CHAPTER-I

INTRODUCTION

1. INTRODUCTION

Endophytes are defined as microorganisms that reside within the plant tissues without causing apparent diseases (Wilson , 1995). Endophytes colonize different internal living healthy tissues such as leaf, bark and root of plant without producing any pathogenic effects (Chanway, 1996). They are usually characterized by the feature that they do not cause any harm to their hosts rather are beneficial (Ludwig-Muller, 2015). The association between the plant and its endophytes is very often mutualistic. Nature of these organisms varies with nature of plants, age, type of tissues, seasons of sampling and environment they are growing in. Plants strictly limit the growth of endophytes and these endophytes use many mechanisms to gradually adapt to their living environments (Dudeja et al., 2012). They are not considered as saprophytes since they remain associated with living tissues, and may in some way contribute to the well being of the host plant. Endophytes are generally not organ-specific and it is likely that many of them may colonize different tissues (Dix and Webster, 1995). Endophytes usually inhabit above ground tissues i.e. leaves, stems, barks, petioles, fruits, flowers, which distinguishes them from mycorrhizal fungi. Some workers, however, are of the opinion that bacterial endophytes are more concentrated in root rather than shoot tissues (Zinniel et al., 2002). Almost all plant species remain colonized by endophytic microorganisms. Under normal circumstances they live asymptomatically within the host tissues (Konig et al., 1999). They affect their hosts positively by growth enhancement and protection against pathogens and feeding damages (Saikkonen et al., 1999). Symptomless nature of endophytes focuses on symbiotic and mutualistic relationship between endophytes and their hosts. It is presumed that hosts provide nutrient to the endophytes, which in turn produce factors that protect the hosts from attack by animals, insects and other microbes (Yang et al., 1994). Some endophytes become borderline pathogenic when the plant is stressed. Endophytic fungi may exist in their host plants in a range of biological associations from near pathogenic to symbiotic (Schulz et al., 2002). Some workers, however, prefer the term aggressive saprophytes or opportunistic pathogens to describe the endophytes. Endophytes make biologically active products, which are selectively active against certain microbes that may be a potential threat to the host plant (Yang et al., 1994). The host plant protects and feeds the endophyte, which in turns produces bioactive metabolites to enhance the growth and competitiveness of the host and consequently protect it from herbivores and plant pathogens (Dreyfuss and Chapela, 1994). Some endophytes have also been used for biocontrol (Lahlali et al., 2013) based on their potential to induce defense responses. Plants can produce these compounds to alter growth of fungi within their tissues before and after colonization. However, the fungi can also produce secondary metabolites for their own benefit, e.g. to get rid of competitors within the host plants. The host plant can then modify either toxic compound to deal with them if these are too harmful, or to use them in alternative processes. It has been observed that the number of endophytes producing these herbicidal substances was three times higher than of soil microbes and twice that of plant pathogenic microorganisms (Ludwing-Muller, 2015). Endophytes also provide advantages to the host plant by producing plant growth regulators, by providing resistance to diseases and also by assisting in phytoremediation (Lodewyckx et al., 2002). The mechanism by which endophytes deal with the ever-changing environmental conditions may provide better survival advantages to host plants. Microorganisms have the ability to utilize various solid substrates as a consequence of diversity of their biological and biochemical evolution; the solid substrates utilized by microorganisms include, among others, live plants. Microorganisms have the ability to utilize various solid substrates as a consequence of diversity of their biological and biochemical evolution; the solid substrates utilized by microorganisms include, among others, live plants. The microorganisms residing within the plant tissues are unique in their adaptations to specific chemical environment of the host. Even some of these microorganisms are shared genetically with the molecular machinery for the synthesis of plant specific compounds. Endophytes therefore, are considered as untapped source of natural products (Rosenblueth and Martinez-Romero, 2006). Endophytes found in healthy tissues of almost all plants investigated so far, therefore, are considered as a rich source of novel bioactive metabolites (Tan and Zou, 2001) for utilization use in agricultural and industry (Strobel and Daisy, 2003) and therapeutic use in medicine (Tejesvi et al., 2005). Fungal endophytes produce various kinds of secondary metabolites which have bioactive potential such as antioxidant, anti-inflammatory, antidiabetic, antitumor and antimicrobial properties (Ruma et al., 2013; Arora et al., 2010). The secondary metabolites produced are associated with the respective biotope or pest (Schulz et al., 2002). The delicate equilibrium between host and endophytic fungi seems to be controlled in part by chemical factors, i.e. herbicidal natural products produced by the fungi versus antifungal metabolites biosynthesized by the host plant. Endophytic microorganisms are an important source of novel metabolites with pronounced antibacterial, antifungal and antiviral activities (Kumar et al. 2016). Some endophytes are capable of developing a biochemical ability to produce compounds similar or identical to those produced by the host plant (Strobel, 2003).

Strobel (2003) rationalized the isolation of endophytes from different plant species. He suggested that the selection of plant species be based on the environment where a specific benefit of the species was observed. These conditions might appear as a consequence of abiotic or biotic stress factors and consequent benefits conferred by endophytes. Besides, plants surrounded by other infected plants but themselves showing no symptoms could be a possible source to look for endophytes. Additional important criteria to be used for plant selection suggested by Yu et al., (2010) include areas with great plant biodiversity.

because these are most likely to contain a large variety of endophytes too. Isolation and characterization of endophytes from traditionally used medicinal plants may lead to production of new bioactive compounds. The isolation of endophytes of endemic plants from ancient land mass might also be promising because of a longer evolutionary period and the potential to develop different types of endophytic organisms (Yu et al., 2010). Endophytes, by residing within the specific chemical environment of host plants, form unique group of microorganisms. Microbially unexplored medicinal plants can have diverse and potential microbial association (Jumponen and Jonnes, 2009).

Different works carried out so far regarding the role of endophytes in the host plants indicate that they can stimulate plants growth, increase disease resistance, improve plant's ability to withstand environmental stresses and recycle nutrients. Besides these, endophytes are also recognized as rich source of secondary metabolites of great importance (Strobel and Daisy, 2003). Many of these compounds are bioactive and the range includes alkaloids, steroids, terpenoids, polyketones, flavonoids, quinols and phenols as well as some chlorinated compounds. Amongst various high value medicinal plants *Taxus baccata* L. has gained considerable importance as a source of the anticancer drug taxol (paclitaxel), a deterpenoid, which was first isolated from the stem bark of *T. brevifolia*. Many fungal endophytes associated with *Taxus* spp have been demonstrated to produce important bioactive metabolites (Strobel et al., 1997; Wang et al., 2000).

Isolation and characterization of endophytes from traditionally used medicinal plants may lead to production of new bioactive compounds. Microbially unexplored medicinal plants can have diverse and potential microbial association (Jumponen and Jonnes, 2009). Endophytes are unique group of microorganisms residing within the specific chemical environment of host plants. Medicinal plants can have potential and diverse microbial association. The rhizome of turmeric is very remarkable due to its metabolite richness and the physiological processes associated with these tissues (Sahadevan et al. 2016)

Curcuma longa L. belongs to family Zingiberaceae is commonly known as "Halodhi" in Assamese. *C. longa* is a small perennial herb bearing many rhizomes on its root system which are the source of its culinary spice known as turmeric and its medicinal extract called Curcumin. it has many medicinal properties like analgesic, antibacterial, anti-inflammatory, anti-tumor, anti-allergic, antioxidant, antiseptic, antispasmodic, appetizer, astringent, cardiovascular, digestive, diuretic, stimulant etc. The mature dried rhizome is most common ingredient of Assamese as well as Indian kitchen as spice and well known antiseptic, antipyretic since ancient times. Traditionally, it has been extensively used by the people of Assam in the treatment of swelling caused by injury. In addition, turmeric also possesses antimicrobial and anticancer properties (Mukhtar et al., 2012). The medicinal properties are assigned due to the presence of curcuminoid and sesquiterpenoid compounds. Metabolites present in *C. longa* are Curcumin-sulphate, Curcumin-glucuronide

Therefore the present study was framed to isolate endophytic microorganisms with antimicrobial properties from a medicinally important plant *C. longa*, available in greater Guwahati with the following objectives:

1. Isolation and identification of endophytic microorganisms from *C. longa* available in greater Guwahati.
2. Diversity of endophytic microorganisms in different tissues and organs of the plant.
3. Antimicrobial activity of endophytes isolated from *C. longa*.