

Engineering of Apocarotenoid Production in *Pseudomonas putida* KT2440 for Promoting Rice Growth and Resilience

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Global food demand requires sustainable strategies to enhance cereal productivity beyond conventional agricultural inputs. The apocarotenoids beta-Cyclocitral (beta-CC) and 3-OH-beta-Cyclocitral (3-OH-beta-CC) are signaling molecules known to promote plant growth and stress tolerance. However, no efficient and environmentally sustainable delivery system currently exists for their agricultural application. This study aims to engineer *P. putida* KT2440 as a rhizosphere-colonizing microbial platform capable of producing those signaling molecules. A carotenoid biosynthetic pathway was introduced, followed by integration of a carotenoid cleavage enzyme to enable apocarotenoid production. Engineered strains were evaluated for metabolite production, growth characteristics, and plant growth-promoting effects in *Oryza sativa* L. Engineered strains successfully accumulated carotenoids, including zeaxanthin and beta-carotene, and produced the apocarotenoid derivatives, beta-apo-8'-carotenal and 3-OH-beta-apo-8'-carotenal, confirming beta-CC and 3-OH-beta-CC formation. Although pathway integration reduced growth rate (OD600 0.623 vs 2.365 in wild-type), colony-forming units increased significantly by 2-fold. Under normal conditions, seedlings treatment increased the freshweight by 85%. And under drought conditions, the treated seedlings again outperformed the controls, with 15% greater freshweight. These results demonstrate that engineered *P. putida* can function as an effective in situ delivery system for apocarotenoid phytoestrogens, significantly enhancing plant growth. This work highlights a scalable and sustainable microbial approach for improving crop productivity, with potential applications under environmentally relevant stress conditions.

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