Genomics of biological control – whole genome sequencing of two mycoparasitic *Trichoderma* spp.

Prasun K. Mukherjee

The filamentous ascomycetous fungi *Trichoderma* spp. are agriculturally and industrially important, being the major source of many commercial enzymes and as biofungicides. More than 60% of all registered biofungicides used for plant disease control are *Trichoderma*-based[1]. Mycoparasitism, wherein one fungus directly kills and obtain nutrients from other fungi is considered to be one of the most important mechanisms of biocontrol. Others being competition for nutrients, antibiosis and induced resistance in plants against invading pathogens. In addition to use as biocontrol, *Trichoderma* spp. are also used as biofertilizers (plant growth promoters) and for the mitigation of abiotic and physiologic stresses[2]. Earlier, an industrial strain of *Trichoderma reesei* was sequenced and analysed[3]. However, this species is not used as biocontrol, being very weakly mycoparasitic. Recently, the whole genomes of two very strong mycoparasitic species of *Trichoderma*, viz. *T. virens* (Gv29-8) and *T. atroviride* (IMI 206040) (teleomorphs *Hypocrea virens* and *H. atroviridis* respectively) have been sequenced (http://genome.jgi-psf.org/TriviGv29_8_2/TriviGv29_8_2.home.html and http://genome.jgi-psf.org/Triat2/Triat2.home.html, respectively). A comparative analysis of these two genome sequences performed by 64 scientists from 22 institutes spread over 14 countries (our laboratory at the Bhabha Atomic Research Centre, Mumbai, being the sole participant from India), with that of weakly mycoparasitic *T. reesei* has provided interesting insights into the genetics of mycoparasitism and biocontrol[4]. The article is freely available online (http://genomebiology.com/2011/12/4/R40).

The whole genome sequencing, performed by shotgun approach, revealed that the genome size of the two mycoparasitic species is greater than that of *T. reesei* (38.8 Mb, 36.1 Mb for *T. virens* and *T. atroviride* respectively, compared to 34 Mb for *T. reesei*). Total gene models predicted also being greater in *T. virens* and *T. atroviride* (12,428, 11,865, against 9143 for *T. reesei*). Approximately one third of the genes are unique to *Trichoderma* which are not shared even in a close relative *Gibberella zeae*. Interestingly, unlike most other filamentous fungi, *Trichoderma* genomes lack a significant repetitive DNA component in the genome and in most cases, the transposable elements were fragmented pointing to the existence of repeat induced point (RIP) mutations. Indeed, *Trichoderma* genome contains all the genes required for RIP in the model fungus *Neurospora crassa*. A comparative analysis with other ascomycetes genome sequences available identified gene families that have expanded in three *Trichoderma* spp. or only in the two mycoparasitic species. Of the 46 gene families that expanded in *Trichoderma* spp., 26 were expanded only in *T. atroviride* and *T. virens*. In a multigenic phylogeny analysis, it was found that *T. atroviride* is the common ancestor for both *T. virens* and *T. reesei* – whereas *T. virens* retained and even enriched the mycoparasitic arsenals; *T. reesei* lost most of this trait during evolution.

Mycoparasitism involves sensing/recognition of the host fungus, attachment and/or coiling around, and lysis brought about by hydrolytic enzymes like chitinases and glucanases (Figure 1). As expected, *Trichoderma* genomes abound in genes for chitinases and glucanases, relative to other ascomycetous fungi. The role of the chitinases in mycoparasitism and biocontrol is well documented in the literature. The chitinolytic enzymes are also strongly expanded in *Trichoderma*, particularly in *T. virens* and *T. atroviride*, which contain the highest number of genes for chitinolytic enzymes of all the described fungi. Secondary metabolites, some of which are antifungal, are known to be involved in mycoparasitism and biocontrol. The two mycoparasitic strains of *Trichoderma* have expanded secondary metabolism arsenal, harbouring more genes than *T. reesei*. For example, *T. virens* genome contains 28 non-ribosomal peptide synthetases (including the gene for strongly antifungal compound gliotoxin), compared to 16 for *T. atroviride* and 10 for *T. reesei*. Many of the secondary metabolism gene clusters are unique to specific *Trichoderma* species whereas some are common in all the three species. Interestingly, two mycoparasitic species also have genes for aegerolysins and the insecticidal Tc toxins, which are not present in the *T. reesei* genome.

The comparative genome analysis thus opens up enormous potential for understanding the fundamental mechanisms of biocontrol, which in turn would help in selecting/generating better strains with improved biocontrol potential.

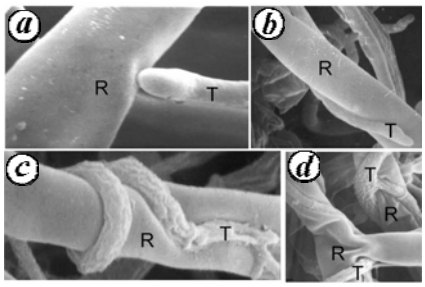

Figure 1. Mycoparasitism of *Trichoderma virens* (T) on *Rhizoctonia solani* (R). ***a***, Attraction; ***b***, Attachment; ***c***, Coiling; ***d***, Lysis.

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Prasun K. Mukherjee is in the Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India; Present address: Central Institute for Cotton Research, PO Box 2, Shankar Nagar (PO), Nagpur 440 010, India.
e-mail: prasunmukherjee1@gmail.com