What is an endophytic fungus?

Since the discovery of the world's first billion-dollar anticancer drug, paclitexel (Taxol) from *Pestalotiopsis microspora*, a fungus that colonizes the Himalayan yew tree *Taxus wallichiana*, without causing apparent injury to the host plant, interest is growing in symptomless parasitic fungi, termed the 'endophytes'[1]. The term endophyte is applied to fungi (or bacteria) which live within plant tissues, for all or part of their life cycle and cause no apparent infections[2–4]. This definition excludes the mycorrhizal fungi but does not imply that endophytic fungi are not cultivable on artificial media.

Some species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal and immunosuppressive compounds[5,6]. Further, endophytic fungi may also produce metabolites with thermoprotective role. For example, plants in some volcanic areas in USA were found colonized by an endophytic fungus *Curvularia* sp.[7]. Whereas the plants grown from surface-sterilized seeds in sterile soil that had been inoculated with *Curvularia* sp. survived constant soil temperature of 50°C, the non-symbiotic plants died. Re-isolation of the fungus demonstrated that thermal protection was also provided to the fungus although the biochemical basis is presently not known. Because of their role in conferring plants the ability to adapt to stress conditions, and because they are proven or perceived sources of secondary metabolites with pharmaceutical importance, the study of fungal endophytes is expected to become an important component of fungal biology in the 21st century. However, the term endophyte has begun to be misapplied. Is an endophytic fungus any species that is isolated on nutrient media from 'surface-disinfected' plant tissues? The practice, which some have conveniently adopted as a 'standard method', is to dip the tissue in 70% alcohol for few seconds, or in 0.5–3.5% sodium hypochlorite for 1 or 2 min, followed by rinses in sterile water before placing it on a nutrient medium for fungal isolation[8–12]. The effectiveness of surface-sterilization procedure is assumed, rarely checked by taking surface prints of tissue or by microscopy.

Fungal spores, ranging from 4 to 20 μm, have a disseminative and resting function[13]. They can gain entry into plant tissues through natural cracks, wounds, lenticels, due to air current or rain water flowing down, or through the agency of insects, beetles, mites and other animals which live and breed in the plants and trees. Even a single spore that escapes the disinfection treatment can yield a mycelial colony, as is commonly experienced by anyone who has attempted to isolate callus tissue from leaves and stem pieces from plants growing in nature. While the occurrence of endophytic fungi is not doubted because of demonstration in thin cut sections or/and their vertical transmission[1], caution is necessary to regard all isolates as 'endophytic'. The doubt arises whether most fungal 'endophytes' isolated from plants growing in nature may not be the spore contaminants that have resisted killing by the disinfection procedure that was used? Not surprisingly, the so-called 'endophytic fungi' often include the species found in air, dust and dirt. The effectiveness of surface sterilization may be checked by taking impressions of leaf surface on nutrient medium, although the absence of growth could be due to unusual triggers for germination[13]. More informative would be to examine the structures on sterile tissues by scanning electron microscopy[6]. It has been suggested that before isolation work, plants may be covered with plastic bags placed over twigs and new leaves used[2].

By definition, an endophytic fungus lives in mycelial form in *biological association* with the living plant, at least for some time[1]. Therefore the minimal requirement before a fungus is termed an 'endophyte' should be the demonstration of its *hyphae in living tissue*. Sathe and Raghukumar[14] *used* a bleaching and a staining technique for demonstrating intracellular hyphae in seagrass and this technique may be generally applicable. Senthilkumar *et al.*[15] used acridine orange fluorescence microscopy for rapid visualization of hyphae in hand cut sections of orchid mycorrhizal roots. Since identification of a fungus from hyphal features alone is rarely possible, the identification techniques will require methods of immunofluorescence detection, DNA sequencing and comparison of sequences to homologous sequences registered in Gene Bank[12]. There is a need also of comparing the biochemical activity of aseptically grown plants with those inoculated with the endophyte. The facultative endophytes would offer the most problem. They might be opportunistic – becoming biotrophic under certain environmental condition. Both types are challenging because of their interactions with the plant, modifications of plant growth, their biosynthetic capabilities and evolutionary implications.

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RAMESH MAHESHWARI

e-mail: ramesh.maheshwari@gmail.com