RESEARCH ARTICLE

Tailocin-mediated interactions in Soft Rot *Pectobacteriaceae*

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Abstract

Bacteria often carry phage-derived elements within their genomes, some of which can produce phage-like particles (tailocins) used as weapons to kill kin strains in response to environmental conditions. This study investigates the production and activity of such particles by plant pathogenic bacteria belonging to the *Pectobacterium*, *Dickeya,* and *Musicola* genera that often compete for the same niche, providing an attractive model to study the ecological role of tailocins. Transmission electron microscopy (TEM) revealed that most analyzed strains (88%) produced tailocins. Tailocin-mediated killing interactions were assessed across 351 strain pairs, showing that *Dickeya* spp. had a higher incidence of killing (57.1%) than *Pectobacterium* spp. (21.6%). Additionally, *Dickeya* spp. strains exhibited inter-genus activity, targeting both *Pectobacterium* spp. and *Musicola* sp., while *Pectobacterium* spp. tailocins were genus-specific. A so-called "bilateral killing" was observed in 33.9% of interactions, predominantly within *Dickeya* spp. Although tailocins were morphologically indistinguishable between producer strains, genomic analyses identified conserved tailocin gene clusters across both genera, with distinct structural differences between *Pectobacterium* spp. and *Dickeya* spp tailocins. This suggests different origins of these particles. Induction experiments demonstrated that tailocin production was significantly boosted by hydrogen peroxide, supporting the potential role of these particles in bacteria-bacteria competition during infection. Lastly, tailocin production was detectable in infected potato tissue but not in river water, highlighting the ecological relevance of tailocins in plant environments.

Keywords:

bacteriophage, phage tail-like particles, hydrogen peroxide, inter-genus, interactions, *Erwinia chrysanthemi*, *Erwinia carotovora*, prophage

Introduction

In highly competitive and resource-limited environments that bacteria colonize and occupy, their survival and welfare often center on the ability to outcompete neighboring rival cells (Bauer et al., 2018;Wagner, 2022). To gain a competitive edge, bacteria evolve diverse strategies, including producing and utilizing phage tail-like particles (tailocins) - structures that directly resemble the tails of bacteriophages but function independently from viral infection and replication (Booth et al., 2023). These particles are highly specialized killing instruments that, by puncturing the cell envelope, lead to the disruption of the cell membrane following the death of the targeted cell (Scholl, 2017). Until recently, the utilization of phage tail-like particles has been primarily studied in *Pseudomonas* species (Carim et al., 2021). However, there are also reports of tailocins isolated from various other Gram-negative and Gram-positive bacterial species, including human, animal, and plant pathogens, as well as saprophytic bacteria that inhabit a wide range of environments (Backman et al., 2024a).

We previously identified and characterized a new type of phage tail-like particles produced by the plant pathogenic bacterium, *Dickeya dadantii* reference strain 3937 (Borowicz et al., 2023). This tailocin, named dickeyocin P2D1, was resistant to various environmental conditions and expressed broad killing potential against members of different *Dickeya* species but was harmless to *Pectobacterium* species and non-toxic to *Caenorhabditis elegans.* We postulated that the dickeyocin P2D1 targets remain phylogenetically closely related strains. Our study was the first to target the presence and conduct an in-depth characterization of a new phage tail-like particle in SRP bacteria. Previously, only one phage tail-like particle, carotovoricin Er from *Pectobacterium carotovorum*, was described, and its nucleotide sequence was analyzed in detail (Yamada et al., 2006). However, since then, large studies have yet to be done to assess the presence of tailocins in SRP bacteria. Moreover, the carotovoricin Er sequence (Yamada et al., 2006) differs from that of dickeyocin P2D1, indicating a distinct evolutionary origin of these two tailocins.

SRP bacteria (consisting of *Pectobacterium* spp., *Dickeya* spp., and *Musicola* spp., previously collectively described as pectinolytic *Erwinia* spp.) are a particularly interesting model

for studying the production and utilization of phage tail-like particles in the environment. *Pectobacterium* spp., *Dickeya* spp., and *Musicola* spp. belong to the same species complex and are often found together in infected hosts. Consequently, due to their metabolic similarities and overlapping niches, they are known to compete against each other (Toth et al., 2021a). Furthermore, these bacteria are known to pass through various ecological niches, including host and non-host plants, natural and agricultural soils, rainwater, surface water, sewage, and insects (Van Gijsegem et al., 2021b). In these diverse environments, SRP bacteria often encounter a diverse array of other species, both closely and distantly related, but their competitive strategies in these alternative biomes remain unknown (Bellieny-Rabelo et al., 2019).

This study aimed to assess the utilization of phage tail-like particles (tailocins) in SRP strains obtained from one selected environment – the Durance River in France (Ben Moussa et al., 2022). Although river water is not recognized as a primary environment where SRP bacteria thrive, this niche can be considered part of the continuum of distinct ecosystems that support the growth and survival of SRP. The tested SRP pool included aquatic and non-aquatic strains, which potentially could interact in water or be transmitted to a common plant host, where they might interact during infection (Van Gijsegem et al., 2021b).

Our understanding of the competition mechanisms in SRP bacteria mediated by phage tail-like particles remains poorly understood (Marquez-Villavicencio et al., 2011;Hugouvieux-Cotte-Pattat, 2016;Hugouvieux-Cotte-Pattat et al., 2023). Specifically, no large studies have been conducted to assess whether the production of tailocins is common in this group of bacteria and how it contributes to their overall ecological success (Borowicz et al., 2023). Addressing this gap remains important for understanding how plant-pathogenic bacteria establish and sustain dominance in diverse environments, particularly in those niches where microbial competition between kin strains is expected to be intense. Additionally, we aimed to characterize the isolated particles and identify gene clusters associated with their synthesis, highlighting the prevalence of tailocin production among environmental SRP strains and their possible role in the intragenus competition.

Materials and Methods

Bacterial strains and culture conditions

Bacterial strains included in this work are listed in Supplementary Table S1. The pool comprised 25 SRP isolates from the waters of Durance River (France), collected between 2015 and 2017 (Ben Moussa et al., 2022), as well as two reference strains: *Dickeya dadantii* 3937 (Pritchard et al., 2013) and *Musicola paradisiaca* NCPPB 2511 (Hugouvieux-Cotte-Pattat et al., 2021). The latter strains were a model in our previous study on tailocins (dickeyocins) from *D. dadantii* 3937 (Borowicz et al., 2023). Routine propagation of bacterial cells was performed at 28 ºC in liquid Trypticase Soy Broth (TSB, Oxoid), with agitation (120 rpm), or on solid Trypticase Soy Agar (TSA, Oxoid) under the same growth conditions (Czajkowski et al., 2010).

Induction and purification of tailocins

Tailocin particles were purified as described previously (Borowicz et al., 2023). Briefly, an overnight culture (*ca.* 16 h) of each tested strain was rejuvenated (1:40) in a fresh aliquot of TSB and cultured for 2.5 hours. Next, tailocin production was induced by the addition of 0.1 µg mL^{-1} of mitomycin C (Abcam, Poland). Twenty-four hours post-induction, tailocins were PEG-purified from 10 mL volumes of filtered (0.2 µm, PES membrane, GoogLab™) culture supernatants. Purified particles were stored in phosphate buffer saline (PBS), pH 7.2, at 4 °C.

Microscopic imaging

The morphology of purified tailocin particles was investigated by transmission electron microscopy (TEM) and atomic force microscopy (AFM). TEM imaging was performed as described earlier (Borowicz et al., 2023) using the Tecnai Spirit BioTWIN microscope (FEI). AFM imaging was performed using JPK NanoWizard® 4 (NanoScience), in non-destructive quantitative imaging (QI™) force spectroscopy mode, employing the SCANASYST-AIR probes (f0 7.0 kHz, diameter <12 nm, k: 0.4 N/m) and the SCANASYST-FLUID+ probes (f0 150 kHz, diameter <12 nm, k: 0.7

129 N/m). Spring constants and sensitivity of the probes were calibrated using the thermal calibration
130 method. Before imaging, the samples were deposited on freshly cleaved mica surfaces and air-
131 dried. Images were processed using JPK Data Processing software. Dimensions of tailocins were
132 calculated based on TEM images. Tailocins from a given strain were considered for measurement
133 only if more than 4 individual particle images were available for analysis. This included 13 out of 18
134 *Pectobacterium* spp. strains, with a median of 10 particles measured per strain, and 7 *Dickeya*
135 spp., with a median of 17 particles measured per strain.

136

137 **Killing spectrum and sensitivity assay**

138 Twenty-seven SRP strains, including 25 water isolates (Supplementary Table S1), were tested,
139 pair by pair, to determine the target range (killing spectrum) of tailocins produced by each group
140 member. Likewise, the opposite was investigated, namely the sensitivity of strains to tailocins
141 produced by all other strains in the pool. Both the killing spectrum and sensitivity were tested using
142 the spot assay as previously described (Hockett and Baltrus, 2017;Yao et al., 2017;Borowicz et al.,
143 2023). Each combination was tested in duplicate in a single setup, and the entire experiment was
144 performed twice using independently-obtained batches of tailocins.

145

146 **SDS-PAGE, ESI LC-MS/MS, and mapping of identified proteins to genomic *loci***

147 Proteins in PEG-purified tailocin preparations were separated by SDS-PAGE, fragmented, and
148 analyzed by mass spectrometry, as described in detail previously (Golebiowski et al.,
149 2022;Borowicz et al., 2023). Briefly, bands were excised from the gel, followed by in-gel trypsin
150 digestion of the embedded proteins, peptide elution, cleanup, and sample concentration (Schmidt
151 and Sinz, 2017;Goldman et al., 2019). Next, the peptides were analyzed by ESI LC-MS/MS. This
152 included separating peptides on the Eksigent microLC column ChromXP C18CL (3 µm, 120 Å, 150
153 × 0.3 mm). The samples were placed onto the column with a CTC Pal Autosampler (CTC Analytics
154 AG, Zwinger, Switzerland), and the injection volume was 5 µl. Solvents A and B consisted of 0.1%
155 (v/v) formic acid (FA) in water and acetonitrile, respectively. LC gradient parameters: 18–50%

156 solvent B in 30 min (Fiołka et al., 2019). Recording of mass spectra with Triple Tof 5600+ mass
157 spectrometer with DuoSpray Ion Source (AB SCIEX, Framingham, MA, USA), and identification of
158 proteins based on the obtained fragmentation spectra using the Peaks Studio 11 (Bioinformatic
159 Solutions) and the appropriate protein database (Supplementary Table S1) (1% FDR). The list of
160 annotations for the MS-identified proteins was searched for phage-associated functions, and the
161 encoding genes were mapped to the genomes of the tested strains. Tailocin-encoding regions
162 were identified based on the clustering of the identified phage/tailocin-related genes within the
163 genomes of the analyzed strains and previous studies concerning the structure of the P2D1 tailocin
164 cluster (Borowicz et al., 2023). As the full sequence of the carotovoricin Er cluster is unavailable,
165 the originally uploaded sequence (accession: AB017338.2) (Yamada et al., 2006) annotated using
166 RAST (https://rast.nmpdr.org/rast.cgi) (Brettin et al., 2015) was used for cluster synteny analysis.

167

168 **Phylogenetic and bioinformatic analyses**

169

170 *Synteny of tailocin clusters*

171 Gene clusters encoding tailocins were extracted from the genomes of *Pectobacterium*, *Dickeya,*
172 and *Musicola* species, except for *Pectobacterium odoriferum* A122-S21-F16, where the putative
173 cluster was split across multiple contigs. For *Pectobacterium* spp. analyzed, the genomic region
174 containing the tailocin cluster was defined as the region between the genes annotated as *tolC*
175 (locus tag in *P. versatile* A69-S13-O15: EG331_00440) and *ybiB* (locus tag in *P. versatile* A69-
176 S13-O15: EG331_00565). In contrast, for *Dickeya* species and a single *Musicola* sp. strain, the
177 genomic region containing the tailocin cluster was identified as the region between the genes
178 annotated as *methyl-accepting chemotaxis protein* (locus tag in *D. dadantii* 3937:
179 DDA3937_RS1200) and *guaD* (locus tag in *D. dadantii* 3937: DDA3937_RS12145).

180 Accession numbers of genomes from which the tailocin regions were extracted are listed in
181 Supplementary Table 1. The extracted sequences were then subjected to synteny analysis and
182 visualization using the Clinker tool (Gilchrist and Chooi, 2021). The analysis was performed
183 separately for *Pectobacterium* and *Dickeya* species, along with a single strain of *Musicola* sp.

184 Genes within the clusters were color-coded based on their putative roles in tailocin production. In
185 the visualization, the presence of links between genes indicates sequence identity percentages
186 greater than 50%. The order of strains in the visualization was determined according to their
187 phylogenetic relationships. The annotation in the synteny analysis was based on *Pectobacterium*
188 *versatile* for *Pectobacterium* spp. and *Dickeya dadantii* 3937 for *Dickeya* spp.

189

190 *Multilocus sequence analysis*

191 A subset of strains was used for the phylogenetic analysis. The selected strains had to possess the
192 following features: (1) the tailocin cluster had to be present on a single genomic contig; (2) genes
193 annotated as fiber and sheath had to be present within the region identified as the tailocin cluster;
194 and (3) the strain had to demonstrate the ability to target at least one other analyzed strain through
195 tailocin interaction. Orthologous sequences were clustered into homologous families using the
196 SiLix software package v1.2.9 (Miele et al., 2011) with an 80% identity threshold and at least 80%
197 overlap. Orthologous sequences (658 coding sequences) were concatenated, aligned using
198 MUSCLE (Edgar, 2004) software v5.1, and filtered using the GBLOCK tool (Castresana, 2000).
199 The alignments were used to build a phylogenetic tree with the BioNJ algorithm with SeaView
200 software v5.0.5 (Gouy et al., 2010), with 200 bootstrap replications.

201

202 *Phenotype-based dendrogram*

203 Agglomerative clustering (Nielsen, 2016) was applied to prepare a dendrogram for tested strains
204 based on the similarity of their killing spectra. The ability to kill a given strain was considered 1, and
205 the lack of this ability was rated 0. The scikit-learn (Virtanen et al., 2020) and SciPy (Virtanen et al.,
206 2020) libraries were used for clustering and dendrogram plotting, respectively. A Phyton script for
207 implementing the above libraries was written with the assistance of ChatGPT-4o (OpenAI)
208 (Supplementary Dataset 3).

209

210 *Prevalence of carotovoricin and dickeyocin clusters in* SRP complete genomes

211 The carotovorocin cluster was blasted against 116 *Pectobacterium* spp. complete, high-quality
212 genomes available on the NCBI database in June 2024 using the NCBI Megablast algorithm with
213 default parameters (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The presence of the cluster was
214 defined according to the 70% sequence coverage and identity thresholds. The available sequence
215 of the originally described carotovoricin Er cluster was incomplete (accession: AB017338.2).
216 Therefore, as a reference carotovoricin sequence for Pectobacterium spp. we used the full
217 nucleotide sequence of the carotovoricin cluster from *P. versatile* A73-S18-O15 (19,528 bp.). For
218 *Dickeya* spp. and the single *Musicola* sp. strain, the full nucleotide sequence of the dickeyocin
219 cluster of *D. dadantii* 3937 strain (26,580 bp) was blasted against a single *Musicola* sp. and 74
220 *Dickeya* spp. complete genomes available on the NCBI database in June 2024. Results were
221 interpreted based on analogous thresholds as described for P2D1 tailocin.

222

223 **Detection of tailocins in river water and potato tubers**

224 To determine whether SRPs produce detectable levels of tailocins in river water and potato tuber
225 tissue, we inoculated these environments with bacteria. After incubation, we purified the tailocins
226 and conducted spot tests as outlined below. Each experiment was performed twice, and detection
227 was considered positive when all replicates gave positive results. To prepare the inoculation
228 suspensions, six strains, namely *P. versatile* A69-S13-O15, *P. quasiaquaticum* A411-S4-F17, *P.*
229 *aquaticum* A212-S19-A16, *D. dadantii* 3937, *D. chrysanthemi* A604-S21-A17, and *D. zeae* A586-
230 S18-A17, were grown in TSB (Oxoid) for 24 h at 28 °C. The cells were harvested by centrifugation
231 (4200 RCF, 5 min) and resuspended in PBS buffer. Turbidity of the suspension was adjusted to
232 0.06 McF (approx. 10^6 CFU mL^{-1}). Five ml of Durance River water filter-sterilized and autoclaved
233 (0.2 µm, PES membrane) were inoculated with 50 µl of bacterial suspension, three replicates per
234 strain. Uninoculated water was used as a control. Samples were incubated for 72 h at 15 °C with
235 shaking (120 rpm) to mimic natural conditions. Following incubation, 2 ml of water from each
236 sample was centrifuged to remove bacterial cells (8000 RCF, 10 min), and the supernatant was
237 processed for purification and detection of tailocins. Tubers cv. Gala were surface sterilized by

238 immersion for 20 min in 5% commercial bleach (ACE, Procter and Gamble), followed by a double
239 rinse in distilled water and air drying under laminar flow. Each tuber was inoculated by inserting a
240 pipette tip containing 50 µl of the test suspension into the tuber (up to the level of liquid within the
241 tip). Tubers inoculated with PBS buffer alone were used as a negative control. Three potato tubers
242 were tested per treatment. Inoculated tubers were placed in humid boxes (85 to 90% relative
243 humidity). Samples were incubated at 28°C for 72 h to enable the development of soft rot
244 symptoms. Next, the tubers were cut at the inoculation site. Tissue macerated by bacteria was
245 spooned out, weighed, and placed in homogenization bags (Bioreba). PBS buffer supplemented
246 with 0.02% diethyldithiocarbamic acid (DIECA) (Sigma-Aldrich) was added to the samples (1:2,
247 w/v) (Perombelon and van Der Wolf, 2002). The samples were homogenized, and 1 mL aliquots
248 were diluted 1:1 with PBS 0.02% DIECA in new tubes. Bacteria and plant debris were pelleted by
249 centrifugation (8000 RCF, 10 min, RT), and the supernatant was processed for purification and
250 detection of tailocins. The supernatants from both experimental setups were filtered (0.2 µm, PES
251 membrane) and transferred to new tubes containing PEG-8000 (final concentration 10 % m/v).
252 Samples were incubated overnight at 4°C with shaking. The putative tailocins were collected by
253 centrifugation (1 h, 16 000 RCF, 4°C) and resuspended in 1/20 volume of the initial sample in PBS.
254 Tailocins were detected using a spot test (Hockett and Baltrus, 2017;Yao et al., 2017). Five µL
255 aliquots of purified tailocins were spotted on soft top agar plates, each inoculated with a strain
256 sensitive to tailocins of the six investigated SRPs (Supplementary Table S2).

257

258 **Semiquantitative assessment of tailocin production depending on the type of inductive**
259 **factor**

260 We investigated whether hydrogen peroxide can induce the production of tailocins in SRPs in a
261 way similar to mitomycin C. The experiment was conducted on the same group of six strains tested
262 for tailocin production in river water and potato tuber tissue. Briefly, an overnight culture (ca. 16 h)
263 of each strain was rejuvenated (1:40) in a fresh aliquot of TSB and cultured for the next 2.5 hours.
264 After that, tailocin production was induced by the addition of 0.1 µg mL^{-1} mitomycin C (Abcam,
265 Poland) or 0.003 % (0.88 mM) hydrogen peroxide (Laboratorium Galenowe Olsztyn Sp. z o. o.,

266 PolandCultures) and cultures with no inductive factor were used as controls. Twenty-four hours
267 post-induction, tailocins were PEG-purified from 2 mL volumes of filtered (0.2 µm, PES membrane)
268 culture supernatants and resuspended in 1/10 of the initial volume in PBS (Borowicz et al., 2023).
269 The concentration of tailocins was estimated by a semiquantitative spot test (Yao et al.,
270 2017;Borowicz et al., 2023) using tailocin-susceptible strains (Supplementary Table S2). The
271 reciprocal of the highest dilution causing a visible plaque was defined as the value of the relative
272 activity in arbitrary units (=1 AU), and the AU per mL of culture was calculated. The experiment
273 was conducted twice, with tree replicas per combination.

274

275 **Competition assay *in vitro***

276 To perform the competition assay, we first selected a spontaneous rifampicin-resistant mutant of *D.*
277 *solani* A623-S20-A17 on TSA with 50 µg mL^{-1} of rifampicin (Sigma-Aldrich)(Glandorf et al.,
278 1992;Berg et al., 2007). Such obtained strain was designated *Ds* RIF and tested in an *in vitro*
279 competition assay against three sets of competitor strains. Each set comprised two *Dickeya* spp.
280 strains which either: 1) were killed by the tailocins of *D. solani* A623-S20-A17 (*D. dianthicola* A260-
281 S21-A16 and *D. zeae* A661-S21-A17), 2) produced tailocins killing *D. solani* A623-S20-A17 (*D.*
282 *zeae* A586-S18-A17 and *D. oryzae* A3-S1-M15) or 3) were killed by the tailocins of *D. solani* A623-
283 S20-A17 but at the same time produced tailocins targeting this strain (bilateral killing)(*D. dadantii*
284 3937 and *D. chrysanthemi* A604-S21-A17).

285 All strains were grown overnight in 0.1 TSB medium. Next, the cells were harvested by
286 centrifugation (3500 RCR, 5 min.), and the pellet was resuspended in a fresh medium to obtain a
287 turbidity of 0.5 McF. This equaled, on average, 1.4×10^8 CFU ml^{-1} ($\pm 4.5 \times 10^7$), verified by dilution
288 plating. 150 µl of 0.1 TSB in a single well of a 96-well plate was inoculated (1:1, 25:25 µl) with the
289 suspension of *Ds* RIF and one of the competing strains to ensure the strains had an even start and
290 avoid tailocins carryover from the initial overnight cultures. Each co-culture was tested under two
291 conditions: 0.1 TSB alone or 0.1 TSB with 0.003 % (0.88 mM) H_2O_2 (tailocin-inductive stress). The
292 cultures were incubated for 22 h at 28°C, with orbital shaking, in the EPOCH2 reader (BioTek).

293 Culture from each well was dilution-plated on two media types: TSB agar (for counting the total
294 number of CFUs) and TSB agar with 50 µg mL^{-1} of rifampicin (for counting the fraction of *Ds* RIF).
295 *Ds* RIF was tested against the wild-type (*wt*) variant of the strain as a control of the mutant's fitness
296 under the applied experimental conditions. Moreover, the ability of *Ds* RIF to produce tailocins was
297 verified in a spot assay, as described above. The competition experiment was performed twice,
298 with two separate co-cultures (replicates) per experiment.

299

300 **Results**

301 ***Pectobacterium* spp. and *Dickeya* spp. strains from Durance River produce phage tail-like**
302 **particles**

303 TEM imaging of preparations obtained from mitomycin C-induced cultures revealed that 22 out of
304 25 studied SRP isolates (88%) produce phage tail-like particles (tailocins) (Fig.1). The three
305 exceptions were *Pectobacterium brasiliense* A143-S20-M16, *Pectobacterium atrosepticum* A597-
306 S4-A17, and *Dickeya dianthicola* A260-S21-A16, for which no such structures could be detected in
307 our experimental setup. All detected tailocins showed similar morphology in TEM and AFM images,
308 regardless of whether they were derived from *Dickeya/Musicola* or *Pectobacterium* spp. (Figures 1
309 and 2). The average length of tailocins produced by *Pectobacterium* spp. was 163±15 nm.
310 Tailocins produced by *Dickeya/Musicola* spp. were slightly shorter, measuring 153±20 nm, with the
311 difference being statistically significant (Mann-Whitney test, $p<0.05$). Similarly, tailocins produced
312 by *Pectobacterium* spp. were, on average, 2 nm wider compared to those of *Dickeya/Musicola*
313 species, with the dimensions amounting to 26±2 nm and 24±2 nm, respectively, with the difference
314 being statistically significant (Mann-Whitney test, $p<0.05$). Three-dimensional images generated
315 based on AFM scans for seven representative tailocin particles are shown in Fig. 2.

316

317 **The killing spectra of SRP phage tail-like particles vary depending on the strain of origin,**
318 **with several cases of inter-genus activity**

319 We evaluated in detail the killing spectra of phage tail-like particles produced by studied SRPs
320 strains. In total, we investigated 702 unique tailocin-mediated interactions (producer-target
321 combinations) for 351 unique pairs (individual producer vs. individual target) of SRPs (Fig. 3). The
322 overall incidence of killing events where a strain was found susceptible to a particular tailocin
323 equaled 16.8% (Table 1). The killing incidence was higher in the tested subset of *Dickeya* strains
324 than in the tested *Pectobacterium* strains, at 57.1% and 21.6%, respectively (Table 1). The two
325 most susceptible strains were *P. versatile* A73-S18-015 and *P. polaris* A641-S16-A17, killed by
326 tailocins from 8 different strains. On the opposite side of the spectrum was *P. carotovorum* A280-
327 S2-N16, which was resistant to tailocins produced by all strains in the study. Twenty-one of the 25
328 environment-derived strains produced tailocins that killed at least one strain from the tested group
329 of 27 SRPs. Out of the 4 strains producing no killing events, three (*P. brasiliense* A143-S20-M16,
330 *P. atrosepticum* A597-S4-A17, and *D. dianthicola* A260-S21-A16) were the ones with no tailocins
331 visible in TEM analyses (Fig. 1), and *P. peruviense* A350-S18-N16 which did not target any of the
332 27 SRPs tested (Fig. 3). On the contrary, *D. oryzae* A3-S1-M15 and *P. odoriferum* A122-S21-F16
333 produced tailocins with the broadest killing range, each targeting 9 out of the 27 strains (33.3%).

334 We also investigated the inter-genus activity of tailocins. *Pectobacterium* strains produced tailocins
335 targeting solely other members of the genus. On the contrary, 5 out of 8 tested *Dickeya* spp.
336 (62.5%) could target at least *one Pectobacterium* sp., in each case, either a *P. versatile* strain or
337 the closely related *P. odoriferum. M. paradisiaca* NCPPB 2511, the single representative of
338 *Musicola* spp., strongly clustered with *Dickeya* spp. both regarding tailocin susceptibility and the
339 specificity of the produced tailocins (Fig. 3, Table 1).

340

341 **Several SRP strains produced tailocins targeting one another – a phenomenon we named**
342 **'bilateral killing'**

343 Analysis of tailocin-mediated interactions in the tested pool of SRPs revealed that 33.9% of the
344 killing events were retaliated (Table 1). Ergo, in the case of 20 of the 351 investigated pairs of
345 SRPs (5.7%), the two strains could target and kill one another with their tailocins. We called the
346 phenomenon 'bilateral killing'. Furthermore, the phenomenon of bilateral killing was more common
347 within the tested subgroup of *Dickeya* spp. (65.6% of killing events retaliated) than within the tested
348 pool of *Pectobacterium* spp. (15.2%), and we observed no bilateral killing between the members of
349 *Dickeya* and *Pectobacterium,* where only unilateral targeting was reported (Table 1). The retaliated
350 killing was very common (80% of killing events) for the mixed pairs of *Dickeya* strains and *M.*
351 *paradisiaca* NCPPB 2511, once again showing clustering between this single NCPPB 2511 strain
352 and *Dickeya* spp.

353

354 **Tailocins of *Dickeya* spp. and *Pectobacterium* spp. are produced from a different ancestral**
355 **origin**

356 Based on the tailocin-associated protein identified by mass spectrometry, we identified gene
357 clusters responsible for the tailocin production in the genomes of the analyzed SRP strains. Among
358 the 25 strains examined, tailocin-encoding clusters were absent only in 2 strains from the analyzed
359 SRP pool, namely in *Pectobacterium brasiliense* A143-S20-M16 and *Pectobacterium atrosepticum*
360 A597-S4-A17. These two strains, together with *Dickeya dianthicola* A260-S21-A16, were also
361 found to be negative for tailocin production in microscopic experiments.

362 We also performed synteny analyses of the tailocin clusters within the *Pectobacterium* and *Dickeya*
363 (including *Musicola*) genera (Fig. 4). These analyses in *Pectobacterium* sp. revealed the homology
364 of the clusters to the one encoding carotovoricin Er (Nguyen et al., 2002) while those in *Dickeya*
365 species and *Musicola* sp. were genetically similar to dickeyocin P2D1 (Borowicz et al., 2023).

366 Furthermore, no SRP strain has been found to possess both two carotovorocin Er and P2D1
367 clusters tailocin simultaneously. The *Pectobacterium* spp. strains exclusively contained

368 carotovorocin Er cluster, while *Dickeya* spp. strains solely harbored cluster encoding P2D1 tailocin.
369 Thus, no strain that contains both types of tailocins has been identified.

370 The overall genomic organization of the tailocin clusters was conserved within each genus (Fig. 4).
371 In *Pectobacterium* species (Fig. 4A), the most prominent difference was a highly variable gene
372 annotated as encoding a tail fiber protein/pyocin knob domain-containing protein, which appeared
373 to contain segments shuffled among different strains (data not shown). Additional, albeit less
374 pronounced, variability was observed in the sequence of the gene coding for a tail tape measure
375 protein. In the case of *P. brasiliense* A143-S20-M16, the cluster region was flanked by genes
376 conserved across other strains; however, instead of a complete tailocin cluster, it contained only a
377 single annotated gene: a mobile element protein gene. In *P. atrosepticum* A597-S4-A17, the region
378 included not only conserved flanking genes but also seven genes present in other clusters and one
379 strain-specific gene. In *Dickeya* species (and *Musicola* sp.) (Fig. 4B), the main differences between
380 strains included (1) the presence of strain-specific genes likely unrelated to tailocin production, (2)
381 the orientation of genes not directly involved in tailocin structural components (such as aromatic
382 acid/H+ transporters and phytase family proteins), (3) sequence variations in intergenic regions
383 (data not shown), and (4) partial sequence differences in tail fiber genes. Notably, for *D. dianthicola*
384 A260-S21-A16, which did not produce tailocins, no significant differences (= the cluster was
385 present in the genome and expressed a high homology to the other Dickeya spp. tailocin clusters)
386 were observed in the tailocin region composition in comparison to other studied *Dickeya* spp.

387

388 **Carotovoricin and dickeyocin clusters are common in the genomes of *Pectobacterium* spp.**
389 **and *Dickeya* spp.**

390 In our pool of analyzed SRPs, not all strains produced tailocins, nor did they harbor the tailocin
391 cluster. This prompted us to determine the prevalence of tailocin clusters across a broader range
392 of SRP strains. We researched 116 complete genomes of *Pectobacterium* spp. for the presence of
393 the carotovoricin cluster and 74 genomes of *Dickeya* and 1 *Musicola* spp. genome for the presence
394 of genes encoding dickeyocin. Within the *Pectobacterium* clade, we identified 96 genomes with

395 homologous regions, meeting the adopted >70% thresholds for both coverage and identity, making
396 it 83% of all tested strains. Twelve genomes showed alignment below the threshold criteria (10% of
397 strains), and 9 (8%) showed no alignment (Dataset S1). Within the joint pool of *Dickeya* spp. and
398 the single *Musicola* spp. genome, we found 52 genomes with an alignment to dickeyocin cluster
399 meeting the 70% threshold (69% of strains), 16 genomes with coverage below 70% (21% of
400 strains), and 7 genomes without alignment (9%) (Dataset S2). Of note, the 6 analyzed complete
401 genomes of the species *P. atrosepticum* present in the NCBI database do not contain a tailocin
402 cluster.
403

404 **Tailocin-killing spectrum activity does not precisely follow the phylogenetic distance of the**
405 **analyzed SRP strains**

406 When comparing the topology of two dendrograms, a phylogenetic tree based on MultiLocus
407 Sequence Analysis (MSLA) and a dendrogram based on the killing spectrum phenotype, our
408 results indicate that the killing spectrum of tailocins does not correlate strictly with the phylogenetic
409 relationships among the tested strains. This suggests that tailocin activity may be influenced by
410 factors other than taxonomic position (Supplementary Fig. S1).

411

412 **Basal level of tailocin production occurs in both *Dickeya* and *Pectobacterium* spp., and a**
413 **significant induction occurs in the presence of hydrogen peroxide**

414 Our previous study reported a basal level of tailocin production in a population of *D. dadantii* 3937
415 not exposed to tailocin-inducive stress (Borowicz et al., 2023). Here, on a pool of six strains,
416 including 3 *Pectobacterium* and 3 *Dickeya* spp. (*P. versatile* A69-S13-O15, *P. quasiaquaticum*
417 A411-S4-F17, *P. aquaticum* A212-S19-A16, *D. dadantii* 3937, *D. chrysanthemi* A604-S21-A17, *D.*
418 *zeae* A586-S18-A17), we aimed to determine whether the basal level of tailocin production occurs
419 in other SRPs. Moreover, we wished to establish whether reactive oxygen species (ROS), namely
420 H_2O_2, induce the production of tailocins in SPRs. As SRPs frequently encounter ROS during
421 infection, with this setup, we wanted to hypothesize whether tailocins may be produced *in situ*
422 during infection (Torres et al., 2006;Reverchon et al., 2016). Basal level of tailocin production was

423 observed for all tested strains. However, the titer of detected tailocins varied between strains, with
424 the highest basal production level observed for our model strain *D. dadantii* 3937 (7×10^4 AU mL^{-1})
425 and the lowest for *P. aquaticum* A411-S4-F17 (5×10^2 AU mL^{-1}) (Fig. 5). For our group of strains,
426 the average tailocin titer was slightly (43%) higher for *Dickeya* than for *Pectobacterium* spp. (Mann-
427 Whitney test, $p<0.05$), with 2.8×10^4 and 1.6×10^4 AU mL^{-1} of culture, respectively.

428 Mitomycin C (0.1 µg mL^{-1}, control) caused a significant induction (approx. 5 to 20-fold) of tailocin
429 production in all strains except *P. quasiaquaticum* A411-S4-F17, for which the increase was not
430 statistically significant compared to no induction (Fig. 5). The highest induction was observed for *D.*
431 *zeae* A586-S18-A17 (19-fold) and *D. chrysanthemi* A604-S21-A17 (20-fold). In the case of *D.*
432 *dadantii* 3937, the induction was relatively low, only 5-fold. However, this low fold change was not
433 connected with low tailocin titer post-induction but resulted from a high basal production level.

434 Hydrogen peroxide-induced tailocin production in all tested strains (approx. 15 to 81-fold) (Fig. 5).
435 For most strains, the applied concentration of hydrogen peroxide (0.88 mM, 0.003%) resulted, on
436 average, in a higher yield of tailocins than when induced by mitomycin C (0.1 µg mL^{-1}). The
437 exceptions were *D. chrysanthemi* A604-S21-A17 and *D. zeae* A586-S18-A17, with comparable
438 results for both inducers. Notably, in the case of *P. quasiaquaticum* A411-S4-F17*,* for which no
439 induction was observed upon treatment with mitomycin C, hydrogen peroxide not only caused
440 induction of tailocin production but also resulted in the highest observed increase compared to
441 basal production levels (81-fold) (Fig. 5).

442

443 **Detectable levels of phage tail-like particles are produced in rotting potato tuber tissue but**
444 **not in river water**

445 Six bacterial strains as described earlier, namely three *Pectobacterium* spp: *P. versatile* A69-S13-
446 O15, *P. quasiaquaticum* A411-S4-F17, *P. aquaticum* A212-S19-A16 and three *Dickeya* spp.: *D.*
447 *dadantii* 3937, *D. chrysanthemi* A604-S21-A17, *D. zeae* (very recently reclassified to *D. parazeae*
448 (Hugouvieux-Cotte-Pattat and Van Gijsegem, 2021)) (A586-S18-A17) were used to inoculate two
449 types of samples: river water (being the isolation source of the strains) and potato tuber tissue

450 (with plant tissue being the primary niche for many SRPs). The goal was to determine whether
451 bacteria in these environments produce noticeable levels of tailocins.

452 Under the applied conditions, tailocin production was not detected in inoculated water samples
453 (Table 2.). On the contrary, tailocins were detected in macerated potato tuber tissue, not just in the
454 case of four strains that caused soft rot symptoms (*P. versatile* A69-S13-O15, *D. dadantii* 3937, *D.*
455 *chrysanthemi* A604-S21-A17, *D. zeae* A586-S18-A17) but also in healthy tuber tissue surrounding
456 the inoculation site in the case of two strains which failed to cause potato tuber rotting (*P.*
457 *aquaticum* A212-S19-A16 and *P. quasiaquaticum* A411-S4-F17) (Table 2) (Supplementary Fig.
458 S2).

459

460 **Producing tailocins alone does not ensure success against a susceptible strain under**
461 **conditions of an 'even start'**

462 We aimed to determine how *D. solani* A623-S20-A17 (*Ds*) performs in two-strain co-cultures with
463 strains susceptible to the strain's tailocins or/and targeted by tailocins of the competitors (Fig. S3).
464 To monitor the proportion of *Ds* relative to competing strains in mixed cultures, we selected a
465 spontaneous rifampicin-resistant mutant of *Ds* (*Ds* RIF). Using *Ds* RIF in the experiments allowed
466 us to determine the percentage of *Ds* in the co-culture using selective media. Prior to the main
467 experiment, rifampicin-resistant mutant *Ds* RIF was tested against the wt strain (*D. solani* A623-
468 S20-A17) to produce tailocins, growth rate, and ability to compete with the wt *in vitro*. The mutant
469 was not compromised regarding the growth rate in 0.1 TSB (Supplementary Fig. S4), was positive
470 for tailocin production, and neither strain achieved dominance in a co-culture with its wt variant.
471 These results indicate that the rifampicin-resistant *Dickeya* spp. strains are not compromised in
472 competitiveness under the applied experimental conditions (Fig. 6).

473 Next, the *Ds* RIF strain was set to compete with three types of competitors: susceptible to the
474 tailocins of *Ds* RIF (SET 1), targeting *Ds* RiF (SET 2), or involved with *Ds* RIF in a bilateral killing-
475 type interaction (SET 3). The success of a strain was defined as its end-point dominance in the co-
476 culture (>90% of total CFU) (Fig. S3). Each strain in a co-culture was ensured an even start (1:1

477 mix). Theoretical prediction of the competition's winner based on tailocin effects was compared to
478 experimental results. We found that two competitor strains targeting *Ds* RIF with their tailocins had
479 prevailed over *Ds* RIF (SET 2), according to what would be expected if tailocins had a key role in
480 the tested setup. However, the Ds RIF outcompeted only a single susceptible strain from SET1
481 and only in the presence of H_2O_2. This shows that in a competition between SRPs, at least under
482 the applied *in vitro* conditions and with an even start scenario, tailocins alone do not guarantee the
483 success of a producer against a susceptible rival.

484

485 **Discussion**

486 Our study revealed that most SRP strains produce phage tail-like particles, and only three
487 SRP strains from the pool were tailocin-negative. The *P. brasiliense* A143-S21-A16 and *P.*
488 *atrosepticum* A597-S4-A17 lacked tailocin clusters, while *D. dianthicola* A260-S21-A16 had a
489 complete cluster but did not produce tailocins. This could be due to reliance on other competitive
490 strategies (Hibbing et al., 2010) or regulation by specific environmental factors not present in the
491 experiment (Bellieny-Rabelo et al., 2019). Furthermore, we assessed the prevalence of tailocin
492 clusters in 190 high-quality, complete genomes of SRP bacteria and found that 83% of
493 *Pectobacterium* spp. and 69% of *Dickeya* spp. genomes contain clusters encoding tailocins. This
494 indicates that tailocins are a very common feature among SRP and may suggest that their
495 production remains important to mediate the ecological success of these bacteria (Ma et al., 2007).
496 The prevalence of tailocins in SRP aligns with the previous observations documenting their
497 presence in various bacterial species, including ubiquitously present *Pseudomonas* spp. (Carim et
498 al., 2021), *Pantoea* spp. (Stice et al., 2023) and *Streptomyces* spp. (Nagakubo et al., 2021). Such
499 global observations have, however, never been done for Soft Rot *Pectobacteriaceae*.

500 It has been repeatedly demonstrated that most tailocins exhibit a narrow killing range
501 typically limited to closely related strains. Still, most of the analysis has been conducted on a small
502 set of strains either at the species level or the genus level (Granato et al., 2019). Some
503 observations, however, are that some phage tail-like particles can cross species boundaries (Yao
504 et al., 2017;Principe et al., 2018). Our work involved a large group of strains encompassing strains

505 belonging to three different genera tested for mutual tailocin-based interactions. It reveals that
506 some SRP strains have a broad range of activity, producing tailocins that can kill more than 30% of
507 the tested strains representing different species. Enlarging the number of tested strains within a
508 given species complex also expands the spectrum of action of each tested tailocin. One should,
509 therefore, remain cautious about the accepted narrow killing range of tailocins previously
510 described.

511 Interestingly, while some *Dickeya* spp. produced tailocins were able to kill *Pectobacterium*
512 spp. cells, the opposite was not observed in our study. Due to their evolutionary association with
513 bacteriophage tails (Ghequire and De Mot, 2015), it is suggested that tailocins might retain some
514 degree of flexibility in the host range, as known from phage-host interactions (Backman et al.,
515 2024a). Inter-genus activity may remain an important trait in competition as mixed bacterial
516 communities involving both genera are frequently observed in the field, and the ability to target a
517 broader range of competitors could confer a selective advantage (Perombelon, 1988; Charkowski,
518 2009;2018; Barny et al., 2024).

519 Likewise, the observation of bilateral killing where two strains reciprocally target each other
520 with tailocins has not been extensively documented. The higher incidence of bilateral killing within
521 *Dickeya* spp. compared to *Pectobacterium* spp. suggests some (yet unknown) ecological factors
522 may more often promote this behavior in *Dickeya* spp. than in *Pectobacterium* spp. This is
523 somehow surprising, given that multiple species of SRP bacteria are often found together in the
524 same environments (Toth et al., 2021a;van der Wolf et al., 2021). In such settings, these various
525 species would be expected to experience similar ecological pressure and benefit from reciprocal
526 tailocin production (Shyntum et al., 2019). It may suggest that *Pectobacterium* spp., differently from
527 *Dickeya* spp., However, this assumption regarding tailocins still requires experimental validation.

528 Phage tail-like particles isolated from both SRP genera exhibit similar morphological
529 characteristics; however, the different origins of tailocin clusters in *Pectobacterium* spp. and
530 *Dickeya* spp. indicates that these phage tail-like particles have been acquired independently in
531 those genera. All analyzed clusters present in *Dickeya* spp. express homology to the one encoding
532 P2D1 tailocin of *D. dadantii* 3937 (Borowicz et al., 2023), whereas all clusters present in

533 *Pectobacterium* spp. express homology to the cluster encoding carotovoricin Er (Yamada et al.,
534 2006). The fact that these phage tail-like particles were observed in all *Dickeya* spp. and most of
535 the *Pectobacterium* spp. strains tested indicated that the tailocin clusters were acquired before
536 species diversification in both genera and were maintained in most species rather untouched. As
537 tailocins are large protein complexes and their synthesis should be a burden for a producer cell,
538 their conservation suggests that tailocin production plays an important role in the ecology of these
539 bacteria (Glasner et al., 2008;Hugouvieux-Cotte-Pattat, 2016).

540 The genes encoding tailocin fiber proteins expressed the lowest homology in the analyzed
541 tailocin clusters both in *Dickeya* spp. and *Pectobacterium* spp. strains analyzed. This confirms that
542 fiber proteins play a pivotal role in determining the specificity of tailocins and their killing range, as
543 reported earlier (Dams et al., 2019). Fiber proteins are the most important determinants in
544 recognizing target bacterial cells (Backman et al., 2024a). This specificity is crucial for the success
545 of tailocins as killing agents, enabling them to discriminate between susceptible and resistant
546 bacterial strains. In bacteriophages, receptor-binding proteins are known to undergo positive
547 selection to adapt to variations in surface receptors of target bacteria, leading to high variability
548 even among closely related strains (Bertozzi Silva et al., 2016). In SRP, the killing spectrum of
549 tailocins, determined by the sequence of fiber proteins, appears to be more dependent on
550 ecological factors and the pressure of host-pathogen interactions encountered by the individual
551 strains than the evolutionary relationships between them.

552 Although mitomycin C is widely used to trigger tailocin induction under laboratory
553 conditions, in natural and agricultural environments, the likely inducers of tailocins remain
554 unknown. In the proof-of-concept experiment, we determined that hydrogen peroxide (a reactive
555 oxygen species agent) is a potent inducer of SRP phage tail-like particles. It is well acknowledged
556 that reactive oxygen species are the common signals for the induction of phage tail-like particles in
557 bacteria (Scholl, 2017). In general, hydrogen peroxide is known to activate the ROS response
558 (Storz et al., 1990;Erill et al., 2007), which is sequentially a recognized tailocin induction pathway.
559 During infection, SRP bacteria often encounter oxidative stress in plant hosts when the plant
560 mounts an immune response (Reverchon and Nasser, 2013;Jiang et al., 2016). Therefore, the

561 induction of tailocins by reactive oxygen species may serve as a mechanism for bacterial survival
562 in their natural settings, aiding in eliminating competing bacteria under stressful conditions.
563 Globally, the ability of plant pathogenic bacteria, including SRP, to produce tailocins both at a basal
564 level and under stress (inducive conditions), such as exposure to ROS, may lead to important
565 ecological consequences. The ability to regulate tailocin production in response to such oxidative
566 stress might give SRP a competitive edge, allowing them to eliminate competitors in the plant
567 environment when they must fight with all other microorganisms to establish a successful infection
568 (Backman et al., 2024b).

569 The variable level of response to mitomycin C and hydrogen peroxide we observed in SRP
570 strains highlights the still unraveled complexity of tailocin regulation. Among the tested strains, *D.*
571 *chrysanthemi* A604-S12-A17, *D. zeae (parazeae*) A586-S18-A17, and *P. versatile* A69-S13-O15
572 showed the highest increase in tailocin production with mitomycin C. In contrast, hydrogen
573 peroxide led to the highest production in *P. quasiaquaticum* A411-S4-F17. This suggests that in
574 SRP, depending on the strain and type of inducer, the production of tailocins is slightly differently
575 regulated. Similar observations were made for prophages, where different environmental factors
576 induced phages with different efficiency (Casjens, 2003;Varani et al., 2013).

577 SRP strains tested in this study were isolated from one selected environment. The set
578 comprised both aquatic and non-aquatic strains, most of which (except *P. aquaticum* and *P.*
579 *quasiaquaticum* strains known to be avirulent on plants) cause symptoms in plants when
580 transmitted to agricultural settings (Ben Moussa et al., 2022). We found that tailocins are produced
581 in infected potato tubers but not in river water. This aligns with the other studies in which tailocins
582 were found to be produced in nutrient-rich, competitive environments (Carim et al., 2021), like
583 decaying plant material, rather than in low-stress environments like river water (Heiman et al.,
584 2023). The finding that tailocins were detected in (rotting) potato tissue but not in river water
585 suggests that the environmental conditions in the potato tubers provide a stronger stimulus for
586 tailocin production, likely due to the presence of ROS, plant exudates, cell wall degradation
587 products, and microbial competition, as opposed to water which for many strains is neither the
588 target niche nor the competitive environment but rather serves as a translocation medium from one

host to another (Pédron and Van Gijsegem, 2019;van der Wolf et al., 2021). This observation may be particularly interesting in the case of SRP bacteria, which compete with each other and other microorganisms for resources present within the plant tissues (Reverchon and Nasser, 2013). The potato tuber tissue represents a primary ecological niche for SRP pathogens (Perombelon and Kelman, 1980;Pérombelon and Salmond, 1995). Consequently, the presence of tailocins in both rotting tissue and the healthy surrounding tissue suggests that tailocin production may be part of the virulence strategy to minimize the risk of competitive infections.

Surprisingly, SRP strains belonging to species that are not found on plants (Portier et al., 2020) and do not provoke evident disease symptoms (*vide*: *P. quasiaquaticum* A411-S4-F17 and *P. aquaticum* A212-S19-A16) still produce tailocins in the plant environment but not in river water. The low nutrient availability, the cost of production of this complex machinery and the few direct competitors of SRP bacteria in river water likely explain the absence of tailocin production in this environment (Lamichhane and Bartoli, 2015) (Bradford et al., 2013;Toth et al., 2021b). The fact that these tailocin clusters were not eliminated from these water-associated species and were induced by the same trigger as plant-associated species suggests that tailocin production is not solely tied to SRP virulence on plants but could also serve in another niche where these bacteria encounter stress. It has been suggested that these water-associated species could be associated with insect larvae in the water stream (Ben Moussa et al., 2023). Whether tailocin production is triggered in such an environment remains to be determined.

Multiple factors play roles in the competition between microbes, with success being the sum of factors/traits of varying importance depending on the environmental context (Hibbing et al., 2010). In our study, in mixed populations of tailocin producers and targets, tailocins were not enough to ensure the dominance of producers over susceptible strains under the scenario of 'even start'. In conjunction with how widespread tailocin clusters are among SRP, this led us to conclude that tailocin-mediated success is often conditional. It is well known that SRP bacteria use diverse weaponry for intra-genus and intra-genus interactions; therefore, tailocin production may be just one possibility to compete with kin microbes.

617 Several factors can influence the outcome, including environmental conditions, strain-
618 specific interactions, and the relative abundance of producers versus susceptible strains. This
619 study explored an 'even start' scenario, where tailocin-producing and susceptible competitors were
620 mixed in equal cell numbers. It was shown that in bacterial populations, even when one strain has
621 an antagonistic advantage (like producing phage tail-like particles), an equal start condition can
622 neutralize this advantage because the susceptible strain is not immediately overwhelmed (Ross-
623 Gillespie and Kummerli, 2014). Moreover, tailocin production, unlike the secretion of most bacterial
624 antimicrobials, requires the lysis of the producer cells. Ergo, it can negatively influence the growth
625 rate of the producer's population. This burden can be assumed negligible when the high producer-
626 to-target ratio is high. Therefore, we speculate that tailocin production may be useful to SRP
627 bacteria to protect already conquered niches from invading competitors, a process named niche
628 exclusion (Bauer et al., 2018).

629 To conclude, our study considerably advances the understanding of the role of phage tail-
630 like particles in the ecology of Soft Rot *Pectobacteriaceae* (SRP). Furthermore, our study expands
631 the knowledge of tailocin function in inter-genus competition among SRPs, their responsiveness to
632 environmental factors, and their potential role in bacterial survival in various ecological niches.

633

Author Contributions

MB: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing, DMK: Conceptualization, Investigation, Supervision, Visualization, Writing – original draft, Writing – review & editing, MS: Investigation, Methodology, Visualization, MN: Investigation, Methodology, Visualization, Writing – original draft, IM: Investigation, Methodology, Writing – original draft, PC: Methodology, Writing – original draft, JP: Conceptualization, Investigation, Methodology, Writing – review & editing, MAB: Conceptualization, Investigation, Methodology, Writing – review & editing, PYC: Investigation, Methodology, JD: Conceptualization, Investigation, Methodology, RC: Conceptualization, Data curation, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing

Competing Interest Statement

The authors declare no competing interests.

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669 **Tables**

670 **Table 1. Tailocin-mediated killing events for the tested pool of 27 SRP strains.**

671

Group	Total interactions[A]	Killing events		Bilateral killing		
		No.	% total	No.	% total	% killing events
All SRPs	702	118	16.8%	40	5.7%	33.9%
Pectobacterium spp.[B]	306	66	21.6%	10	3.3%	15.2%
Dickeya spp.[C]	56	32	57.1%	21	37.5%	65.6%
Mixed genera[D]	340	19	5.6%	9	2.6%	47.37%
Psp vs *Dsp*	162	0	0%	0	0%	0%
Dsp vs *Psp*	162	6	3.7%	0	0%	0%
Dsp vs *M. par.*	8	5	62.5%	4	50%	80%
M. par vs *Dsp*	8	6	75%	5	62.5%	83.3%
M. par vs *Psp*	18	2	11.1%	0	0%	0%
Psp vs *M. par*	18	0	0%	0	0%	0%

672 [A] total interactions - the number of combinations where each strain from a subset was tested both for the
673 potential to produce tailocins and for tailocin susceptibility (potential target)
674 [B] both strains in the tested interaction assigned to *Pectobacterium* spp. (total of 18 strains tested)
675 [C] both strains in the tested interaction assigned to *Dickeya* spp. (total of 8 strains tested)
676 [D] each strain of the pair representing a different genus; *Psp* – *Pectobacterium* spp.; *Dsp* – *Dickeya* spp.; *M.*
677 *par* – *Musicola paradisiaca* NCPPB 2511. The first strain in each combination is the tailocin producer.
678
679
680

681 **Table 2. Production of tailocins in river water and potato tubers.** Qualitative tailocin
682 production in different conditions for various bacterial strains. The strains tested include *P.*
683 *versatile* A69-S13-O15, *P. quasiaquaticum* A411-S4-F17, *P. aquaticum* A212-S19-A16, *D.*
684 *dadantii* 3937, *D. chrysanthemi* A604-S21-A17, and *D. zeae* A586-S18-A17. Tailocin
685 production was assessed in infected potato tubers, in Durance River water, and as a positive
686 control following Mitomycin C and H_2O_2 induction in TSB (Tryptic Soy Broth). The symbols
687 represent the following: "+" indicates detectable tailocins in the sample with the applied
688 method; "-" indicates undetectable tailocins in the sample with the applied method; "*"
689 indicates that tailocins were detectable if tuber tissue in direct contact with inoculated
690 bacteria was pooled from three potato tubers.

691

Strain	**Tailocins production**			
	In Durance River water	**In infected potato tuber**	**Mitomycin C induction in TSB**	**H_2O_2 induction in TSB**
P. versatile A69-S13-O15	-	+	+	+
P. quasiaquaticum A411-S4-F17	-	+*	+	+
P. aquaticum A212-S19-A16	-	+*	+	+
D. dadantii 3937	-	+	+	+
D. chrysanthemi A604-S21-A17	-	+	+	+
D. zeae A586-S18-A17	-	+	+	+

+ tailocins detectable in the sample in the applied method

- tailocins undetectable in the sample in the applied method

** tubers were asymptomatic, but tailocins were detectable in tuber tissue at the inoculation site; the material was collected and pooled from three potato tubers.*

692

693

694 **Figures**

695 **Fig. 1 TEM images picturing tailocins isolated from the 27 analyzed SRP strains.**

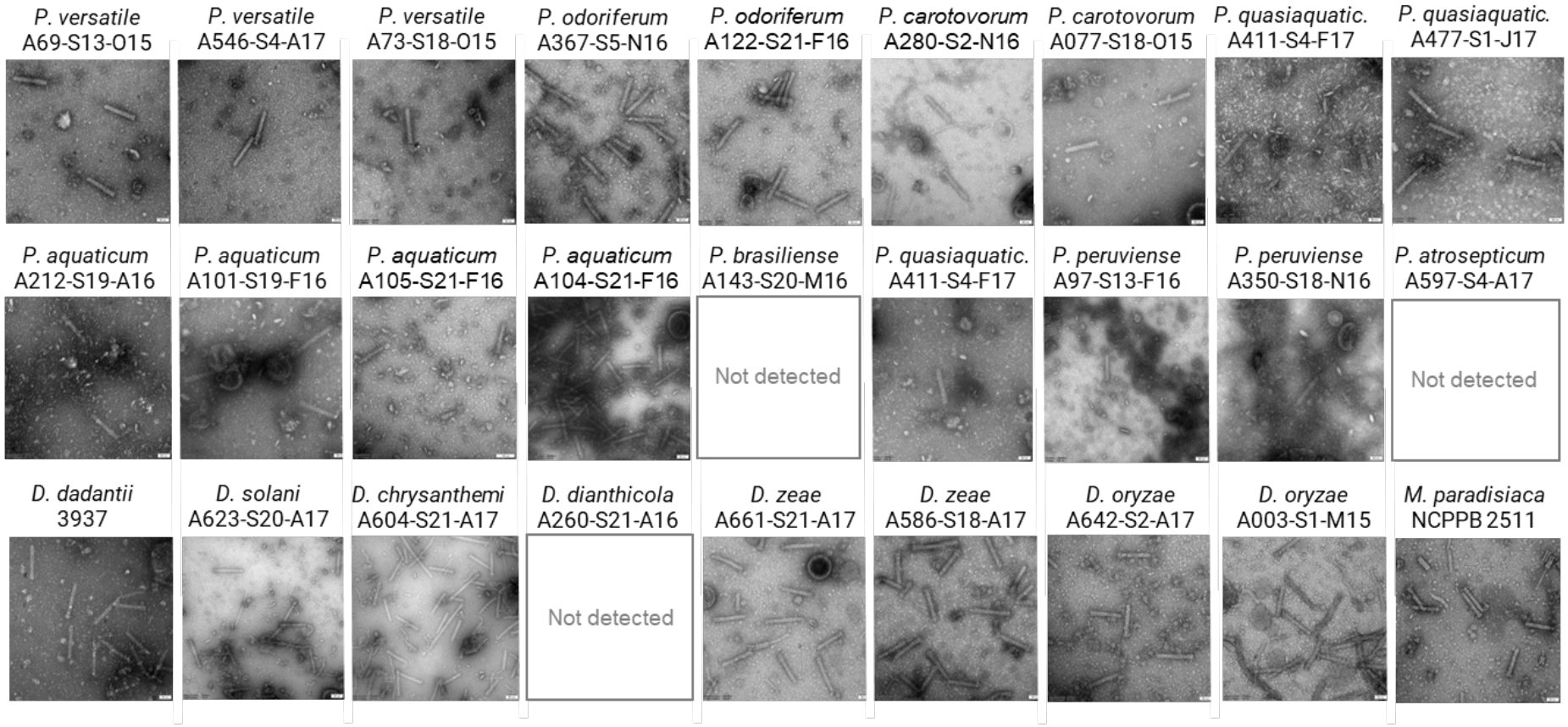

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Fig. 2 Images of the representative tailocins generated based on AFM scans. AFM imaging was performed using the JPK NanoWizard® 4 (NanoScience) in QI™ in the quantitative imaging (QI)™ force spectroscopy mode. Images were processed using JPK Data Processing software. Panel A shows the AFM scan of Dickeyocin P2D1 produced by *D. dadant*ii 3937, panels B-H. show the highest quality 3D visualizations of representative tailocin particles, generated based on AFM scans, for the following strains: *D. dadantii* 3937*, D. solani* A623-S20-A17, *D. zeae* A661-S21-A17. *M. paradisiaca* NCPPB 2511, *P. odoriferum* A367-S5-N16, *P. aquaticum* A105-S21-F16, *P. aquaticum* A104-S21-F16.

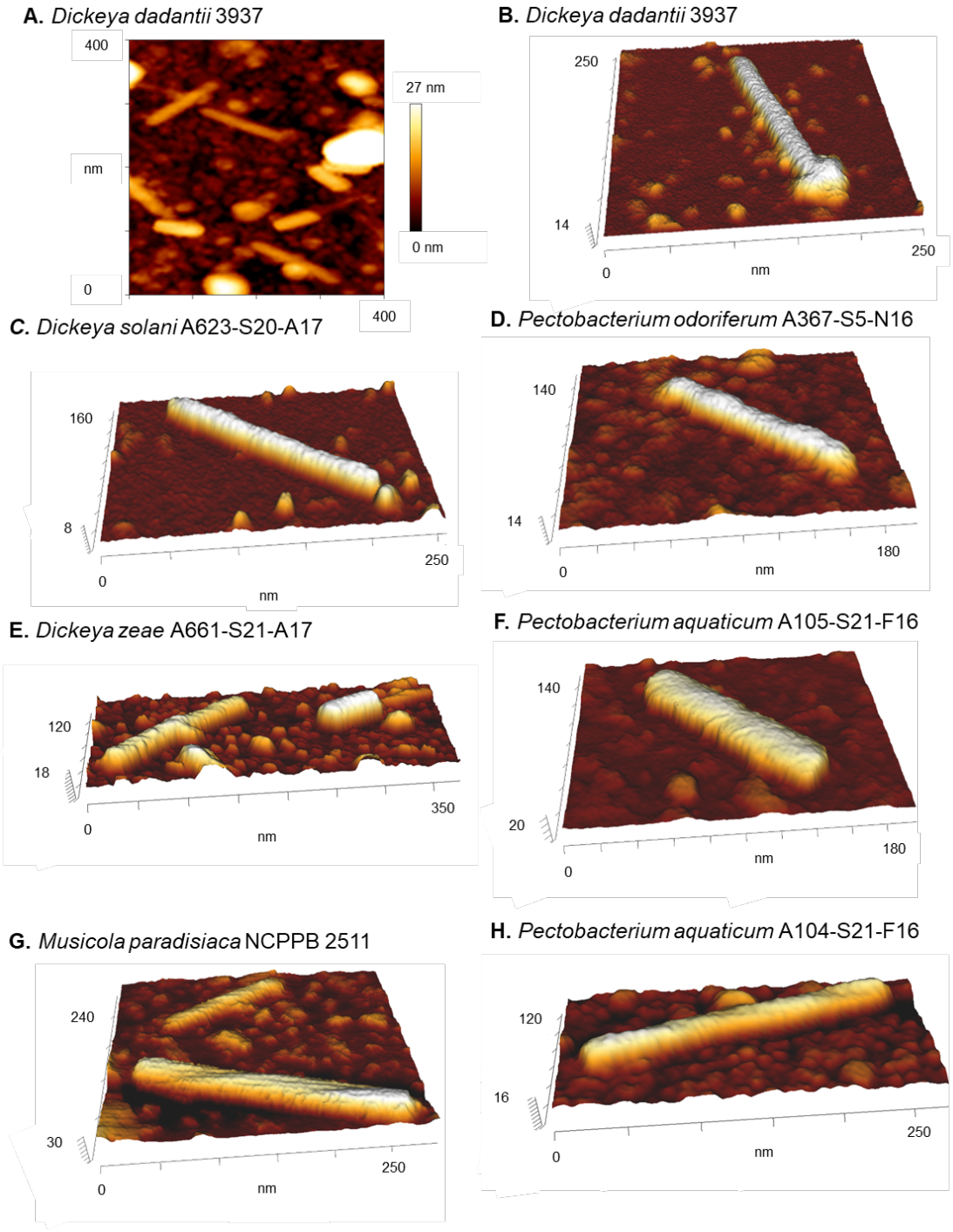
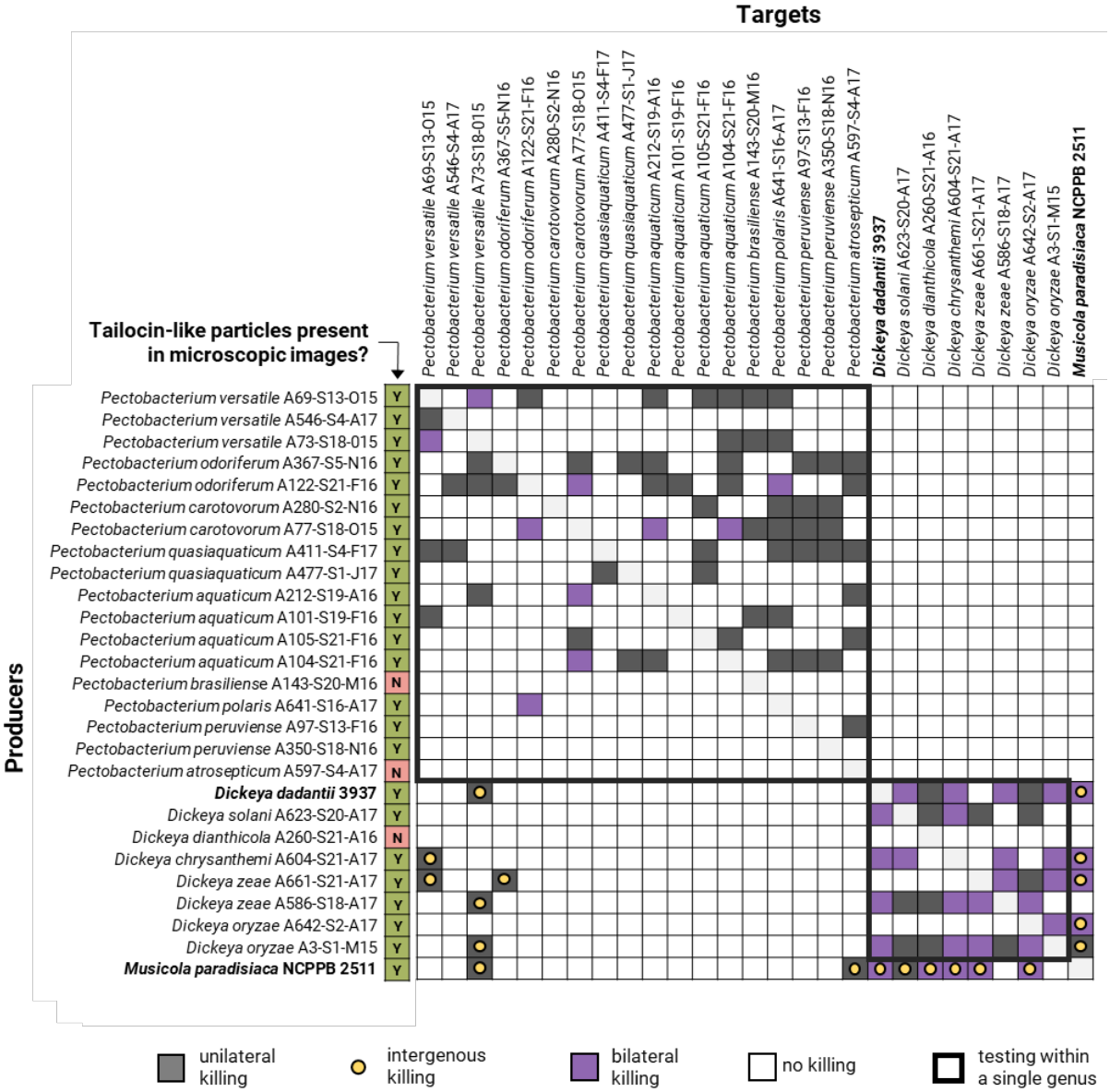
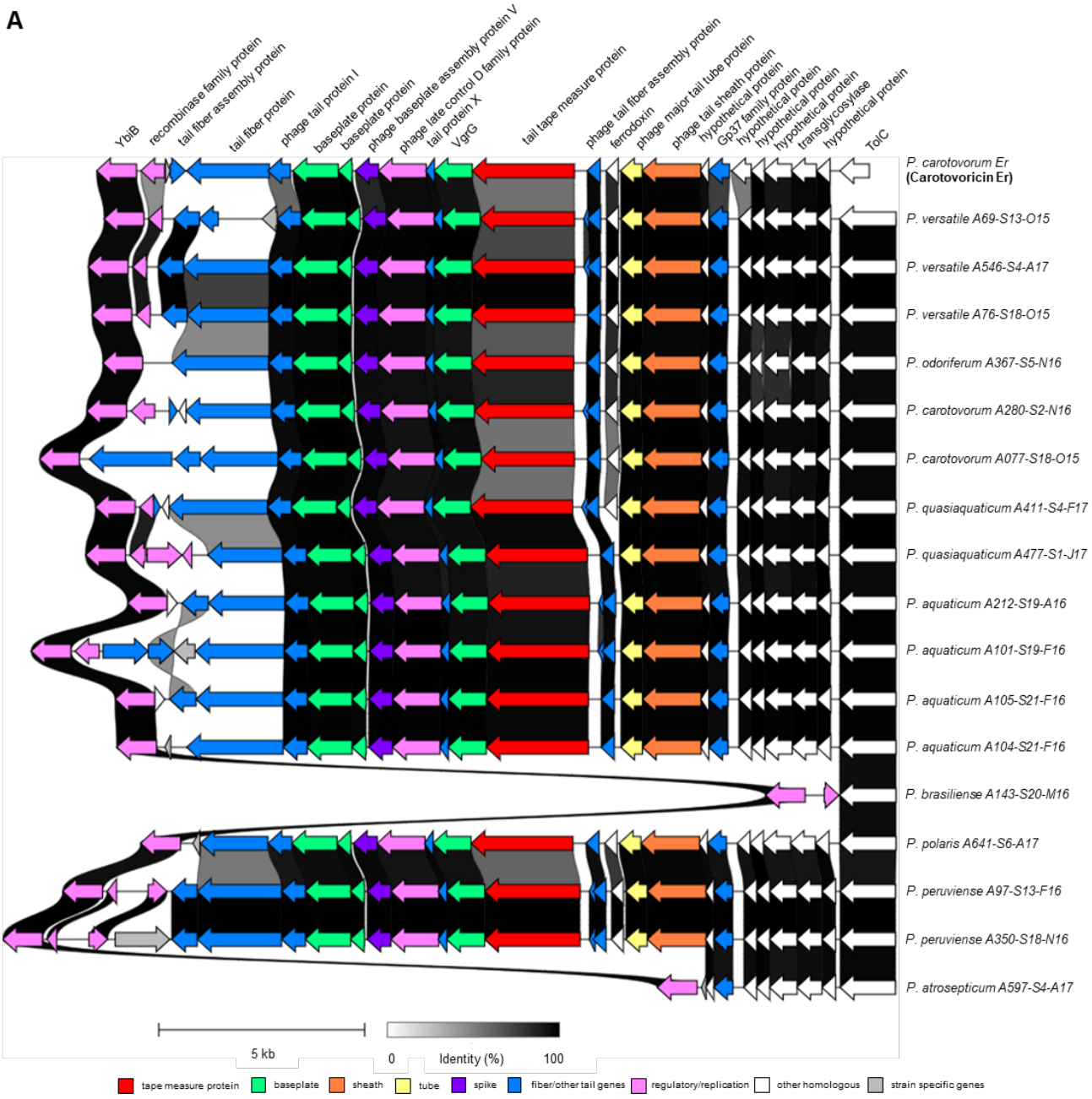


Fig. 3 Matrix of tailocin-mediated interactions within the group of tested SRP strains. The tested pool comprised 27 strains: 25 isolates from the Durance River water and two type strains: *D. dadantii* 3937 and *M. paradisiaca* NCPPB 2511 (in bold). Grey-shaded cells indicate the susceptibility of a target strain to the tailocins of a given producer strain (unilateral killing). Unilateral killing where the producer and the target belong to different genera, is additionally marked by a yellow dot. Lillac cells also indicate the susceptibility of the target strain to the investigated tailocin, but in particular situations where the target strain was observed to produce 'retributive' tailocins against the producer (bilateral killing). A dedicated column indicates whether tailocins were (Y) or were not (N) detected by microscopic imaging in culture supernatants of the tested strains following treatment with mitomycin C.



722 **Fig. 4 Comparison of tailocin clusters in the analyzed group of SRPs.** The upper panel
723 (A) depicts regions homologous to the carotovoricin-encoding cluster typical for
724 *Pectobacterium* spp. (reference: Carotovoricin Er accession: AB045036.1, *P. versatile* A69-
725 S13-O15 RMBQ01000001.1, positions in the genome: 94551-113781). The lower panel (B)
726 shows regions homologous to dickeyocin P2D1, typical for *Dickeya* spp. and also present in
727 *Musicola paradisiaca* NCPPB 2511 (reference: *D. dadantii* 3937 NC_014500.1, positions in
728 the genome 2730482-2757061).



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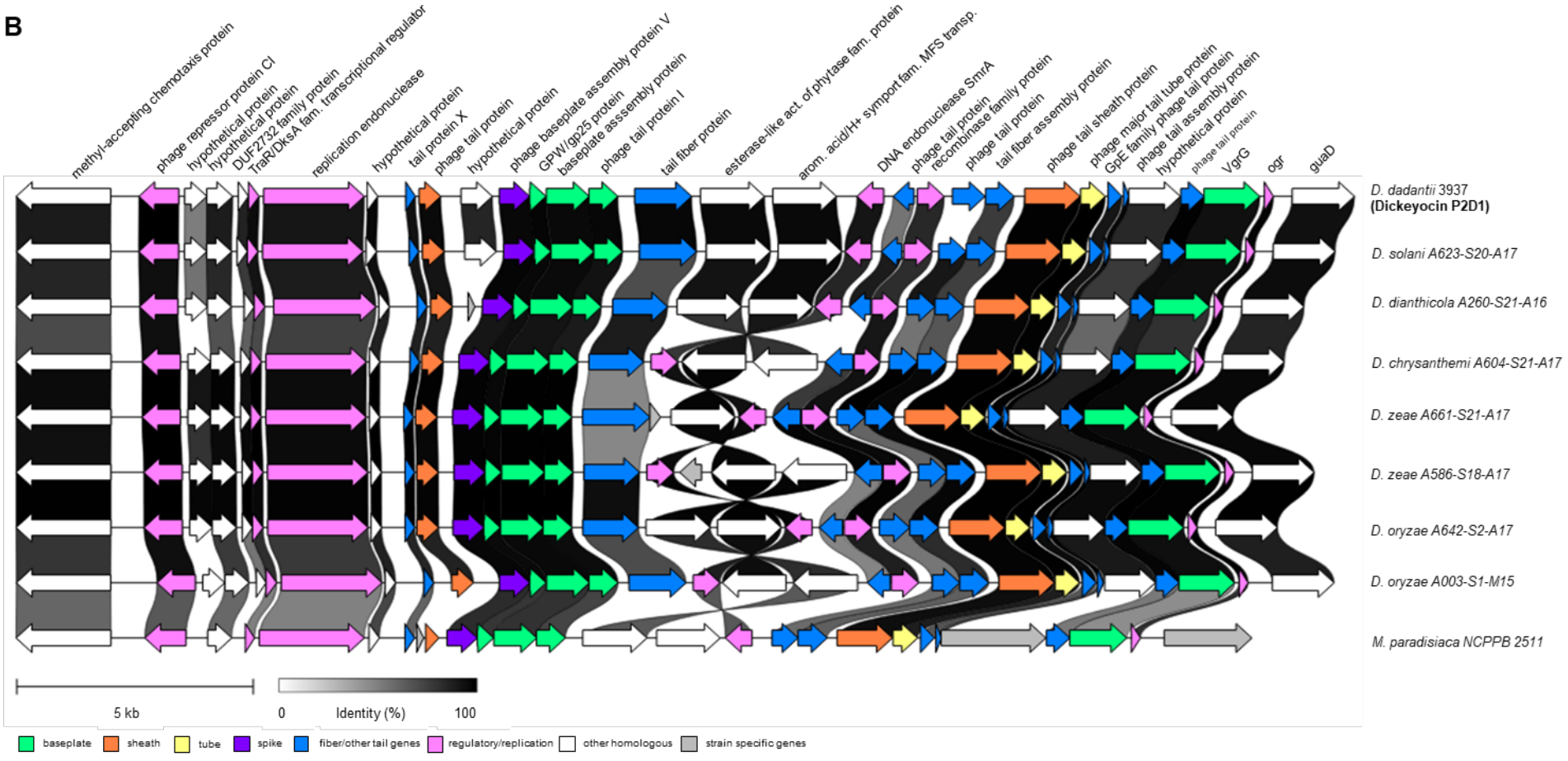
B
methyl-accepting chemotaxis protein
phage repressor protein CI
hypothetical protein
hypothetical protein
DUF2732 family protein
TraR/DksA fam. transcriptional regulator
replication endonuclease
hypothetical protein
tail protein X
phage tail protein
hypothetical protein
phage baseplate assembly protein V
GPW/gp25 protein
baseplate asssembly protein
phage tail protein I
tail fiber protein
esterase-like act. of phytase fam. protein
arom. acid/H+ symport fam. MFS transp.
DNA endonuclease SmrA
phage tail protein
recombinase family protein
phage tail protein
tail fiber assembly protein
phage tail sheath protein
phage major tail tube protein
GpE family phage tail protein
phage tail assembly protein
hypothetical protein
phage tail protein
VgrG
ogr
guaD
D. dadantii 3937
(Dickeyocin P2D1)
D. solani A623-S20-A17
D. dianthicola A260-S21-A16
D. chrysanthemi A604-S21-A17
D. zeae A661-S21-A17
D. zeae A586-S18-A17
D. oryzae A642-S2-A17
D. oryzae A003-S1-M15
M. paradisiaca NCPPB 2511
5 kb
0
Identity (%)
100
baseplate
sheath
tube
spike
fiber/other tail genes
regulatory/replication
other homologous
strain specific genes

732

733 **Fig. 5 Basal and induced tailocin production levels in six *Pectobacterium* spp. and**
734 ***Dickeya* spp strains.** The graph shows the logarithm of the arbitrary tailocin units per mL of
735 culture (Log AU mL^{-1}) for three strains of *Pectobacterium* spp. (*P. versatile* A69-S13-O15, *P.*
736 *quasiaquaticum* A411-S4-F17, *P. aquaticum* A212-S19-A16) and three strains of *Dickeya*
737 spp. (*D. dadantii* 3937, *D. chrysanthemi* A604-S21-A17, *D. zeae* A586-S18-A17). The basal
738 production levels (no induction) are compared to those with mitomycin C or hydrogen
739 peroxide after induction. Averages from 2 experiments, each consisting of 3 separate
740 inductions, are shown, and error bars represent the standard deviation.

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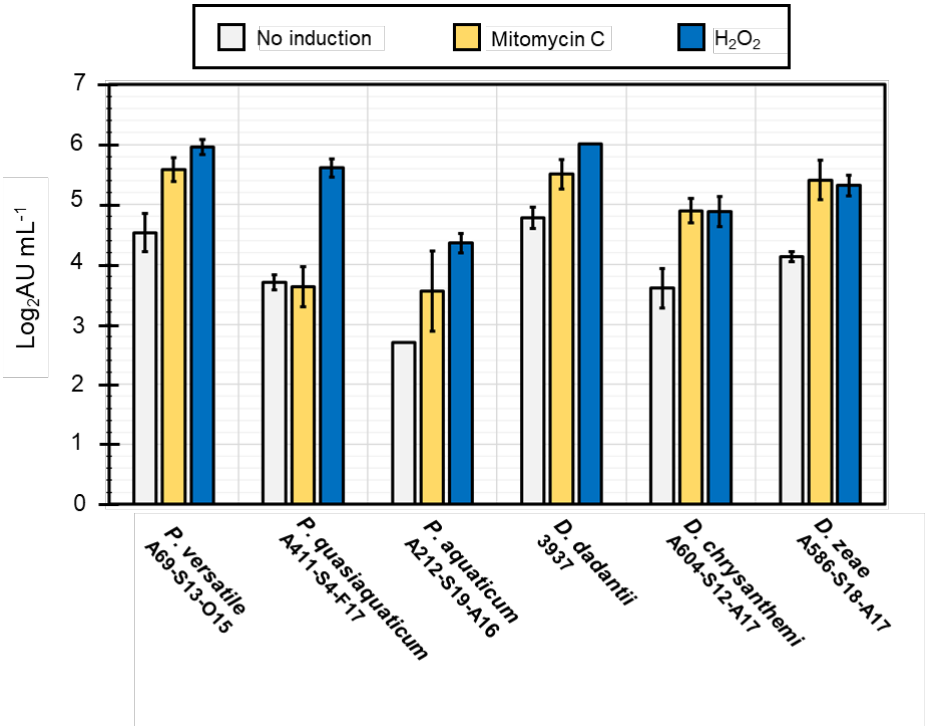

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745 **Fig. 6 Competition between *D. solani* A623-S20-A17 RIF^R (*Ds* RIF) and other SRP**
746 **strains in two-strain co-cultures.** The success was defined as the end-point dominance of
747 a given strain in the co-culture (>90% of total CFU), with both strains ensured with an even
748 start (1:1 mix; ca. 10^7 CFU/ml/strain). The theoretical winners, predicted based on tailocin-
749 mediated interactions, were compared with those that were experimentally established. Co-
750 cultures were conducted for 22 h in 0.1 TSB alone or supplemented with 0.88 mM H_2O_2
751 (tailocin-inductive conditions). SET 1: strains sensitive to tailocins of *Ds* RIF. SET 2: strains
752 producing tailocins against *Ds* RIF. SET 3: strains sensitive to tailocins of *Ds* RIF and
753 producing tailocins targeting this strain (bilateral killing). *ctrl* – control experiment; competition
754 between *Ds* RIF and the *wt* variant of this strain.

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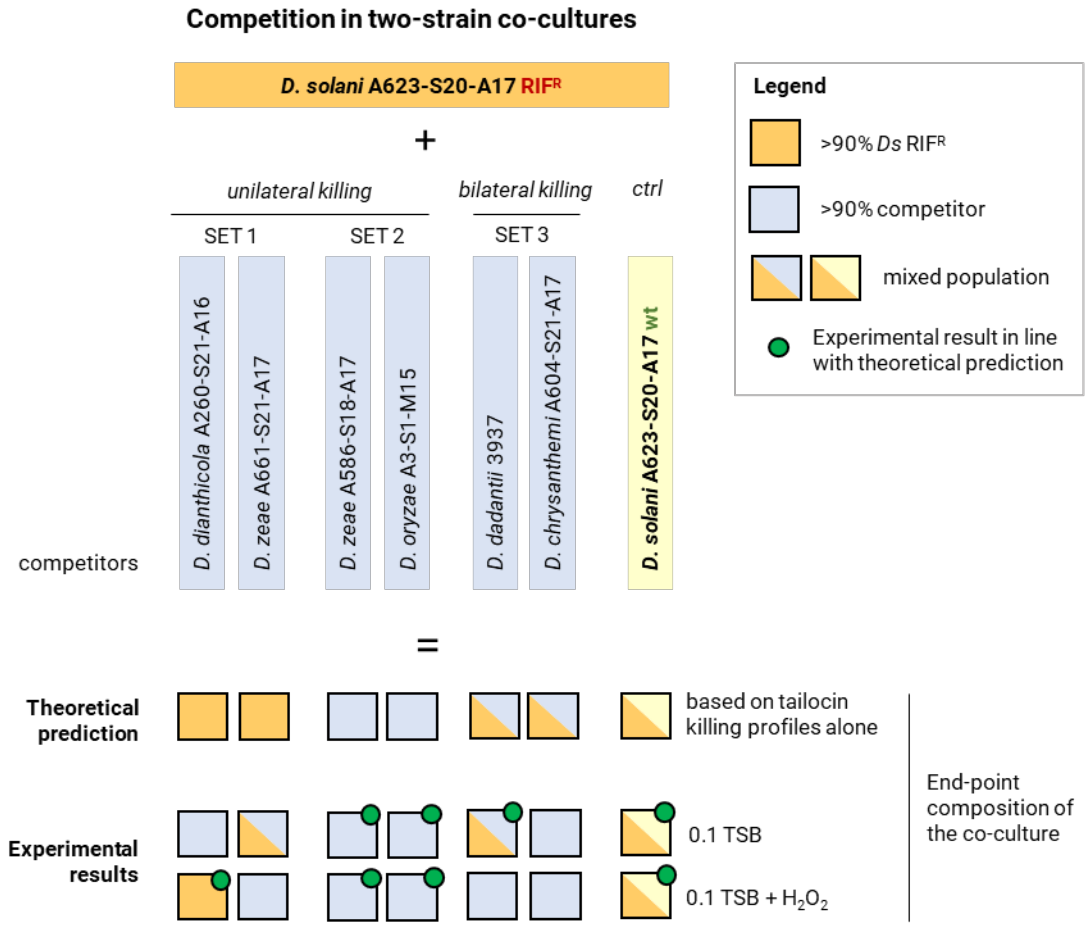

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760 **Supplementary Tables**

761 **Table S1. List of bacterial strains used in this study.**

Strain	Isolation source, country, year	Reference	GenBank accession no. for genome
Dickeya chrysanthemi A604-S21-A17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_020406775.2
Dickeya dianthicola A260-S21-A16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_020406895.2
Dickeya oryzae A003-S1-M15	Durance river. France. 2015	(Ben Moussa et al., 2022)	GCA_020406815.2
Dickeya oryzae A642-S2-A17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_020406685.2
Dickeya solani A623-S20-A17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_020406975.1
Dickeya zeae A586-S18-A17 recently reclassified to *D. parazeae*)	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_020520245.1
Dickeya zeae A661-S21-A17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_020406575.2
Pectobacterium aquaticum A101-S19-F16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_003382625.2
Pectobacterium aquaticum A104-S21-F16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_003382595.2
Pectobacterium aquaticum A105-S21-F16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_003382585.2
Pectobacterium aquaticum A212-S19-A16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_003382565.3
Pectobacterium atrosepticum A597-S4-A17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_020406655.2
Pectobacterium brasiliense A143-S20-M16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_013449685.1
Pectobacterium carotovorum A077-S18-O15	Durance river. France. 2015	(Ben Moussa et al., 2022)	GCA_020520265.1
Pectobacterium carotovorum A280-S2-N16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_020406635.1
Pectobacterium odoriferum A122-S21-F16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_013450045.1
Pectobacterium odoriferum A367-S5-N16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_022507245.1
Pectobacterium peruviense A350-S18-N16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_003312355.2
Pectobacterium peruviense A97-S13-F16	Durance river. France. 2016	(Ben Moussa et al., 2022)	GCA_003312345.1
Pectobacterium polaris A641-S6-A17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_020406835.1
Pectobacterium quasiaquaticum A477-S1-J17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_014946775.2
Pectobacterium quasiaquaticum A411-S4-F17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_014946845.1
Pectobacterium versatile A546-S4-A17	Durance river. France. 2017	(Ben Moussa et al., 2022)	GCA_020406615.1
Pectobacterium versatile A69-S13-O15	Durance river. France. 2015	(Ben Moussa et al., 2022)	GCA_004296725.1
Pectobacterium versatile A73-S18-O15	Durance river. France. 2015	(Ben Moussa et al., 2022)	GCA_020865565.1
Dickeya dadantii 3937	*Saintpaulia* sp. France. 1972	(Kotoujansky et al., 1982)	GCA_000147055.1
Musicola paradisiaca NCPPB 2511	*Musa paradisiaca*. Colombia. 1973 *	(Hugouvieux-Cotte-Pattat et al., 2021)	GCA_000400505.1

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763 **Table S2. List of sensitive strains used in a spot assay to detect tailocins from the**
764 **given producer strains.**

765

Tailocin producer	Sensitive strain
P. versatile A69-S13-O15	*P. aquaticum* A105-S21-F16
P. quasiaquaticum A411-S4-F17	*P. atrosepticum* A597-S4-A17
P. aquaticum A212-S19-A16	*P. aquaticum* A105-S21-F16
D. dadantii 3937	*M. paradisiaca* NCPPB 2511
D. chrysanthemi A604-S21-A17	*D. zeae* A586-S18-A17
D. zeae A586-S18-A17 (recently reclassified to *D. parazeae*)	*M. paradisiaca* NCPPB 2511

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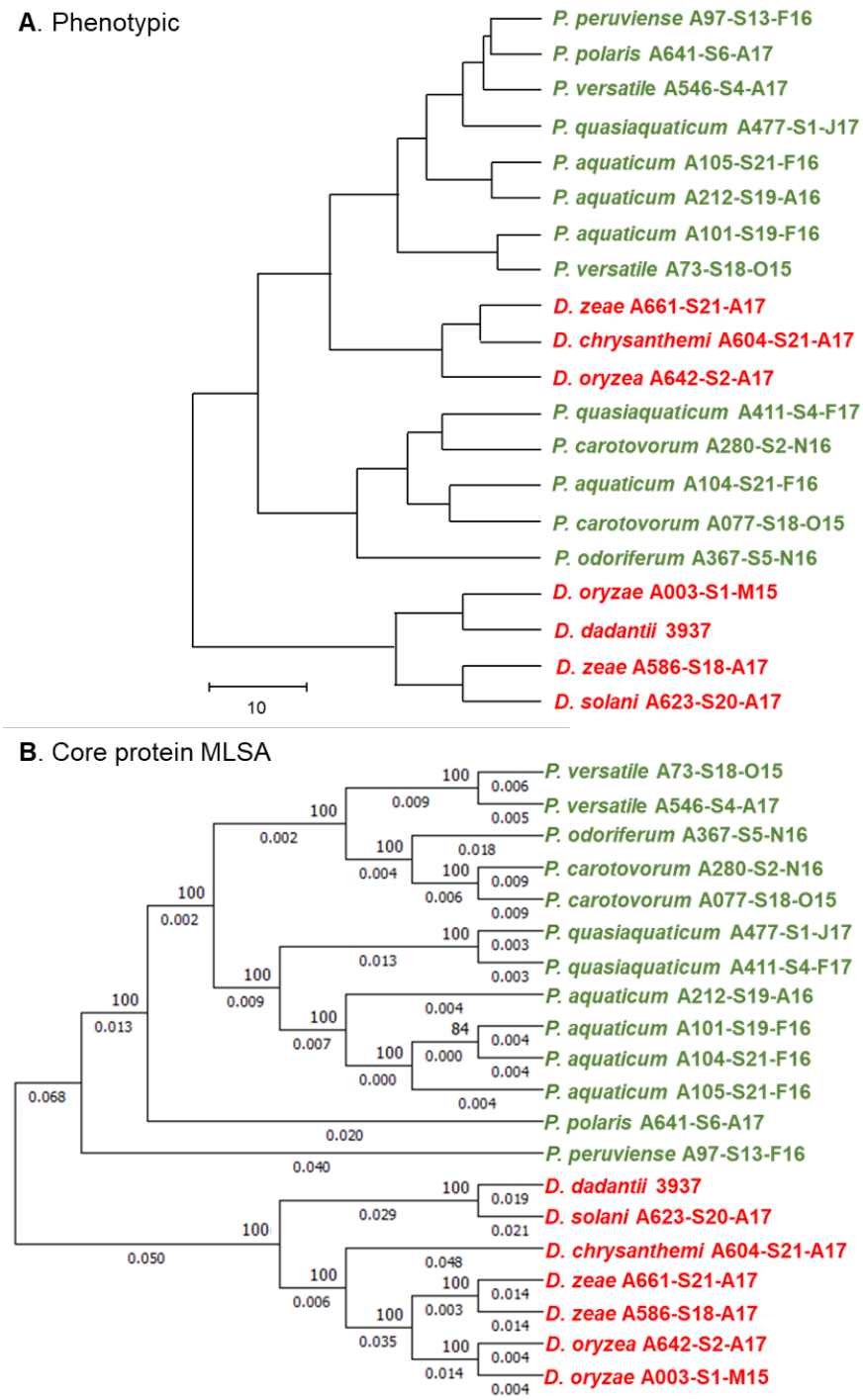
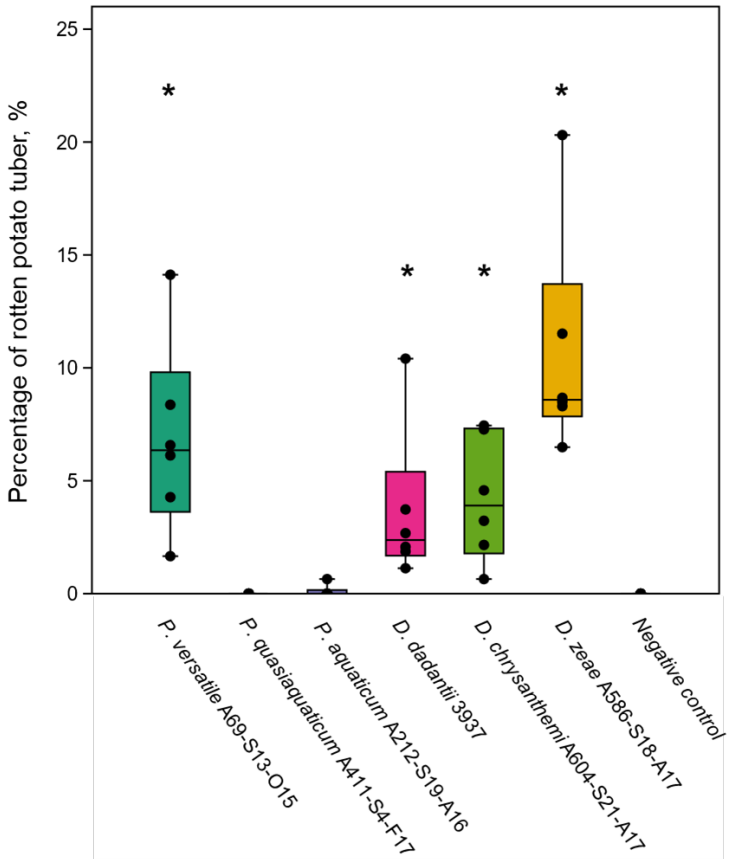
Supplementary Figures

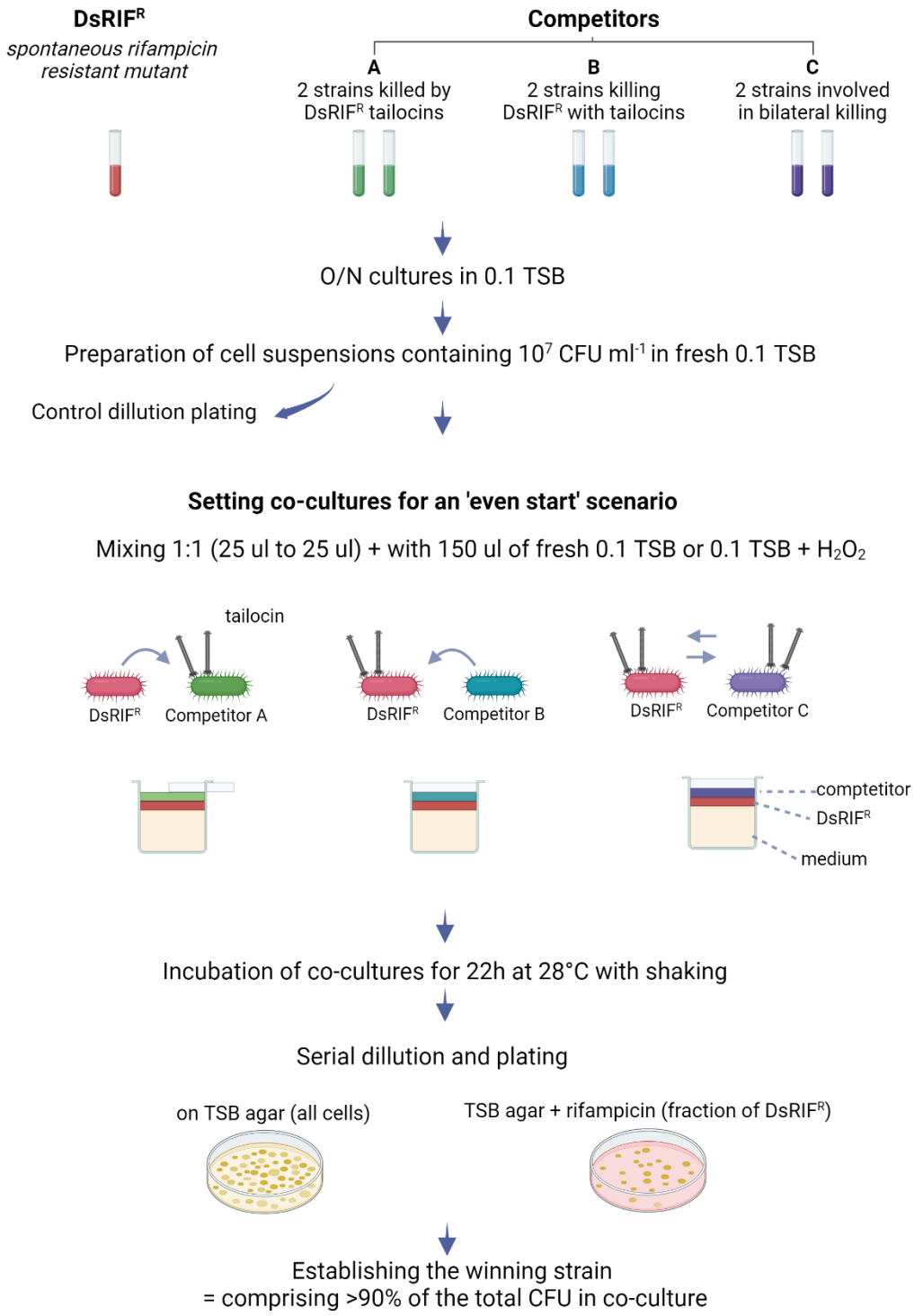
Supplementary Fig. S1. Comparison of phenotypic and phylogenetic trees. (A) dendrogram calculated based on the similarity of killing spectra of investigated strai; the tree is drawn to scale, with branch lengths measured in the distance; (B) multilocus sequence analysis of core nucleotide coding sequences using the BioNJ algorithm. Branch lengths and bootstrap values are shown. *Pectobacterium* spp. strains are marked in green, and *Dickeya* spp. strains are marked in red.



Supplementary Fig. S2. Severity of soft rot symptoms caused on potato tubers by six SRP strains tested for production of tailocins *in planta*. The results are expressed as a percentage of rotten tissue relative to the total mass of each potato tuber. In the box plot, whiskers reflect the maximum and minimum values; the box shows the interquartile range (Q1 to Q3), and the bars inside the boxes represent medians. Points indicate results for individual samples/tubers (n=6). The asterisks (*) denote statistically significant differences ($p < 0.05$) between a sample inoculated with the given strain and the negative control. Significance was calculated using the one-sample t-test for data sets with normal distribution and the Wilcoxon test for data not following a normal distribution. Negative control – tubers inoculated with sterile PBS buffer.



792 **Supplementary Fig. S3 Schematic representation of the steps included in the**
793 **competition experiment.** Created with BioRender.com.



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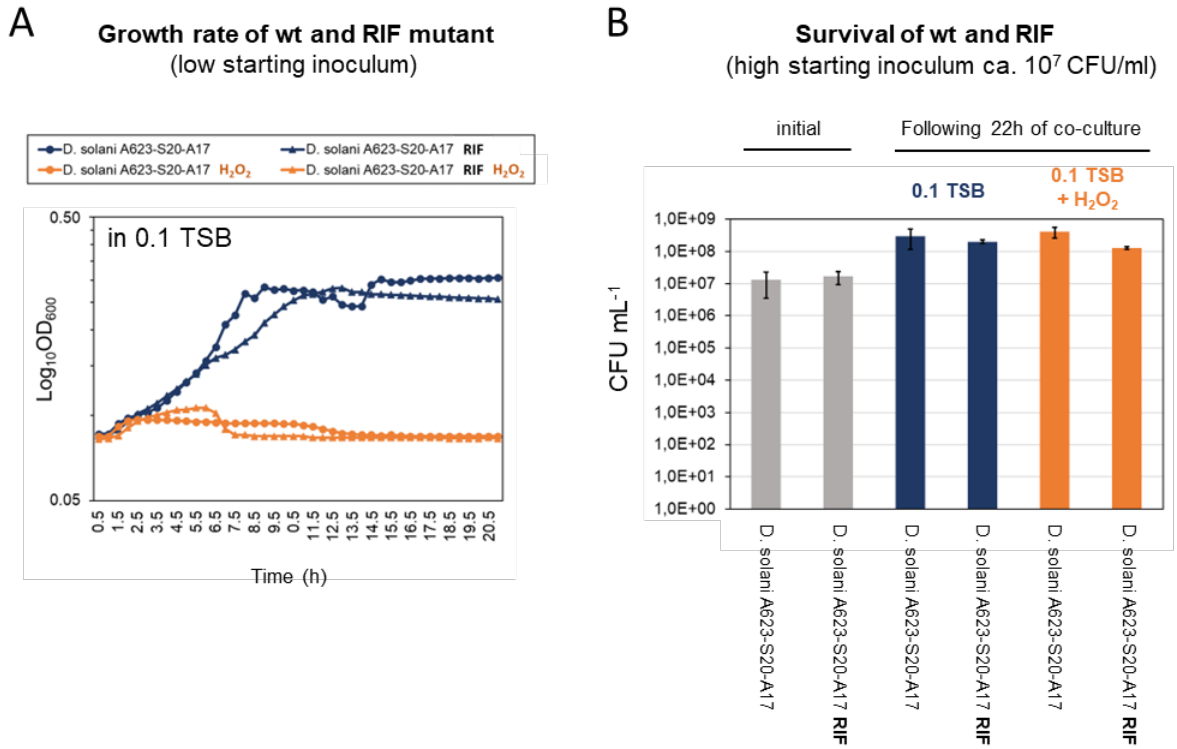
Supplementary Fig. S4 Comparison of growth rates (A) and the survival (B) of the *D. solani* A623-S20-A17 wt and the rifampicin-resistant mutant of this strain in 0.1 TSB alone and supplemented with 0.003 % hydrogen peroxide (H_2O_2). The test was performed in 96-well plates incubated at 28˚C, with shaking, in a multiwell plate reader (Biotek). To assess growth rates (A), 150 µl aliquots of 0.1 TSB medium were inoculated with 3 µl of suspension containing o/n culture diluted to 0.5 McF (low inoculum). Each time point is an average of two replicates. For cell survival (B), the medium was inoculated with a high initial number of cells (ca. 10^7 CFU/ml) applied in the co-culture competition experiments (Fig. 5). The results are the average of two separate experiments, 2 technical replicates each. The obtained results indicate that the wt and RIF^R variants of *D. solani* A623-S20-A17 have similar growth characteristics in the applied conditions and that 0.003% H_2O_2 prevents the growth of the strains when low initial inoculum is used but does not negatively affect the survival of cells in high-density cultures.



Supplementary datasets

Dataset S1 Results of a megablast search for carotovoricin clusters within 116 closed genomes of *Pectobacterium* spp. The carotovoricin sequence from *P. versatile* A73-S18-O15 was used as a query (genome accession: NZ_CP086369.1).

Dataset S2 Results of a megablast search for dickeyocin clusters within 75 closed *Dickeya* and *Musicola* spp. genomes. Dickeyocin P2D1 from *D. dadantii* 3937 was used as a query (genome accession: NC_014500.1).

Dataset S3 Python script for analyses (ChatGPT-4o)

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