

Towards synthetic nitrogen-fixing symbioses in grasses

Abstract

Too much nitrogen (N)-fertilizer is used in many agricultural systems, at great environmental cost, while too little is used in the poorest systems, jeopardising food security. As a step towards solving these contrasting N-related problems, we aim to build synthetic nitrogen-fixing symbioses between bacteria and grasses, based on knowledge gained from decades of research on natural nitrogen-fixing symbioses in legumes. Key steps in this synthetic biology project include engineering of: signal compound production in bacteria and signal recognition in plants; concomitant biosynthesis of a specialized C-source by the plant for use by the bacteria; catabolism of this specialized C-source for energy production, as well as nitrogen fixation, respiratory protection of nitrogenase, and conditional suppression of ammonia assimilation in bacteria; and, finally, ammonium uptake by plant cells. Chassis' for the bacterial synthetic biology are natural endophytes or epiphytes of grasses, while the target model and crop species are barley and maize. Significant progress has been made in each of these areas. Ultimately, substantial synthetic associative nitrogen-fixation in staple food crops could increase yields of resource-poor farmers and decrease the need for industrial N-fertilizers in resource-rich agricultural systems.

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Introduction

Levels of mineral and organic nitrogen (N) in most soils limit primary production in natural ecosystems and agriculture (Elser et al. 2007; O'Neill et al. 2004). Industrial production of N-fertilizers fuelled the Green Revolution and today inject over 100 million tons of reactive-N per year into agricultural systems (Canfield et al. 2010). Without N-fertilizers there would likely be 2 billion fewer people alive today, yet massive use of fertilizers over much of the globe is compromising human health and natural ecosystems, and challenging the sustainability of modern agriculture (Rockstrom et al. 2009; Sutton et al. 2011). In contrast, millions of resource-poor farmers lack sufficient N-fertilizer to ensure good harvests, especially in Africa where yields are often only 10-20% of yield potential for staples like maize.

To address the grand challenges related to N-use in agriculture through biological research, the BBSRC of the UK and the NSF of the USA hosted a nitrogen ideas lab in the UK to develop plausible alternatives to industrial N-fertilizers. Several projects were funded, including this one on Synthetic Symbioses, SynSym.

Strategy

Key steps in this synthetic biology project include engineering of: signal compound production in bacteria and signal recognition in plants; concomitant biosynthesis of a specialized C-source by the plant for use by the bacteria; catabolism of this specialized C-source for energy production, as well as nitrogen fixation, respiratory protection of nitrogenase, and conditional suppression of ammonia assimilation in bacteria; and, finally, ammonium uptake by plant cells. Chassis' for the bacterial synthetic biology are natural endophytes or epiphytes of grasses, while the target model and crop species are barley and maize.

Progress

Rhizobium sp. IRBG74 has been shown to promote growth of rice (Biswas et al. 2000) and was of interest to us as a potential growth-promoting root endophyte of other grasses and as a chassis for engineering nitrogen fixation and other genes. We found that this strain IRBG74 is able to colonize roots of some barley and maize genotypes. Work is underway to identify barley and maize genes that respond to microbial signals, including those from IRBG74, using RNA-seq(encing). Rhizopine was chosen as the target C-compound for biosynthesis in plants for use by associated N-fixing bacteria. After clarifying/correcting the biosynthetic

pathway to rhizopine in bacteria, progress has been made in expressing bacterial genes in plants to produce scyllo-inosamine. Natural metabolites of maize roots and root exudates have been characterized by gas chromatography and mass spectrometry (GC-MS), and include sugars and sugar alcohols, including myo-inositol a precursor to rhizopine. A novel rhizopine biosensor has been produced to detect and measure rhizopine release from plant roots. Rhizopine catabolism genes have been transferred successfully to IRBG74. Large gene-part libraries, including various inducible promoters, ribosome binding sites and terminators, have been constructed for expression of rhizopine catabolism, nitrogen fixation (*nif*) and other genes in chassis bacteria for this project. Natural *nif* gene clusters have been transferred to non-nitrogen-fixing chassis to achieve nitrogen fixation in these, and we are in the process of testing synthetic *nif* clusters to the same end. Progress has been made in understanding how bacteria protect nitrogenase from oxygen, for example through respiratory oxygen-consumption, as a prelude to achieving conditions suitable for nitrogen fixation in synthetic symbioses. Several strategies are currently being pursued to limit assimilation of ammonia during nitrogen fixation in synthetic bacteria, in order to make ammonium available to the plant for growth. Ammonium uptake and growth on low ammonium have been characterized in the model grass, *Setaria viridis*, as well as maize, and these species like others appear to be well-equipped to capture ammonium that would be released by ammonia-exporting endophytes.

Outlook

This project tests our current ideas about what it takes to achieve successful nitrogen fixing symbiosis. These ideas were shaped by work on legume-rhizobium systems. We aim to achieve proof-of-concept that non-legume plants and bacteria can be engineered to communicate and exchange reduced-C from the plant for reduced-N (ammonium) from nitrogen-fixing bacteria, within the space of 5 years. We expect that a similar amount of time will be required, in addition, to optimize C- and N-exchange to achieve agriculturally-significant N-supply to target crop species. If synthetic nitrogen-fixing systems can be engineered for grasses, especially major food species such as maize, it could be a game-changer for agriculture with respect to sustainable inputs of N, with benefits for resource-poor farmers as well as resource rich, environmentally-challenged agricultural systems. Ultimately, the challenge of gaining societal acceptance of a synthetic biology solution to the world's N-problems may be more difficult to achieve than synthetic nitrogen-fixing symbioses.

References

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