

OPTICAL CONTROL OF EXOPOLYSACCHARIDE PRODUCTION IN *SINORHIZOBIUM MELILOTI* FOR STUDYING BIOFILM FORMATION AND WATER RETENTION

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The rhizosphere contains many types of microbes interacting with plant roots, creating a complex symbiotic system. Microbial processes occurring in the rhizosphere are essential to the productivity of terrestrial ecosystems. In particular, exopolysaccharide produced by soil microbes allows dynamic regulation of soil moisture by modulating water transport. We have demonstrated that purified microbial exopolysaccharide (EPS) impacts soil water retention through enhancing the variability of water distributions in the soil microstructure. However, the impact of EPS on water transport in soil is not understood due to complex interaction of microbial EPS with soil microstructure and particle surface properties. To decipher the causal role of EPS in soil microstructures, we set out to develop engineered soil bacteria with spatially regulated EPS biosynthesis capabilities. Here we report genetic engineering of soil bacterium *Sinorhizobium meliloti* to enable *in situ* spatial control of EPS production. We show that the photo-sensitive transcription factor EL222, derived from *Erythrobacter litoralis*, allows robust control of gene expression in *S. meliloti*. Essential genes in the type II EPS (a major component of EPS from *S. meliloti* in the soil) production pathway were identified, and deletion strains were generated. Complementation of the essential gene using a synthetic promoter controlled by EL222 led to robust light-activated production of EPS. Optimization of the engineered genetic construct was performed by varying promoters, ribosome binding sites, and using alternative start codons. Using the engineered EPS production strain, we observed rapid settlement of EPS producing *S. meliloti* in liquid culture, and selective biofilm formation quantified by a crystal violet staining assay. This approach enables spatially regulated EPS production and biofilm formation. We will demonstrate control of gene expression in a synthetic soil microsystem that emulates aggregated sandy loam soil. We will also report our current progress on using these new strains of soil bacteria to study the impact of EPS production on water drying rate in the synthetic soil microsystem. We anticipate that the engineered genetic constructs will be broadly applicable for dissecting gene function in a defined population of microbes in the rhizosphere.