The ISME Journal (2010) 4, 1081–1098

www.nature.com/ismej

COMMENTARIES

'Designer' mycorrhizas?: Using natural genetic variation in AM fungi to increase plant growth

Ian R Sanders

The ISME Journal (2010) **4,** 1081–1083; doi:10.1038/ismej.2010.109; published online 8 July 2010

The arbuscular mycorrhizal symbiosis formed between arbuscular mycorrhizal fungi (AMF; Glomeromycota) and plant roots is probably the most abundant symbiosis in the world. This symbiosis is formed by the majority of plant species and contributes to improving plant growth and promotes plant diversity (Smith and Read, 2008). Mycorrhizal fungi increase plant growth by improving acquisition of phosphate, an essential nutrient for plant growth. With the rapidly expanding human population, world phosphate reserves are at a critical level, and improved strategies of phosphate use and cycling in agro-ecosytems are essential (Gilbert, 2009; Gross, 2010). Furthermore, producing superphosphate fertilizer is energy intensive and expensive. Clearly, AMF have the potential to improve the sustainable use of phosphate in agro-ecosystems, especially in tropical acidic soils. The fungi can be applied to the soil in the form of spores. The spores are normally applied with a substrate carrier, most often soil, and the fungus with the carrier is known as inoculum. However, for several reasons they have had limited use in agriculture. First, most crops are naturally colonized by AMF in the soil and so adding more AMF may not seem necessary. Second, a given AMF may improve the growth of some plant species but not others. So, there is unlikely to be any universal inoculum that is effective for all crops. Third, and economically the most significant, is that most AMF species have to be grown in unsterile conditions with plants. It is labour intensive, expensive and does not guarantee that the inoculum is free of other soil microorganisms that could be potential pathogens. However, by coupling recent advances in understanding the ecology, natural genetic diversity and genetics of AMF with technological advances in inoculum production, it may be possible in the future to create 'designer' mycorrhiza; using inoculum that have been manipulated for a desired growth effect with a given crop and that can be produced in an economically viable way.

For years, researchers working on the mycorrhizal symbiosis have been able to cultivate AMF *in vitro* in sterile growth conditions on artificial media with plant roots transformed with *Agrobacterium rhizogenes* (Declerck *et al.*, 2005). This culture system could potentially provide the solution to producing clean inoculum in an economically viable way. The problem is that fungal growth in that culture system is generally quite slow and researchers have had difficulty getting many different AMF species into, or to grow well, in that culture system. One of the few AMF species that seems well adapted to growing in that system is known as *Glomus intraradices*. It is widely found in agricultural soils. So the question is whether the genetic variation within an AMF species, which is cultivable *in vitro*, is large and can lead to large variation in how the fungi affect plant growth? Furthermore, is it possible to manipulate this genetic variation within the fungus in an environmentally acceptable way to develop new AMF lines that have improved effects on plant growth with a given crop plant. All these features should be interesting for commercial AMF inoculum producers.

Studies have revealed surprisingly high levels of within-species genetic variability in the AMF *G. intraradices*. A recent study of *G. intradices* isolates available worldwide demonstrated very high diversity in rDNA sequences (Stockinger *et al.*, 2009). In that study, two distinct clades were identified, but even within each clade extremely high levels of sequence variation were observed. Amplified fragment length polymorphism and sequence-based markers revealed a very large number of polymorphisms among *G. intraradices* isolates all originating from one small field in Switzerland (Koch *et al.*, 2004). To obtain very clean DNA, those studies were restricted to a collection of isolates that had been put into the sterile *in vitro* culture system with transformed carrot roots. Importantly, within that collection, genetic differences were shown to translate into different fungal phenotypes and differential effects on plant growth (Koch *et al.*, 2006; Croll *et al.*, 2008). Thus, natural genetic variation within an AMF species is indeed interesting for development of improved inoculum, both in terms of the growth rate of the fungus in culture and because of its effects on plant growth.

However, an exciting new study reveals even higher levels of genetic diversity within field

populations of *G. intraradices* (Börstler *et al*., 2010). Börstler *et al*. revisited the same Swiss field. Instead of restricting themselves to *G. intraradices* from the *in vitro* cultivated collection, they looked at the diversity of mitochondrial large-subunit rDNA haplotypes of *G. intraradices* colonizing roots. Their study predicts much higher levels of diversity in *G. intraradices* populations than previously thought. Furthermore, the study also included another agricultural field and two grassland sites. This revealed that genetic variability in *G. intraradices* was also very high among sites, with higher diversity in the agricultural fields than the grasslands. This new level of genetic diversity within one AMF species, which can be cultured in a clean *in vitro* system, is exciting news for the potential of this fungus for inoculum development. It seems that there is, indeed, a very high level of diversity in this fungus that could be used to select for improved inoculum growth and for its symbiotic effects on plants.

So, how could this genetic variability be used to develop effective new inoculum with a specific growth effect on a given crop? One way is to manipulate the genetics of AMF. Until recently, it was assumed that AMF are completely clonal and that no genetic exchange takes place between genetically different AMF. However, recently genetic exchange has been shown to take place between genetically different *G. intraradices* and that this gives rise to 'hybrid' progeny with phenotypes that are sometimes novel compared with those of the two parents (Croll *et al*., 2009). AMF are able to harbour genetically different nuclei within a common cytoplasm (Hijri and Sanders, 2005). In a recent study, Angelard *et al.* (2010) took lines of *G. intraradices* that actually gave a negative growth effect on rice and manipulated their genetics. These manipulations used naturally existing biological processes in the fungus; namely, genetic exchange and segregation rather than any laboratory-engineered gene insertion. They produced genetically novel *G. intraradices* lines that could induce up to fivefold growth increases in rice. The manipulations involved taking pairs of genetically distant *G. intraradices* isolates, allowing them to fuse to produce crossed lines. The crossed lines did not improve rice growth. However, by making single spore lines from crossed lines, genetically different nuclei of the fungus were partitioned in newly forming spores in different proportions; a type of partial segregation. Thus, siblings from one AMF line are genetically different from the parental line and from each other. The genetically different fungal lines induced strong differences in rice growth. Interestingly, some of the segregated AMF lines that did not alter rice growth gave different growth effects on another plant, *Plantago lanceolata*, showing that genetic changes in the fungus can have specific effects on different plant species. Rice is a globally important food crop. The ability to take an AMF that is not beneficial and produce an AMF line that is highly beneficial on such a plant using 'environmentally acceptable' manipulations of the genetics of the fungus is exciting. Perhaps more exciting is that this was carried out with *G. intraradices*, which, as we now know, is a genetically very diverse AMF. Furthermore, because segregated lines have specific effects on crops, this means that inoculum producers may now be in a position to manipulate natural genetic variation in AMF to make 'designer' mycorrhizas, with fungi selected for their symbiotic effects on a particular crop.

Although geneticists have been making advances in understanding genetic variability in AMF and how AMF genetics contributes to the symbiotic effects with plants, biotechnologists have made significant breakthroughs increasing the efficiency of AMF inoculum production in the *in vitro* system. Some companies are now able to produce AMF *in vitro* in very large quantities at relatively low cost. As the demand for phosphate fertilizer goes hand –in hand with the growth in the world's population, prices of this important resource will certainly rise. Initial field trials in Colombia with such mass *in vitro*-produced *G. intraradices* inoculum show promising results for maintaining food crop yields while greatly reducing phosphate inputs, making the cost of inoculum a minor factor in the production costs (A Rodriguez; personal communication). With the exciting possibly for geneticists and inoculum producers to now manipulate the fungus to produce effective inoculum for a given crop or soil type, the mass-produced 'designer' mycorrhiza appears to be a step closer and more of an economic reality.

IR Sanders is at Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland
E-mail: ian.sanders@unil.ch

References

Angelard C, Colard A, Niculita-Hirzel H, Croll D, Sanders IR. (2010). Segregation in a mycorrhizal fungus alters rice growth and symbiosis-specific gene transcription. *Curr Biol* **20**; doi: 10. 1016/ j.cub.2010.05.031.

Börstler B, Thiery O, Sykorova Z, Berner A, Redecker D. (2010). Diversity of mitochondrial large subunit rDNA haplotypes of *Glomus intraradices* in two agricultural field experiments and two semi-natural grasslands. *Mol Ecol* **19**: 1497–1511.

Croll D, Giovannetti M, Koch AM, Sbrana C, Ehinger M, Lammers PJ *et al.* (2009). Nonself vegetative fusion and genetic exchange in the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytol* **181**: 924–937.

Croll D, Wille L, Gamper HA, Mathimaran N, Lammers PJ, Corradi N *et al.* (2008). Genetic diversity and host

plant preferences revealed by simple sequence repeat and mitochondrial markers in a population of the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **178**: 672–687.
Declerck S, Strullu DG, Fortin JA. (2005). *In Vitro Culture of Mycorrhizas*. Springer: Heidelberg, Germany.
Gilbert N. (2009). The disappearing nutrient. *Nature* **461**: 716–718.
Gross G. (2010). Fears over phosphorus supplies. *Curr Biol* **20**: 386–387.
Hijri M, Sanders IR. (2005). Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. *Nature* **433**: 160–163.
Koch AM, Croll D, Sanders IR. (2006). Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. *Ecol Lett* **9**: 103–110.
Koch AM, Kuhn G, Fontanillas P, Fumagalli L, Goudet J, Sanders IR. (2004). High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. *Proc Natl Acad Sci USA* **101**: 2369–2374.
Smith SE, Read DJ. (2008). *The Mycorrhizal Symbiosis*. Academic Press: San Diego.
Stockinger H, Walker C, Schussler A. (2009). '*Glomus intraradices* DAOM197198', a model fungus in arbuscular mycorrhizal research, is not *Glomus intraradices*. *New Phytologist* **183**: 1176–1187.

Big sulfur bacteria

Bo Barker Jørgensen

The ISME Journal (2010) **4,** 1083–1084; doi:10.1038/ismej.2010.106; published online 15 July 2010

Cultivated bacteria generally have small cell sizes and are adapted to rapid growth and efficient substrate uptake of ambient solutes. Most bacteria in the environment, however, in particular in the sub-surface soil or seabed, live under strongly substrate-limited conditions. They also have small cell sizes but grow only very slowly, with mean generation times of many years. The lithotrophic and mostly autotrophic bacteria of the genera *Beggiatoa*, *Thioploca* and *Thiomargarita* have followed a very different path of prokaryotic evolution. These big sulfur bacteria show unique and fascinating specializations to a life in gradients between sulfide and oxygen or nitrate.

Beggiatoa are best known from visible mass occurrences on sheltered coastal sediments or decaying algal debris in which they aggregate as white films at the oxygen-sulfide interface. They also form distinct benthic mats around cold seeps and hydrothermal vents, which are discovered in large numbers through the increasing access to the deep sea by manned submersibles and remotely operated vehicles (Jørgensen and Boetius, 2007). The most widespread occurrence of *Beggiatoa*, however, is in the oxidized but anoxic zone of sediments in which neither their electron donor nor their electron acceptor seems to be available. The *Beggiatoa* are rarely detected here because they are too few to be discovered by normal cell counting techniques and their 16S ribosomal RNA (rRNA) genes have been difficult to amplify by PCR. Owing to their large individual cell size *Beggiatoa* may constitute a significant fraction of the entire prokaryotic biomass and yet belong to the rare biosphere in surface sediments. Techniques generally used for meiofauna studies are required to find and quantify the mm-long filaments of *Beggiatoa* (Jørgensen *et al.*, 2010).

So what is the unique adaptation of *Beggiatoa*, *Thioploca* and *Thiomargarita*, which makes large size of selective advantage? A majority of the big sulfur bacteria studied so far seem to have different variants of the same general specialization. They are storage tanks for a soluble electron acceptor, nitrate, and a solid electron donor, elemental sulfur. With sufficient storage capacity they are no longer dependent on the concurrent presence of their substrates or bound to their diffusion interfaces. The bacteria may occasionally visit these interfaces but can otherwise move around freely in between them. During anaerobic periods when they reduce internal nitrate to ammonium they seem to save energy and accumulate elemental sulfur, but when they reach oxygen they increase respiration rates and grow at the expense of internal sulfur oxidation.

The following example from Preisler *et al.* (2007) illustrates this mode of life. The common marine *Beggiatoa* of 25–30 μm diameter were found to have 100–300 mM nitrate in their vacuoles and 300–400 mM elemental sulfur in cytoplasmic invaginations, both concentrations calculated per total cell volume. With a measured metabolic rate consuming 13 mM nitrate and 15 mM S^0 per day they have energy reserves for almost a month. Even with their slow gliding speed of 2 μm per second (Dunker *et al.*,