Continuous directed evolution of soybean (*Glycine max*) SBPase

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Protein engineering for crop applications can be limited by a lack of understanding of the sequence-structure-function relationship. Directed evolution can overcome this by combining gene-specific hypermutation and selection. Continuous directed evolution does this within the cell by linking survival to a desired protein activity, amplifying the search of sequence space for gene variants with improved or novel functions. It therefore has the potential to guide gene editing efforts in plant or crop enzymes where little is known about their function. However, this application is currently lacking. Here, a continuous directed evolution technique, OrthoRep, is applied to soybean (*Glycine max*) SBPase, a photosynthetic enzyme implicated in the rate limiting step of carbon assimilation under current and future atmospheric CO2 concentrations and whose activity has been shown to determine plant yield.

We establish and validate an SBPase activity dependent yeast (*Saccharomyces cerevisiae*) selection platform and introduce OrthoRep into it. Ninety-six independent evolution campaigns across a range of population sizes and replicates show that SBPase is readily and rapidly evolvable, reaching native yeast SBPase-like performance in a timescale of 50-70 generations in most populations.

A well-defined set of genotypes appearing multiple times in populations with evolved improved growth was identified, with the high degree of convergence and evolution speed suggesting a smooth fitness landscape. Assays are currently underway to validate growth-enhancing mutation properties, determine causality in multi-substitution mutants, and biochemically characterize the most promising mutants. Additionally, we are working on solving the first crystal structure of a higher plant SBPase to understand the structural and mechanistic basis of improved enzymatic performance. This work showcases the power of synthetic biology for improving crops for sustainability or bioproducts.