

Endophytes to the rescue of plants!

Bhavdish N. Johri

It is now apparent that majority of healthy and symptomless plants harbour bacteria and fungi endophytically in various tissues, seed, root, stem and leaf. The relative preponderance of fungi as endophytes has completely changed the number game by way of the microbial diversity status since such endophytes have now been predicted to reach 1 million! For some time now, there were doubts that endophytic fungi might represent the existing fungal species described from other habitats. In a detailed investigation of white pine alone, new endophytic fungal forms have been observed to be genetically distinct from the existing diversity of such fungi.

Why do plants harbour such a load of bacteria and fungi internally? Would it not be an undue stress on plant metabolism? Are there favours extended by the endophyte to the parent plant? These and other questions are slowly being answered.

Major investigations of fungal endophytes in particular have taken root from the reports of grasses including 'tall fescue' (*Festuca arundinacea*), which were introduced in North America from Europe and were associated with systemic, seed-associated endophyte, *Neotyphodium epophialum*. This fungal endophyte colonizes the above-ground plant parts intercellularly, without any external manifestation of its presence in the plant. In fact, endophyte-carrying plants grow more vigorously and are toxic to herbivores. Furthermore, such plants are more drought-tolerant than non-infected plants. Clay and Holah¹ have carried out a detailed analysis of this endophyte on plant diversity in successional fields and observed that in a four-year period, plant species richness declined whereas tall fescue dominance increased in infected plots, even though there was no distinct difference in total plant productivity. What is of concern here is the fact that an endophyte with near negligible biomass relative to plant, possessed the capacity to alter plant community structure, and this process could have been in operation throughout its expanding range.

Unlike the tall fescue fungal endophyte that moves through plants via vertical transmission, several plant species including woody trees harbour such endo-

phytes through horizontal transmission and can help plants sustain thermal resistance and attack by phytopathogens^{2,3}.

In an interesting study of mutualistic endophyte and associated plant adaptation, Redman *et al.*² collected 200 plants of *Dichanthelium lanuginosum* from geothermal sites in Lassen Volcanic National Park (LVNP) and Yellowstone National Park (YNP), where average annual temperature varied between 20 and 50°C. A fungal endophyte, characterized by rDNA sequencing as *Curvularia* sp., was consistently recovered from the roots, crowns, leaves and seed-coats of all plants examined. Since soils in the study region were devoid of a *Curvularia* sp., this indicated horizontal entry of the fungus in the host plant. The fungus did not grow axenically and thus appeared exclusively mutualistic with *D. lanuginosum*. Artificially inoculated plants with spores of this *Curvularia* sp. could resist constant soil temperatures of 50°C for 3 days and intermittent temperatures as high as 65°C for 10 days! The endophyte could be recovered from surface-sterilized roots and leaves, suggesting protection from thermal stress to both plants and the fungus. Reassessment of this property in field at YNP showed that fungus-infected plants performed better by way of root and leaf mass compared to non-infected plants ≤ 40°C; infected plants survived higher temperatures, whereas non-infected plants collapsed. The beneficial influence of this *Curvularia* sp. was much more discernible at higher temperatures and the mutualistic fungus could be reisolated at 45°C, the field soil temperature. Authors of this report have suggested operation of several mechanisms of thermotolerance, including formation of cell-wall melanin pigment in the endophyte that can dissipate heat along the hyphae and/or complex with oxygen radicals generated during heat stress. Alternately, they have suggested that the presence of a mutualistic endophyte can act as a 'biological trigger' to activate the stress response system more rapidly and strongly than non-symbiotic plants.

Fungal endophytes have been recovered from all plant species examined until now and thus they represent a ubiquitous component of the terrestrial plant com-

munity. The above reports show that horizontally transmitted endophytes develop into as close a partnership as that of the vertically transmitted partners, such as cool grasses of the temperate region. In a study of fungal endophytes of *Theobroma cacao*, Arnold *et al.*³ observed that leaves carrying fungal endophyte decreased both leaf necrosis and leaf mortality when challenged with the phytopathogen *Phytophthora* sp., but the protection was localized. Mature leaves showed greater endophyte-mediated protection than the young leaves. This report opens up immense new possibilities not only in the domain of natural biological control but also in breeding for resistance, once the molecular mechanisms of such endophyte-mediated protection strategies are known.

Several new developments in the field of fungal endophytes, particularly woody plants have appeared on the scene. However, estimation of their diversity has not been simple, since many of these endophytic forms match the diversity known from the non-endophytic fungi. This raises a pertinent question: Are 'endophytes' largely known parasites that may have colonized the host in a 'cryptic' or 'latent' manner? To answer this question, Ganley *et al.*⁴ investigated the community of unknown endophytic fungi in *Pinus monticola*, the Western white pine, employing a sequence-based approach of identification and characterization. According to them, approximately 90% of the 2019 cultures belonged to a single family Rhytismataceae. Further analysis showed that none of the endophytes matched the sequences of three known rhytismataceous parasites, *Bifusella linearis*, *Lophodermella arcuata* and *Meloderma desmazieresii*. It has, therefore, been argued that if most fungal endophytes represent unknown taxa, then estimates of 1 million endophytes are only reasonable. What has also come to light is the presence of considerable genetic variability in endophytic fungi such as *Guignardia citricarpa*⁵.

In India, various facets of endophytic fungal diversity and biology are being investigated by Suryanarayanan and his group⁶⁻⁸. Currently, attempts are also underway at characterization of their metabolites in India, an area of considerable

biotechnological interest ever since Gary Strobel and his team described a functionalized diterpenoid and the famed anticancer agent paclitaxel, from species of *Taxus* across the globe⁹⁻¹¹. A novel fungus *Taxomyces andreanae* was recovered from *Taxus brevifolia*, which also produced paclitaxel. With these beginnings, endophytic, bacterial and fungal diversity has been extensively screened for antibiotics, antivirals and anticancer agents, as antioxidants, anti-insecticidal activity, and antidiabetic and anti-immunosuppressive compounds, etc.^{12,13}.

While antimicrobial and anticancerous searches continue with renewed vigour, novel technologies based on endophytic bacteria have recently emerged on the scene. Barc *et al.*¹⁴ have utilized genetically engineered endophytic bacterium, *Burkholderia cepacia* for phytoremediation of water-soluble and volatile organic pollutants such as toluene. This was achieved through the introduction pTOM plasmid of *B. cepacia* G4 into the natural bacterial endophyte of the yellow lupine (*B. cepacia* L. S. 2.4), and introduction of the modified strain (*B. cepacia* VM 1330) into surface-sterilized seeds. Besides considerable degradation of toluene which resulted in marked reduction of phyto-

toxicity, there was 50–70% reduction in transpiration through the leaves. Endophytic colonization strategy had earlier been attempted with the biocontrol bacterium *Bacillus subtilis* BB for vegetable brassicas following seed inoculation¹⁵.

Endophytic habitat appears to provide a protective environment that helps a potentially exploitable bacterium with reduced competition from the indigenous microbial populations. In view of their widespread application in plant and human health and environment, concerted efforts at endophytic diversity searches coupled with exploitation are necessary in the country on account of the varied and rich plant diversity.

1. Clay, K. and Holah, J., *Science*, 1999, **285**, 1742–1744.
2. Redman, R. S., Sheehan, K. B., Stout, R. G., Rodriguez, R. J. and Henson, J. M., *Science*, 2002, **298**, 1581.
3. Arnold, A. E., Meffa, L. C., Kyollo, D., Rojas, E. I., Maynard, Z., Robbins, N. and Herre, E. A., *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 15649–15654.
4. Ganley, R. J., Brunfeld, S. J. and Newcombe, G., *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 10107–10112.
5. Glienke-Blanco, C., Aguilar-Vildoso, C. I., Vieira, M. L. C., Barroso, P. A. V. and

- Azevedo, J. L., *Genet. Mol. Biol.*, 2002., **25**, 251–255.
6. Suryanarayanan, T. S. and Kumaresan, V., *Mycol. Res.*, 2000, **104**, 1465–1467.
7. Suryanarayanan, T. S., Ravishankar, J. P., Venkatesan, G. and Murali, T. S., *Mycol. Res.*, 2004, **108**, 1–5.
8. Girivasan, K. P. and Suryanarayanan, T. S., *Czech. Mycol.*, 2004, **56**, 33–43.
9. Strobel, G. A. and Long, D., *ASM News*, 1998, **64**, 263–268.
10. Strobel, G. A., Ford, E., Li, J. Y., Sears, J., Sidhu, R. S. and Hess, W. M., *Syst. Appl. Microbiol.*, 1999, **22**, 426–433.
11. Shreshtha, K., Strobel, G. A., Prakash, S. and Gewali, M., *Planta Med.*, 2001, **67**, 374–376.
12. Strobel, G. A., *Crit. Rev. Biotechnol.*, 2002, **22**, 315–333.
13. Strobel, G. and Daisy, B., *Microbiol. Mol. Biol. Rev.*, 2003, **67**, 491–502.
14. Barc, T. *et al.*, *Nature Biotechnol.*, 2004, **22**, 583–588.
15. Wuelff, E. G., van Vuurdes, J. W. L. and Hockenhull, J., *Plant Soil*, 2003, **255**, 463–474.

*Bhavdish N. Johri is in the Department of Biotechnology and Bioinformatics Centre, Barkatullah University, Bhopal 462 026, India.
e-mail: bhavdishnjohri@rediffmail.com*

Indo-US nuclear agreement: expectations and concerns

M. R. Srinivasan

The Indo-US Agreement of 18 July 2005 and the subsequent agreement of 2 March 2006, following the visit of President Bush to India, have been discussed extensively in the Indian and US media for the past ten months. At present, the US Congress is holding hearings on the proposed legislation to enable the US to enter into civil nuclear energy cooperation with India and to allow the US administration to approach the nuclear suppliers' group to adjust its policies to make an exception in the case of India. We shall certainly be hearing of both support and opposition from various Senators and Congressmen. As of now it is not clear if the US Congress will accord its approval before the June or July deadline. There will be a recess thereafter and on reconvening, the US Congress is expected to be busy with new elections. There is also a possibility that the US Congress, even if it were to

approve the legislation, may include some additional conditions that India would have to accept. The Indian Government's position is that it stands by the agreements of 18 July 2005 and 2 March 2006 and that no new conditions would be acceptable to the Government.

To understand the whole gamut of issues involved, it is important to recall the background to the 18 July 2005 agreement. This agreement itself sought to redress the anomalous situation India enjoyed in the global non-proliferation regime. India refused to join the nuclear non-proliferation treaty (NPT), which came into being in 1968, at the initiative of USA, USSR and the UK. The NPT defined a cut-off date of 1 January 1967 and recognized those countries which had carried out a nuclear test prior to that date as nuclear weapon states (NWS), and all other countries as non-nuclear weapon states

(NNWS). Initially, the NPT was conceived to deny the countries that launched the second world war, namely Germany, Japan and Italy permanently of the ability to make nuclear weapons. As it turned out, the NPT legitimized USA, USSR, the UK, France and China as NWS (incidentally, the same five states are also the five permanent members of the United Nations Security Council with veto powers) and required other states to give up their rights to acquire nuclear weapons for all times.

India termed the NPT discriminatory and refused to join it from the very beginning. Pakistan, although receiving substantial military and economic assistance from USA, also refused to join the NPT, on the ground that India had chosen to keep itself out of it. Israel also kept itself out of the NPT and managed to build up a nuclear weapon capability during