Genetically engineering *Pseudomonas* strains for T6SS-dependent controlled release of effectors

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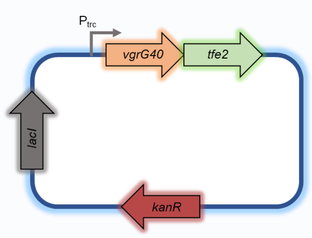
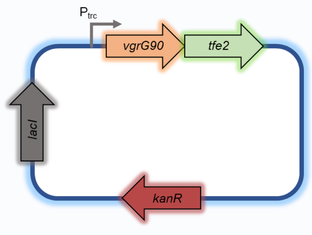
Aims of the study

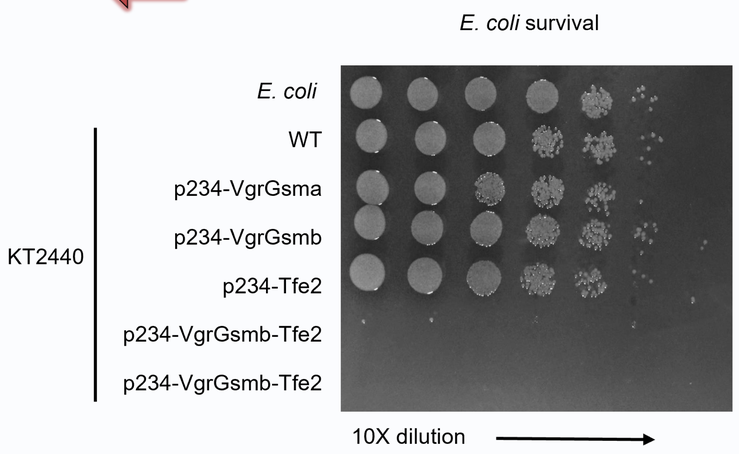
Type VI secretion systems (T6SS) are Gram-negative bacteria's syringe-like nanomachine that injects effectors into prokaryotic and eukaryotic cells leading to the death of their target. These systems have the potential to be genetically engineered for their application in different biotechnological fields such as medicine or agriculture. As proof of concept, in this work we have genetically engineered the well-known soil-dwelling *Pseudomonas putida* KT2440 strain, achieving the controlled expression of related and non-related T6SS effectors. These results open a window of opportunities to secrete a wide range of molecules to different targets using this *Pseudomonas* strain as bacterial chassis. Specifically, this work shows promising results in the agriculture field as genetically modified *P. putida* KT2440 strains overexpressing T6SS-related effector Tfe2 and T6SS-non-related effector chitosanase can now inhibit bacterial and fungal strains such as the phytopathogen *Botrytis cinerea*.

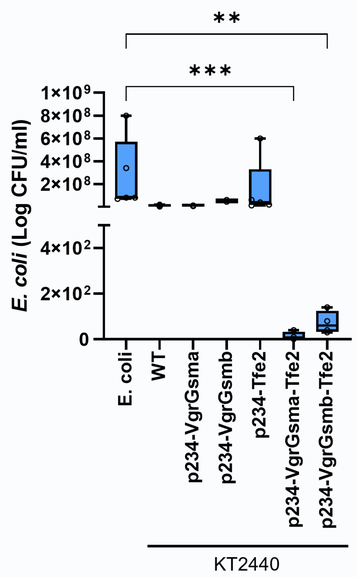
Results

T6SS-dependent antibacterial activity from Tfe2 effector

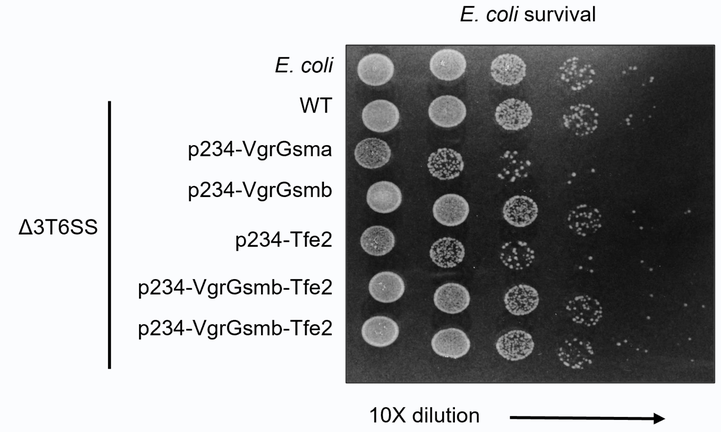


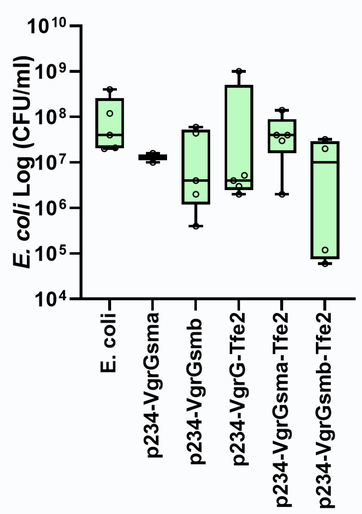




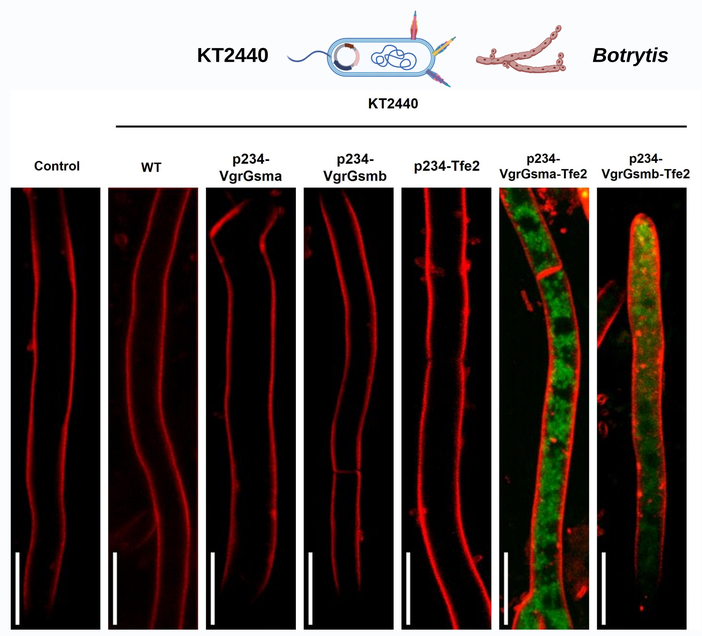


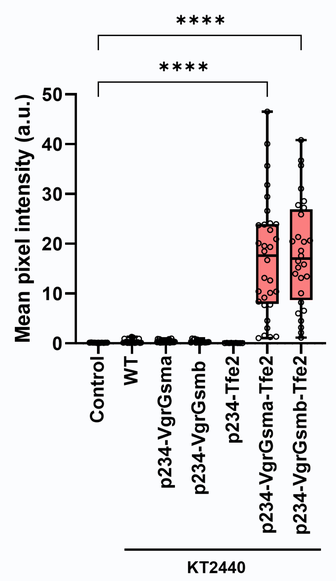
And the importance of T6SS...



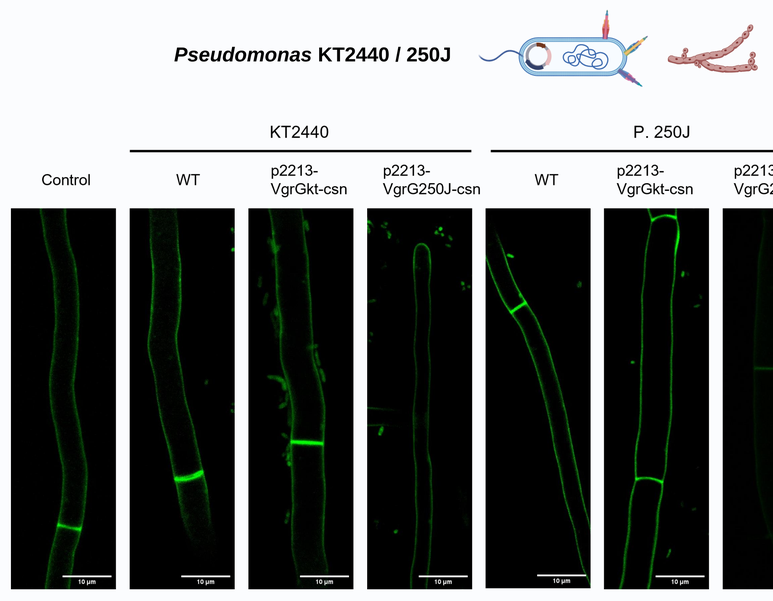


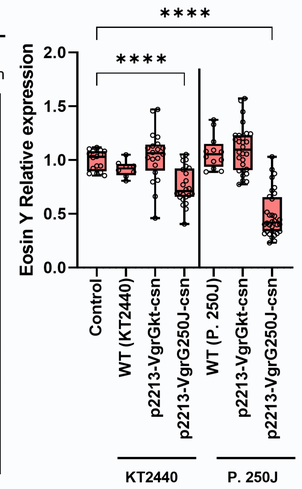
T6SS-dependent antifungal activity from Tfe2 effector





Engineered T6SS to express chitosanase





Conclusions

- Combining efectors with VgrG proteins permit the T6SS-dependent release of those effectors.
- Genetic engineering can be used to express exogenous effectors in *Pseudomonas* to module the growth of different microorganisms.
- The non-dependent T6SS protein chitosanase can be genetically engineered to degrade chitosan in a T6SS dependent way.

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