Bacterial pathogens dynamic during multi-species infections

Marie-Anne Barny*, Sylvia Thieffry, Christelle Gomes de Faria, Elisa Thebault and Jacques Pédron

Sorbonne Université, INRAE, IRD, CNRS, UPEC, Institute of Ecology and Environmental Sciences-Paris (iEES-Paris), 4 place Jussieu, F-75252 Paris, France

**E-mail : marie-anne.barny@sorbonne-universite.fr*

Abstract

Soft rot *Pectobacteriacea* (SRP) gathers more than 30 bacterial species that collectively rot a wide range of plants by producing and secreting a large set of plant cell wall degrading enzymes (PCWDEs). Worldwide potato field surveys identified 15 different SRP species on symptomatic plants and tubers. The abundance of each species observed during outbreaks varies over space and time and the mechanisms driving species shift during outbreak are unknown. Furthermore, multi-species infections are frequently observed and the dynamics of these coinfections are not well understood.

To understand the dynamics of coinfections, we set up 16 different synthetic communities of 6 SRP strains to mimic coinfections. The bacteria present in each tested community were representative of 2 different species, with 3 strains per species. These communities were inoculated in potato tubers or on synthetic media and their outcome was followed by amplification and Illumina sequencing of the discriminatory housekeeping gene *gap*A. We also compared disease incidence and bacterial multiplication in potato tubers during mixed-species infection and single-species infection. A species that was unable to induce disease in potato was efficiently maintained and eventually became dominant in some of the communities tested, indicating that cheating can shape dominant species. Modeling indicates that the cost of PCWDEs production and secretion, the rate of potato degradation and the diffusion rate of degraded substrate could favor the cheater species. Interaction outcomes differed between potato tuber and synthetic medium, highlighting the driving effect of environmental conditions, with higher antagonistic interactions observed in potato tubers. Antagonistic interactions were strain specific and not species specific. Toxicity interference was also observed within some communities, allowing the maintenance of strains otherwise sensitive to toxic compounds. Overall, the results indicate that intraspecific competition, cooperation through trophic interaction and toxicity interference contribute to the maintenance of SRP diversity. The implications of these processes for epidemiological surveillance are discussed.

Introduction

Pathogenic bacteria developed specialized weapons to attack their host, subvert host defenses and gain nutrient to develop inside their host. Among pathogens, some are specialists with a narrow host range and infect a single host while others have the capacity to infect many different hosts. A mirrored situation occurred with disease, while many diseases are defined by a single well known pathogenic species, other diseases could be triggered by a complex of bacterial species that share common bacterial weapons and could act either individually and/or collectively during disease development. When a disease is triggered by a species complex many questions concerning the dynamic of the complex are still unsolved. Notably, it is unclear to what extend the different species involved compete, cheat, and cooperate within the symptoms. As well it is also unclear if cheating, competition and cooperation behavior mainly occurs at the strain level or at the species level. Furthermore, as each disease cycle involved many different steps, it is unclear to which extend the competition/cooperation game change with changing environmental conditions and to what extend these interactions are shaping the bacterial diversity of pathogens.

Plant pathogens with a broad host range, such as the soft rot *Pectobacteriacea* (SRP) species complex, provide an interesting model to address these questions. SRP pathogens belong to three genera, *Pectobacterium, Dickeya* and *Musicola*, of the family *Pectobacteriacea* within the order *Enterobacteral*. Taxonomic studies recognize 38 species within the SRP group, of which 23 belong to the genus *Pectobacterium*, 12 belong to the *Dickeya* genus and 2 were formerly assigned to the *Dickeya* genus and now belong to the newly described genus *Musicola* (Charkowski 2018; Hugouvieux-Cotte-Pattat et al. 2021). All SRP attack the host plant by secreting a cocktail of plant cell wall degrading enzymes through the type 2 secretion system (T2SS), resulting in rotting and maceration of plant tissues on which the bacteria actively develop (Charkowski 2018; Hugouvieux-Cotte-Pattat et al. 2014). Due to their collective large host spectrum, SRP plant infections lead to severe economic losses and are collectively recognized as one of the ten most important bacterial plant pathogens (Mansfield et al. 2012; Ma et al. 2007; Portier et al. 2020). Among SRP species, some species like *Pectobacterium brasiliense*, *Pectobacterium carotovorum* or *Pectobacterium versatile* are described as broad range species isolated from a large number of plant species while other like *Pectobacterium parmentieri*, *Pectobacterium atrosepticum* or *Dickeya solani* have been mainly recorded on a single host (Ma et al. 2007; Portier et al. 2020; Hugouvieux-Cotte-Pattat et al. 2023). *P. versatile* is the most frequently isolated taxon among the SRP species, both on plants and in the environment, a characteristic that might be linked with its higher resistance to antibiotics than

other *Pectobacterium* spp. (Royer et al. 2022; Smoktunowicz et al. 2022; Ben Moussa et al. 2022; Portier et al. 2020) but this species is currently considered as a minor pathogen not responsible for severe outbreaks but frequent companion species during disease or on symptomless plants (Curland et al. 2021; Ma et al. 2022; Smoktunowicz et al. 2022). Some newly described species like *Pectobacterium aquaticum* or *Pectobacterium quasiaquaticum* have been isolated solely from river water and their potential host plants (if any) are currently unknown raising the possibility that these species act are merely saprophytes on plant debris or evolved toward hosts alternative to plant, for example as gut species of plant feeding insect or water microfauna (Pédron et al. 2019; Ben Moussa et al. 2022, 2023). Species belonging to the *Dickeya* genus are well known to infect ornamentals of high economical value but several species could also be observed on major crops such as maize (*Dickeya zeae*) rice (*Dickeya oryzae*) or potato (*Dickeya dianthicola* or *D. solani*) where they have been responsible of severe outbreaks (Hugouvieux-Cotte-Pattat et al. 2023).

Potato production, estimated according to FAO to over 376 million tons worldwide in 2021, is particularly exposed to SRP species. In potato field, SRP triggers a disease called blackleg. This name referred to the necrosis observed at the stem basis of infected potato plant. Harvested potato tubers could also develop a severe rotting, called soft rot, that could destroy the whole harvest. Given its economic importance, potato is the most intensively surveyed crop attacked by SRP and 15 different SRP species have been recorded on potato worldwide (Toth et al. 2021). In Europe and North America, *P. atrosepticum* was initially considered to be the primary pathogen causing blackleg and soft rot in potatoes. However, from the 1970s, *D. dianthicola* increased in prevalence, until the appearance of *D. solani* in the early 2000s while more recently the spread of *P. parmentieri*, and *P. brasiliense* was observed (van der Wolf et al. 2021). The succession of these prevalent species is not yet understood. Notably, aggressiveness of the species is likely not the main factor explaining the prevalence of species in potato field (de Werra et al. 2021). Other traits such as survival ability in the environment or competition/cooperation abilities within the host plant are likely involved in the observed turn over. Importantly, mix of SRP spp. during infection could represent up to 20% of recorded symptoms (Ge et al. 2021; Motyka-Pomagruk et al. 2021; de Werra et al. 2021). These mixed infections probably contribute to the evolution of epidemic clones through HGT transfer of trait associated to the bacterial fitness (Khayi et al. 2015; Royer et al. 2022). Furthermore mix of SRP spp. are also frequently reported on asymptomatic potato tubers (Degefu 2021; Smoktunowicz et al. 2022). These latent infections likely influence the dynamic of disease through cooperation and competition but this has not been investigated in details.

The aim of the present work was to experimentally assess the fate of mix infection during soft rot within potato tubers. To understand the dynamic of coinfections, we set up 16 different synthetic communities of 6 SRP strains to mimic coinfection. Bacteria present in each synthetic community were representative of 2 different species, with 3 strains per species. These communities were inoculated on potato tuber or on growth medium and the outcome of each synthetic community was followed by amplification and Illumina sequencing of the discriminative housekeeping gene *gap*A (Cigna et al. 2017; Ben Moussa et al. 2022). We also compared disease rating and bacterial multiplication on potato tuber during coinfection and single species infection. Overall, the results indicate that competition, cooperation and trophic interactions shape the bacterial SRP community and allow for the maintenance of SRP diversity.

Materials and Methods

Strains and media used

The strain used in this study are described in table S1. For each species, where possible, the bacterial strains were selected to allow strain discrimination using the gapA barcode (see below). For some species, such as *D. dianthicola*, *D. solani* and *P. polaris/P. parvum*, we did not find discriminating strains in our collection (Table S2). This is probably due to the fact that these latter species are quite homogeneous (Hugouvieux-Cotte-Pattat et al. 2023; Pasanen et al. 2020). The bacterial media used were LB medium without NaCl (hereafter LB: per liter 10 g tryptone, 5 g yeast extract) and TSB (per liter: 1,7 g casein peptone, 0.3 g soya peptone, 0.3 g NaCl, 0.25 g K_2HPO_4, 0.25 g glucose). When needed, 15 g agar per liter were added for solid media.

Inocula preparation

The bacteria were plated on LB plates and incubated overnight at 28°C. A single colony was then used to inoculate 2 ml of LB medium which was incubated overnight at 28°C, with agitation at 120 rpm. This liquid culture (100 µl) was used to inoculate a 10% TSB agar plate, which was incubated overnight at 28°C. The grown bacterial layer was then suspended in 50 mM phosphate buffer (pH 7) and adjusted to an OD_{600nm}=1. The 3 strains of the same species were then mixed in a 1/1/1 ratio. An exception was the inoculum composed of *P. parvum* (2 strains) and *P. polaris* (1 strain), which were mixed together as the experiment was set up before the description of the *P. parvum* species, a close relative of *P. polaris* (Pasanen et al. 2020).

137 The bacterial load of each 3 strains-mixtures was check by serial dilution and plating on TSB
138 agar plates. The species mixtures were then prepared by mixing two of the prepared strain-
139 mixtures in volumes 1/1. Each final species-mixture is thus the mixture of 6 strains adjusted to
140 DO_{600nm}=1 in the ratio 1/1/1/1/1/1, where the first strain-mixture corresponds to one of the nine
141 following species: *P. atrosepticum, P. carotovorum, P. parmentieri, P. versatile, P.*
142 *polaris/P.parvum, D. dianthicola, D. solani, P. versatile, P. aquaticum*, and the second strain-
143 mixture corresponds either to the species *P. aquaticum* or to the species *P. versatile*. A 2 ml
144 aliquot of each time zero species-mix inoculum was stored at -20°C until DNA extraction.
145 These 18 species-mixtures were then used to inoculate triplicates of 2 ml TSB liquid medium
146 (1 µl in 2 ml) or 10 potato tubers (10 µl per tuber). The inoculated TSB medium was incubated
147 at 26°C with shaking during 48h. Serial dilution and plating was performed to check the
148 bacterial growth at the end of the culture. The bacterial load at time zero (inoculum) and at the
149 end of the experiment were used to calculate the number of bacterial generation achieved (log_{10}
150 (cfu end experiment/cfu T0)/log_{10} (2)). Cultures were then centrifuged and the pellet was kept
151 at -20°C until DNA extraction.
152
153 **In vitro bacterial competition assays**
154 Pairwise in vitro bacterial competition assays were performed between the *P. brasiliense, P.*
155 *versatile* and *P. aquaticum* strains (3 strains per species, 81 combinations tested). Briefly,
156 overnight TSB cultures of each strain were adjusted to an DO_{600nm} = 0.15. Samples of 5 µl of
157 the strain 1 were spotted onto freshly poured agar lawn inoculated with strain 2 (1 ml with 15
158 ml TSB agar medium) and plates were allowed to grow for 24 h at 28°C. If strain 1 inhibits the
159 growth of strain 2, a clear halo is observed around the spot of strain 1. Experiment was repeated
160 twice with similar results.
161
162 **Potato inoculation**
163 Three experiments were performed, the first one was devoted to analyze the mono-species
164 infection with the mix of the 3 strains/species. The second and third one involved mix of two
165 different species (6 strains) with either *P. aquaticum* species or *P. versatile* species challenged
166 by one of the other 8 species.
167 For each mix, 10 potato tubers were thoroughly washed, and surface disinfected in a 1 %
168 hypochlorite solution bath prior inoculation and each tuber was punctured with pipette tips
169 containing 10 µl of the inoculum. The tip was left in the tuber. As a negative control, a pipette
170 tip with 10µl sterile water was inserted into one tuber. Inoculated tubers were placed on paper

171 towel in plastic boxes and 50 mL of distilled water was poured into the bottom of the boxes and
172 the boxes were carefully closed to achieve relative humidity above 90 %. After 5 days of
173 incubation at 26°C, the tubers were cut and the entire macerating symptom was collected and
174 weighed. Part of the symptom (50 to 300 mg) was also weighed and suspended in 1 ml of sterile
175 phosphate buffer in order to count by serial dilution and plating the bacterial load present in the
176 symptom. To recover the bacteria within the symptoms, the rest of the symptom was suspended
177 with 7 ml of 50 mM phosphate buffer and stirred for 4 hours at ambient temperature. The tubes
178 were then left without shaking for 10 minutes to allow the excess starch to settle and 2 ml at the
179 surface of the suspension was recovered and centrifuged for 10 minutes at 10,000 g. The formed
180 pellet was kept at -80°C until DNA extraction.
181
182 **Statistical analysis**
183 One specie mix (*P. aquaticum/P. versatile*) was shared between the second and the third
184 experiments and this allowed to eliminate inter-experiment variation unrelated to the involved
185 species mix before statistical analysis. To do so we corrected the maceration weight observed
186 in the third experiment with the following coefficient X = (mean of *P. aquaticum/P.versatile*
187 10 potato tubers maceration weight of the 2ndexperiment)/ mean of *P. aquaticum/P.versatile* 10
188 potato tubers maceration weight results of the 3rd experiment) before statistical analysis.
189 Statistical analyses were performed in R version 4.0.2 using a Kruskal-Wallis test with p-value
190 < 0.05 followed by a post-hoc test (Dunn test with Bonferroni correction). Spearman correlation
191 calculation were performed on line (https://biostatgv.sentiweb.fr/?module=tests/spearman).
192
193 **DNA extraction, DNA amplification and sequencing**
194 For each of the 18 species-mix analyzed, DNA extraction was performed on 10 ml of the time
195 zero inoculum, on 2 ml of each of the TSB triplicate after 48h of culture and on 2 ml of 3 to 6
196 potato macerated tubers symptoms. The bacteria were pelleted by centrifugation and DNA
197 extraction was performed with the Wizard® genomic DNA purification kit (Promega).
198 Amplification and sequencing were performed at MR DNA (www.mrdnalab.com, Shallowater,
199 TX, USA). Briefly, the *gap*A 376 partial gene sequence was amplified using PCR primers
200 *gap*AF376: GCCCGTCTCACAAAGA and *gap*AR: TCRTACCARGAAACCAGTT) with
201 barcode on the forward primer. PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA)
202 was performed under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C
203 for 30 s, 57°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min
204 was performed. Amplified PCR products were checked in 2 % agarose gel to determine the

205 success of amplification and the relative intensity of bands. Multiple samples were pooled
206 together in equal proportions based on their molecular weight and DNA concentrations. Pooled
207 samples were purified using calibrated Ampure XP beads and used to prepare Illumina DNA
208 library. Sequencing was performed on a MiSeq sequencer following the manufacturer's
209 guidelines. Sequence data were processed using MR DNA analysis pipeline (MR DNA,
210 Shallowater, TX, USA). In summary, sequences were joined, depleted of barcodes and
211 sequences <150bp or with ambiguous base calls were removed. Reads were filtered based on
212 Q score and expected error probability and any read with a number of expected errors greater
213 than 1.0 were discarded.

214

215 **Illumina sequencing analysis**

216 After quality trimming, a total of 2,230,817 and 3,215,158 reads were obtained for *in vitro*
217 (TSB, 64 samples) and potato tuber (100 samples) assays respectively, with an average of
218 34,857 ± 963 and 32,152 ± 7538 reads per samples respectively.
219 In order to quantify the distribution of the *Pectobacterium* and *Dickeya* strains after *in vitro*
220 growth or potato inoculation, reads were aligned to the corresponding amplified sequences of
221 the *gap*A gene, using the nucleotide-nucleotide Blastn tool (version 2.11.0+, e-value threshold
222 10^{-5}). Only reads with 100% full-length identity were used (547,866 reads, 24.6% of the total
223 reads and 1,213,517 reads, 37.7% of the total reads for *in vitro* and potato tuber assays
224 respectively. A few potato tubers were withdrawn of the analysis since unexpected *P.*
225 *parmentieri* sequences were observed, suggesting an initial presence of this pathogen as latent
226 species hosted on the tuber prior to the inoculation.

227 Bacterial distribution analysis was performed at the species level for all assays (sum of all
228 strains of the same species), and at the strain level when it was possible. In fact, for some
229 species, certain strains cannot be distinguished due to sequence similarities: *P. versatile*
230 CFBP6051 and A73.1, all *D. dianthicola* strains, all *D. solani* strains, *P. parmentieri* CFBP1338
231 and CFBP5382, *P. atrosepticum* CFBP1527 and CFBP1453, *P. carotovorum* CFBP6074 and
232 CFBP1402, all *D. polaris* strains.

233

234 **Model presentation**

235 The equations of the model are:

236 $dDX/dt = k(D_R - D_X) - (gA - \epsilon)D_X X + cX/z + X$

237 $dDA/dt = k(D_R - D_A) - g_A D_A A$

$dDR/dt = -k(2D_R - D_X - D_A) - \lambda D_R$

$dX/dt = (g_A - \epsilon)\, D_X X - mX$

$dA/dt = g_A D_A A - mA$

with X the density of the species that produces enzymes able to degrade the substrate, A the density of the cheater species that only consumes the degraded substrate without being able to produce it, D_X is the concentration of degraded substrate in the vicinity of species X (i.e. local pool of degraded substrate of X), D_A is the corresponding local substrate pool for A, and D_R is the concentration of degraded substrate in the regional environmental (hereafter regional pool of degraded substrate), k is the diffusion rate of degraded substrate between local and regional pools, c the maximum rate of enzymatic substrate degradation, z half-saturation constant of enzymatic substrate degradation, gA consumption rate of degraded substrate without cost, ϵ cost of enzyme PCWDEs production and secretion on consumption rate of degraded substrate, m Mortality rate of X and A, λ Loss of degraded substrate from regional pool. The mathematical analysis of the model together with the parameter's values used in numerical simulations are provided in supplementary file S1.

Search for carbapenem synthesis and resistance

The beta-lactam carbapenem is synthesized and degraded by the *car*ABCDEF genes while the *car*GHR genes are involved in resistance to the produced carbapenem (Shyntum et al. 2019). These genes were blast searched with *car*ABCDEFGHR genes of *P. brasiliense* CFBP6617 as query among the 27 strains used to build up the synthetic communities using the genoscope Microbial Genome Annotation & Analysis Platform (https://mage.genoscope.cns.fr/microscope/).

Results:

Set up of the experiment

A general view of the experimental set up is shown in Figure 1. In brief, each synthetic SRP community was a mixture of 6 strains belonging to two different species. The mixture of two species was preferred because the mixture of two different species is often observed in the field (Degefu 2021; de Werra et al. 2021; Ge et al. 2021; Motyka-Pomagruk et al. 2021; Smoktunowicz et al. 2022). The addition of three strains per species allows to disentangle the "species" effects from the "strain" effects. The species involved were 8 species commonly

isolated from potato tubers, *P. versatile, P. atrosepticum, P. carotovorum, P. parmentieri, P. polaris, P. brasiliense, D. solani, D. dianthicola*, and one species *P. aquaticum* that was only isolated from fresh surface water. Synthetic communities were set up with either *P. versatile* or *P. aquaticum* facing one of the others 8 species. *P. versatile* and *P. aquaticum* were chosen as challenging species because of their very different distribution aera. *P. versatile* that occurs on many different plants, is the main SRP species represented in bacterial collections and is also frequently isolated from non-host environments, such as surface freshwater highlighting its ecological success among SRP (Portier et al. 2019; Ben Moussa et al. 2022). In contrast, *P. aquaticum* has not been isolated from plants and is frequently isolated from water (Portier et al. 2020; Ben Moussa et al. 2022). SRP communities were inoculated on TSB medium or on potato tubers and the proportion of each species at the beginning and end of the experiment was assessed by Illumina sequencing of the *gap*A discriminative gene marker (Cigna et al. 2017; Ben Moussa et al. 2022). Symptoms occurring on potato tubers were also recorded for each 6 strains community and compared with symptoms observed with the mix of 3 strains of single-species inoculation.

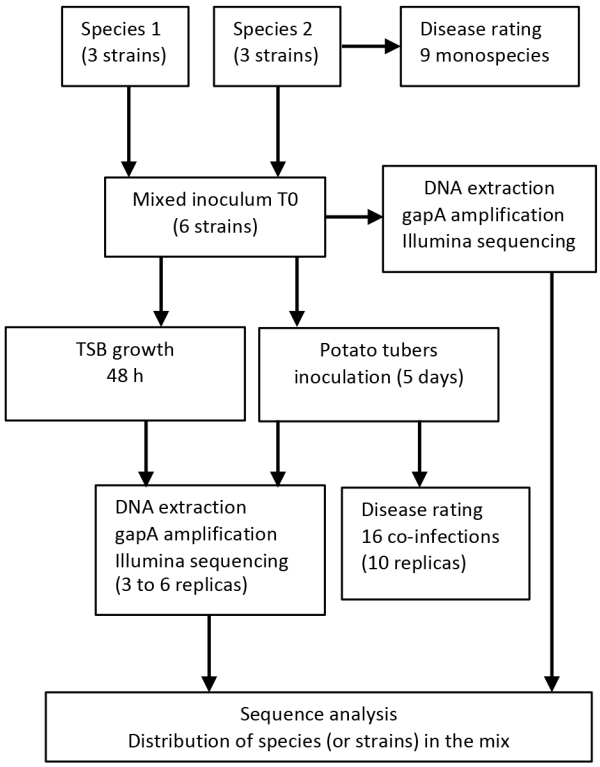


Figure 1: Set up of the experiment.

Symptoms observation and bacterial multiplication in potato tubers

291 Single-species infection resulted in various symptom development in potato tubers depending
292 on the inoculated species tested. Outliers were frequently observed and there was considerable
293 variability in response between the 10 inoculated potato tubers (Fig. 2A). The 10 potato tubers
294 inoculated with *P. aquaticum* showed hardly any symptoms and statistical analysis confirmed
295 that *P. aquaticum* developed less severe symptoms than *P. versatile, P. parmentieri, P. brasil-*
296 *iense* and *P. atrosepticum* (Fig. 2A). Bacterial multiplication was assessed and the number of
297 bacterial generations achieved within each inoculated tuber was calculated (Fig. 2B). It varied
298 from 0.9 to 13.7 with a median of 8.9 depending on the tuber inoculated. This multiplication
299 rate correlated with the weight of macerating symptoms (Spearman correlation 0.451, p-value
300 8.87E-14). After inoculation with *P. aquaticum*, bacterial multiplication within the tubers was
301 much lower than with other single inoculated single species, confirming *P. aquaticum* poor
302 ability to rot potato tuber (Fig. 2B) as already described (Ben Moussa et al, 2023). The addition
303 of either *P. versatile* or *P. aquaticum* to the inoculation mix did not affect the severity of the
304 symptoms observed compared to the other single species inoculation but *P. aquaticum* (Fig.
305 2A). Similarly, the severity of rot symptoms was similar for all co-inoculations, except for *P.*
306 *parmentieri* in competition with *P. versatile*, which showed a significantly higher maceration
307 rate than *P. aquaticum* in competition with *P. versatile* (Fig. 2A). Bacterial multiplication was
308 also unaffected by the mixed-species inoculation (Fig. 2B). In the following experiments 3 to 6
309 tubers from each SRP community were analyzed by amplification and Illumina sequencing of
310 the *gap*A barcode.
311

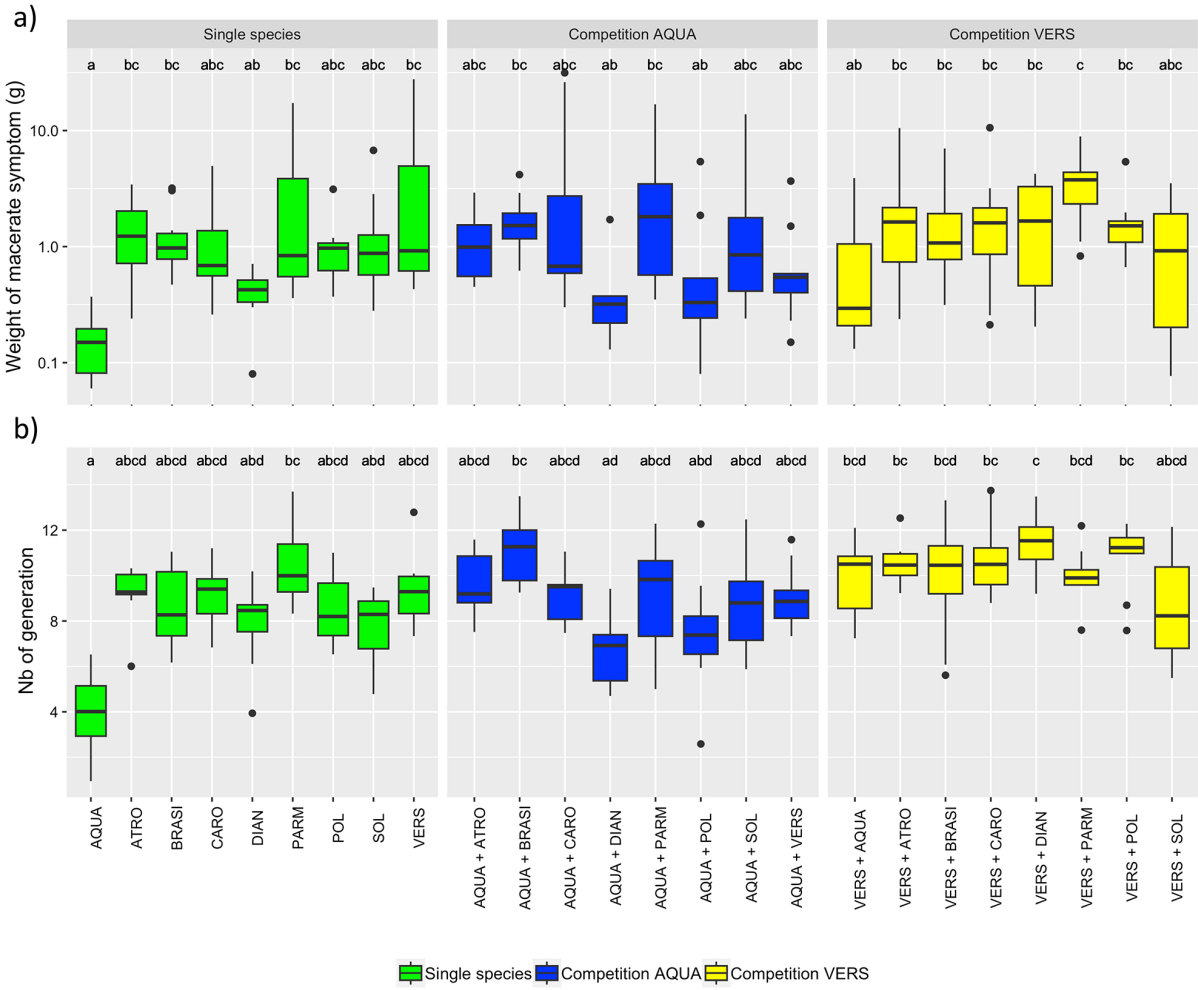


Figure 2: Symptoms and bacterial growth of the SRP synthetic communities on potato tubers. a) Weight of macerate symptom b) bacterial growth within potato tuber. Single-species mixtures (3 strains) are shown in green. Two-species mixtures (6 strains) are shown in blue and yellow for *P. aquaticum*, and *P. versatile* consortia respectively. AQUA : *P. aquaticum* ; ATRO: *P. atrosepticum ;* BRASI: *P. brasiliense* ; CARO: *P. carotovorum*; PARM: *P. parmentieri* ; POL: *P. polaris* ; SOL: *D. solani*; VERS: *P. versatile* . The result of the statistical analysis is represented by the letters (a, b, c) at the top of the boxplots. The bacterial mixtures sharing the same letter(s) are not statistically different from each other (P>0.05 Kruskal Wallis followed by Dunn test with the Bonferroni correction). The strains used in each bacterial mixture are listed in Table S1.

Species outcome within SRP communities inoculated in TSB medium

Bacterial multiplication was assessed after 48 hours of growth on TSB medium and the number of bacterial generations obtained was calculated. It ranged from 4.2 to 9 generations and was repeatedly higher in the mixture containing *P. aquaticum* compared to the mixture containing *P. versatile* (Fig. S1). A total of 2,230,817 sequences were obtained after Illumina sequencing,

328 with an average of 34,857 ± 963 sequences per sample. The 376 pb *gapA* sequence allowed us
329 to distinguish all the inoculated species and to calculate the percentage of sequences assigned
330 to *P. aquaticum* or *P. versatile* within each SRP community at time zero and after 48 hours of
331 growth (Fig 3 A and B). At time zero, *P. aquaticum* or *P. versatile* represented 30% to 60% of
332 the inoculum in each mix containing another *Pectobacterium* species as expected for an even
333 inoculation of each strain. However, in the SRP community containing *Dickeya spp.*, *P.*
334 *aquaticum* and *P. versatile* made up more than 60% of the mixture. This deviation was
335 particularly important for the mix containing *D. dianthicola* as *P. versatile* and *P. aquaticum*
336 represented 78% and 79% respectively. A similar deviation was observed after counting the cfu
337 of each individual 3 species mix at time zero indicative of a higher inoculation of *P. versatile*
338 and *P. aquaticum* compared to the *D. dianthicola* species in the initial mix (data not shown).
339 This bias is likely due to the fact that *D. dianthicola* is highly mucoid impacting the optical
340 density-cfu relation. For all the others synthetic communities tested, no obvious bias was
341 detected and the strains are correctly equilibrated in the initial mix.
342 After 48 hours of growth, with an average number of generations of 6.7 after 48 hours of
343 growth, the initial population size represents only around 1% of the final population size. The
344 *gap*A barcode allowed to analyzed the proportion of each SRP species within the synthetic
345 community (Fig. 3 A and B). *P. aquaticum* and *P. versatile* were considered to drastically
346 outcompete the other SRP species if the mean percentage for the three replicates reached at
347 least 90% at the end of the experiment. They were considered to be drastically outcompeted by
348 the other SRP species if their mean percentage for the three replicate was inferior to 10%.
349 Finally, if the mean percentage of *P. aquaticum* or *P. versatile* was between 10% and 90%, *P.*
350 *aquaticum* and *P. versatile* were considered to be coexisting with their challenging SRP species.
351 Using this criterion most of the SRP community (12 out of 16) allowed coexistence of both
352 species after 2 days of growth in TSB medium (Fig. 3A and B). *P. aquaticum* outcompeted *D.*
353 *dianthicola* and coexisted with all the other challenging species. *P. versatile* was outcompeted
354 when co-inoculated with *P. brasiliense, P. carotovorum* or *D. solani* and it coexisted with the
355 other species at various levels.
356

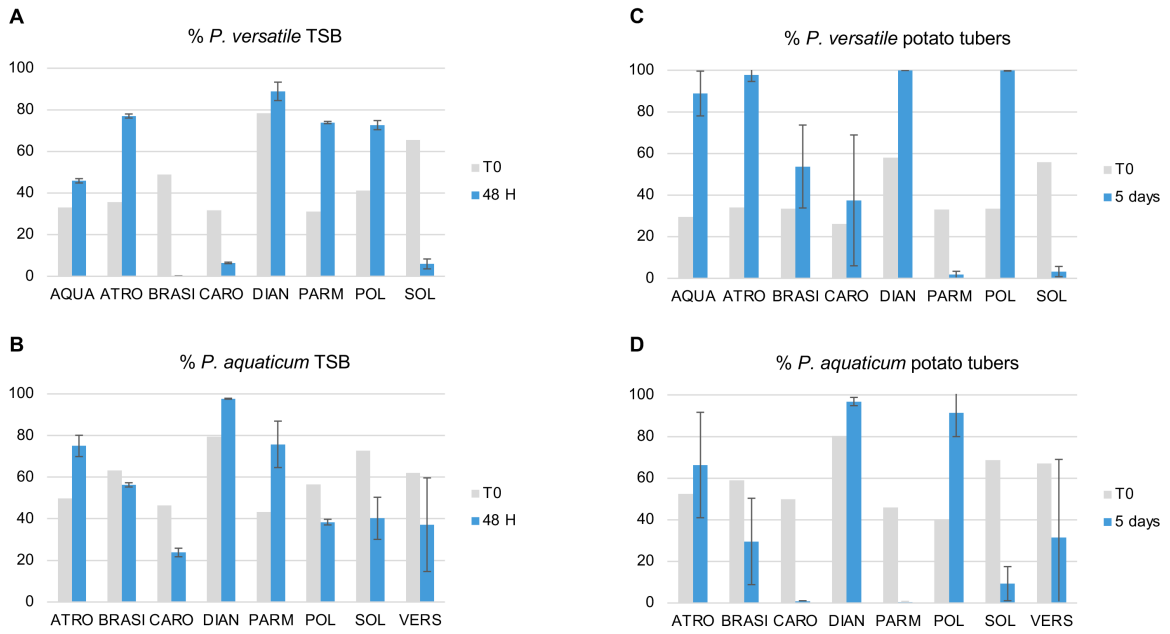


Figure 3: Species outcome within SRP communities.

Percentage of *P. versatile* or *P. aquaticum* in mixed-species SRP communities after 48h TSB growth (A and B) or 5 days post inoculation within potato tuber (C and D). Grey bars: % of *P. aquaticum* or *P. versatile* within the inocula at time zero, Blue: bars % of *P. aquaticum* or *P. versatile* after 48 hours growth in TSB or 5 days within potato tubers. Three replicates were analysed after 48h growth in TSB and 3 to 5 potato tubers were analysed 5 days post inoculation. Bars : standard deviation. Species's acronym: AQUA : *P. aquaticum* ; ATRO: *P. atrosepticum ;* BRASI: *P. brasiliense* ; CARO: *P. carotovorum*; PARM: *P. parmentieri* ; POL: *P. polaris* ; SOL: *D. solani*; VERS: *P. versatile.*

Species outcome within SRP communities inoculated within potato tubers

A total of 3,215,158 sequences were obtained after Illumina sequencing, with an average of 32,152 ± 7538 sequences per sample. We calculated the percentage of reads assigned to *P. aquaticum* or *P. versatile* in the mixed-species SRP communities at time zero and after growth inside the potato tubers (Fig. 3 C and D). Using the same criteria as after TSB growth, coexistence was observed 5 days post infection in 6 out of the 16 mixed-species SRP communities inoculated in potato tubers. Within potato tubers, *P. aquaticum* was outcompeted in presence of *P. carotovorum, P. parmentieri* and *D. solani*, it outcompeted *D. dianthicola* and *P. polaris* and coexisted with *P. atrosepticum, P. brasiliense* and *P. versatile*. *P. versatile* was outcompeted in the presence of *P. parmentieri* and *D. solani*, it outcompeted *P. atrosepticum, D. dianticola* and *P.polaris/P.parvum* and coexisted with *P. aquaticum, P. brasiliense and P. carotovorum*.

379 The outcome of the competitions in potato tubers was different from that observed after growth
380 on TSB for 10 out of the 16 SRP communities tested (Table 1). For example, within potato
381 tubers *P. aquaticum* was outcompeted by *P. carotovorum, P. parmentieri* and *D. solani* while
382 it coexisted with each of these three species in TSB. Similarly, while *P. versatile* coexisted with
383 *P. brasiliense* and *P. carotovorum* in potato tubers, it was outcompeted by both species in the
384 TSB medium. As well, *P. aquaticum* and *P. versatile* were outcompeted by *P. parmentieri* in
385 potato tubers but both species coexisted with *P. parmentieri* in the TSB medium. Overall, out
386 of the 16 tested combinations, the two species coexisted twice as much in TSB medium (12/16
387 SRP communities tested) compared to within potato tuber (6/16 SRP communities tested).
388
389 **Table 1:** Comparison of species outcome after growth in TSB or potato tuber.

Species mix	Potato tuber	TSB
AQUA-ATRO	65.02	75
AQUA-BRASI	29.57	56.28
AQUA-CARO	0.77	23.8
AQUA-DIAN	96.81	94.88
AQUA-PARM	0.32	75.76
AQUA-POL	91.37	38.39
AQUA-SOL	9.29	40.21
AQUA VERS	38.29	37.14
VERS-ATRO	97.64	77.02
VERS-BRASI	53.72	0.13
VERS-CARO	37.44	6.42
VERS-DIAN	99.94	88.87
VERS-PARM	1.72	73.89
VERS-POL	99.81	72.64
VERS-SOL	3.23	5.93
VERS-AQUA	88.8	45.88

390
391 Blue: coexistence. Green: *P. versatile or P. aquaticum* dominates. Red: *P. versatile* or *P. aquaticum* was
392 outcompeted. The number indicated the % mean of *P. aquaticum* or *P. versatile* at the end of the
393 experiment
394
395 **Modelling of cheater persistence**
396 The SRP species *P. aquaticum* has been isolated from water and is not recorded on plant
397 (Pédron et al. 2019; Portier et al. 2020). We noticed here that the *P. aquaticum* strains have a
398 poor capacity to rot potato tubers and multiply within symptoms (Fig. 2). This is a previously

399 described phenotypic trait that could be linked to a reduced production of PCWDEs (Ben
400 Moussa et al. 2023). We observed nevertheless, that *P. aquaticum* when co-inoculated with
401 another species, could be maintained and even become dominant in several synthetic
402 communities tested (Fig. 3C and D). To investigate the potential mechanisms by which *P.*
403 *aquaticum* might persist when co-inoculated with other species and become even dominant, we
404 analysed a model describing the dynamics of two species in competition for one common
405 resource originating from the degradation of a substrate by the enzymatic activity of only one
406 of the two species (species X, Fig. 4A, Appendix model). The other species (A) is a cheater as
407 it does not contribute to the production of degraded substrate, and thus has no cost related to
408 enzyme production in that context. The model considers heterogeneous distribution of the
409 degraded substrate, with specific concentrations in the vicinity of each species (species local
410 pool of degraded substrate, Fig. 4A) resulting from the depletion of degraded substrate through
411 consumption for both species X and A, and from the creation of degraded substrate by enzyme
412 production for species X. Degraded substrate diffuses at a given rate between the two species-
413 specific local pools and a regional pool, determining the interactions between the species A and
414 X through their indirect effect on the concentration of degraded substrate in the regional pool.
415 The analysis of the model (Fig. 4 B and C, supplementary file S1) suggests that the cheater
416 species A can persist when it has sufficiently higher growth rate than the producer species X
417 (i.e. sufficient cost of enzyme production on the growth rate of species X) and when species X
418 is sufficiently efficient in producing degraded substrate (i.e. high maximum rate of substrate
419 degradation). Higher diffusion rate of degraded substrate between the local and regional pools
420 also favours species A persistence for lower enzyme production cost of species X (Fig. 4B and
421 C). The cheater species can become dominant (i.e. proportion of abundance of A higher than
422 0.5) when A has significantly higher growth rate than X (intermediate to high cost of enzyme
423 production) and when the rate of substrate degradation by X is high, especially when the
424 diffusion of degraded substrate in the environment are high. However, in such cases the
425 abundance of X is low (see supplementary file S1) and we are at the limit of the coexistence
426 domain between X and A. Indeed, as A has relatively very high growth rate in comparison to
427 X, this can lead to the exclusion of X by A through competition for the consumption of degraded
428 substrate, further leading to the extinction of A due to the loss of production of degraded
429 substrate by X (see supplementary file S1).

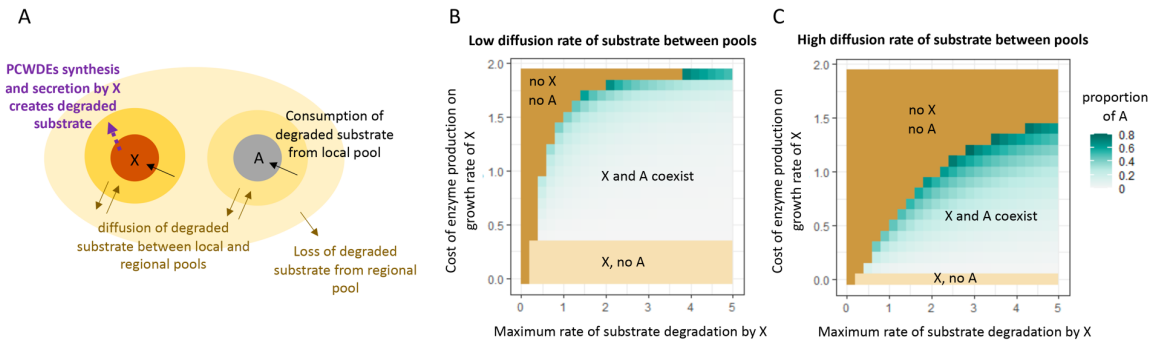


Figure 4: Modeling of cheater abundance.

A: Schematic description of the model analysing the coexistence between a species X that produces degraded substrate and a cheater species A that only consumes such substrate. The producer species X and its local pool of degraded substrate are represented by a brown circle and a dark yellow circle respectively. The cheater species A and its local pool are represented by a grey and a light-yellow circle respectively. The oval shape corresponds to the regional pool of degraded substrate in the environment. B and C: effects of the rate of substrate production and the cost of degradation for X on coexistence and relative abundance of A for two scenarios of flows of degraded substrate in the environment.

Strains outcome within the SRP communities

Beyond the species level, the 376 pb *gap*A Illumina sequenced fragment allowed the differentiation of the 3 inoculated strains of *P. brasiliense* or *P. aquaticum* and one of the three strains for *P. versatile*, *P. carotovorum*, *P. parmentieri* and *P. atrosepticum* (Table S2). This allowed us to analyze the fate of these 10 strains within the 16 tested SRP communities within potato tubers or TSB (Table 2, Fig. S2 and S3). Comparison of the fate of these 10 strains showed that the outcome was mainly determined at the strain level (Fig. 4, see Fig. S2, Fig. S3 for the 32 tested combinations). In 20 cases, within the 3 strains of a single species, the fate of the 3 strains was different. For example, within the *P. aquaticum/P. brasiliense* community in which the six strains could be distinguished, after growth in TSB, one strain belonging to the *P. aquaticum* species (A212) and two strains belonging to the *P. brasiliense* species (CFBP5381 and CFBP3230) were outcompeted, while two strains of *P. aquaticum* (A101 and A127) and one strain of *P. brasiliense* (CFBP6617) coexisted (Fig. 5). The result of the same community grown on potatoes was different, allowing the coexistence of one *P. brasiliense* strain (CFBP6617) and only one *P. aquaticum* strain (A101) (Fig. 5). Also, within the *P. aquaticum/P. parmentieri* community, the *P. parmentieri* strain CFBP8475 outcompeted the 5 other strains on potato tubers, but this was not the case on TSB (Fig. 5).

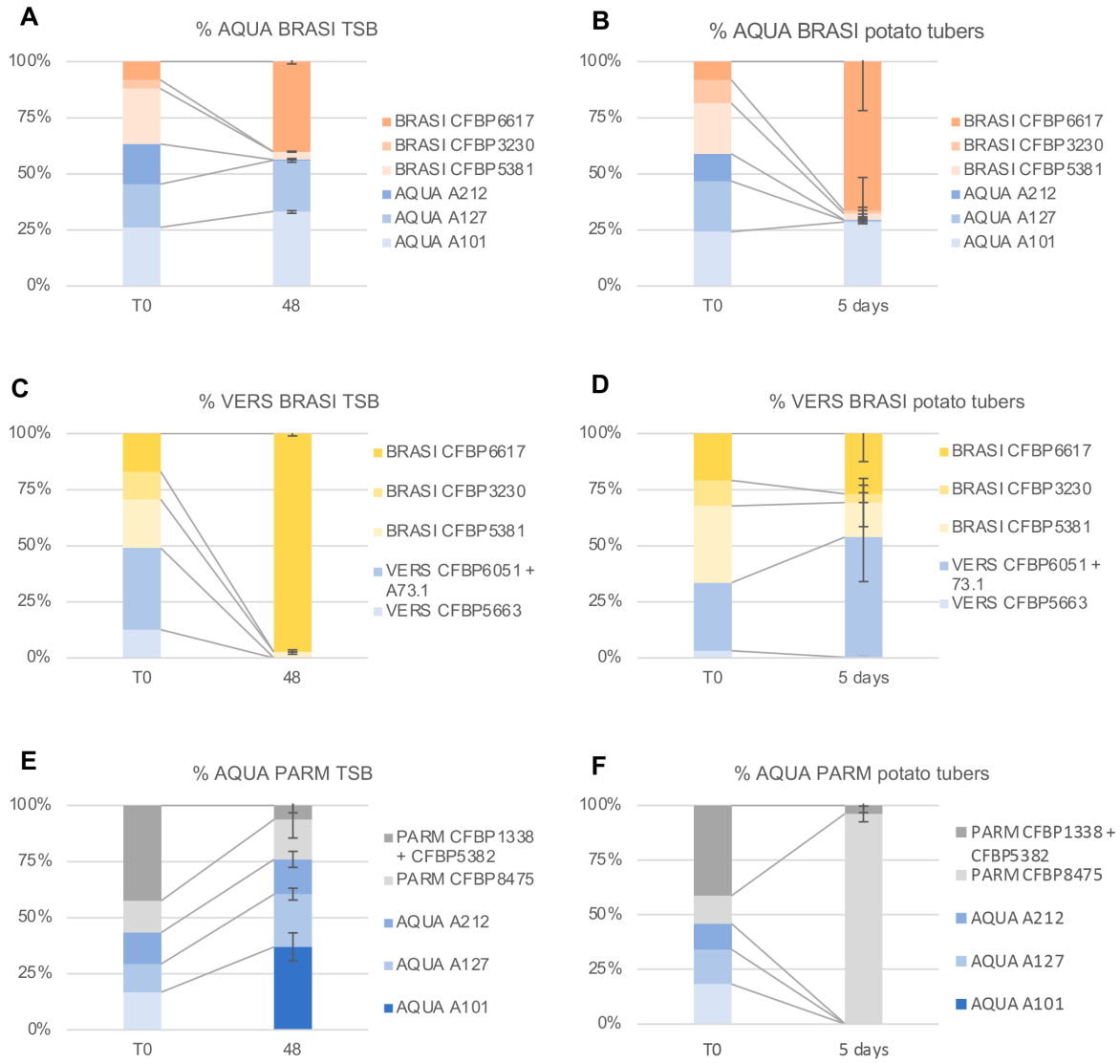


Figure 5: Examples of strains outcome within SRP communities.

The proportion of each strain in 3 different mixed-species SRP communities after 48h growth in TSB growth or 5 days post inoculation within potato tubers is indicated in the legend of each graph. *P. aquaticum-P. brasiliense* strains consortium in TSB (A) or within potato tuber (B) *P. versatile* - *P. brasiliense* strains consortium in TSB (C) or within potato tuber (D). *P. aquaticum* - *P. parmentieri* strains consortium in TSB (E) or within potato tuber (F). In each graph the first column represents the % of each strain within the inoculum (T0), the second column represents the outcome of the inoculated community after 48h in TSB or 5 days post potato tubers inoculation. Three replicates were analysed after 48h growth in TSB and 3 to 5 potato tubers were analysed 5 days post inoculation. Bars: standard deviation. Species's acronym: AQUA : *P. aquaticum* ; ATRO: *P. atrosepticum ;* BRASI: *P. brasiliense* ; CARO: *P. carotovorum*; PARM: *P. parmentieri* ; POL: *P. polaris* ; SOL: *D. solani*; VERS: *P. versatile.* The names of each strain follow the species'names.

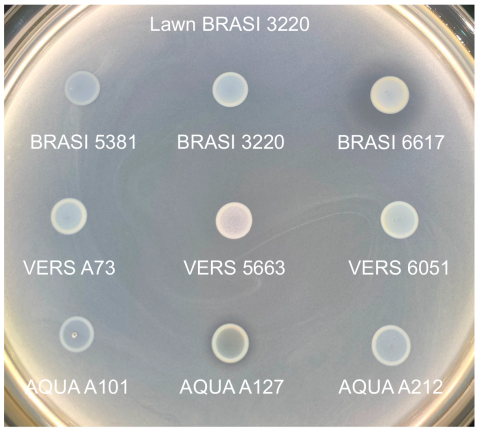
471 The strains had different outcomes when grown on potato tubers and TSB. Overall, the
472 outcompeted strains made up a higher percentage within the potato tuber (66%) than after
473 growth on TSB (32%) (Table 2).
474
475 **Table 2:** Outcome of individual strains within the tested SRP communities.

Single strain discriminated with the gapA barcode	nb of tested synthetic communities	potato tubers			TSB		
		>90%	<10%	10-90%	>90%	<10%	10-90%
VERS CFBP5663	9	0	9	0	0	3	6
AQUA A101	9	0	4	5	0	0	9
AQUA A127	9	0	7	2	0	2	7
AQUA A212	9	0	7	2	0	4	5
ATRO CFBP7375	2	0	1	1	0	2	0
BRASI CFBP5381	2	0	1	1	0	2	0
BRASI CFBP3230	2	0	2	0	0	2	0
BRASI CFBP6617	2	0	0	2	1	0	1
CARO CFBP2046	2	0	2	0	0	1	1
PARM CFBP8475	2	1	0	1	0	0	2
Total	48	1	33	14	1	16	31
%		2%	66%	28%	2%	32%	62%

476 VERS: *P. versatile*; AQUA: *P. aquaticum*; ATRO: *P. atrospecticum*; BRASI: *P. brasiliense*; CARO:
477 *P. carotovorum*; PARM: *P. parmentieri*.
478
479 However, the dominance of a single strain over the other 5 strains of the SRP community was
480 rarely observed both within tubers and in TSB medium: only the *P. parmentieri* strain
481 CFBP8575 dominates in potato tubers when associated with *P. aquaticum* and, in TSB medium,
482 the *P. brasiliense* strain CFBP6617 dominates when associated with *P. versatile* (Table 2, Fig.
483 5). In the other synthetic communities tested, on TSB or within the potato tubers, coexistence
484 of at least two strains was observed in 23 of the 32 combinations tested, the remaining 7
485 combinations were inconclusive because we could not differentiate some strains with the *gap*A
486 barcode (Fig. S2 and S3).
487
488 **Focus on the SRP communities involving *P. brasiliense***
489 The strain *P. brasiliense* CFBP6617, also called 1692, is a model strain known to produce,
490 among other toxic compounds, a carbapenem antibiotic (Holden et al. 1998; Shyntum et al.
491 2019). We set up pairwise *in vitro* competition assays between the 6 strains of *P. brasiliense*
492 and *P. aquaticum* to check for carbapenem production. This showed that the *P. brasiliense*
493 strain CFBP6617 inhibited the growth of the other two *P. brasiliense* strains, CFBP3220 and

494 CFBP5381, and of the two *P. aquaticum* strains, A212 and A127 (Fig. 6). The *P. aquaticum*
495 strain A101 was not inhibited by the *P. brasiliense* strain CFBP6617. Consistent with this latter
496 result, the *car*G, *car*H and *car*F genes conferring resistance to carbapenem (Coulthurst et al.
497 2005) were only detected in the genome of the strain *P. aquaticum* A101. During our pairwise
498 inhibition tests, we also observed that the *P. aquaticum* strain A127 produces an unknown
499 compound that inhibits the growth of the strain *P. brasiliense* CFBP6617. The radius of action
500 of this unknown toxin is smaller than the one induced by the carbapenem production by the *P.*
501 *brasiliense* strain CFBP6617 (Fig. 6).

A



B

		Spot	
Lawn	strain	BRASI 6617	AQUA A127
	BRASI 6617	R	s
	BRASI 3220	S	s
	BRASI 5381	S	s
	VERS 6051	R	s
	VERS 5663	R	R
	VERS A73	R	s
	AQUA A212	S	R
	AQUA A127	S	R
	AQUA A101	R	R

502

503 **Figure 6:** Pairwise competition assays.
504 Pairwise competition assays were setup between the 9 strains belonging to the species *P. brasiliense*, *P.*
505 *aquaticum* and *P. versatile*. A: example of inhibition assay showing that the *P. brasiliense* strain
506 CFBP3230 (lawn BRASI 3220) is inhibited by the *P. brasiliense* strain CFBP6617 (spot BRASI 6617)
507 and also by the *P. aquaticum* strain A127 (spot AQUA A127). Note that the radius of inhibition triggered
508 by the *P. aquaticum* strain A127 is smaller than the one triggered by the *P. brasiliense* strain CFBP6617.
509 B: inhibition observed for the 81 pairwise tested competitions. R: no inhibition; S: the strain in the lawn
510 is inhibited by the strain in the spot with a large radius of inhibition; s: the strain in the lawn is inhibited
511 by the strain in the spot with a small radius of inhibition; BRASI: strains belonging to the *P. brasiliense*

512 species; AQUA: strains belonging to the *P. aquaticum* species; VERS: strains belonging to *the P.*
513 *versatile* species.
514
515 These observed pairwise inhibitions could probably explain the outcome of the *P. aquaticum/P.*
516 *brasiliense* community after growth on TSB medium (Fig. 5), as the 3 strains CFBP 6617, A127
517 and A101 were efficiently maintained after growth on this medium, while the 3 other strains
518 were outcompeted. The result of the same community on potato tubers was slightly different as
519 only 2 strains, CFBP6617 and A101, were efficiently maintained (Fig. 5). This could probably
520 be explained by the fact that the unknown toxic compound that counteract the growth of *P.*
521 *brasiliense* strain CFBP6617 is not produced by strain A127 or inefficient within potato tuber,
522 while the carbapenem is still produced within potato tuber. Surprisingly, within the *P.*
523 *versatile/P. brasiliense* community, we observed that the *P. brasiliense* strain CFBP5381 was
524 maintained within the potato tuber despite its sensitivity to the carbapenem produced by the *P.*
525 *brasiliense* strain CFBP6617 (as revealed with the *P.aquaticum/P. brasiliense* SRP community)
526 (Fig. 5). This suggests that at least one of the two *P. versatile* strains CFBP6051 or A73 still
527 observed in the potato tubers at the end of the experiment is interfering with the toxic effect of
528 *P. brasiliense* CFBP6617 to allow the growth of *P. brasiliense* CFBP5381. The mechanism that
529 allows the *P. versatile* strains to resist to carbapenem toxicity of *P. brasiliense* CFBP6617 is
530 unknown, as the two strains do not carry the carbapenem resistance genes *car*G, *car*H and *car*F
531 in their genomes (Coulthurst et al. 2005). Whatever the mechanism involved, it is either not
532 induced or not efficient in TSB medium, as CFBP6617 dominates over the other 5 strains of
533 the *P.brasiliense/P. versatile* community TSB medium.
534
535 **Discussion:**
536
537 The dynamic of microbial communities is an important research field. Theoretical studies
538 showed that cooperative trophic interaction generally leads to the steady coexistence of distinct
539 bacterial species, that organize themselves to create a community-level metabolism that best
540 exploits the nutrients present (Gralka et al. 2020; Taillefumier et al. 2017). This is exemplified
541 by synthetic metabolic consortia set up for various biotechnological applications that regroup
542 highly diverse bacteria with different metabolic capacity that could be stably maintained. As
543 well, metabolic cross feeding could allow maintenance of bacterial species otherwise
544 outcompeted in natural environment (Franza et al, 2016). The maintenance of microbial
545 diversity within pathogenic bacterial species complex cannot follow the same rule as, in that

546 case, the microbes within these species complex are closely phylogenetically related with
547 highly similar metabolic properties. This metabolic closeness implies a strong substrate overlap
548 likely associated with strong competition between species within the same niche (Weiss et al.
549 2022). It is therefore not straightforward to understand which mechanisms allow the
550 maintenance of diversity within these pathogenic species complexes. The bacterial SRP species
551 complex is probably one of the most diverse species complexes of plant pathogenic bacteria. It
552 gathers 38 accepted or proposed species and at least 15 SRP species have been identified on the
553 potato host, with frequent co-infection with at least 2 species (Toth et al. 2021). The work
554 presented here takes advantage of *gap*A barcoding Illumina sequencing to analyze the bacterial
555 dynamics of 16 different synthetic SRP communities involving 6 strains belonging to two
556 different species.
557 The outcome of the 16 synthetic communities was highly dependent on environmental
558 conditions and strongly differed between potato tubers and TSB stirred liquid medium. The
559 striking outcome differences of the synthetic community in the two different environments
560 should warns us on the use of synthetic liquid medium to test consortia stability when these
561 consortia should be used in a different environment. Notably, coexistence between the two
562 species was twice more frequent in TSB liquid medium than within potato tubers and
563 outcompeted strains were more numerous within the potato tubers than after growth on TSB
564 liquid medium. Competition through production of secreted toxic compounds, such as
565 carbapenem (Coulthurst et al. 2005; McGowan et al. 1997), carotovoricin (Nguyen et al. 1999;
566 Itoh et al. 1978), bacteriocins (Chuang et al. 2007; Chan et al. 2011; Roh et al. 2010; Grinter et
567 al. 2012), NRPS-PKS secondary metabolites (Brual et al. 2023; Cheng et al. 2013) have been
568 described in SRP. The production of many of these compounds is enhanced by plant extracts
569 or in potato tubers (Bellieny-Rabelo et al. 2019; Mattinen et al. 2007) and this could explain
570 that more strains are outcompeted during growth in potato tubers. Furthermore, competition
571 mechanisms relying on cell to cell contact, like the type 6 secretion system (T6SS), are also
572 described in SRP (Shyntum et al. 2019; Arizala and Arif 2019) but are likely inefficient in liquid
573 stirred medium. Modelling studies showed that in a structured environment such as that found
574 in potato tubers, the production of antimicrobial molecules could be beneficial even if the
575 producer is rare (Chao and Levin, 1981; Durrett and Levin, 1997; Gardner et al., 2004). The
576 enhanced production of antimicrobial molecules and their higher efficiency within a structured
577 environment, such as potato tubers, likely explain the higher competition observed within
578 potato tubers compared to liquid TSB medium.

579 As the outcome of the community is highly dependent on the environment, it is likely that each
580 of the communities studied will evolve as the infection progresses in the field under natural
581 conditions. In particular, each of the analysed communities is likely to evolve differently within
582 the stem during blackleg infection in the field, as the environmental conditions within the potato
583 tuber and within the potato stem are drastically different. Contrasting SRP fitness in stem and
584 tuber has been reported previously (Blin et al. 2021) and further work is required to analyse
585 how our synthetic consortia will evolve in potato stem.
586 Our results also illustrate that the competition is strain-specific rather than species-specific, as
587 in many combinations we observed that the fate of the 3 strains of the same species was
588 different. This is likely related to the fact that toxin production and toxin resistance are
589 functional traits associated with strains rather than with species and are generally encoded
590 within the accessory genomes of each species, as exemplified here by biosynthesis and
591 resistance to carbapenem (Shyntum et al. 2019). In most combinations, the *P. brasiliense* strain
592 CFBP6617 outcompeted the other two *P. brasiliense* strains, consistent with the observed
593 pairwise *in vitro* competition and the presence of the carbapenem cluster in the strain genomes.
594 However, within the *P. versatile/P. brasiliense* synthetic community, we observed an
595 interference towards the toxic effect of *P. brasiliense* CFBP6617 on *P. brasiliense* strain
596 CFBP5381, likely mediated by *P. versatile* strains. This interference may involve the secreted
597 β-lactamase commonly found in *P. versatile* strains which may inactivates the carbapenem
598 antibiotic (Royer et al. 2022). Further work is needed to test this hypothesis. *In vitro* pairwise
599 inhibition indicated that "face-to-face" competition may also occur in some of the synthetic
600 SRP communities tested, as for example *P. brasiliense* strain CFBP6617 is toxic to *P.*
601 *aquaticum* strain A127, but the reverse is also true. The complexity of the competitive interplay
602 between strains is enhanced by the number of toxic compounds and resistance mechanisms
603 known to be produced by SRP. Theoretical models highlight that chemical warfare between
604 microbes promote diversity (Kelsic et al. 2015; Czárán et al. 2002). The fact that each SRP
605 strains carries its own set of toxic compounds and resistance mechanisms could therefore
606 contribute to the maintenance of multiple strains as observed with the majority of the 16
607 synthetic communities tested. The complexity of the competition between SRP strains rather
608 than species probably also explains the temporal and spatial succession of different SRP species
609 in epidemic outbreaks.
610 Beyond the competition mechanisms, the virulence factors of SRP may also contribute to the
611 maintenance of SRP diversity, as the main virulence factors of SRP, i.e. the ability of SRP to
612 degrade the plant cell wall through the secretion of PCWDEs, can be considered as a public

613 good used to feed the whole community. In potato tubers, the important release of nutrients
614 once SRP express their main virulence factors allows the development of associated
615 commensals (Kõiv et al. 2015). Here, we observed that *P. aquaticum* strains were efficiently
616 maintained in the population within potato tubers in 5 out of 8 synthetic communities tested.
617 We also observed that *P. aquaticum* strains were unable to produce soft rot disease symptoms
618 and to multiply efficiently in the potato tubers when inoculated alone. The inability of *P.*
619 *aquaticum* strains to produce soft rot symptoms when inoculated alone strongly suggests that
620 the T2SS and associated virulence factors are not properly induced within the potato tuber and,
621 as such, *P. aquaticum* does not share the cost of producing virulence factors. However, when
622 mixed with other species, *P. aquaticum* strains could eventually become dominant, suggesting
623 that they can behave as cheaters, benefiting greatly from nutrient sharing. Several independent
624 mechanisms could be proposed to explain this efficient cheating behavior. Firstly, the low
625 expression of T2SS and the associated virulence factors and the smaller genome of *P.*
626 *aquaticum* strains compared to that of other SRP species may allow a more rapid multiplication
627 at the expense of the other SRP species. In support of this hypothesis, we observed here that in
628 TSB medium the 8 synthetic communities with *P. aquaticum* have a tendency to grow at higher
629 density in TSB medium than the 8 synthetic communities with *P. versatile*. It has also been
630 reported that *P. aquaticum* strains have increased mobility compared to that of other SRP
631 species (Ben Moussa et al. 2023). This may also influence the ability of *P. aquaticum* strains to
632 access nutrients within the symptoms. *P. aquaticum* has never been isolated from potatoes in
633 the field or in storage, and their cheating behavior observed here could therefore be considered
634 an artefact. Nevertheless, the observation of this cheating behavior suggests that other SRP
635 species could also behave as cheaters in the field, as plants infected with several SRP species
636 are often observed. This is supported by the fact that the repertoire and regulation of virulence
637 factors is variable among SRP species (Babujee et al. 2012; Jonkheer et al. 2021; Arizala and
638 Arif 2019; Li et al. 2018), likely allowing for a trade-off between growth and the ability to
639 cheat. Interestingly, our modelling suggests that the more efficient the SRP strain is at
640 decomposing potato tubers, the higher the abundance of the cheaters in the symptoms. This
641 greater abundance of the cheaters may affect the ability to detect the SRP strain responsible for
642 the symptoms and increase the risk of not identifying the initial SRP strain responsible for the
643 epidemic. Therefore, field sampling plans should be calibrated to account for this risk.
644 In summary, the dynamics of the 16 synthetic SRP communities presented here highlight some
645 possible mechanisms allowing both the maintenance of high SRP diversity within the SRP
646 species complex and the regular species shifts responsible for outbreaks observed in the field.

Cooperation for virulence, exemplified by cheating behavior of *P. aquaticum*, and the complexity of competition between strains, are two mechanisms that likely contribute to the maintenance of the SRP complex diversity. The fact that competition occurs at the strain level rather than the species level probably also explains the regular appearance of new species responsible for epidemic outbreaks. This suggests that deep sequencing or *gap*A barcoding of plant symptoms are important to understand epidemic dynamic and rapidly identify new strains responsible for outbreaks in the field.

Authors contributions

Jacques Pédron: Conceptualization (equal); supervision (equal): data curation (lead): formal analysis (equal), methodology (equal), visualization (equal), writing original draft (equal), writing-review and editing (equal)

Christelle Gomes de Faria: investigation (equal), writing-review and editing (equal)

Sylvia Thieffry: Methodology (equal), conceptualization (equal), writing-review and editing (equal)

Elisa Thebault: Supervision (equal), conceptualization (lead), methodology (equal), visualization (equal), writing-review and editing (equal).

Marie-anne Barny: Funding acquisition (lead), supervision (equal), investigation (equal), visualization (equal), writing original draft (equal), writing-review and editing (equal)

Acknowledgements:

We thank Denis Faure and Jérémy Cigna for helpful discussions during the setup of the experiment. We also thank Angel Alfon for performing the pairwise competition tests and Hajar Ben Moussa for statistical analysis and vizualisation of Fig. 2. This work was possible due to funding contract ANR-17-CE32-0004.

Conflict of interest statement

The authors declare they have no conflict of interest relating to the content of this article.

Data, script and code availability

Supplemental data are available in the tables S1 and S2, the figures S1, S2, S3 and the file S1 for model details. Sequenced data and the script used to analyse the sequences. could be found in the zenodo file DOI 10.5281/zenodo.10212828 (https://zenodo.org/records/10212829). The

scripts used to perform the statistical analysis could be found in the zenodo file https://doi.org/10.5281/zenodo.10404740.

Supplementary information

FigS1: Bacterial multiplication in TBS medium

FigS2: Result at strain level of the 16 synthetic communities after growth on TSB.

FigS3: Result at strain level of the 16 synthetic communities 5 days post inoculation on potato tubers.

File S1: supplemental modeling

Table S1: strains description

Table S2: Intra-species strain discrimination with the 341 nt gapA barcode

All supplementals are available in the zenodo file

https://zenodo.org/doi/10.5281/zenodo.10886378

695 **References**:

696 1. Arizala, D., and Arif, M. 2019. Genome-wide analyses revealed remarkable heterogeneity in pathogenicity determinants, antimicrobial compounds, and CRISPR-Cas systems of complex phytopathogenic genus *Pectobacterium*. Pathogens. 8:247. DOI:10.3390/pathogens8040247

701 2. Babujee, L., Apodaca, J., Balakrishnan, V., Liss, P., Kiley, P. J., Charkowski, A. O., et al. 2012. Evolution of the metabolic and regulatory networks associated with oxygen availability in two phytopathogenic enterobacteria. BMC Genomics. 13:110. DOI:10.1186/1471-2164-13-110

706 3. Bellieny-Rabelo, D., Tanui, C. K., Miguel, N., Kwenda, S., Shyntum, D. Y., and Moleleki, L. N. 2019. Transcriptome and comparative genomics analyses reveal new functional insights on key determinants of pathogenesis and interbacterial competition in *Pectobacterium* and *Dickeya* spp. Appl. Environ. Microbiol. 85 DOI: 10.1128/AEM.02050-18

712 4. Ben Moussa, H., Bertrand, C., Rochelle-Newall, E., Fiorini, S., Pédron, J., and Barny, M.-A. 2022. The diversity of soft rot *Pectobacteriaceae* along the Durance River stream in the south-east of France revealed by multiple seasonal surveys. Phytopathology DOI :10.1094/PHYTO-12-21-0515-R

717 5. Ben Moussa, H., Pédron, J., Hugouvieux-Cotte-Pattat, N., and Barny, M. 2023. Two species with a peculiar evolution within the genus *Pectobacterium* suggest adaptation to a new environmental niche. Environ. Microbiol. :1462-2920.16479. DOI: 10.1111/1462-2920.16479

722 6. Blin, P., Robic, K., Khayi, S., Cigna, J., Munier, E., Dewaegeneire, P., et al. 2021. Pattern and causes of the establishment of the invasive bacterial potato pathogen *Dickeya solani* and of the maintenance of the resident pathogen *D. dianthicola*. Mol. Ecol. 30:608–624. • DOI: 10.1111/mec.15751

727 7. Brual, T., Effantin, G., Baltenneck, J., Attaiech, L., Grosbois, C., Royer, M., et al. 2023. A natural single nucleotide mutation in the small regulatory RNA ArcZ of *Dickeya solani* switches off the antimicrobial activities against yeast and bacteria ed. Pascale Romby. PLOS Genet. 19:e1010725. DOI: 0.1371/journal.pgen.1010725

732 8. Chan, Y.-C., Wu, J.-L., Wu, H.-P., Tzeng, K.-C., and Chuang, D.-Y. 2011. Cloning, purification, and functional characterization of Carocin S2, a ribonuclease bacteriocin produced by *Pectobacterium carotovorum*. BMC Microbiol. 11:99. DOI: 10.1186/1471-2180-11-99

737 9. Charkowski, A. O. 2018. The changing face of bacterial soft-rot diseases. Annu. Rev. Phytopathol. 56:269–288. DOI: 10.1146/annurev-phyto-080417-045906

740 10. Cheng, Y., Liu, X., An, S., Chang, C., Zou, Y., Huang, L., et al. 2013. A Nonribosomal peptide synthase containing a stand-alone condensation domain is essential for

phytotoxin zeamine biosynthesis. Mol. Plant-Microbe Interactions. 26:1294–1301. DOI:10.1094/MPMI-04-13-0098-R

11. Chuang, D., Chien, Y., and Wu, H.-P. 2007. Cloning and expression of the *Erwinia carotovora* subsp. *carotovora* gene encoding the low-molecular-weight bacteriocin Carocin S1. J. Bacteriol. 189:620–626. DOI: 10.1128/JB.01090-06

12. Cigna, J., Dewaegeneire, P., Beury, A., Gobert, V., and Faure, D. 2017. A gapA PCR-sequencing assay for identifying the *Dickeya* and *Pectobacterium* potato pathogens. Plant Dis. 101:1278–1282. DOI: 10.1094/PDIS-12-16-1810-RE

13. Coulthurst, S. J., Barnard, A. M. L., and Salmond, G. P. C. 2005. Regulation and biosynthesis of carbapenem antibiotics in bacteria. Nat. Rev. Microbiol. 3:295–306. DOI:10.1038/nrmicro1128

14. Curland, R. D., Mainello, A., Perry, K. L., Hao, J., Charkowski, A. O., Bull, C. T., et al. 2021. Species of *Dickeya* and *Pectobacterium* isolated during an outbreak of blackleg and soft rot of potato in northeastern and north central united states. Microorganisms. 9:1733. DOI: 10.3390/microorganisms9081733

15. Czárán, T. L., Hoekstra, R. F., and Pagie, L. 2002. Chemical warfare between microbes promotes biodiversity. Proc. Natl. Acad. Sci. 99:786–790. DOI: 0.1073/pnas.01239989

16. Degefu, Y. 2021. Co-occurrence of latent *Dickeya* and *Pectobacterium* species in potato seed tuber samples from northern Finland. Agric. Food Sci.. DOI:10.23986/afsci.101446

17. Ge, T., Ekbataniamiri, F., Johnson, S. B., Larkin, R. P., and Hao, J. 2021. Interaction between *Dickeya dianthicola* and *Pectobacterium parmentieri* in Potato Infection under Field Conditions. Microorganisms. 9:316. DOI: 10.3390/microorganisms9020316

18. Gralka, M., Szabo, R., Stocker, R., and Cordero, O. X. 2020. Trophic interactions and the drivers of microbial community assembly. Curr. Biol. 30:R1176–R1188. DOI:10.1016/j.cub.2020.08.007

19. Grinter, R., Milner, J., and Walker, D. 2012. Ferredoxin containing bacteriocins suggest a novel mechanism of iron uptake in *Pectobacterium* spp ed. Pierre Cornelis. PLoS ONE. 7:e33033. DOI: 10.1371/journal.pone.0033033

20. Holden, M. T. G., McGowan, S. J., Bycroft, B. W., Stewart, G. S. A. B., Williams, P., and Salmond, G. P. C. 1998. Cryptic carbapenem antibiotic production genes are widespread in *Erwinia carotovora*: facile trans activation by the carR transcriptional regulator. Microbiology. 144:1495–1508. DOI: 10.1099/00221287-144-6-1495

21. Hugouvieux-Cotte-Pattat, N., des-Combes, C. J., Briolay, J., and Pritchard, L. 2021. Proposal for the creation of a new genus *Musicola* gen. nov., reclassification of

Dickeya paradisiaca (Samson et al. 2005) as *Musicola paradisiaca* comb. nov. and description of a new species *Musicola keenii* sp. nov. Int. J. Syst. Evol. Microbiol. 71 DOI:
22. 735 10.1099/ijsem.0.005037

23. Hugouvieux-Cotte-Pattat, N., Condemine, G., and Shevchik, V. E. 2014. Bacterial pectate lyases, structural and functional diversity: Bacterial pectate lyases. Environ. Microbiol. Rep. 6:427–440. DOI: 10.1111/1758-2229.12166

24. Hugouvieux-Cotte-Pattat, N., Pédron, J., and Van Gijsegem, F. 2023. Insight into biodiversity of the recently rearranged genus *Dickeya*. Front. Plant Sci. 14:1168480. DOI: 10.3389/fpls.2023.1168480

25. Itoh, Y., Izaki, K., and Takahashi, H. 1978. Purification and characterization of a bacteriocin from *Erwinia carotovora*. J. Gen. Appl. Microbiol. 24:27–39.DOI:10.2323/jgam.24.27

26. Jonkheer, E. M., Brankovics, B., Houwers, I. M., van der Wolf, J. M., Bonants, P. J. M., Vreeburg, R. A. M., et al. 2021. The *Pectobacterium* pangenome, with a focus on *Pectobacterium brasiliense*, shows a robust core and extensive exchange of genes from a shared gene pool. BMC Genomics. 22:265. DOI: 10.1186/s12864-021-07583-5

27. Kelsic, E. D., Zhao, J., Vetsigian, K., and Kishony, R. 2015. Counteraction of antibiotic production and degradation stabilizes microbial communities. Nature. 521:516–519. DOI:10.1038/nature14485

28. Khayi, S., Blin, P., Jacques Pédron, J., Chong, T.-M., Chan, K.-G., Mohieddine Moumni, M., et al. 2015. Population genomics reveals additive and replacing horizontal gene transfers in the emerging pathogen *Dickeya solani*. BMC Genomics. 16:788. DOI:10.1186/s12864-015-1997-z

29. Li, X., Ma, Y., Liang, S., Tian, Y., Yin, S., Xie, S., et al. 2018. Comparative genomics of 84 *Pectobacterium* genomes reveals the variations related to a pathogenic lifestyle. BMC Genomics. 19 DOI: 10.1186/s12864-018-5269-6

30. Ma, B., Hibbing, M. E., Kim, H.-S., Reedy, R. M., Yedidia, I., Breuer, J., et al. 2007. Host range and molecular phylogenies of the soft rot Enterobacterial genera *Pectobacterium* and *Dickeya*. Phytopathology. 97:1150–1163. DOI: 10.1094/PHYTO-97-9-1150

31. Ma, X., Brazil, J., Rivedal, H., Perry, K. L., Frost, K., and Swingle, B. 2022. First Report of *Pectobacterium versatile* causing potato soft rot in Oregon and Washington. Plant Dis. 106:1292. DOI: 10.1094/PDIS-08-21-1635-PDN

32. Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., et al. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. 13:614–629. DOI: 10.1111/j.1364-3703.2012.00804.x

33. Mattinen, L., Nissinen, R., Riipi, T., Kalkkinen, N., and Pirhonen, M. 2007. Host-extract

838 induced changes in the secretome of the plant pathogenic bacterium
839 *Pectobacterium atrosepticum* . PROTEOMICS. 7:3527–3537. DOI:
840 10.1002/pmic.200600759
841
842 34. McGowan, S. J., Sebaihia, M., O'Leary, S., Hardie, K. R., Williams, P., Stewart, G. S. A.
843 B., et al. 1997. Analysis of the carbapenem gene cluster of *Erwinia carotovora* :
844 definition of the antibiotic biosynthetic genes and evidence for a novel β-lactam
845 resistance mechanism. Mol. Microbiol. 26:545–556. DOI:10.1046/j.1365-
846 2958.1997.6001974.x
847
848 35. Motyka-Pomagruk, A., Zoledowska, S., Sledz, W., and Lojkowska, E. 2021. The
849 occurrence of bacteria from different species of *Pectobacteriaceae* on seed potato
850 plantations in Poland. Eur. J. Plant Pathol. 159:309–325. DOI:10.1007/s10658-020-
851 02163-x
852
853 36. Nguyen, A. H., Tomita, T., Hirota, M., Sato, T., and Kamio, Y. 1999. A simple
854 purification method and morphology and component analyses for carotovoricin Er, a
855 Phage-tail-like bacteriocin from the plant pathogen *Erwinia carotovora* Er. Biosci.
856 Biotechnol. Biochem. 63:1360–1369. DOI: 10.1271/bbb.63.1360
857
858 37. Pasanen, M., Waleron, M., Schott, T., Cleenwerck, I., Misztak, A., Waleron, K., et al.
859 2020. *Pectobacterium parvum* sp. nov., having a *Salmonella* SPI-1-like Type III secre-
860 tion system and low virulence. Int. J. Syst. Evol. Microbiol. 70:2440–2448. DOI:
861 38. 10.1099/ijsem.0.004057
862
863 39. Pédron, J., Bertrand, C., Taghouti, G., Portier, P., and Barny, M.-A. 2019.
864 *Pectobacterium aquaticum* sp. nov., isolated from waterways. Int. J. Syst. Evol.
865 Microbiol. 69:745–751. DOI: 10.1099/ijsem.0.003229
866
867 40. Portier, P., Pédron, J., Taghouti, G., Dutrieux, C., and Barny, M.-A. 2020. Updated Tax-
868 onomy of *Pectobacterium* Genus in the CIRM-CFBP Bacterial Collection: When Newly
869 Described Species Reveal "Old" Endemic Population. Microorganisms. 8:1441. DOI:
870 41. 10.3390/microorganisms8091441
871
872 42. Portier, P., Pédron, J., Taghouti, G., Fischer-Le Saux, M., Caullireau, E., Bertrand, C., et
873 al. 2019. Elevation of *Pectobacterium carotovorum* subsp. *odoriferum* to species level
874 as *Pectobacterium odoriferum* sp. nov., proposal of *Pectobacterium brasiliense* sp.
875 nov. and *Pectobacterium actinidiae* sp. nov., emended description of *Pectobacterium*
876 *carotovorum* and description of *Pectobacterium versatile* sp. nov., isolated from
877 streams and symptoms on diverse plants. Int. J. Syst. Evol. Microbiol. 69:3207–3216.
878 DOI: 10.1099/ijsem.0.003611
879
880 43. Roh, E., Park, T.-H., Kim, M., Lee, S., Ryu, S., Oh, C.-S., et al. 2010. Characterization of
881 a new bacteriocin, Carocin D, from *Pectobacterium carotovorum* subsp. *carotovorum*
882 Pcc21. Appl. Environ. Microbiol. 76:7541–7549. DOI: 10.1128/aem.03103-09
883
884 44. Royer, G., Dixit, Z., Pédron, J., Pierrat, G., Demontant, V., Berçot, B., et al. 2022.
885 Genetic and Phenotypic Study of the *Pectobacterium versatile* Beta-Lactamase, the

Enzyme Most Similar to the Plasmid-Encoded TEM-1 ed. Christopher A. Elkins. Appl. Environ. Microbiol. 88:e00220-22. DOI: 10.1128/aem.00220-22

45. Shyntum, D. Y., Nkomo, N. P., Shingange, N. L., Gricia, A. R., Bellieny-Rabelo, D., and Moleleki, L. N. 2019. The impact of Type VI secretion system, bacteriocins and antibiotics on bacterial competition of *Pectobacterium carotovorum* subsp. *brasiliense* and the regulation of carbapenem biosynthesis by iron and the ferric-uptake regulator. Front. Microbiol. 10 DOI: 10.3389/fmicb.2019.02379

46. Smoktunowicz, M., Jonca, J., Stachowska, A., May, M., Waleron, M. M., Waleron, M., et al. 2022. The international trade of ware vegetables and ornamental plants—an underestimated risk of accelerated spreading of phytopathogenic bacteria in the era of globalisation and ongoing climatic changes. Pathogens. 11:728. DOI:10.3390/pathogens11070728

47. Taillefumier, T., Posfai, A., Meir, Y., and Wingreen, N. S. 2017. Microbial consortia at steady supply. eLife. 6:e22644. DOI: 10.7554/eLife.22644

48. Toth, I. K., Barny, M., Brurberg, M. B., Condemine, G., Czajkowski, R., Elphinstone, J. G., et al. 2021. *Pectobacterium* and *Dickeya*: environment to disease development. In *Plant Diseases Caused by Dickeya and Pectobacterium Species*, eds. Frédérique Van Gijsegem, Jan M. van der Wolf, and Ian K. Toth. Cham: Springer International Publishing, p. 39–84. http://link.springer.com/10.1007/978-3-030-61459-1_3

49. Weiss, A. S., Burrichter, A. G., Durai Raj, A. C., Von Strempel, A., Meng, C., Kleigrewe, K., et al. 2022. In vitro interaction network of a synthetic gut bacterial community. ISME J. 16:1095–1109. DOI: 10.1038/s41396-021-01153-z

50. de Werra, P., Debonneville, C., Kellenberger, I., and Dupuis, B. 2021. Pathogenicity and Relative Abundance of *Dickeya* and *Pectobacterium* species in Switzerland: an epidemiological dichotomy. Microorganisms. 9:2270. DOI:10.3390/microorganisms9112270

51. van der Wolf, J. M., Acuña, I., De Boer, S. H., Brurberg, M. B., Cahill, G., Charkowski, A. O., et al. 2021. Diseases Caused by *Pectobacterium* and *Dickeya* Species Around the World. In *Plant Diseases Caused by Dickeya and Pectobacterium Species*, eds. Frédérique Van Gijsegem, Jan M. van der Wolf, and Ian K. Toth. Cham: Springer International Publishing, p. 215–261. http://link.springer.com/10.1007/978-3-030-61459-1_7