How promising are endophytic fungi as alternative sources of plant secondary metabolites?

V. Priti, B. T. Ramesha, Shweta Singh, G. Ravikanth, K. N. Ganeshaiah, T. S. Suryanarayanan and R. Uma Shaanker

Endophytic fungi, which colonize plants internally without apparent adverse effects, occur ubiquitously in plants[1]. In contrast to their pathogenic fungal counterparts, the endophytic fungi exist in a mutualistic association with their host plants, and in few cases, enhance the ability of plants to tolerate abiotic[2] and biotic[3] stresses. In culture, outside of their host tissue, endophytic fungi are also known to produce a number of important secondary metabolites including anti-cancer, anti-fungal, anti-diabetic and immunosuppressant compounds[1,4]. Occasionally, these compounds are the same as those produced by the respective host plants, thus triggering the expectation that endophytic fungi can serve as an alternative source of important plant secondary metabolites. This possibility was perhaps first realized and mooted by Stierle and his co-workers, following their discovery that the endophytic fungus *Taxomyces andreanae* of the yew plant *Taxus brevifolia* could also produce taxol (generic name: paclitaxel), the multi-billion dollar anti-cancer compound produced by the yew plant[5]. Since the discovery of taxol from *Taxus brevifolia*[6], the world's supply of taxol comes from these trees. However, because of the destructive felling of trees for their bark coupled with their slow growth, the existing natural stocks of trees is fast depleting, and it is feared that it would not sustain the global demand. In this context, the discovery of Stierle and his co-workers[5] was heralded as very significant and it raised the hope of using the endophytic fungus as a sustainable alternative source of taxol. But, how far has this hope been realized? Here, we critically address this question.

Spurred by Stierle *et al.*[5] discovery, scores of studies have been made to identify endophytic fungal sources of a number of important plant secondary metabolites as revealed by Strobel and Daisy[7]. In fact, since the publication of the report by Stierle and his co-workers[5], there has been a monotonic increase in the number of US patents filed on endophytic fungi producing important metabolites with diverse biological activities (Figure 1).

It would be illustrative to narrate a few studies, especially those that have been prospected for anti-cancer compounds. For example, besides *Taxomyces andreanae*, a number of other endophytic fungi including *Seimatoantlerium tepuiense* and *S. nepalense* have been reported to produce paclitaxel[8]. Another anti-cancer agent, a selective cytotoxic quinine dimer, torreyanic acid was isolated from the endophyte *Pestalotiopsis microspora* associated with the endangered tree *Torreya taxifolia* (*Florida torreya*)[9]. Torreyanic acid is 5–10 times more potent than taxol, and causes cell death by apoptosis[9]. Three novel cytochalasins including 22-oxa-(12)-cytochalasins with anti-tumour activity were reported from *Rhinocladiella* sp., an endophye of *Tripterygium wilfordii*[10]. Extracts of *Curvularia* sp., an endophytic fungus isolated from *Ocotea corymbosa*, yielded two new benzopyran derivatives and two known compounds that were tested in cell proliferation and anti-fungal assays. Compound (2′S)-2-(propan-2′-ol)-5-hydroxybenzopyran-4-1 induced a potent anti-proliferative stimulus in two mammalian cell lines[11]. *Aspergillus niger* IFB-E003, an endophyte from *Cynodon dactylon*, was found to produce rubrofusarin B which is cytotoxic to the colon cancer cell line[12] SW1116. *Entrophospora infrequens* associated with *Nothapodytes foetida*, a medicinal plant native to the Western Ghats, India, was found to produce camptothecin[13]. In summary, many of these efforts have led to the discovery of endophytic fungi as a source of a number of important metabolites and have raised the prospect of using such organisms as alternative sources of these metabolites.

Notwithstanding these discoveries, however, to date there has been no known published breakthrough in exploiting the potential of the endophytic fungi as a source of important secondary metabolites. None of the identified endophytic fungal isolates has had any industrial application as yet. In 2002, Strobel who started it all said, 'efforts are underway by several pharmaceutical companies to determine the feasibility of making microbial taxol a commercial reality'[14]. Seven years later, we have no confirmation of taxol being commercially produced from its endophytic fungal source. In India, taxol continues to be extracted from the bark of the rapidly dwindling

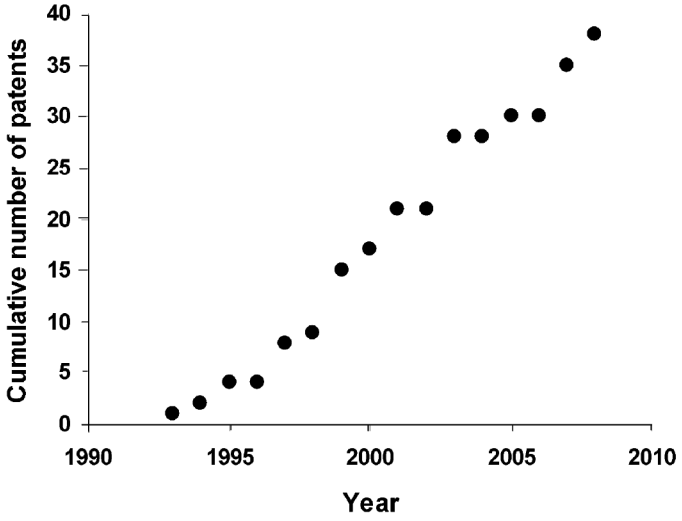

Figure 1. Cumulative number of US patents granted on endophytic fungi producing important metabolites and exhibiting biologically important activity (data compiled from www.patentsonline.com and www.patentstorm.us).

natural stocks of *Taxus* from the Himalayan belt region. The picture is the same for other compounds produced by endophytic fungi. For example, in yet another discovery, Strobel's team[15] claimed that an endophytic fungus, *Muscudor albus*, producing volatile organic compounds (VOCs) could have significant commercial use in numerous agricultural applications[15]. However, these are yet to be demonstrated as commercial successes.

We believe that the failure of exploiting the endophytic fungi rests on our current poor understanding of the evolutionary significance of these organisms and their dynamic interaction with their respective hosts (for example, Bailey *et al.*[16]). The mechanism or process underlying the production of the plant secondary metabolites by the endophytic fungi remains enigmatic. Even today it is believed and not proven that the reason why some endophytes produce certain phytochemicals, originally characteristic of the host, might be due to a genetic recombination of the endophyte with the host in evolutionary time[17]. In fact, there is less evidence for the horizontal transfer of genes coding for secondary metabolites between plant and fungi[18]. Furthermore, very little is known about the biochemistry and physiology of the interactions of an endophyte with its host plant. It would seem that many factors changing in the host as related to the season, age, environment and location may influence the biology and metabolism of the endophytes (for example, Moricca and Ragazzi[19]). It is pertinent to mention that secondary metabolite production in many fungi is regulated by environmental factors[20]. Thus not surprisingly, though a number of studies have shown the production of a specific metabolite by endophytic fungi in culture independent of the host, most often the production of biomolecules by a nascent endophyte isolate is severely attenuated through subculturing[21]. Consequently, most of the endophytic fungi do not lend themselves for up-scaling the production through fermentation engineering approaches. Although the reasons for such attenuation are not extensively studied, it is conjectured that it could be due to a lack of host stimulus in the culture media; continuous and some critical signaling from the host may be required by the endophytic fungi to sustain the production of such metabolites[22]. Recent gene sequencing exercise has shown that the secondary metabolism of fungi in general has been little understood and that gene clusters of many secondary metabolites in fungi are not expressed in culture[23]. Though a few studies have looked at the possibility of horizontal gene transfers between the host and endophytic fungus[24], it is far from clear how the different regulations are imposed. In the few elicitation studies conducted so far, there is very meagre success, and none of these has lead to the recovery of economically feasible levels of yield of bioactive compounds.

Despite the highly heralded report of endophytic fungi producing taxol in 1993 and the scores of studies that followed it, little progress has been made in harnessing the metabolic potential of endophytic fungi. Clearly, as mentioned above, more research is required to understand the biology of these fungi and their intricate relationship with their hosts to unravel their metabolic pathways and thereby to realise their potential utility.

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V. Priti, Shweta Singh and K. N. Ganeshaiah are in School of Ecology and Conservation, University of Agricultural Sciences, GKVK, Bangalore 560 065, India; K. N. Ganeshaiah is also in Department of Forestry and Environmental Sciences, GKVK, Bangalore 560 065, India; B. T. Ramesha and R. Uma Shaanker are in the Department of Crop Physiology and School of Ecology and Conservation, University of Agricultural Sciences, GKVK, Bangalore 560 065, India; G. Ravikanth is associated with Ashoka Trust for Research in Ecology and the Environment, Hebbal, Bangalore 560 024, India; T. S. Suryanarayanan is in the Vivekananda Institute of Tropical Mycology, RKM Vidyapith, Chennai 600 004, India.*

**e-mail: umashaanker@gmail.com*