P. 2010.** Studies on bioprospecting of endophytic bacteria from the medicinal plant of Andrographis paniculate for their antimicrobial activity and antibiotic susceptibility pattern, International journal of current pharmaceutical research, 2(3), 63-68.
16) **Strobel G, Daisy B. 2003.** Bioprospecting for microbial endophytes and their natural products, *Microbiol Mol Biol Rev*, 67(4), 491-502.
17) **Clarridge JE. 2004.** Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev. 2004; 17 4: 840–862.