Title: IMAGINE BioSecurity: Biocontainment of Genetically Engineered Cyanobacteria.

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Project Goals: This SFA project task will develop active biocontainment modules that prevent growth of cyanobacteria in the natural environment should they escape the lab or industrial setting. We will also assess the efficacy of a second strategy for biocontainment in which genes responsible for synthesis of storage molecules are knocked out to reduce fitness in the natural environment where fluctuations in nutrient concentrations are common.

Abstract Text: Algae (including eukaryotic microalgae and cyanobacteria) have been genetically engineered to convert light and carbon dioxide to many industrially and commercially relevant chemicals including biofuels, materials, and nutritional products. At industrial scale, genetically engineered algae may be cultivated outdoors in open ponds or in closed photobioreactors. In either case, industry must address the potential risk that accidental release of the engineered algae into the natural environment may result in a variety of negative impacts to the environment. Genetic biocontainment strategies are therefore under development to reduce the probability that these engineered bacteria can survive outside of the laboratory or industrial setting. As part of the IMAGINE SFA project, we are testing strategies for biocontainment of strains of *Synechocystis* sp. PCC6803 that have been engineered to produce ethylene. We are combining two strategies: 1) knockout of storage molecule synthesis genes, and 2) active lethal genes induced by an environmental signal.

Knockout of storage molecule synthesis genes is expected to reduce fitness of engineered cyanobacteria if they escape to the natural environment where fluctuations in nutrient concentrations are common and elemental storage is important for surviving periods of low nutrient concentrations. We have initially targeted the genes responsible for synthesizing storage molecules of carbon (glycogen), phosphorous (polyphosphate), nitrogen (cyanophycin), and iron (bacterioferritin) as well as a gene essential for salt acclimation. In addition to these knockouts, we have designed active biocontainment modules which can sense that the cells are no longer in the lab setting and induce the expression of lethal genes. Initially, we are testing a biocontainment module in which expression of the RNase, *mazF*, is controlled by the promoter, PisiAB, which is induced by iron limitation. Biocontainment efficacy will be assessed in conditions that are representative of what the cells are subject to in the natural environment. These strains engineered for biocontainment will be assessed for fitness and bioproductivity, including ethylene production, as a function of biocontainment constraints. The resultant data will be integrated into genome-scale models to establish a predictive framework for optimal biodesigns.

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