Beating rhizobial ineffectiveness

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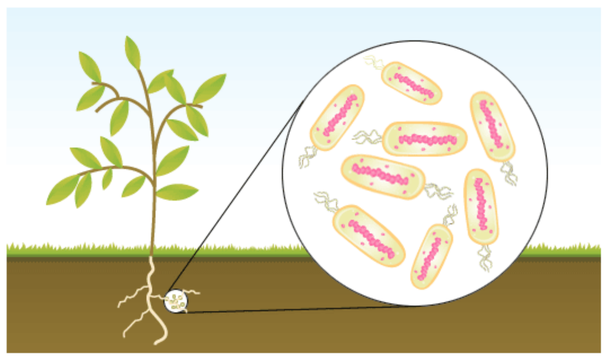
Background

Nitrogen is essential for all living organism on earth for synthesis of proteins, nucleic acid and other nitrogen-containing compounds. The atmosphere contains approximately 80% of nitrogen gas (N_2), which is still useless in that form for many organisms. Therefore N_2 needs to be fixed by bacteria to be useful.

Plants of the legume family form a symbiotic relationship with nitrogen fixing bacteria called rhizobia, which fix atmospheric nitrogen after becoming established inside root nodules of legumes. This symbiosis is globally of major importance in agricultural systems. Establishment of a rhizobial population inside a nodule usually results from a clonal infection by a single highly competitive strain from a complex population in soil.



Competition amongst rhizobial strains in the soil leads to the most competitive strains infecting the legume. Unfortunately, competitiveness and effectiveness in terms of nitrogen fixation are separate rhizobial traits that are not closely linked. Therefore, legumes tend to become nodulated by highly competitive yet ineffective rhizobial strains, causing poor plant growth. This is an acute problem in beans (*Phaseolus vulgaris)*.

As such it is a major problem for nutrition in countries where beans are an important high nitrogen crop such as parts of Africa, India and South America. Research to identify competitive highly efficient nitrogen-fixing strains has been limited because the only way to screen for rhizobial strains that are both competitive and effective has been to isolate individual strains and compare them one at a time in large-scale plant growth assays.

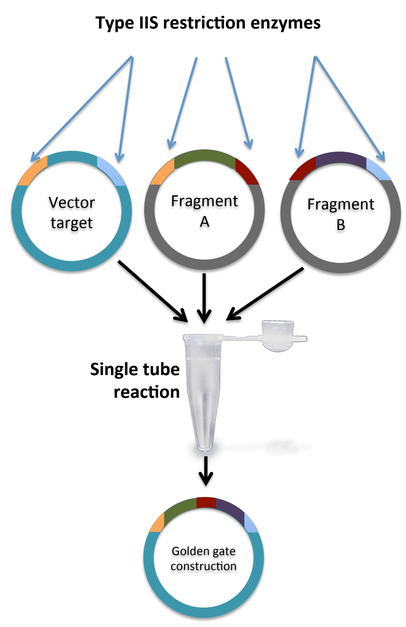
Aim

The principal aim of this project is to design a tool to assess strain competitiveness and effectiveness in an integrated high-throughput strategy. The novelty of this research is the use of the latest techniques in molecular biology as Golden Gate Assembly, bioreporters, and bar-coding of plasmids to enable rapid identification of successful strains, allowing us to compare multiple strains at once and thus screen efficiently for competitive and efficient strains.

Experimental Procedure

Golden Gate Assembly

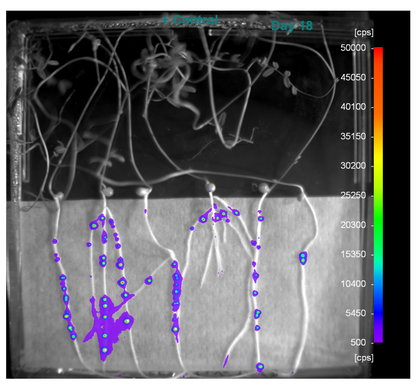
This method allows parallel assembly of multiple DNA fragments in a one-tube reaction.



Bioreporters

These are living microbial cells that have been genetically engineered to produce a measurable signal in response to a specific chemical or physical agent in their environment. To monitor the competitiveness between strains in this project we will use the use *lux*, *gusA* and *celB* marker genes.

A



B

A) *luxCDABE, B) gusA* and *celB* marker genes in *Rhizobium leguminosarum*

Bar-coding

We are developing high-density bar-coding of plasmids so that the rhizobial strains can be individually tagged. We will read the barcodes by sequencing allowing us to quantify infection in each plant.

Summary

The basic experimental procedure will be to assess marked strains against a large population of native soil rhizobia. These elite strains must be both competitive and highly effective at fixing nitrogen.

This work can contribute to obtain high yields of crops, to cover the minimum necessary of the global food demand practicing sustainable agriculture.

Acknowledgments

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