Title: Disentangling the molecular mechanisms of disease suppression by endophytic *Flavobacterium* sp. 98

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Abstract:
Endophytic microorganisms that colonize and thrive inside plant tissues can provide protection to plants against pathogen infection. Among the beneficial endophytes, we previously showed that *Flavobacterium* sp. 98 (Fl98) protects sugar beet seedlings against the fungal root pathogen *Rhizoctonia solani.* To date, however, the underlying molecular mechanisms of disease control by Fl98 remain elusive. In this study, we investigated the role of biosynthetic gene cluster 298 (BGC298) in disease protection by Fl98. BGC298 was enriched in the endosphere microbiome of sugar beet seedlings grown in *Rhizoctonia*-suppressive soil and the BGC298 knockout mutant of Fl98 was less effective in disease control (Carrión et al., Science 366:606-612, 2019). Comparative metabolomics conducted here revealed that BGC298 is involved in regulating the biosynthesis of 5,6-dimethybenzimidazole (DMB), an antifungal compound that inhibits hyphal growth of *R. solani*. Subsequent site-directed mutagenesis of the DMB-synthase gene *bluB* abolished DMB production by Fl98. Greenhouse bioassays further showed that both mutants (ΔBGC298, Δ*bluB*) were compromised in protection of the sugar beet seedlings against infections by *R. solani*. Moreover, bioinformatic analyses suggested that *bluB* is widely distributed in the *Flavobacterium* clade, while BGC298 is less conserved and only present in a small subset of plant-associated *Flavobacterium* genomes. These findings revealed the pivotal role of BGC298 and DMB biosynthesis in plant protection by endophytic *Flavobacterium* sp. 98.

Keywords:
Flavobacterium, Disease-suppressive soil, Endophytic microbiome, 5,6-dimethylbenzimidazole, Molecular mechanisms, Comparative genomics

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