CHAPTER 1

INTRODUCTION

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Endophytic bacteria colonize the interior tissue of the plant without causing any negative effects on their host (Schulz and Boyle, 2006), and of the nearly 300 000 plant species that exist on the earth, each individual plant is host to one or more endophytes (Strobel *et al*., 2004). Endophytic microorganisms enter primarily through root zone; however flowers, stems and cotyledons may also be used as a route of entry. The bacteria may also enter tissues via germinating radicles. Endophytes inside a plant may either become localized at the point of entry or may spread throughout the plant tissues (Hallmann *et al.*, 1997). Endophytic bacteria have been isolated from different plant tissues such as roots (Li *et al*., 2016; Abbamondi *et al.*, 2016), stem (Andreolli *et al*., 2016; Ji *et al*., 2014), barks (Khan *et al*., 2016; Doley and Jha, 2014), shoots (Taghavi *et al*., 2009), leaves (Ji *et al*., 2014), fruits (Abdallah *et al*., 2016), rhizomes (Kumar *et al*., 2016) and seeds (Herrere *et al*., 2016).

Endophytes are ubiquitous with a rich biodiversity and unexplored biosynthetic potential (Strobel and Daisy, 2003; Ryan *et al*., 2009). Endophytes produce a group of bioactive compounds and enzymes to survive in the unique chemical environment of the host plant (Strobel, 2003). Their metabolic activities also help in increasing the growth and development of plants. This is because of direct growth promotion effects through the production of plant growth regulators, N-fixation, synthesis of ACC (1 aminocyclopropane-1-carboxylate) deaminase, phosphate solubilization, and/ or by indirect mechanism by providing resistance to diseases through the production of antimicrobial metabolites or siderophores to inhibit pathogenic microorganisms (Khan *et al*., 2016; Ji *et al*., 2014; Sun *et al*., 2009; Abbamondi *et al*., 2016).

Microorganisms which are mainly considered as plant growth promoting endophytes include the strains in the bacterial genera of *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arthrobacter, Azospirillium, Azotobacter, Azomonas, Bacillus, Beijerinckia, Burkholderia, Clavibacter, Enterobacter, Erwinia, Flavobacterium, Klebsiella, Methylobacterium, Microbacterium, Pantoea, Plantibacter, Pseudomonas, Rhodococcus, Rhizobium, Serratia, Sphingomonas* and *Stenotrophomonas*. (Abbamondi *et al*., 2016; Andreolli *et al*., 2016; Khan *et al*., 2016; Kumar *et al*., 2016; Li *et al*., 2016; Lumactud *et al*., 2016). The precise role of endophytes in plants is not yet known. However, their capability to thrive within the host tissues away from microbial competition and environmental degradation has made endophytes potential candidates for use in agriculture.

Most of endophytic bacteria are beneficial to plant because they are able to act as biocontrol agents, available more nutrient, produce plant growth hormones and natural product resources for medicine, agriculture and industries (Bacon and Hintol, 2006; Strobel and Daisy, 2003). Endophytic bacteria have been shown to prevent the development of disease through endophyte-mediated de novo synthesis of novel compounds and antifungal metabolites. New drugs were identify from the investigation of the biodiversity of endophytic strains which may have effective in treatment of diseases in humans, plants and animals (Strobel *et al*., 2004). Although the majority of research on plant-associated bacteria has been focused on rhizobacteria, interest in the diversity and role of endophytic bacteria is increasing. The main reason for the interest in endophytes is the realization that, if these bacteria can be re-introduced in the endophytic stage, a more stable relationship can be established between plant-beneficial endophytic bacteria and plants than for rhizospheric or epiphytic bacteria and plants. Therefore, endophytes with the plant-beneficial traits are potentially excellent plant growth promoters and/or biological control agents for sustainable crop production (Strobel, 2003).

Bacterial endophytes have been reported to promote plant growth by a number of different mechanisms. These mechanisms include phosphate solubilization activity (Verma *et al*., 2001; Wakelin *et al*., 2004; Thamizh Vendan *et al*., 2010; Audipudi *et al*., 2014), production of phytohormones (Lee *et al*., 2004; Chimwamurombe *et al*., 2016; Khan *et al*., 2016; Yu *et al*., 2016), nitrogen fixation (Compant *et al*., 2005; Balachandar *et al*., 2006; Ji *et al*., 2014), siderophore biosynthesis (Lodewyckx *et al*., 2002; Rungin *et al*., 2012; Abbamondi *et al*., 2016) and supplying essential nutrients to the host plant (Costa and Loper, 1994; Puente *et al*., 2009). Bacterial endophytes may also promote plant growth as a consequence of the bacterium expressing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which cleaves ACC to α-ketobutyrate and ammonia and thereby decreases ethylene levels in host plants (Sessitsch *et al*., 2005; Sun *et al*., 2009). Some studies have indicated that the plant growth-promoting potential of endophytes is higher than that of rhizosphere microbes (Reiter *et al*., 2003) but the role of bacterial endophytes in plant growth are not yet fully implicit. Nearly all of these microorganisms are not pathogenic to the host plant. Moreover, the association between the plant and its endophytes is very often mutualistic. The presence of bacterial endophytes in plants is variable and, occasionally transient (Van Overbeek *et al*., 2008), they are also often capable of eliciting drastic physiological changes that modulate the growth and development in the plant (Conrath, 2006). Endophytic bacteria are better than their rhizospheric and rhizoplanic counterparts in terms of benefiting their host through nitrogen fixation as they can provide fixed nitrogen directly to their host (Cocking, 2003). As low partial oxygen pressure is necessary for the expression of the O_2 sensitive enzyme, nitrogenase, endosphere of plant root is more amenable for N_2 -fixation reaction. Moreover, endophytic bacteria are less vulnerable to competition with other soil microbes for scarce resources and remain protected to various abiotic and biotic stresses (Reinhold-Hurek and Hurek 1998). The ability of PGPB to solubilize mineral phosphate has been of immense interest to agricultural microbiologists as it can lead to enhanced availability of phosphorus for effective plant growth. PGPB have been reported to mobilize precipitated phosphate to plants, representing a possible mechanism of plant growth promotion under field conditions (Bhattacharyya and Jha, 2012).

Most of endophytes produced bioactive metabolites that helps in the host-endophyte relationship (Strobel, 2003). These metabolites may serve as sources of novel natural products for exploitation in medicine, agriculture, and industry (Bacon and White 2000, Strobel & Daisy 2003). Much relationship exists between endophytes and its host plants which ranges from mutualism or symbiosis to antagonism (Schulz and Boyle 2005).

The methods are being used to evaluate the presence and location of endophytic bacteria which include immunological detection of bacteria, fluorescence tags, and confocal laser scanning microscopy (Chelius and Triplett 2000; Hartmann *et al*., 2000; Elbeltagy *et al*., 2001; Verma *et al*., 2004; Sandhiya *et al*., 2005). In addition, specific oligonucleotide probes could also be of use to visualized bacteria which presence inside the plants (Hartmann *et al*., 2000; Toumatia *et al.*, 2016).

The study of plant-associated microorganisms is of great importance for biotechnological applications, for example, biological control of plant pathogens, plant growth promotion or isolation of active compounds. Understanding the diversity of plant-bacterial associations and their role in plant development is necessary if these associations are to be manipulated to increase crop production, conserve biodiversity and sustain agro-ecosystems in relation to as well as under dry farming conditions which may help in overcoming abiotic stress.

Medicinal plants have beneficial entophyte-plant relationship that may be explored and utilized. Endophytes are considered to be a promising source of novel secondary metabolites (Shultz *et al*., 2002; Strobel, 2003; Puri *et al*., 2006) with the potential to be medically useful as well as important in agriculture and industry (Strobel and Daisy, 2003). The anticancer drug lead compound podophyllotoxin (Puri *et al*., 2006), and the natural insecticide azadirachtin (Kusari *et al*., 2012) produced by endophyte is a good example.

The plant growth promoting properties of endophytes are unique, and therefore it is significant to study such properties from microbial populations linked with medicinally important and economically important plants. *A. aspera* L (Latjeera; Rough Chaff tree) is a known medicinal plant that is used for the dilation of the blood vessels, lowering of the blood pressure, depression of the heart and increases the rate and amplitude of respiration (Neogi *et al*., 1970). This plant have been found effective in treating disorders like piles, renal dropsy, pneumonia, cough, kidney stone, skin eruption, snake bites, dysentery (Aziz *et al*., 2005). The plant is also reported to have antiperiodic, antiasthmatic (Charyulu, 1982), diuretic (Subramaniam, 1961), purgative, laxative, hepatoprotective (Katewa *et* *al*., 2001), anti-allergic, and various other important medicinal properties. The pharmacological effects of *A. aspera* L are attributed to the presence of active compound like alkaloids, saponins, sterols. Other active constituents are D-Glucuronic Acid, β-Dgalactopyranosylester of D-Glucuronic Acid, oleanolic acid, amino acids, hentriacontane, sapogenin (Khastgir *et al*., 1958), ecdysterone (Ikan *et al*., 1971), betaine (Kapoor *et al*., 1966), p- benzoquinone, hydroquinone, spathulenol, nerol, α-ionone, asarone and eugenol (Rameshwar, 2007). Considering the medicinal importance of *A. aspera*, it's pertinent to understand the role of endophytic bacteria on its growth and other properties.

A renewed interest in the internal colonization of healthy plants by endophytic bacteria has arisen as their potential for exploitation in agriculture becomes apparent. Exploitation of endophyte–plant interactions may result in the promotion of plant health and play a significant role in low-input sustainable agriculture applications for both food and non-food crops. Considering the enormous potential of the endophytic microorganisms, this research work was envisaged to study the role of endophytic bacteria in plant growth promotion of *Achyranthes aspera* L. with the following objectives.

OBJECTIVE OF RESEARCH WORK:

- Collection of *Achyranthes aspera* L. plants from different area of Manipur.
- Isolation and screening of endophytic strains, subculturing and preservation.
- Primary screening through *in vitro* bioassays for growth promotion (biofertilizer action).
- Selection of promising strains and their characterization:

i) Traditional methods: morphological, physiological and biochemical tests.

ii) Molecular characterization using 16S r DNA sequence analysis of strains.

- Secondary screening of the selected strains through pot/greenhouse tests for phytostimulation.
- Effect of inoculation on medicinal property of plant.