NITROGEN FIXING ENDOPHYTIC MICROBES FROM CEREAL CROPS AND THEIR BIOTECHNOLOGICAL APPLICATIONS

SYNOPSIS

Submitted to Eternal University
In the partial fulfillment
For the Degree of
Doctor of Philosophy in Biotechnology

By

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Under the Supervision
of
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Title: Nitrogen Fixing Endophytic microbes from Cereal Crops and their Biotechnological Applications

I. INTRODUCTION

The endophytic microbes are referred to as those microorganisms, which colonize in the interior of the plant parts, viz: root, stem or seeds without causing any harmful effect on host plant. The word endophyte means ‘in the plant’ and is derived from the Greek words endon (within) and phyton (plant). The usage of this term is as broad as its literal definition and spectrum of potential hosts and inhabitants, e.g. bacteria (Kobayashi and Palumbo 2000), fungi (Stone et al 2000), plants (Marler et al 1999) and insects of plants (Feller 1995). Endophytes have been defined by various authors in somewhat different ways (Schulz and Boyle 2005, Rosenblueth and Martínez-Romero 2006, Mercado-Blanco and Lugtenberg 2014). Microbial endophytes can be isolated from surface-disinfected plant tissue or extracted from internal plant tissue (Hallmann et al 1997). Endophytes inside a plant may either become localized at the point of entry or spread throughout the plant. These microorganisms can reside within cells (Jacobs et al 1985), in the intercellular spaces (Patriquin and Döbereiner 1978) or in the vascular system (Bell et al 1995). Endophytic microbes enter plants mainly through wounds, naturally occurring as a result of plant growth or through root hairs and at epidermal conjunctions. Besides providing entry avenues, wounds also create favorable conditions for the bacteria by allowing leakage of plant exudates that serve as a nutrient source for the bacteria (Quadt-Hallmann et al 1997). Other entry sites for endophytic microbes include flowers, stomata and lenticels (Kluepfel 1993). Once in the plant, the endophytic microbes have an ecological advantage over the epiphytic microbes in that they are protected from adverse external environmental conditions such as those associated with
temperature, salinity, drought, pH, osmotic potentials, and ultraviolet radiation, which are major factors limiting long-term microbial survival. The population density of endophytic microbes is highly variable, depending mainly on the microbial species and host genotypes but also on the host developmental stage, inoculum density, and environmental conditions. Molecular analysis has shown that plant defense responses also limit bacterial populations inside plants.


These microbes may promote plant growth in terms of increased germination rates, biomass, leaf area, chlorophyll content, nitrogen content, protein content, roots and shoot length, yield and tolerance to abiotic stresses like drought, temperature, flood, salinity etc. Endophytic microbes can promote plant growth directly through biological nitrogen fixation (BNF); phytohormone production (Indole acetic acid and Gibberellic acids); solubilization of phosphorus (P), potassium (K) and zinc (Zn); inhibition of ethylene biosynthesis in response to biotic or abiotic stress (induced systemic tolerance) etc., or indirectly through inducing resistance to pathogen by production of ammonia, hydrogen cyanide and siderophores (Hallmann et al 1997, Rosenblueth and Martínez-Romero 2006, Verma et al 2015). Biological nitrogen fixation is the process of conversion of atmospheric nitrogen into ammonia. The process of BNF is mostly done by nitrogen fixing microbes. In the recent
agriculture system, much attention has been paid on the biological nitrogen fixation by several endophytic diazotrophic bacteria, such as, *Acetobacter*, *Azoarcus*, and *Herbaspirillum* in gramineous plants. They are mostly found within the plant tissues and contribute to significant nitrogen fixation (James and Olivares 1998, Reinhold-Hurek and Hurek 1998). More than 60% of the nitrogen uptake (about 150 kg N ha/year) from BNF, in Sugarcane cultivars was demonstrated with N\(^{15}\) isotope and N balance studies (Urquiaga et al 1992). *Acetobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas* have been reported as BNF in different crops including wheat, barley, maize, sugarcane etc. (Hartmann et al 1995, Baldani et al 1996, Verma et al 2013, Suman et al 2016, Verma et al 2016)

Looking at the importance of endophytic bacteria which increase the crop yield of cereal crops by biological nitrogen fixation and others plant growth promoting attributes, the present investigation would be carried out with the following objectives:

1. Isolation and characterization of endophytic microbes from cereal crops
2. Characterization of endophytic microbes for nitrogen fixation and other plant growth promoting attributes
3. Molecular characterization of selected endophytic microbes
4. Evaluation and validation of selected endophytic microbes for growth and yield of crops

The rationale of the proposed study is to utilize efficient and effective endophytic microbes isolates for developing bio-fertilizers to increase the yield of cereal crop plants. The great importance of the nitrogen resides in the fact that it is an essential constituent of the proteins, nucleic acids and other compounds of nitrogen base essential to the vital processes of all live beings. In this regards, targeting endophytic microbes, a specific group of microbes colonizing cereal crops, could be even more beneficial to be used in the case of different crops, if they possess desired PGP attributes. Therefore, the study of diversity, potential and mechanism of plant growth promotion by endophytic microbes is of immense significance and contribution towards sustainable agriculture
II. REVIEW OF LITERATURE

The endophytic microbe has been reported from different types such as archaea, eubacteria and fungi. Among bacteria, endophytic bacteria were isolated from different phylum mainly: Actinbacteria, Firmicutes, proteobacteria, Bacteroidetes and Deinococcus-Thermus. The Proteobacteria were further grouped as α β and γ-proteobacteria. Distribution of endophytic bacteria varied in all bacterial phyla, Proteobacteria were the most dominant followed by actinobacteria. Least number of endophytic bacteria was reported from phylum Deinococcus-Thermus and Acidobacteria followed by Bacteroidetes, such bacteria were include both Gram-positive and Gram-negative bacteria (Lodewyckx et al 2002, Yadav et al 2015).

Endophytic microbes were reported as by both culture-dependent and culture in-dependent approaches. It is possible to assess only a small fraction of the bacterial diversity associated with plants using the isolation methods described above because only a few endophytic bacterial species can be cultivated using traditional laboratory methods. The sizes of bacterial communities as determined using culture-independent methods might be 100- to 1,000-fold larger than communities uncovered via traditional isolation (Yashiro et al 2011).

Endophytic bacteria have been reported in almost every plant studied (Ryan et al 2008). On review of different plants, it was found that endophytic microbes were the most predominant and the studied belonged to three major phyla Actinobacteria, Proteobacteria and Firmicutes. Among 116 reported genera from seventeen different plants (Suman et al 2016), twenty three microbes were reported as the most predominant namely Achromobacter, Acinetobacter, Agrobacterium, Bacillus, Brevundimonas, Burkholderia, Cladosporium, Clavibacter, Enterobacter, Flavobacterium, Hannaella, Herbaspirillum, Klebsiella, Methylobacterium, Microbacterium, Microspora, Micrococcus, Paenibacillus, Pantoea, Pseudomonas, Rhizobium, Rhodococcus, Sphingomonas, Staphylococcus, Stenotrophomonas and Streptomyces (Mcinroy and Kloepper 1995, Hallmann et al 1997, Reinhold-Hurek and Hurek 1998, Rosenblueth and Martínez-Romero 2006, Ryan et al 2008, Pageni et al 2013, Verma et al 2014, 2015, Mercado-Blanco 2015). Among twenty three genera most predominant were Bacillus, Burkholderia, Enterobacter, Methylobacterium Pantoea, Pseudomonas and Streptomyces were most dominant and reported on more than five host plants. There were many endophytic bacteria found to be common in more than three host plants. Along with common endophytic microbial genera, many niche specific microbial genera have been reported from all seventeen host plants such as Mycobacterium, Planobispora, Planomonospora and Thermomonospora from wheat; Aeromonas, Alkanindiges, Caulobacter, Chlamydomyces, Chryseobacterium, Comamonas,
Coniothyrium, Gallionella, Geobacter, and Trichodermaviridae from rice; Bradyrhizobium and Raoultella from sugarcane; Azotobacter, Haloferax, Methanospirillum, Rhanella, and Thermoplasma from maize; Curtobacterium and Guignardia from citrus; Frateuria, Janthinobacterium, Ralstonia and Sporosarcina from chilli; Coryneform frigoribacterium and Variovorax from potato; Candida from tomato; Alternaria, Drechslera, Leuconostoc, and Tsukamurella from soybean; Mesorhizobium from chickpea; Lactobacillus and Sphingopyxis from strawberry (Suman et al. 2016).

Most studies on the occurrence of endophytic microbes have been performed using culture-dependent approaches. The genus Bacillus has been consistently described as culturable and endophytic, and these bacteria can colonize rice (Sun et al. 2008), mustard (Sheng et al. 2008), chilli (Rasche et al. 2006), citrus (Araújo et al. 2001), potato (Sessitsch et al. 2004), soybean (Hung and Annapurna 2004), bean (Figueiredo et al. 2008), chickpea (Saini et al. 2015) and strawberry (Dias et al. 2009). The member Bacillus and Bacillus derived genera (BBDG) associated with different plant show different plant growth promoting attributes such as solubilization of P, K and Zn; production of phytohormones and biocontrol against different pathogens (Tilak et al. 2005, Yadav et al. 2015). The genus Burkholderia has been reported as endophytic in different plant, but most dominant in sugarcane and associated mainly for nitrogen fixation (Suman et al. 2001, Castro-González et al. 2011). Additionally, other studies have described the importance of members of the genus Burkholderia in the cultivation of rice (Govindarajan et al. 2008, Rangjaroen et al. 2014), maize (Bevivino et al. 1998), citrus (Araújo et al. 2002) and cotton (Quadt-Hallmann et al. 1997). The member of Enterobacter bacteria have been reported as endophytic bacteria in different plant such as rice (Piromyou et al. 2015), maize (Montanez et al. 2012), citrus (Araújo et al. 2002), pea (Tariq et al. 2014), and strawberry (de Melo Pereira et al. 2012). The pink-pigmented facultative methylotrophs (PPFMs) have been reported from diverse host plants including wheat (Yadav et al. 2015), rice, citrus, (Dourado et al. 2015), bean (de Oliveira Costa et al. 2012). In plant colonization, the frequency and distribution may be influenced by plant genotype or by interactions with other associated microorganisms, which may result in increasing plant fitness. The Methylobacterium have potential capacity to fix nitrogen, nodule the host plant, produce cytokinins, auxin and enzymes involved in the induction of systemic resistance, such as pectinase and cellulase, and therefore plant growth promotion. The different species of Pantoea have been described as cosmopolitan endophytes found in wheat (Verma et al. 2014), rice (Rangjaroen et al. 2014), sugarcane (Muangthong et al. 2015), maize (Ikeda et al. 2013), citrus (Araújo et al. 2002), chilli (Kang et al. 2007) and potato (Reiter et al.
Members of *Pantoea* are ubiquitous in plant tissues; they are able to influence plant growth through the production of auxins or cytokinins and induce systemic resistance against diseases. The *Pseudomonas* are ubiquitous in nature, and the member of γ-proteobacteria have been reported from different plant tissues such as wheat, rice (Sun et al 2008), sugarcane (Suman et al 2001), maize (Thanh and Diep 2014, Szilagyi-Zecchin et al 2014), chilli (Kang et al 2007), tomato (Kumar et al 2011), potato (Reiter et al 2003, Sessitsch et al 2004), pearl millet (Gupta et al 2013) and strawberry (de Melo Pereira et al 2012). *Streptomyces* has been reported from shoot, root and seeds of different plant such as wheat (Coombs and Franco 2003a, b), rice (Tian et al 2004), maize (Araújo et al 2000) and chilli (Rasche et al 2006).

Endophytic microbes are of agriculturally important as they can enhance plant growth; improve plant nutrition through nitrogen fixation and other mechanisms. (Araújo et al 2000, Lodewyckx et al 2002, Sun et al 2008, Yanni et al 2011, Pavlo et al 2011, Rangjaroen et al 2014, Verma et al 2015). Endophytes may increase crop yields, remove contaminants, inhibit pathogens, and fixed nitrogen or novel substances (Quadt-Hallmann et al 1997). In endophytic relationships, growth promoting microbes reside within the apoplastic spaces in the host plants. There is direct evidence for the existence of endophytes in the apoplastic intercellular spaces of parenchymal tissue (Dong et al 1997) and the xylem vessels (James et al 1994, Lacava and Azevedo 2013, Glick 2015). Endophyte-infected plants often grow faster than the non infected ones (Cheplick et al 1989). The growth stimulation by endophytes can be a consequence of nitrogen fixation (de Bruijn et al 1997, Suman et al 2001, Iniguez et al 2004, Taulé et al 2012, Pankievicz et al 2015), production of phytohormones, such as IAA and cytokines (Rashid et al 2012, Nath et al 2013, Lin and Xu 2013, Jasim et al 2014, Yadav et al 2015), biocontrol of phytopathogens through the production of antifungal or antibacterial agents, siderophore production, nutrient competition and induction of acquired host resistance, or enhancing the bioavailability of minerals. Several studies have indicated that endophytic colonization can also result in increased plant vigor, and it confers tolerance to biotic and abiotic stresses, enhanced drought tolerance, and improved phosphorus utilization (Yadav et al 2015). Although the interaction between endophytic microbes and their host plants is not fully understood, many isolates showed beneficial effects on their hosts and may play an important role in the physiology of these plants. Several bacterial endophytes have been reported to support plant growth by providing phytohormones, low- molecular weight compounds, or enzymes.
Sustainable agriculture requires the use of strategies to increase or maintain the current rate of food production while reducing damage to the environment and human health. The use of microbial plant growth promoters is an alternative to conventional agricultural technologies. Plant growth-promoting microbe can affect plant growth directly or indirectly. The direct promotion of plant growth by PGPM, for the most part, entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment. The indirect promotion of plant growth occurs when PGPM decrease or prevent the deleterious effects of one or more phytopathogenic organisms.

Nitrogen is the major limiting factor for plant growth. The application of N$_2$-fixing endophytic bacteria as biofertilizer has emerged as one of the most efficient and environmentally sustainable methods for increasing the growth and yield of crop plants. Biological nitrogen fixation (BNF) is one of the possible biological alternatives to N-fertilizers and could lead to more productive and sustainable agriculture without harming the environment. Many associative and endophytic bacteria are now known to fix atmospheric nitrogen and supply it to the associated host plants. A variety of nitrogen fixing bacteria like Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Enterobacter, Gluconoacetobacter, Herbaspirillum, Klebsiella, Pseudomonas, and Serratia have been isolated from the rhizosphere of various crops, which contribute fixed nitrogen to the associated plants (James et al 1994, Olivares et al 1996, Giller 2001, Elbeltagy et al 2001, Boddey et al 2003, Wei et al 2014, Reis and Teixeira 2015, White Jr et al 2014).

In recent years, application of endophytic microbial inoculants supplying N requirement efficiently to the various host plants including cereal crops have drawn attention for increasing plant yield in sustainable manner. In terms of benefiting through nitrogen fixation, endophytic microbes are considered to be better than that of rhizospheric one as they provide fixed nitrogen directly to their host plant and fix nitrogen more efficiently due to lower oxygen pressure in the interior of plants than that of soil. The concept of BNF by endophytes (Dobereiner 1992), has led to investigations on the potential uses of endophytic nitrogen-fixing bacteria that colonize graminaceous plants. Gluconoacetobacter diazotrophicus is the main contributor of endophytic BNF in sugarcane, which according to nitrogen balance studies fix as high as 150 Kg N ha$^{-1}$yr$^{-1}$ (Muthukumarasamy et al 2005).

Studies of endophytes in sugarcane have focused on isolation and characterization using morphological and physiological studies of diazotrophic bacteria as well as molecular characterization of nif genes and 16S rRNA sequence analysis. Magnani et al 2010, reported that endophytic bacteria live inside sugarcane plant tissues without causing disease; these
bacteria belonged to five groups, based on the 16S rRNA sequences. Group I comprised 14 representatives of the Enterobacteriaceae; group II was composed of Bacilli; group III contained one representative, Curtobacterium sp; group IV contained representatives of the Pseudomonadaceae family, and group V had one isolate with an uncultured bacterium. Four isolates were able to reduce acetylene to ethylene. Most of the bacteria isolated from the sugarcane stem and leaf tissues belonged to Enterobacteriaceae and Pseudomonaceae, respectively, demonstrating niche specificity.

Quecine et al (2012), reported that sugarcane growth promotion by the endophytic bacterium Pantoea agglomerans 33.1 showing nitrogen fixation. Tam and Diep (2014), isolated and characterized 27 isolates on LGI medium from sugarcane, and all of them have ability of nitrogen fixation and P solubilization together with IAA biosynthesis but there were 10 isolates having the best characteristics. All the endophytic bacteria belonged to Proteobacteria and 3 isolates belonged to Alpha-proteobacteria (30%), 2 isolates were Beta-proteobacteria (20%) 5 isolates were Gamma-proteobacteria (50%). Enterobacter oryzae LT7, Achromobacter xylosidans T16, Achromobacter insolitus R15b and Pantoea agglomerans T12 revealed promising candidates with multiple beneficial characteristics and they have the potential for application as inoculants or bio-fertilizer adapted to poor latosols and acrisols because they are not only famous strains but also are safety strains for sustainable agriculture. Burkholderia, Herbaspirillum, Azospirillum and Rhizobium leguminosarum are contributor of endophytic BNF in rice (Biswas et al 2000, Baldani and Baldani 2005, Govindarajan et al 2008, Isawa et al 2009, Doty 2011, Estrada et al 2013, Choudhury et al 2014, Aon et al 2015). As all of these assumptions seem to support the conditions for nitrogen fixation by endophytic bacteria, there is still no direct evidence that endophytic bacteria actually are the responsible agents of biological nitrogen fixation. Although some agriculturally important crops, such as rize, wheat and maize contain numerous endophytic bacteria such as Acetobacter diazotrophicus, Herbaspirillum sp., and Azospirillum sp., there is little evidence that these bacteria actually fix N₂ in their host plants (James et al 1994).

Ji et al (2014), have isolated 576 endophytic bacteria from the leaves, stems, and roots of 10 rice cultivars and identified 12 of them as diazotrophic bacteria using a specific primer set of nif gene. Through 16S rDNA sequence analysis, nif H genes were confirmed in the two species of Penibacillus, three species of Microbacterium, three Bacillus species, and four species of Klebsiella. Rice seeds treated with these plant growth-promoting bacteria (PGPB) showed improved plant growth, increased height and dry weight and antagonistic effects
against fungal pathogens. In addition, auxin and siderophore producing ability, and P solubilizing activity were studied for the possible mechanisms of plant growth promotion. The widespread use of synthetic fertilizers has resulted in environmental degradation, a decline in beneficial micro- and macroorganisms, and accumulation of chemical residues in the food system. For sustainable agriculture, the use of biologically derived fertilizers would be ecologically sound and economically viable alternatives. These crop-associated indigenous nitrogen fixers may be agronomically important because they could supply part of the nitrogen that the crop requires. Inoculation with N-fixing endophytic bacterium may represent an alternative to the use of chemical N fertilizers and is associated with decreased production costs as well as a considerable increase in crops production.

Verma et al (2014, 2015), reported psychrotolerant and drought tolerant endophytic bacteria from wheat showing nitrogen fixation activity by different genera of *Arthrobacter, Flavobacterium, Bacillus, Methylobacterium, Providencia, Pseudomonas, Stenotrophomonas* and *Enterobacter*. These bacteria also possess solubilization of P, K, Zn; produced IAA, siderophore, ACC deaminase, HCN and ammonia and showed antifungal activity against plant pathogens. Verma et al (2016), have isolated and characterized a total 264 bacteria associated with wheat, which have been isolates from peninsular zone of India, among all isolated bacteria 26 were identified as endophytic of which thirteen diverse groups of bacteria were characterized for nitrogen fixation, such as *Achromobacter, Alkaligens, Bacillus, Delftia, Providencia, Pseudomonas, Rhodobacter* and *Salmonella*. These biological nitrogen fixing bacteria also solubilized P as well as K at high temperatures. Selected nitrogen fixing, P and K-solubilizing bacteria could be effectively used as biofertilizers at place of chemical fertilizers. NPK could be increase soil productivity to improve sustainability of agriculture production.
III. Methodology

1. Isolation and characterization of endophytic microbes from cereal crops
   - Survey and collection of samples from cereal crops (Wheat, Maize, Barley and Oats) growing in Sirmaur district, Himachal Pradesh.
   - Isolation of endophytic microbes using different growth media such as: Nutrient agar medium, King’s B agar, Luria-Bertani agar, Jensen agar, Nitrogen free bromothymol Medium (NFB), Tryptic soy agar, Potato dextrose agar, Rose bengal agar etc.
   - Morphological and cultural characterization of isolated microbes on the basis of Size (in mm), Shape (spherical/rod-like/spiral/filamentous/irregular), Colour (Pigmented/Non-pigmented), Margin (Lobate/Wavy/Smooth), Surface (Smooth/Rough/Wrinkled) and Polysaccharides producer.

2. Characterization of endophytic microbes for nitrogen fixation and other plant growth promoting attributes
   - Nitrogen fixing attributes using Acetylene Reduction Assay
   - Screening of endophytic microbes for other plant growth promoting attributes including P, K and Zn solubilization, production of siderophore and phytohormones

3. Molecular characterization of endophytic microbes
   - Isolation of genomic DNA from selected endophytic microbes
   - PCR amplification of 16S rDNA and candidate nitrogen fixation gene (s)
   - Sequencing of 16S rRNA genes and nitrogen fixation gene (s) for identification
   - Phylogenetic analyses will be carried out of 16S rRNA genes using MEGA 6.

4. Evaluation and validation of selected endophytic microbes for growth and yield of crops
   - Seed germination bioassay of selected microbial isolates with N₂-fixtion and other PGP attributes
   - Evaluation of endophytic microbes in vitro and in vivo for PGP and yield of selected crops
IV. Plan of Research Work

Time schedule of activities and activity milestones

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<td>Isolation and characterization of endophytic microbes from cereal crops</td>
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<td>Characterization of endophytic microbes for N\textsubscript{2}-fixation and other PGP attributes</td>
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<td>Molecular characterization of selected endophytic microbes</td>
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<td>Evaluation and validation of selected endophytic microbes for growth and yield of crops</td>
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V. WORK DONE

Objective 1: Isolation and characterization of endophytic microbes from cereal crops
- Plant samples from cereal crops (wheat, oat and barley) were collected from Baru sahib, Sirmaur, Himachal Pradesh, India.
- Endophytic microbes has been isolated using thirteen different selective and complex growth media
- A total of 237 microbes have been isolated from wheat (159), Barley (43) and oat (35).
- Isolated microbes have been characterized for their morphological characteristic.

Objective 2: Characterization of endophytic microbes for nitrogen fixation and other plant growth promoting attributes
- All isolates have been screened for plant growth promoting attributes (solubilization of P, K and Zn; production of ammonia, siderophores, hydrogen cyanide and indole acetic acid).
VI. EXPECTED OUTCOMES OF RESEARCH

- Advancement in taxonomy of endophytic microbes
- Mapping of extent of total genetic variability of endophytic microbial isolates.
- Identification of novel species/strain of endophytic microbes
- Development of N$_2$-fixing bio- inoculants for cereal crops

References


**ADVISORY COMMITTEE**

Name of the Student: Mrs Kusam Lata Rana

Reg. No.: BS15PSBT003

Department: Biotechnology

College: Agriculture

Major Advisor: Dr. Ajar Nath Yadav

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<td>Dr. Ajar Nath Yadav, Biotechnology</td>
<td>Major Advisor</td>
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<td>Dr. Vinod Kumar, Biotechnology</td>
<td>Co-Major Advisor</td>
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<td>Dr. H.S. Dhaliwal, Agriculture</td>
<td>Member</td>
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<td>Dr. Pritesh Vyas, Biotechnology</td>
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<td>Dr. N. Ramu, Agronomy</td>
<td>Member</td>
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HOD 18/11/2016  (Signature)

Major Advisor  (Signature)

Dean PGS  (Signature)

Dean of College  (Signature)

For Approval  

Vice Chancellor
ADVISORY COMMITTEE MEETING

Name of the Student: MRS KUSAM LATA RANA
Major Subject: BIOTECHNOLOGY
Major Advisor: DR. AJAR NATH YADAV
Name of Department: BIOTECHNOLOGY
Name of College: AKAL COLLEGE OF AGRICULTURE
Venue: DEPARTMENT OF BIOTECHNOLOGY
Date: 25-10-2016
Time: 3:0 pm

Students Work Load Teaching

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<td>MIC-201</td>
<td>Fermentation and Industrial Microbiology</td>
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Title of Thesis: Nitrogen Fixing Endophytic Microbes from Cereal Crops and their Biotechnological Applications

Research Work Carried out in the present Semester
- Plant samples from cereal crops (wheat, oat and barley) were collected from Baru sahib, Sirmaur, Himachal Pradesh, India.
- A total of 237 endophytic microbes have been isolated using thirteen different selective and complex growth media.
- Isolated microbes have been characterized for their morphological characteristic.
- All isolates have been screened for plant growth promoting attributes (solubilization of phosphorus, potassium and zinc, production of ammonia, siderophores, hydrogen cyanide and indole acetic acid).

Research Work to be carried out in the next semester
- Characterization of microbes for nitrogen fixation attributes
- Molecular characterization of selected endophytic microbes

Signature of the Student

Signature of Major Advisor

Committee Members:

1. Dr. Ajar Nath Yadav
2. Dr. Vinod Kumar
3. Dr. H.S. Dhaliwal
4. Dr. Pritesh Vyas
5. Dr. N. Ramu