An Electrochemical Model of Rhizosphere ROS Generation to Analyze Commensal Engineering Strategies

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Project goals: Our goal is to develop robust and generalizable cellular and population level containment mechanisms for soil microbes that are robust to environmental perturbation

A common source of plant dysbiosis caused by pathogenic signaling within the rhizosphere is overproduction of reactive oxygen species (ROS). This appears to be in part due to unregulated or pathogenic redox signaling between local microbial species and the root of the plant. One solution to this problem is to develop commensal microbial species that sense root ROS production and in turn alter the composition of the microbiome. In pursuit of this goal, we have adapted electrochemical methods to study the response of a community of bacteria to localized hydrogen peroxide stimulation as a model of root ROS production. Within these experiments, a standard gold electrode is used to synthesize hydrogen peroxide at the electrode surface through a 2-electron oxygen reduction reaction (ORR). The hydrogen peroxide is then rapidly degraded by bacterial mechanisms with distance away from the electrode. We found that within a simple electrochemical cell, only a marginal number of bacteria are stimulated by the hydrogen peroxide produced by the electrode due to spatial limitations. However, when using a "transmitter/receiver" coculture of *E. coli,* we found that the transmitter population, which produces a quorum sensing signal in response to hydrogen peroxide, could activate gene circuits within a large and homogenous population of receiver cells (see Figure 1). Using this system, we have demonstrated two methods that could be used to alter microbiome composition in response to ROS while simultaneously containing the engineered populations. First, we demonstrate how coculture signaling can be used to upregulate tyrosine synthesis, which can be used to selectively enrich auxotrophic populations. Second, we demonstrate the ability to cause the receiver population to increase its growth rate, thereby increasing its overall composition within the microbial community. Lastly, we show that these two methodologies can be combined to enrich the population of cells producing an auxotrophic nutrient. We believe these electrochemical methodologies can be useful for exploring strategies to alter rhizosphere microbial composition while also preventing the spread of engineered species.

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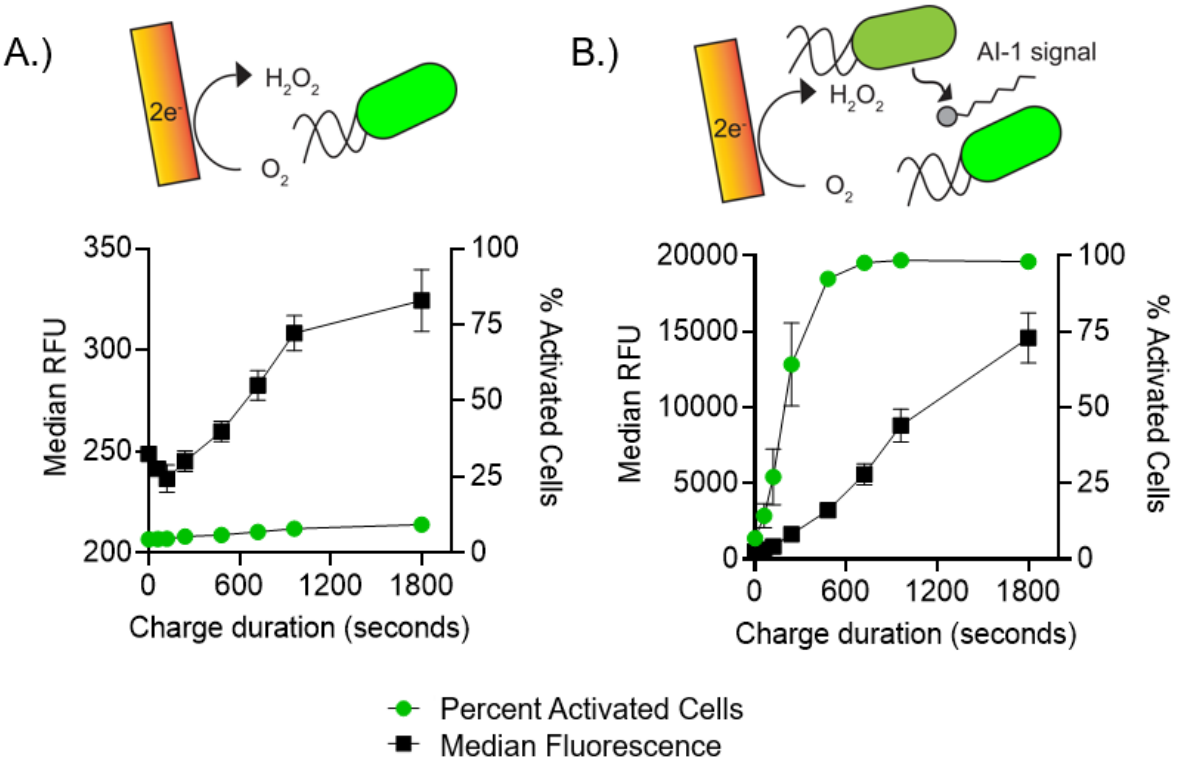

Figure 1: Comparison of Monoculture and Coculture Activation via Localized Hydrogen Peroxide Generation. In this model system we tested two separate models of root microbe activation from ROS: First, a monoculture model in which an engineered population expresses GFP in response to hydrogen peroxide (A); Second, a "transmitter/receiver" coculture model in which a small fraction of the culture is comprised of "transmitter" cells which produce a quorum sensing autoinducer-1 (AI-1) signal in response to hydrogen peroxide. The AI-1 signal in turn activates GFP expression in the second population, termed "receiver" cells (B). In each, the charge duration is the amount of time the electrode was biased to produce hydrogen peroxide. We also defined cell activation as the percent of the analyzed population that had fluorescence exceeding two standard deviations away from a null control. A.) We found that while increasing the charge duration did increase the median fluorescence of the monoculture as measured by flow cytometry, only a small portion of the population was activated. B.) Within the coculture model, however, the percent of activated cells rose rapidly with increasing charge duration indicating the quorum sensing molecule homogenized the response of the entire system.