A Novel Synthetic Biology Approach to Enabling Soil Bacteria to Produce Powerful Chlorinated Auxins

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Enhanced crop production on a sustainable basis is necessary for solving the global food crisis. However, many agricultural practices to enhance crop growth are detrimental to the environment. Soil bacteria are a great alternative, and benefit plants in various ways, including releasing plant auxins. One of such auxins is indole-3-acetic acid (IAA), a molecule that stimulates crop growth and participates in many physiological processes in plants. It was found that the chlorinated derivatives of IAA, such as 5-Cl-IAA and 4-Cl-IAA, are much stronger growth promoters than IAA. Unfortunately, soil bacteria don't naturally produce chlorinated IAAs, which has limited their applicability in agriculture. The objective of this project was to engineer soil bacteria for the ability to produce powerful chlorinated auxins. A microbial L-tryptophan 5-halogenase (PyrH) gene was cloned and functionally verified in Escherichia coli. It was then ligated into the Pseudomonas expression vector pMiSI. The resulting plasmid was introduced into the model soil bacterium Pseudomonas putida KT2440. This engineered strain can efficiently chlorinate L-tryptophan at C-5. Furthermore, P. putida KT2440/pMiSI-pyrH can produce 5-Cl-IAA from exogenously supplied IAA, which was confirmed by MS and NMR analyses. PyrH was next engineered into Pseudomonas chlororaphis O6, a plant-benefiting soil bacterium capable of producing IAA naturally. LC-MS analysis revealed that the engineered strain can produce 5-Cl-IAA without exogenous supply of the substrate. This work thus provides a novel approach to enabling soil bacteria to produce powerful plant growth hormones, which can be used in agriculture to promote crop growth and enhance food production.