GENERAL INTRODUCTION

Fungal endophytes

Literally, the word endophyte means “in the plant” (endon Gr. =within, phyton = plant). The usage of the term “endophyte” is as broad as its literal definition and spectrum of potential hosts and inhabitants. The most common usage of the term “endophyte” for organisms whose infections are internal and inconspicuous, and in which the infected host tissues are at least transiently symptomless, is equally applicable to bacterial prokaryotes and fungal eukaryotes (Stone et al., 2000; Schulz and Boyle, 2005). Endophytes include an assemblage of microorganisms with different life history strategies; those that follow an endophytic growth phase, grow saprophytically on dead or senescing tissue, avirulent microorganisms, incidentals, also latent and virulent pathogens at early stages of infection (Schulz and Boyle, 2006).

Endophytic fungi, a polyphyletic group of highly diverse, primarily ascomycetous fungi defined functionally by their occurrence within asymptomatic tissues of plants, are found in above-ground tissues of liverworts, hornworts, mosses, lycophytes, equisetopsids, ferns, and seed plants from the arctic to the tropics, and from agricultural fields to the most diverse tropical forests (Arnold, 2007). Endophytic fungal associations differ from mycorrhizas primarily by the absence of a localised interface of specialised hyphae, the absence of synchronised plant-fungus development, and the lack of plant benefits from nutrient transfer – the three key defining features of mycorrhizas (Brundrett, 2004). However, plants may benefit indirectly from endophytes by increased resistance to herbivores, pathogens or stress, or by other unknown mechanisms (Schulz, 2006). Stoyke and Currah (1991) introduced the form taxon “dark septate endophyte” (DSE) and used it
for fungi that form partly or entirely melanised and having septate thalli within healthy root tissues. The taxon “DSE” serves primarily to differentiate these fungi from endophytes with septate and hyaline hyphae, from fungi having sparse septation and hyaline hyphae that are characteristic of arbuscular mycorrhizal fungi. Taxonomic assignment of many DSE is problematic because sexual and asexual reproductive structures are either absent, rare, or are produced only under specific conditions (Arnold, 2007). Non-mycorrhizal and non-DSE fungi also occur within plant tissues and such endophytic fungi were found to lack the distinctive morphology of both mycorrhizal symbionts and DSE (Suryanarayanan and Vijaykrishna, 2001; Kumar and Hyde, 2004).

Fungal endophytes, excluding the mycorrhizal forms, consist of two basic ecological groups: the balansiaceous or ‘grass endophytes’, and the non-balansiaceous taxa. The ‘grass endophytes’ form a unique group of closely related fungi with ecological requirements and adaptations distinct from those of other endophytes (Petrini, 1996). They belong to the ascomycetous genera *Epichloe* and *Balansia*, and their anamorphs *Neotyphodium* and *Ephelis*. In contrast to the balansiaceous endophytes, the non-balansiaceous fungal endophytes are diverse, both phylogenetically and with respect to life-history strategy (Schulz and Boyle, 2005). Colonisation can be inter- or intracellular, localised or systemic. In the case of the non-balansiaceous, the term ‘endophyte’ generally refers to a fungus capable of cryptic occupation of plant tissue and describes a momentary status (Schulz and Boyle, 2005).

**Rationales of plant selection for the isolation of novel endophytic fungi**

Tropical endophytes synthesise more active natural products than temperate endophytes (Bills *et al*., 2002). This observation suggests the importance of the host plant in
influencing the metabolism of endophytic fungi. The rationales for plant selection, which increases the probability of isolating novel endophytic fungi as suggested by Strobel and Daisy (2003), are as follows:

(i) Plants from unique environmental settings, especially those with an unusual biology, and possessing novel strategies for survival are considered for study.

(ii) Plants that have an ethno-botanical history (use by indigenous people) and are related to specific uses or applications of interest are selected for study.

(iii) Plants that are endemic, have an unusual longevity, or that have occupied a certain ancient landmass, such as Gondwanaland, are also more likely to lodge endophytes with active natural products than other plants.

(iv) Plants growing in areas of great biodiversity also have the prospect of housing endophytes with great biodiversity.

Based on these rationales and their extensive ethnomedicinal usage by the ethnic communities, five plants were selected for the study namely, *Potentilla fulgens* L., *Osbeckia stellata* Buch.-Ham. ex D. Don, *Osbeckia chinensis* L., *Camellia caduca* CL. Ex. Brandis and *Schima khasiana* Dyer.

The ‘Sacred Forests’ of Meghalaya, India

Protecting the patches of forests on religious grounds is an age-old practice in Meghalaya. In the olden days almost all the villages in Khasi, Jaintia and Garo hills had well-preserved sacred forests in the vicinity of the village habitations. In spite of the remarkable changes that have taken place in socio-cultural, religious and economic spheres of the people over the past few decades, these forests have been able to withstand the anthropogenic pressure and a number of them are still found in good condition
throughout the state. Based on the ownership pattern and management control and the
tribe that preserves them, these forests popularly called as sacred groves, are variously
The sacred forests of Meghalaya are unique in many ways. Unlike their counterparts in
the rest of India and abroad, these forests are quite large in size and some of them being
as big as 900 hectares in area. These forests have definite legal status since they are
considered as classified under the United Khasi-Jaintia Hills Autonomous District
(Management and Control of Forests) Act, 1958 and enjoy protection under such acts,
rules and regulations. Owing to their large size, they perform a number of ecosystem
services including protection of water sources and maintenance of clean environment.
The traditional village level institutions in this part of India exercise a strong control and
influence over the society. Thus, the involvement of such institutions in protecting the
sacred forests, has contributed immensely to their preservation. Sacred forests represent
the remnants of the climax vegetation of the area and are a natural treasure house of
biodiversity. The vegetation of the sacred forests, in general, is usually different from that
of the adjoining areas. Many of the endangered species described for the state of
Meghalaya (India) are presently confined to these forests only (Haridasan and Rao,
1985). The vegetation of most of the undisturbed sacred groves can be distinguished into
four major strata viz., canopy layer, sub-canopy layer, shrub layer and ground flora.
Emergent trees generally constitute the canopy layer, while tree species like
Rhododendron arborea and Myrica esculenta constitute the sub-canopy layer. Shrub
layer is composed of a number of shrubs and saplings of tree species. Ground flora
consists of grasses, herbs, ferns and bryophytic species along with the seedlings of the trees (Tiwari et al., 1999).

The need for the present study

Areas having an exceptional concentration of endemic species and experiencing loss of habitat have been termed as biodiversity hot-spots (Myers et al., 2000). Together, they occupy about 1.4% of the earth’s surface and contain about 44% of the world’s total flora (Jamir and Pandey, 2003). Out of the 34 biodiversity hot spots identified on the earth, three of them, namely Himalaya, Indo-Burma and the Western Ghats, are located in the Indian subcontinent (Anonymous, 2008). Due to the presence of a large number of primitive and ancient flowering plants in the region, the Indo-Burma region of northeast India was considered as the cradle of ancient angiosperms (Takhtajan, 1969). Meghalaya, in northeast India, which falls under the realms of the Indo-Burma biodiversity hotspot, is reported to have 3128 species of flowering plants including 1236 endemic species (Khan et al., 1997).

The World Health Organization (WHO) has listed 20,000 plants globally having medicinal usage (Gupta and Chadha, 1995). To this list, the share of India’s contribution is 15–20% (Singh, 2000). According to the WHO estimate, about 80% of the populations in the developing countries depend directly on plants for medicines (Pareek, 1996; Mukhopadhyay, 1998). In India, about 2000 drugs used for medication are of plant origin (Dikshit, 1999). The local communities residing in the biodiversity-rich areas of the north-eastern region of India have traditionally used and relied on herbs for treating various ailments (Kayang et al., 2005). There are nearly 250 plants of north-eastern region including herbs, shrubs and trees reported to have been used as drug or
medicaments. These plants have been used as medicines for the treatment of asthma, arthritis, diabetes, diarrhea, cancer, dysentery, hypertension, malaria, leucoderma, rheumatism, skin disease, jaundice, spondolysis, tuberculosis etc. (Kamboj, 2004). There are 876 medicinal plant species in Meghalaya, out of which 188 are commercially exploited (Anonymous, 2000).

It is conceivable that medicinal plants have the association of microbes as endophytes that mimic the chemistry of their respective host plants and make the same bioactive natural product(s) or derivatives that are more bioactive (Strobel, 2002). Plants having an ethno-botanical history and being used by the traditional medical practitioners for the treatment of various ailments are the best candidates for the search of novel endophytes producing pharmaceutically relevant metabolites. It is often found out, that the healing powers of the botanical source, in fact, may have nothing to do with the natural products of the plant, but is directly related with the microbial endophyte(s) residing inside the plant (Strobel and Daisy, 2003). It seems obvious that endophytes are a rich and reliable source of genetic diversity including novel and undescribed species. (Strobel, 2006).

Novel microbes usually have associated with them novel natural products (Strobel and Long, 1998). It is also hypothesized that some endophytes could have co-evolved with their respective host plant and, as a result, already exists compatibly with a higher life form (Fletcher and Easton, 2001). Thus, closer biological associations may have developed between these endophytes in their respective hosts than is the case with epiphytes or soil inhabitant microorganisms. The result of these associations may be the production, by the endophyte, of bioactive chemicals that are involved in the relationship (Strobel and Daisy, 2003).
It is obvious that a number of economically and medicinally important plant species will soon be extinct, considering the rate at which plant resources are depleting globally. In the last few decades, over-exploitation of forest resources has led to an enormous species loss. As a result, 20–25% of existing plant species in India has become endangered (Dhar et al., 2002). Medicinal plants are now under great pressure due to their excessive collection/exploitation or habitat loss. The degree of threat to natural populations of medicinal plants has increased, because more than 90% of medicinal plant raw material for herbal industries in India and also for export is drawn from natural habitat (Dhar et al., 2002). The extinction of rare and endemic medicinal plants or of economically important plants, will not only wipe out the plants from the face of earth, but will also lead to the extinction of numerous microbial endophytes associated with respective plants. Therefore, it is felt that prior to the screening of the endophytic metabolites, it is important to characterize the endophytic microbes as such, from the medicinally and economically important plants of the biodiversity-rich areas of the north-eastern region of India.

Unique examples of such areas with a very rich biodiversity are the traditionally preserved sacred forests (groves) in Meghalaya. Despite all the anthropogenic disturbances caused to natural environment in Meghalaya, an estimated forest cover of about 1000 sq km, accounting for about five percent area of the state, has been left undisturbed since time immemorial due to religious beliefs (Anonymous, 1984). These forests known as ‘sacred forests’ or ‘sacred groves’ constitute a substantial portion of the private and community forests and are scattered all over the hill regions of Meghalaya.
The importance of the biodiversity of sacred forests, its inventorization and conservation has long been recognized (Gadgil and Vartak, 1975; Haridasan and Rao, 1985). Although there has been some work on the faunal and floral components of sacred forests (Boojh and Ramakrishnan, 1983; Rodgers, 1994), the microbial components especially the plant endophytes have not received any attention as yet. The traditionally conserved sacred forests of Meghalaya are rich in plant biodiversity (Laloo et al., 2006) and this richness may lead to the discovery of an even larger diversity of novel endophytes, which as a whole or their metabolites may have great promise for use in medicine, agriculture and industry. Keeping these facts in view, the present study was undertaken to search for novel endophytic fungi from ethno-medicinally important plants growing in traditional sacred forests of Meghalaya.

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