GENOMICS

Fungal symbiosis unearthed

Dan Cullen

Associations between plant roots and fungi are a feature of many terrestrial ecosystems. The genome sequence of a prominent fungal partner opens new avenues for studying such mycorrhizal interactions.

Plants and fungi often form marriages of convenience. In one form of this symbiotic relationship — an ectomycorrhizal association — long, branching fungal filaments known as hyphae ramify between cells of the root's outer layers, form a sheath around the root, and radiate outwards into the surrounding soil and litter. This transport network then allows the fungus to derive photosynthetically produced sugars from the host and in turn to transfer nitrogen and phosphorus to the plant. The ectomycorrhizal fungus *Laccaria bicolor* has been widely studied, in part because it is easy to grow in culture and establishes mycorrhizal associations with tree roots in laboratory experiments (Fig. 1). In a giant step forward, an international team of investigators report the genome analysis of this fungus. The analysis, published by Martin *et al.* on page 88 of this issue[1], reveals a mix of the intriguing and the unexpected.

At 65 million base pairs, the genome of *L. bicolor* is bigger than that of previously published fungal genomes. The size may be partly explained by the large number of mobile DNA sequences, known as transposable elements, that constitute more than 20% of the genome. Using a combination of gene-prediction tools, the authors[1] identify 20,614 protein-encoding genes. Of these, about 70% (14,464) show significant similarity to sequences in protein databases, particularly those from other homobasidiomycetes, the major fungal taxon to which *L. bicolor* belongs. Compared with other fungal genomes, the *L. bicolor* genome contains both more and larger gene families. Most of them have clear orthologues in other fungi — that is, they are genes that evolved from a common ancestor. Others, however, are unique to *L. bicolor*. Perhaps reflecting the complex exchange of nutrients between *L. bicolor* and its hosts, there is an especially large number of predicted membrane-bound transporter proteins.

An expectation of fungi that colonize forest litter is that they should secrete enzymes that break down cellulose and perhaps also lignin, two of the main components of plant cell walls. A voluminous literature and recent genome analysis of cellulolytic organisms[2–4] and aggressive plant pathogens[5,6] affirm a common strategy for efficient cellulose degradation.

YDgespc bcjcrcb dmp amnwpgefr am k njg_lac[

Figure 1 | *Laccaria bicolor* in action. This sequence of micrographs shows the colonization of poplar (*Populus* sp.) roots by this fungus. The images, from left to right, were taken 3, 10 and 28 days after initial fungus–root contact. By day 28, hyphal cells (green) form a dense external sheath and penetrate between host cells. (Photos are ×120 and are courtesy of J. Richter and V. Legué, INRA-Nancy.)

Minimally, this strategy involves a synergistic attack by exocellobiohydrolases and endoglucanases, and the activities of these extracellular enzymes are often enhanced by their having cellulose-binding structural domains. Unexpectedly, given the capacity of *L. bicolor* to persist in litter, the genome of this fungus reveals only a single gene encoding an endoglucanase with a cellulose-binding domain, and no genes for exocellobiohydrolases. There is also little evidence of the oxidative systems necessary for lignin degradation; genes encoding lignin-depolymerizing peroxidases, which generally occur as multigene families in efficient lignin-degrading fungi, are absent.

Thus, *L. bicolor* seems to be poorly adapted for efficient degradation of carbon-rich lignocellulose, which may reflect a reliance on host-supplied carbon. In contrast, the high number of genes encoding various glycoside hydrolases and proteinases that are predicted by the authors may indicate a capacity to use alternative nutrient sources (such as insects, bacteria and decomposed organic matter) and to transfer nitrogen and phosphorus to the host. In this connection, interactions within the broad microbial community, including bacteria present inside the fungal cells[7], may have an important role in mobilizing soil nutrients.

A paucity of predicted enzymes that degrade plant cell walls has also been observed in the genome of *Ustilago maydis*[8], a distantly related basidiomycete that is the causal agent of corn smut. The hyphae of this amazing pathogen proliferate within the plant, but rather than causing rapid cell death and necrosis, the fungus induces spectacular, spore-filled tumours. Kämper *et al.*[8] presciently suggested parallels between such pathogens and "plant-growth-promoting mycorrhizal fungi"; the absence of conventional cellulases in *L. bicolor* and *U. maydis* supports this view.

Another intriguing similarity with *U. maydis* is the impressive number of sequences predicted to encode secreted proteins of fewer than 300 amino acids in length. In *U. maydis*, the genes for these 'secreted small proteins' are extensively clustered and the proteins are implicated in pathogenesis. Gene clustering is not as pronounced in *L. bicolor*, but it is tempting to speculate that in *L. bicolor* some of these proteins are involved in establishing and maintaining symbiotic interactions. Consistent with this possibility, the whole-genome expression microarrays carried out by Martin *et al.*[1] show that several genes encoding secreted small proteins are expressed during symbiosis. Another analytical technique used by the authors was immunofluorescence microscopy, through which one such secreted small protein was localized to hyphae within colonized roots, but was not evident in the free-living fungus.

With the genome of *L. bicolor* in hand, we should see rapid progress in elucidating the molecular processes involved in symbiotic interactions[9]. In addition to the expression microarrays and immunofluorescence studies described by Martin *et al.*[1], direct genetic manipulation of *L. bicolor* may be within reach[10]. Further, the ability of *L. bicolor* to form mycorrhizae with *Populus trichocarpa*, which is the only tree whose genome has been sequenced to date[11], offers unique opportunities for comprehensive investigations of a complete ectomycorrhizal system. ■

Dan Cullen is at the USDA Forest Products Laboratory, One Gifford Pinchot Drive, Madison, Wisconsin 53726, USA.
e-mail: dcullen@facstaff.wisc.edu

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