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# Bacterial pathogens dynamic during multi-species infections

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**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.

101 The aim of the present work was to experimentally assess the fate of mix infection during soft
102 rot within potato tubers. To understand the dynamic of coinfections, we set up 16 different
103 synthetic communities of 6 SRP strains to mimic coinfection. Bacteria present in each synthetic
104 community were representative of 2 different species, with 3 strains per species. These
105 communities were inoculated on potato tuber or on growth medium and the outcome of each
106 synthetic community was followed by amplification and Illumina sequencing of the
107 discriminative housekeeping gene *gap*A (Cigna et al. 2017; Ben Moussa et al. 2022). We also
108 compared disease rating and bacterial multiplication on potato tuber during coinfection and
109 single species infection. Overall, the results indicate that competition, cooperation and trophic
110 interactions shape the bacterial SRP community and allow for the maintenance of SRP
111 diversity.
112
113 **Materials and Methods**
114
115 **Strains and media used**
116 The strain used in this study are described in table S1. For each species, where possible, the
117 bacterial strains were selected to allow strain discrimination using the gapA barcode (see
118 below). For some species, such as *D. dianthicola*, *D. solani* and *P. polaris/P. parvum*, we did
119 not find discriminating strains in our collection (Table S2). This is probably due to the fact that
120 these latter species are quite homogeneous (Hugouvieux-Cotte-Pattat et al. 2023; Pasanen et al.
121 2020). The bacterial media used were LB medium without NaCl (hereafter LB: per liter 10 g
122 tryptone, 5 g yeast extract) and TSB (per liter: 1,7 g casein peptone, 0.3 g soya peptone, 0.3 g
123 NaCl, 0.25 g $K_2HPO_4$, 0.25 g glucose). When needed, 15 g agar per liter were added for solid
124 media.
125
126 **Inocula preparation**
127 The bacteria were plated on LB plates and incubated overnight at 28°C. A single colony was
128 then used to inoculate 2 ml of LB medium which was incubated overnight at 28°C, with
129 agitation at 120 rpm. This liquid culture (100 µl) was used to inoculate a 10% TSB agar plate,
130 which was incubated overnight at 28°C. The grown bacterial layer was then suspended in 50
131 mM phosphate buffer (pH 7) and adjusted to an $OD_{600nm}$=1. The 3 strains of the same species
132 were then mixed in a 1/1/1 ratio. An exception was the inoculum composed of *P. parvum* (2
133 strains) and *P. polaris* (1 strain), which were mixed together as the experiment was set up before
134 the description of the *P. parvum* species, a close relative of *P. polaris* (Pasanen et al. 2020).

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

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

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

212

213 **Illumina sequencing analysis**

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

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

231

232 **Model presentation**

233 The equations of the model are:

234 $dDX/dt = k(D_R - D_X) - (gA - \epsilon)D_X X + cX/z + X$

235 $dDA/dt = k(D_R - D_A) - g_A D_A A$

236 $dDR/dt = -k(2D_R - D_X - D_A) - \lambda D_R$

237 $dX/dt = (g_A - \epsilon)\, D_X X - mX$

238 $dA/dt = g_A D_A A - mA$

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

251

252 **Search for carbapenem synthesis and resistance**

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

259

260 **Results:**

261

262 **Set up of the experiment**

263 A general view of the experimental set up is shown in Figure 1. In brief, each synthetic SRP
264 community was a mixture of 6 strains belonging to two different species. The mixture of two
265 species was preferred because the mixture of two different species is often observed in the field
266 (Degefu 2021; de Werra et al. 2021; Ge et al. 2021; Motyka-Pomagruk et al. 2021;
267 Smoktunowicz et al. 2022). The addition of three strains per species allows to disentangle the
268 "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.

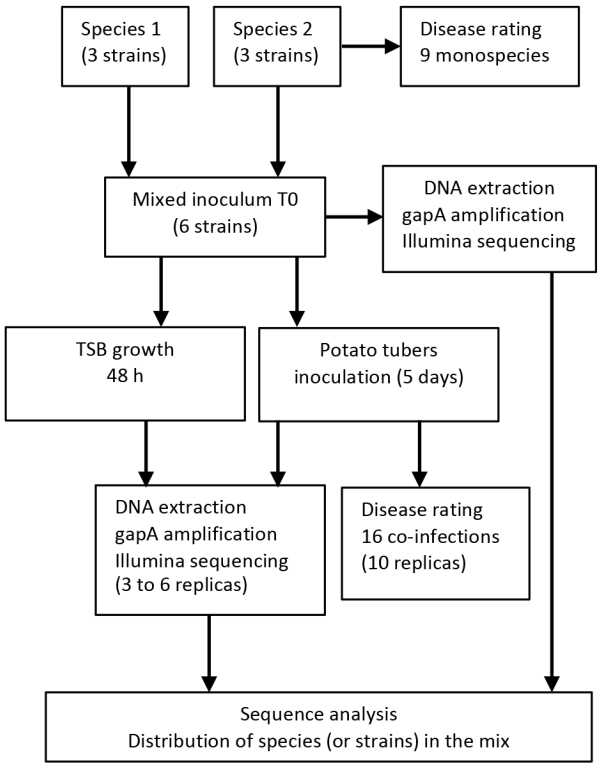



**Figure 1:** Set up of the experiment.

**Symptoms observation and bacterial multiplication in potato tubers**

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

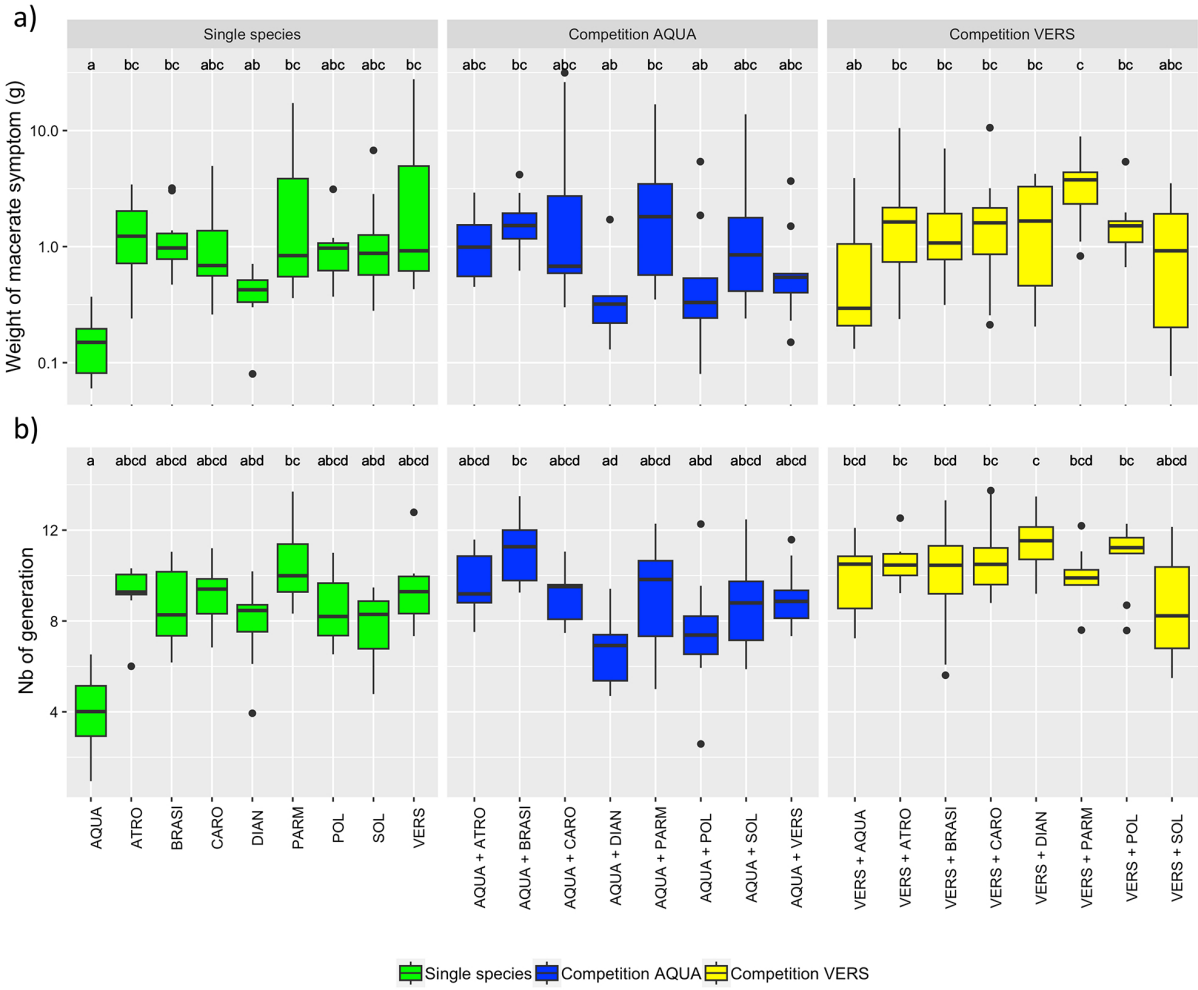



**Figure 2:** Symptoms and bacterial growth of the SRP synthetic communities on potato tubers. a) Weight of macerate symptom b) bacterial growth within potato tuber. Single-species mixtures (3 strains) are shown in green. Two-species mixtures (6 strains) are shown in blue and yellow for *P. aquaticum*, 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,

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

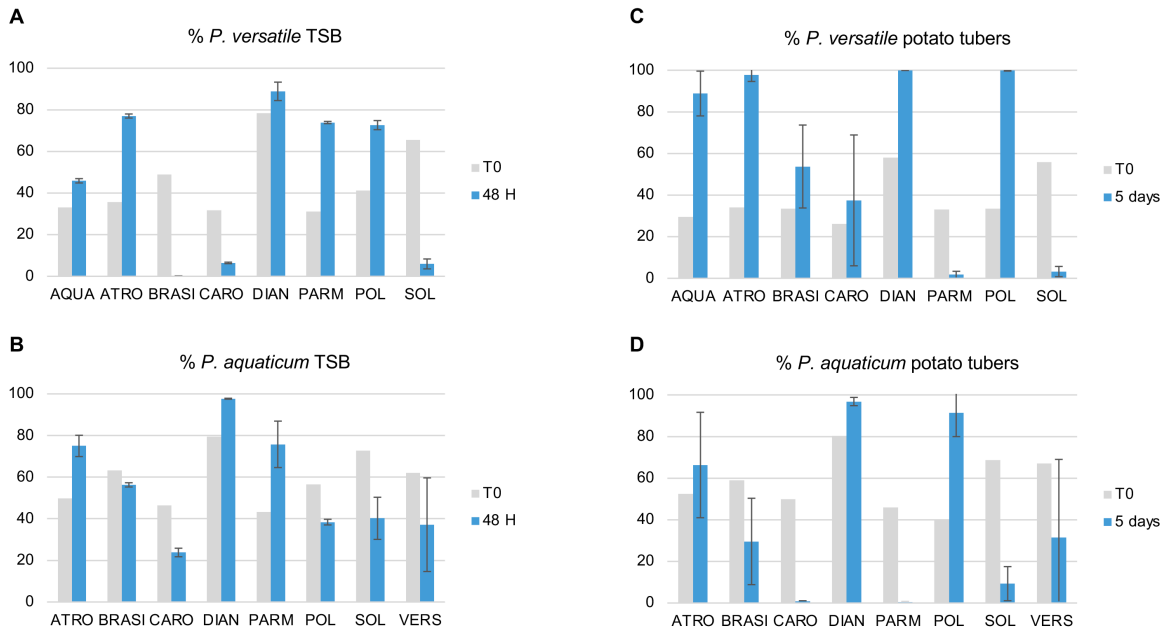



**Figure 3:** Species outcome within SRP communities.

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*.

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

388
389 Blue: coexistence. Green: *P. versatile or P. aquaticum* dominates. Red: *P. versatile* or *P. aquaticum* was
390 outcompeted. The number indicated the % mean of *P. aquaticum* or *P. versatile* at the end of the
391 experiment
392
393 **Modelling of cheater persistence**
394 The SRP species *P. aquaticum* has been isolated from water and is not recorded on plant
395 (Pédron et al. 2019; Portier et al. 2020). We noticed here that the *P. aquaticum* strains have a
396 poor capacity to rot potato tubers and multiply within symptoms (Fig. 2). This is a previously

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

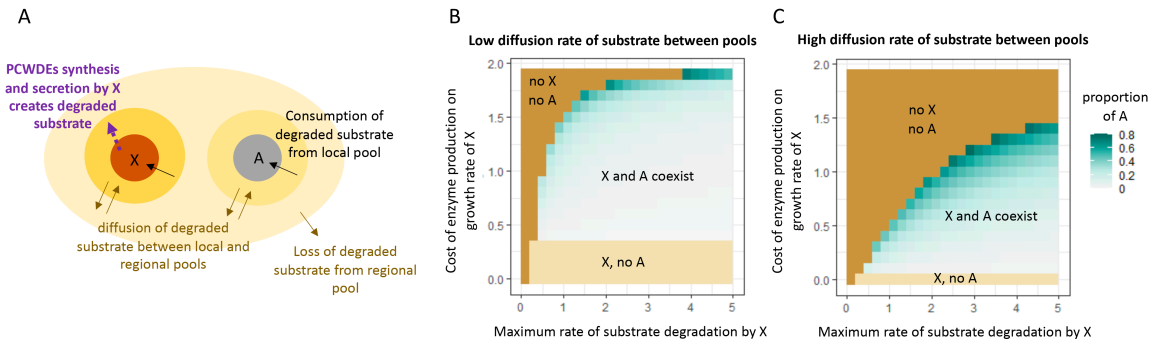



**Figure 4:** Modeling of cheater abundance.

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).

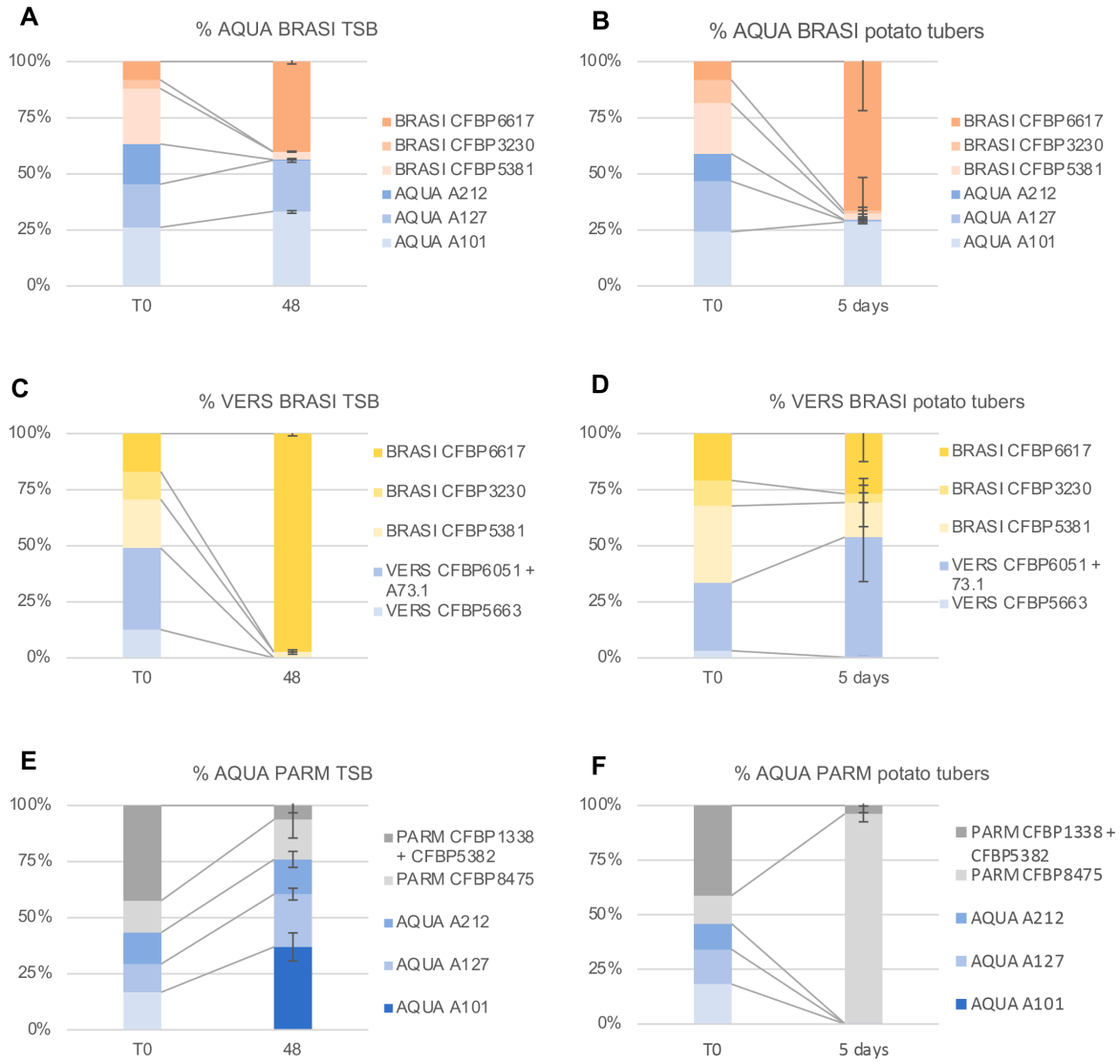



**Figure 5:** Examples of strains outcome within SRP communities.

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.

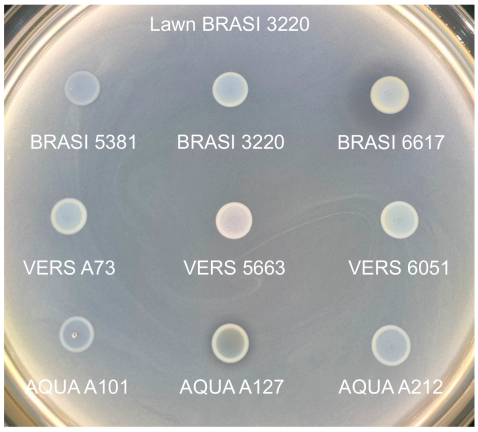
469 The strains had different outcomes when grown on potato tubers and TSB. Overall, the
470 outcompeted strains made up a higher percentage within the potato tuber (66%) than after
471 growth on TSB (32%) (Table 2).
472
473 **Table 2:** Outcome of individual strains within the tested SRP communities.

| 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% |

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

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

500

501 **Figure 6:** Pairwise competition assays.

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

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

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

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

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

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**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

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