PhD THESIS ABSTRACT

Ecological role of tailocins (phage tail-like particles) in Soft Rot Pectobacteriaceae

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Bacteria colonising plant surfaces and tissues face intense competition for space and nutrients and therefore deploy a diverse arsenal of interference mechanisms to inhibit rivals. Among plant-associated bacteria, Soft Rot Pectobacteriaceae (SRP) are particularly exposed to such competitive pressure. These bacteria – *Pectobacterium*, *Dickeya*, and *Musicola* species that cause soft rot and other maceration-associated diseases in economically important crops – are found across habitats ranging from surface water and agricultural soil to infected plant tissue, where they encounter both closely related SRP and taxonomically distant competitors. Under competitive pressure, these bacteria have evolved several antagonistic mechanisms, including tailocins – phage tail–like bacteriocins encoded by domesticated prophage regions in their genomes. Tailocins are bactericidal particles that kill susceptible cells by puncturing their cell envelope. What makes tailocins unusual among bacterial weapons is that their production requires the death of the producing cell: particles are assembled in the cytoplasm and can only be released through lysis. Despite this cost, tailocin gene clusters are found across diverse bacterial taxa, which raises the question of what selective advantage sustains such expensive weapons. To date, among SRP, only one type of tailocin has been described in detail – carotovoricin Er from *Pectobacterium carotovorum* – and the selective advantage of tailocins in this group remains largely unexplored.

This thesis, which consists of four published studies, characterises tailocin-mediated interactions in SRP, from the molecular properties of individual particles to their potential ecological consequences in plant-associated habitats. To establish a model system, I characterised the first R-type tailocin from *Dickeya dadantii* strain 3937, designated dickeyocin P2D1. The gene cluster encoding P2D1 shares sequence homology with the genes encoding the tail of *Peduovirus* P2 – a lineage phylogenetically distinct from that of carotovoricin Er – indicating that the two SRP genera acquired their tailocins independently from different phage ancestors. Further, to identify the conditions and timing of tailocin production, the temporal dynamics of P2D1 release were resolved, showing that production follows a tightly coordinated sequence and can be triggered by structurally unrelated DNA-damaging agents, including mitomycin C, hydrogen peroxide, ciprofloxacin, and norfloxacin. The finding that H_2O_2 induces tailocin release is of particular ecological relevance, because SRP bacteria encounter plant-derived reactive oxygen species during the oxidative burst that accompanies infection.

To determine how widespread tailocin production is among environmental SRP, I surveyed 27 strains isolated from the Durance River in France. This river connects ecosystems in which different SRP species co-occur, making it a source of strains that are likely to encounter one another in nature. Of the 27 strains tested, 24 produced tailocin particles. A broader genomic survey, including screening of 190 complete SRP genomes, confirmed that tailocin clusters are present in 83% of *Pectobacterium* and 69% of *Dickeya* genomes. All *Dickeya* clusters are homologous to the P2D1 locus and all *Pectobacterium* clusters to the carotovoricin Er locus, with no genome carrying both types, confirming independent acquisition after the divergence of the two genera. Systematic pairwise testing among these strains revealed that *Dickeya* tailocins kill more frequently and across broader taxonomic boundaries than *Pectobacterium* tailocins, including activity against *Pectobacterium* strains – an asymmetry not observed in the opposite direction. Tailocin particles were also detected in experimentally *Dickeya*-infected potato tissue, indicating that these particles are present during plant infection.

Tailocins are generally considered to target kin strains. In this work, to test whether the ecological reach of tailocins extends beyond SRP, I screened 480 soil and rhizosphere bacterial isolates for sensitivity to P2D1. Nine strains were found susceptible, all spanning different phylogenetic clades within genus *Pseudomonas*. Tests involving a P2D1-deficient mutant of *D. dadantii* confirmed that the activity is tailocin-mediated. Six of the nine susceptible *Pseudomonas* are non-pathogenic and can suppress *D. dadantii* soft rot on potato, whereas three cause soft rot themselves. P2D1 can therefore eliminate bacteria with contrasting ecological roles relative to its producer – both direct antagonists and organisms that exploit the same host.

Together, these findings indicate that tailocins are a widespread and functional component of the competitive arsenal of SRP. The convergent maintenance of tailocin clusters of independent phage origin in both genera, the detection of particles in infected plant tissue, and the demonstration that H_2O_2 triggers their production all point toward a selective advantage that has sustained tailocin carriage despite the lethal cost of deployment. While direct evidence for tailocin-mediated competition under natural conditions remains to be obtained, this thesis establishes the molecular, genomic, and ecological foundations on which such studies can now be built.