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 steam 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, aggressivity 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 latent mix of SRP spp. are also reported on asymptomatic potato tuber and could represent up to 18% detected latent infection (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. 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. 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 dipotassium hydrogen phosphate, 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 DO_{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). The bacterial load of each 3 strains-mixtures was check by serial dilution and plating on TSB agar plates. The species mixtures were then prepared by mixing two of the prepared strain-mixtures in volumes 1/1. Each final species-mixture is thus the mixture of 6 strains adjusted to DO_{600nm}=1 in the ratio 1/1/1/1/1/1, where the first strain-mixture corresponds to one of the nine

136 following species: *P. atrosepticum, P. carotovorum, P. parmentieri, P. versatile, P.*
137 *polaris/P.parvum, D. dianthicola, D. solani, P. versatile, P. aquaticum*, and the second strain-
138 mixture corresponds either to the species *P. aquaticum* or to the species *P. versatile*. A 2 ml
139 aliquot of each time zero species-mix inoculum was stored at -20°C until DNA extraction.
140 These 18 species-mixtures were then used to inoculate triplicates of 2 ml TSB liquid medium
141 (1 µl in 2 ml) or 10 potato tubers (10 µl per tuber). The inoculated TSB medium was incubated
142 at 26°C with shaking during 48h. Serial dilution and plating was performed to check the
143 bacterial growth at the end of the culture. The bacterial load at time zero (inoculum) and at the
144 end of the experiment were used to calculate the number of bacterial generation achieved ($\log_{10}$
145 (cfu end experiment/cfu T0)/$\log_{10}$ (2)). Cultures were then centrifuged and the pellet was kept
146 at -20°C until DNA extraction.
147
148 **In vitro bacterial competition assays**
149 Pairwise in vitro bacterial competition assays were performed between the *P. brasiliense, P.*
150 *versatile* and *P. aquaticum* strains (3 strains per species, 81 combinations tested). Briefly,
151 overnight TSB cultures of each strain were adjusted to an DO_{600nm} = 0.15. Samples of 5 µl of
152 the strain 1 were spotted onto freshly poured agar lawn inoculated with strain 2 (1 ml with 15
153 ml TSB agar medium) and plates were allowed to grow for 24 h at 28°C. If strain 1 inhibits the
154 growth of strain 2, a clear halo is observed around the spot of strain 1. Experiment was repeated
155 twice with similar results.
156
157 **Potato inoculation**
158 Three experiments were performed, the first one was devoted to analyze the mono-species
159 infection with the mix of the 3 strains/species. The second and third one involved mix of two
160 different species (6 strains) with either *P. aquaticum* species or *P. versatile* species challenged
161 by one of the other 8 species.
162 For each mix, 10 potato tubers were thoroughly washed, and surface disinfected in a 1 %
163 hypochlorite solution bath prior inoculation and each tuber was punctured with pipette tips
164 containing 10 µl of the inoculum. The tip was left in the tuber. As a negative control, a pipette
165 tip with 10µl sterile water was inserted into one tuber. Inoculated tubers were placed on paper
166 towel in plastic boxes and 50 mL of distilled water was poured into the bottom of the boxes and
167 the boxes were carefully closed to achieve relative humidity above 90 %. After 5 days of
168 incubation at 26°C, the tubers were cut and the entire macerating symptom was collected and
169 weighed. Part of the symptom (50 to 300 mg) was also weighed and suspended in 1 ml of sterile

170 phosphate buffer in order to count by serial dilution and plating the bacterial load present in the
171 symptom. To recover the bacteria within the symptoms, the rest of the symptom was suspended
172 with 7 ml of 50 mM phosphate buffer and stirred for 4 hours at ambient temperature. The tubes
173 were then left without shaking for 10 minutes to allow the excess starch to settle and 2 ml at the
174 surface of the suspension was recovered and centrifuged for 10 minutes at 10,000 g. The formed
175 pellet was kept at -80°C until DNA extraction.
176
177 **Statistical analysis**
178 One specie mix (*P. aquaticum/P. versatile*) was shared between the second and the third
179 experiments and this allowed to eliminate inter-experiment variation unrelated to the involved
180 species mix before statistical analysis. To do so we corrected the maceration weight observed
181 in the third experiment with the following coefficient X = (mean of *P. aquaticum/P.versatile*
182 10 potato tubers maceration weight of the 2ndexperiment)/ mean of *P. aquaticum/P.versatile* 10
183 potato tubers maceration weight results of the 3rd experiment) before statistical analysis.
184 Statistical analyses were performed in R version 4.0.2 using a Kruskal-Wallis test with p-value
185 < 0.05 followed by a post-hoc test (Dunn test with Bonferroni correction). Spearman correlation
186 calculation were performed on line (https://biostatgv.sentiweb.fr/?module=tests/spearman).
187
188 **DNA extraction, DNA amplification and sequencing**
189 For each of the 18 species-mix analyzed, DNA extraction was performed on 10 ml of the time
190 zero inoculum, on 2 ml of each of the TSB triplicate after 48h of culture and on 2 ml of 3 to 6
191 potato macerated tubers symptoms. The bacteria were pelleted by centrifugation and DNA
192 extraction was performed with the Wizard® genomic DNA purification kit (Promega).
193 Amplification and sequencing were performed at MR DNA (www.mrdnalab.com, Shallowater,
194 TX, USA). Briefly, the *gap*A 376 partial gene sequence was amplified using PCR primers
195 *gap*AF376: GCCCGTCTCACAAAGA and *gap*AR: TCRTACCARGAAACCAGTT) with
196 barcode on the forward primer. PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA)
197 was performed under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C
198 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
199 was performed. Amplified PCR products were checked in 2 % agarose gel to determine the
200 success of amplification and the relative intensity of bands. Multiple samples were pooled
201 together in equal proportions based on their molecular weight and DNA concentrations. Pooled
202 samples were purified using calibrated Ampure XP beads and used to prepare Illumina DNA
203 library. Sequencing was performed on a MiSeq sequencer following the manufacturer's

guidelines. Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences were joined, depleted of barcodes and sequences <150bp or with ambiguous base calls were removed. Reads were filtered based on Q score and expected error probability and any read with a number of expected errors greater than 1.0 were discarded.

Illumina sequencing analysis

After quality trimming, a total of 2,230,817 and 3,215,158 reads were obtained for *in vitro* (TSB, 64 samples) and potato tuber (100 samples) assays respectively, with an average of 34,857 ± 963 and 32,152 ± 7538 reads per samples respectively.

In order to quantify the distribution of the *Pectobacterium* and *Dickeya* strains after *in vitro* growth or potato inoculation, reads were aligned to the corresponding amplified sequences of the *gap*A gene, using the nucleotide-nucleotide Blastn tool (version 2.11.0+, e-value threshold 10^{-5}). Only reads with 100% full-length identity were used (547,866 reads, 24.6% of the total reads and 1,213,517 reads, 37.7% of the total reads for *in vitro* and potato tuber assays respectively. A few potato tubers were withdrawn of the analysis since unexpected *P. parmentieri* sequences were observed, suggesting an initial presence of this pathogen as latent species hosted on the tuber prior to the inoculation.

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

Model presentation

The equations of the model are:

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

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

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

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

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

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. Briefly, each synthetic SRP community was a mixture of two species with three strains per species. 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;

270 Ben Moussa et al. 2022). In contrast, *P. aquaticum* has not been isolated from plants and is
271 frequently isolated from water (Portier et al. 2020; Ben Moussa et al. 2022). SRP communities
272 were inoculated on TSB medium or on potato tubers and the proportion of each species at the
273 beginning and end of the experiment was assessed by Illumina sequencing of the *gap*A
274 discriminative gene marker (Cigna et al. 2017; Ben Moussa et al. 2022). Symptoms occurring
275 on potato tubers were also recorded for each 6 strains community and compared with symptoms
276 observed with the mix of 3 strains of single-species inoculation.
277
278 **Symptoms observation and bacterial multiplication in potato tubers**
279 Single-species infection resulted in various symptom development in potato tubers depending
280 on the inoculated species tested. Outliers were frequently observed and there was considerable
281 variability in response between the 10 inoculated potato tubers (Fig. 2A). The 10 potato tubers
282 inoculated with *P. aquaticum* showed hardly any symptoms and statistical analysis confirmed
283 that *P. aquaticum* developed less severe symptoms than *P. versatile, P. parmentieri, P.*
284 *brasiliense* and *P. atrosepticum* (Fig. 2A). Bacterial multiplication was assessed and the number
285 of bacterial generations achieved within each inoculated tuber was calculated (Fig. 2B). It
286 varied from 0.9 to 13.7 with a median of 8.9 depending on the tuber inoculated. This
287 multiplication rate correlated with the weight of macerating symptoms (Spearman correlation
288 0.451, p-value 8.87E-14). After inoculation with *P. aquaticum*, bacterial multiplication within
289 the tubers was much lower than with other single inoculated single species, confirming *P.*
290 *aquaticum* poor ability to rot potato tuber (Fig. 2B) as already described (Ben Moussa et al,
291 2023). The addition of either *P. versatile* or *P. aquaticum* to the inoculation mix did not affect
292 the severity of the symptoms observed compared to the other single species inoculation but *P.*
293 *aquaticum* (Fig. 2A). Bacterial multiplication was also unaffected by the mixed-species
294 inoculation (Fig. 2B). In the following experiments 3 to 6 tubers from each SRP community
295 were analyzed by amplification and Illumina sequencing of the *gap*A barcode.
296
297 **Species outcome within SRP communities inoculated in TSB medium**
298 Bacterial multiplication was assessed after 48 hours of growth on TSB medium and the number
299 of bacterial generations obtained was calculated. It ranged from 4.2 to 9 generations and was
300 repeatedly higher in the mixture containing *P. aquaticum* compared to the mixture containing
301 *P. versatile* (Fig. S1). A total of 2,230,817 sequences were obtained after Illumina sequencing,
302 with an average of 34,857 ± 963 sequences per sample. The 376 pb *gapA* sequence allowed us
303 to distinguish all the inoculated species and to calculate the percentage of sequences assigned

304 to *P. aquaticum* or *P. versatile* within each SRP community at time zero and after 48 hours of
305 growth (Fig 3 A and B). At time zero, *P. aquaticum* or *P. versatile* represented 30% to 60% of
306 the inoculum in each mix containing another *Pectobacterium* species as expected for an even
307 inoculation of each strain. However, in the SRP community containing *Dickeya spp.*, *P.*
308 *aquaticum* and *P. versatile* made up more than 60% of the mixture. This deviation was
309 particularly important for the mix containing *D. dianthicola* as *P. versatile* and *P. aquaticum*
310 represented 78% and 79% respectively. A similar deviation was observed after counting the cfu
311 of each individual 3 species mix at time zero indicative of a higher inoculation of *P. versatile*
312 and *P. aquaticum* compared to the *D. dianthicola* species in the initial mix (data not shown).
313 This bias is likely due to the fact that *D. dianthicola* is highly mucoid impacting the optical
314 density-cfu relation.
315 After 48 hours of growth, with an average number of generations of 6.7 after 48 hours of
316 growth, the initial population size represents only around 1% of the final population size. The
317 *gap*A barcode allowed to analyzed the proportion of each SRP species within the synthetic
318 community (Fig. 3 A and B). *P. aquaticum* and *P. versatile* were considered to drastically
319 outcompete the other SRP species if the mean percentage for the three replicates reached at
320 least 90% at the end of the experiment. They were considered to be drastically outcompeted by
321 the other SRP species if their mean percentage for the three replicate was inferior to 10%.
322 Finally, if the mean percentage of *P. aquaticum* or *P. versatile* was between 10% and 90%, *P.*
323 *aquaticum* and *P. versatile* were considered to be coexisting with their challenging SRP species.
324 Using this criterion most of the SRP community (12 out of 16) allowed coexistence of both
325 species after 2 days of growth in TSB medium (Fig. 3A and B). *P. aquaticum* outcompeted *D.*
326 *dianthicola* and coexisted with all the other challenging species. *P. versatile* was outcompeted
327 when co-inoculated with *P. brasiliense, P. carotovorum* or *D. solani* and it coexisted with the
328 other species at various levels.
329
330 **Species outcome within SRP communities inoculated within potato tubers**
331 A total of 3,215,158 sequences were obtained after Illumina sequencing, with an average of
332 32,152 ± 7538 sequences per sample. We calculated the percentage of reads assigned to *P.*
333 *aquaticum* or *P. versatile* in the mixed-species SRP communities at time zero and after growth
334 inside the potato tubers (Fig. 3 C and D). Using the same criteria as after TSB growth,
335 coexistence was observed 5 days post infection in 6 out of the 16 mixed-species SRP
336 communities inoculated in potato tubers. Within potato tubers, *P. aquaticum* was outcompeted
337 in presence of *P. carotovorum, P. parmentieri* and *D. solani*, it outcompeted *D. dianthicola* and

338 *P. polaris* and coexisted with *P. atrosepticum, P. brasiliense* and *P. versatile*. *P. versatile* was
339 outcompeted in the presence of *P. parmentieri* and *D. solani*, it outcompeted *P. atrosepticum,*
340 *D. dianticola* and *P.polaris/P.parvum* and coexisted with *P. aquaticum, P. brasiliense and P.*
341 *carotovorum*.
342 The outcome of the competitions in potato tubers was different from that observed after growth
343 on TSB for 10 out of the 16 SRP communities tested (Table 1). For example, within potato
344 tubers *P. aquaticum* was outcompeted by *P. carotovorum, P. parmentieri* and *D. solani* while
345 it coexisted with each of these three species in TSB. Similarly, while *P. versatile* coexisted with
346 *P. brasiliense* and *P. carotovorum* in potato tubers, it was outcompeted by both species in the
347 TSB medium. As well, *P. aquaticum* and *P. versatile* were outcompeted by *P. parmentieri* in
348 potato tubers but both species coexisted with *P. parmentieri* in the TSB medium. Overall, for
349 the 16 tested combinations, coexistence between the two species twice was more frequent in
350 TSB medium (12/16 SRP community tested) than within potato tuber (6/16 SRP community
351 tested).
352
353 **Modelling of cheater persistence**
354 The SRP species *P. aquaticu*m has been isolated from water and is not recorded on plant
355 (Pédron et al. 2019; Portier et al. 2020). We noticed here the poor capacity of the *P. aquaticum*
356 strains to rot potato tuber and to multiply within symptoms (Fig. 2), a phenotypic trait that was
357 already described previously (Ben Moussa et al. 2023). We observed nevertheless, that *P.*
358 *aquaticum* when co-inoculated with another species, could be maintained and even become
359 dominant in several synthetic communities tested (Fig. 3C and D). To investigate the potential
360 mechanisms by which *P. aquaticum* might persist when co-inoculated with other species and
361 become even dominant, we analysed a model describing the dynamics of two species in
362 competition for one common resource originating from the degradation of a substrate by the
363 enzymatic activity of only one of the two species (species X, Fig. 4A, Appendix model). The
364 other species (A) is a cheater as it does not contribute to the production of degraded substrate,
365 and thus has no cost related to enzyme production in that context. The model considers
366 heterogeneous distribution of the degraded substrate, with specific concentrations in the vicinity
367 of each species (species local pool of degraded substrate, Fig. 4A) resulting from the depletion
368 of degraded substrate through consumption for both species X and A, and from the creation of
369 degraded substrate by enzyme production for species X. Degraded substrate diffuses at a given
370 rate between the two species-specific local pools and a regional pool, determining the
371 interactions between the species A and X through their indirect effect on the concentration of

372 degraded substrate in the regional pool. The analysis of the model (Fig. 4 B and C,
373 supplementary file S1) suggests that the cheater species A can persist when it has sufficiently
374 higher growth rate than the producer species X (i.e. sufficient cost of enzyme production on the
375 growth rate of species X) and when species X is sufficiently efficient in producing degraded
376 substrate (i.e. high maximum rate of substrate degradation). Higher diffusion rate of degraded
377 substrate between the local and regional pools also favours species A persistence for lower
378 enzyme production cost of species X (Fig. 4B and C). The cheater species can become dominant
379 (i.e. proportion of abundance of A higher than 0.5) when A has significantly higher growth rate
380 than X (intermediate to high cost of enzyme production) and when the rate of substrate
381 degradation by X is high, especially when the diffusion of degraded substrate in the
382 environment are high. However, in such cases the abundance of X is low (see supplementary
383 file S1) and we are at the limit of the coexistence domain between X and A. Indeed, as A has
384 relatively very high growth rate in comparison to X, this can lead to the exclusion of X by A
385 through competition for the consumption of degraded substrate, further leading to the extinction
386 of A due to the loss of production of degraded substrate by X (see supplementary file S1).
387
388 **Strains outcome within the SRP communities**
389 Beyond the species level, the 376 pb *gap*A Illumina sequenced fragment allowed the
390 differentiation of the 3 inoculated strains of *P. brasiliense* or *P. aquaticum* and one of the three
391 strains for *P. versatile*, *P. carotovorum*, *P. parmentieri* and *P. atrosepticum* (Table S2). This
392 allowed us to analyze the fate of these 10 strains within the 16 tested SRP communities within
393 potato tubers or TSB (Table 2, Fig. S2 and S3). Comparison of the fate of these 10 strains
394 showed that the outcome was mainly determined at the strain level (Fig. 4, see Fig. S2, Fig. S3
395 for the 32 tested combinations). In 20 cases, within the 3 strains of a single species, the fate of
396 the 3 strains was different. For example, within the *P. aquaticum/P. brasiliense* community in
397 which the six strains could be distinguished, after growth in TSB, one strain belonging to the
398 *P. aquaticum* species (A212) and two strains belonging to the *P. brasiliense* species (CFBP5381
399 and CFBP3230) were outcompeted, while two strains of *P. aquaticum* (A101 and A127) and
400 one strain of *P. brasiliense* (CFBP6617) coexisted (Fig. 5). The result of the same community
401 grown on potatoes was different, allowing the coexistence of one *P. brasiliense* strain
402 (CFBP6617) and only one *P. aquaticum* strain (A101) (Fig. 5). Also, within the *P. aquaticum/P.*
403 *parmentieri* community, the *P. parmentieri* strain CFBP8475 outcompeted the 5 other strains
404 on potato tubers, but this was not the case on TSB (Fig. 5). The outcome of each strain was
405 different between potato tubers and TSB, and overall, the outcompeted strains were more

406 numerous within the potato tuber (72%) than after growth on TSB (32%) (Table 2). However,
407 the dominance of a single strain over the 5 other strains of the SRP community was rarely
408 observed both within tubers and in TSB medium: only *P. parmentieri* dominates in potato
409 tubers when associated with *P. aquaticum* and, in TSB medium, *P. brasiliense* dominates when
410 associated with *P. versatile* (Table 2, Fig. 5). In the other synthetic communities tested, on TSB
411 or within the potato tubers, coexistence of at least two strains was observed in 23 of the 32
412 combinations tested, the remaining 7 combinations were inconclusive because we could not
413 differentiate some strains with the *gap*A barcode (Fig. S2 and S3).
414
415 **Focus on the SRP communities involving *P. brasiliense***
416 The strain *P. brasiliense* CFBP6617, also called 1692, is a model strain known to produce,
417 among other toxic compounds, a carbapenem antibiotic (Holden et al. 1998; Shyntum et al.
418 2019). We set up pairwise *in vitro* competition assays between the 6 strains of *P. brasiliense*
419 and *P. aquaticum* to check for carbapenem production. This showed that the *P. brasiliense*
420 strain CFBP6617 inhibited the growth of the other two *P. brasiliense* strains, CFBP3220 and
421 CFBP5381, and of the two *P. aquaticum* strains, A212 and A127 (Fig. 6). The *P. aquaticum*
422 strain A101 was not inhibited by the *P. brasiliense* strain CFBP6617. Consistent with this latter
423 result, the *car*G, *car*H and *car*F genes conferring resistance to carbapenem (Coulthurst et al.
424 2005) were only detected in the genome of the strain *P. aquaticum* A101. During our pairwise
425 inhibition tests, we also observed that the *P. aquaticum* strain A127 produces an unknown
426 compound that inhibits the growth of the strain *P. brasiliense* CFBP6617. The radius of action
427 of this unknown toxin is smaller than the one induced by the carbapenem production by the *P.*
428 *brasiliense* strain CFBP6617 (Fig. 6).
429 These observed pairwise inhibitions could probably explain the outcome of the *P. aquaticum/P.*
430 *brasiliense* community after growth on TSB medium (Fig. 5), as the 3 strains CFBP 6617, A127
431 and A101 were efficiently maintained after growth on this medium, while the 3 other strains
432 were outcompeted. The result of the same community on potato tubers was slightly different as
433 only 2 strains, CFBP6617 and A101, were efficiently maintained (Fig. 5). This could probably
434 be explained by the fact that the unknown toxic compound that counteract the growth of *P.*
435 *brasiliense* strain CFBP6617 is not produced by strain A127 or inefficient within potato tuber,
436 while the carbapenem is still produced within potato tuber. Surprisingly, within the *P.*
437 *versatile/P. brasiliense* community, we observed that the *P. brasiliense* strain CFBP5381 was
438 maintained within the potato tuber despite its sensitivity to the carbapenem produced by the *P.*
439 *brasiliense* strain CFBP6617 (as revealed with the *P.aquaticum/P. brasiliense* SRP community)

(Fig. 5). This suggests that at least one of the two *P. versatile* strains CFBP6051 or A73 still observed in the potato tubers at the end of the experiment is interfering with the toxic effect of *P. brasiliense* CFBP6617 to allow the growth of *P. brasiliense* CFBP5381. The mechanism that allows the *P. versatile* strains to resist to carbapenem toxicity of *P. brasiliense* CFBP6617 is unknown, as the two strains do not carry the carbapenem resistance genes *car*G, *car*H and *car*F in their genomes. Whatever the mechanism involved, it is either not induced or not efficient in TSB medium, as CFBP6617 dominates over the other 5 strains of the *P.brasiliense/P. versatile* community TSB medium.

Discussion:

The dynamic of bacterial microbial communities is an important research field. Theoretical studies showed that cooperative trophic interaction generally leads to the steady coexistence of distinct bacterial species, that organize themselves to create a community-level metabolism that best exploits the nutrients present (Gralka et al. 2020; Taillefumier et al. 2017). This is exemplified by synthetic metabolic consortia set up for various biotechnological applications that regroup highly diverse bacteria with different metabolic capacity that could be stably maintained. As well, metabolic cross feeding could allow maintenance of bacterial species otherwise outcompeted in natural environment (Franza et al, 2016). The maintenance of microbial diversity within pathogenic bacterial species complex cannot follow the same rule as, in that case, the microbes within these species complex are closely phylogenetically related with highly similar metabolic properties. This metabolic closeness implies a strong substrate overlap likely associated with strong competition between species within the same niche (Weiss et al. 2022). It is therefore not straightforward to understand which mechanisms allow the maintenance of diversity within these pathogenic species complexes. The bacterial SRP species complex is probably one of the most diverse species complexes of plant pathogenic bacteria. It gathers 38 accepted or proposed species and at least 15 SRP species have been identified on the potato host, with frequent co-infection with at least 2 species (Toth et al. 2021). The work presented here takes advantage of *gap*A barcoding Illumina sequencing to analyze the bacterial dynamics of 16 different synthetic SRP communities involving 6 strains belonging to two different species.

The outcome of the 16 synthetic communities was highly dependent on environmental conditions and strongly differed between potato tubers and TSB stirred liquid medium. The striking outcome differences of the synthetic community in the two different environments

474 should warns us on the use of synthetic liquid medium to test consortia stability when these
475 consortia should be used in a different environment. Notably, coexistence between the two
476 species was twice more frequent in TSB liquid medium than within potato tubers and
477 outcompeted strains were more numerous within the potato tubers than after growth on TSB
478 liquid medium. Competition through production of secreted toxic compounds, such as
479 carbapenem (Coulthurst et al. 2005; McGowan et al. 1997), carotovoricin (Nguyen et al. 1999;
480 Itoh et al. 1978), bacteriocins (Chuang et al. 2007; Chan et al. 2011; Roh et al. 2010; Grinter et
481 al. 2012), NRPS-PKS secondary metabolites (Brual et al. 2023; Cheng et al. 2013) have been
482 described in SRP. The production of many of these compounds is enhanced by plant extracts
483 or in potato tubers (Bellieny-Rabelo et al. 2019; Mattinen et al. 2007) and this could explain
484 that more strains are outcompeted during growth in potato tubers. Furthermore, competition
485 mechanisms relying on cell to cell contact, like the type 6 secretion system (T6SS), are also
486 described in SRP (Shyntum et al. 2019; Arizala and Arif 2019) but are likely inefficient in liquid
487 stirred medium. Modelling studies showed that in a structured environment such as that found
488 in potato tubers, the production of antimicrobial molecules could be beneficial even if the
489 producer is rare (Chao and Levin, 1981; Durrett and Levin, 1997; Gardner et al., 2004). The
490 enhanced production of antimicrobial molecules and their higher efficiency within a structured
491 environment, such as potato tubers, likely explain the higher competition observed within
492 potato tubers compared to liquid TSB medium.
493 Our results also illustrate that this competition is strain-specific rather than species-specific, as
494 in many combinations we observed that the fate of the 3 strains of the same species was
495 different. This is likely related to the fact that toxin production and toxin resistance are
496 functional traits associated with strains rather than with species and are generally encoded
497 within the accessory genomes of each species, as exemplified here by biosynthesis and
498 resistance to carbapenem (Shyntum et al. 2019). In most combinations, the *P. brasiliense* strain
499 CFBP6617 outcompeted the other two *P. brasiliense* strains, consistent with the observed
500 pairwise *in vitro* competition and the presence of the carbapenem cluster in the strain genomes.
501 However, within the *P. versatile/P. brasiliense* synthetic community, we observed an
502 interference towards the toxic effect of *P. brasiliense* CFBP6617 on *P. brasiliense* strain
503 CFBP5381, likely mediated by *P. versatile* strains. This interference may involve the secreted
504 β-lactamase commonly found in *P. versatile* strains which may inactivates the carbapenem
505 antibiotic (Royer et al. 2022). Further work is needed to test this hypothesis. *In vitro* pairwise
506 inhibition indicated that "face-to-face" competition may also occur in some of the synthetic
507 SRP communities tested, as for example *P. brasiliense* strain CFBP6617 is toxic to *P.*

508 *aquaticum* strain A127, but the reverse is also true. The complexity of the competitive interplay
509 between strains is enhanced by the number of toxic compounds and resistance mechanisms
510 known to be produced by SRP. Theoretical models highlight that chemical warfare between
511 microbes promote diversity (Kelsic et al. 2015; Czárán et al. 2002). The fact that each SRP
512 strains carries its own set of toxic compounds and resistance mechanisms could therefore
513 contribute to the maintenance of multiple strains as observed with the majority of the 16
514 synthetic communities tested. The complexity of the competition between SRP strains rather
515 than species probably also explains the temporal and spatial succession of different SRP species
516 in epidemic outbreaks.
517 Beyond the competition mechanisms, the virulence factors of SRP may also contribute to the
518 maintenance of SRP diversity, as the main virulence factors of SRP, i.e. the ability of SRP to
519 degrade the plant cell wall through the secretion of PCWDEs, can be considered as a public
520 good used to feed the whole community. In potato tubers, the important release of nutrients
521 once SRP express their main virulence factors allows the development of associated
522 commensals (Kõiv et al. 2015). Here, we observed that *P. aquaticum* strains were efficiently
523 maintained in the population within potato tubers in 5 out of 8 synthetic communities tested.
524 We also observed that *P. aquaticum* strains were unable to produce soft rot disease symptoms
525 and to multiply efficiently in the potato tubers when inoculated alone. The inability of *P.*
526 *aquaticum* strains to produce soft rot symptoms when inoculated alone strongly suggests that
527 the T2SS and associated virulence factors are not properly induced within the potato tuber and,
528 as such, *P. aquaticum* does not share the cost of producing virulence factors. However, when
529 mixed with other species, *P. aquaticum* strains could eventually become dominant, suggesting
530 that they can behave as cheaters, benefiting greatly from nutrient sharing. Several independent
531 mechanisms could be proposed to explain this efficient cheating behavior. Firstly, the low
532 expression of T2SS and the associated virulence factors and the smaller genome of *P.*
533 *aquaticum* strains compared to that of other SRP species may allow a more rapid multiplication
534 at the expense of the other SRP species. In support of this hypothesis, we observed here that in
535 TSB medium the 8 synthetic communities with *P. aquaticum* have a tendency to grow at higher
536 density in TSB medium than the 8 synthetic communities with *P. versatile*. It has also been
537 reported that *P. aquaticum* strains have increased mobility compared to that of other SRP
538 species (Ben Moussa et al. 2023). This may also influence the ability of *P. aquaticum* strains to
539 access nutrients within the symptoms. *P. aquaticum* has never been isolated from potatoes in
540 the field or in storage, and their cheating behavior observed here could therefore be considered
541 an artefact. Nevertheless, the observation of this cheating behavior suggests that other SRP

species could also behave as cheaters in the field, as plants infected with several SRP species are often observed. This is supported by the fact that the repertoire and regulation of virulence factors is variable among SRP species (Babujee et al. 2012; Jonkheer et al. 2021; Arizala and Arif 2019; Li et al. 2018), likely allowing for a trade-off between growth and the ability to cheat. Interestingly, our modelling suggests that the more efficient the SRP strain is at decomposing potato tubers, the higher the abundance of the cheaters in the symptoms. This greater abundance of the cheaters may affect the ability to detect the SRP strain responsible for the symptoms and increase the risk of not identifying the initial SRP strain responsible for the epidemic. Therefore, field sampling plans should be calibrated to account for this risk.

In summary, the dynamics of the 16 synthetic SRP communities presented here highlight some possible mechanisms allowing both the maintenance of high SRP diversity within the SRP 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).

Figure legends

Figure 1: Set up of the experiment.

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.

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.*

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.

Figure 5: Example 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 grap 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.

Figure 6: Pairwise competition assays.

Pairwise competition assays were setup between the 9 strains belonging to the species *P. brasiliense*, *P. aquaticum* and *P. versatile*. A: example of inhibition assay showing that the *P. brasiliense* strain CFBP3230 (lawn BRASI 3220) is inhibited by the *P. brasiliense* strain CFBP6617 (spot BRASI 6617) and also by the *P. aquaticum* strain A127 (spot AQUA A127). Note that the radius of inhibition triggered by the *P. aquaticum* strain A127 is smaller than the

one triggered by the *P. brasiliense* strain CFBP6617. B: inhibition observed for the 81 pairwise tested competitions. R: no inhibition; S: the strain in the lawn is inhibited by the strain in the spot with a large radius of inhibition; s: the strain in the lawn is inhibited by the strain in the spot with a small radius of inhibition; BRASI: strains belonging to the *P. brasiliense* species; AQUA: strains belonging to the *P. aquaticum* species; VERS: strains belonging to *the P. versatile* species.

Table 1: Comparison of species outcome after growth in TSB or potato tuber

Table 2: Outcome of individual strains discriminated with gapA barcoding within the 16 tested SRP communities

Figure S1: Bacterial multiplication in TBS medium

For each tested communities (3 replicates), the bacterial load at time zero (inoculum) and at the end of the experiment were used to calculate the number of bacterial generation achieved ($\log_{10}$ (cfu end experiment/cfu T0)/$\log_{10}$ (2)). The *P. aquaticum* and *P. versatile* communities are compared. The result of the statistical analysis is represented by the letters (a, b) 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).

Figure S2: Result at strain level of the 16 synthetic communities after growth on TSB.

The proportion of each strain (or mix of strains when strains could not be differentiated by the *gap*A barcode) is indicated in the legend of each graph. The 2 columns represent the proportion of strains observed in the inoculum (T0) and after 48h of growth in TSB medium (48) for each tested synthetic community. Error bar on the second column (48) represent the standard error observed for the 3 replicates. The 16 different tested communities are as follow: AQUA-ATRO: mix of 3 *P. aquaticum* strains and 3 *P. atrosepticum* strains; AQUA-BRASI: mix of 3 *P. aquaticum* strains and 3 *P. brasiliense* strains; AQUA-CARO: mix of 3 *P. aquaticum* strains and 3 *P. carotovorum* strains; AQUA-DIAN: mix of 3 *P. aquaticum* strains and 3 *D. dianthicola* strains: AQUA-PARM: mix of 3 *P. aquaticum* strains and 3 *P. parmentieri* strains; AQUA-POL: mix of 3 *P. aquaticum* strains, 1 *P.polaris* and 2 *P. parvum* strains: AQUA-SOL: mix of 3 *P. aquaticum* strains and 3 *D. solani* strains, AQUA-VERS: mix of 3 *P. aquaticum* strains and 3 *P. versatile* strains; VERS-AQUA: mix of 3 *P. versatile* strains and 3 *P. aquaticum* strains; VERS-ATRO: mix of 3 *P. versatile* strains and 3 *P.*

677 *atrosepticum* strains; VERS-BRASI: mix of 3 *P. versatile* strains and 3 *P. brasiliense* strains;
678 VERS-DIAN: mix of 3 *P. versatile* strains and 3 *D. dianthicola* strains; VERS-CARO: mix of
679 3 *P. versatile* strains and 3 *P. carotovorum* strains; VERS-PARM: mix of 3 *P. versatile*
680 strains and 3 *P. parmentieri* strains; VERS-POL: mix of 3 *P. versatile* strains, 1 *P. polaris* and
681 2 *P. parvum* strains; VERS-SOL: mix of 3 *P. versatile* strains and 3 *D. solani* strains. The
682 strains used in each bacterial mixture are listed in Table S1.
683
684 Figure S3: Result at strain level of the 16 synthetic communities 5 days post inoculation on
685 potato tubers.
686 The proportion of each strain (or mix of strains when strains could not be differentiated by
687 the *gap*A barcode) is indicated in the legend of each graph. The 2 columns represent the
688 proportion of strains observed in the inoculum (T0) and after 5 days after inoculation within
689 potato tubers (5 dpi) for each tested synthetic community. Error bar on the second column (5
690 dpi) represent the standard error observed for the 3 to 6 inoculated potato tubers. The 16
691 different tested communities are as follow: AQUA-ATRO: mix of 3 *P. aquaticum* strains and
692 3 *P. atrosepticum* strains; AQUA-BRASI: mix of 3 *P. aquaticum* strains and 3 *P. brasiliense*
693 strains; AQUA-CARO: mix of 3 *P. aquaticum* strains and 3 *P. carotovorum* strains; AQUA-
694 DIAN: mix of 3 *P. aquaticum* strains and 3 *D. dianthicola* strains: AQUA-PARM: mix of 3
695 *P. aquaticum* strains and 3 *P. parmentieri* strains; AQUA-POL: mix of 3 *P. aquaticum*
696 strains, 1 *P. polaris* and 2 *P. parvum* strains: AQUA-SOL: mix of 3 *P. aquaticum* strains and
697 3 *D. solani* strains, AQUA-VERS: mix of 3 *P. aquaticum* strains and 3 *P. versatile* strains;
698 VERS-AQUA: mix of 3 *P. versatile* strains and 3 *P. aquaticum* strains; VERS-ATRO: mix of
699 3 *P. versatile* strains and 3 *P. atrosepticum* strains; VERS-BRASI: mix of 3 *P. versatile*
700 strains and 3 *P. brasiliense* strains; VERS-DIAN: mix of 3 *P. versatile* strains and 3 *D.*
701 *dianthicola* strains; VERS-CARO: mix of 3 *P. versatile* strains and 3 *P. carotovorum* strains;
702 VERS-PARM: mix of 3 *P. versatile* strains and 3 *P. parmentieri* strains; VERS-POL: mix of
703 3 *P. versatile* strains, 1 *P. polaris* and 2 *P. parvum* strains; VERS-SOL: mix of 3 *P. versatile*
704 strains and 3 *D. solani* strains. The strains used in each bacterial mixture are listed in Table
705 S1.
706
707 Table S1: strains description
708
709 Table S2: Intra-species strain discrimination with the 341 nt gapA barcode
710

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Figure 1: Set up of the experiment.

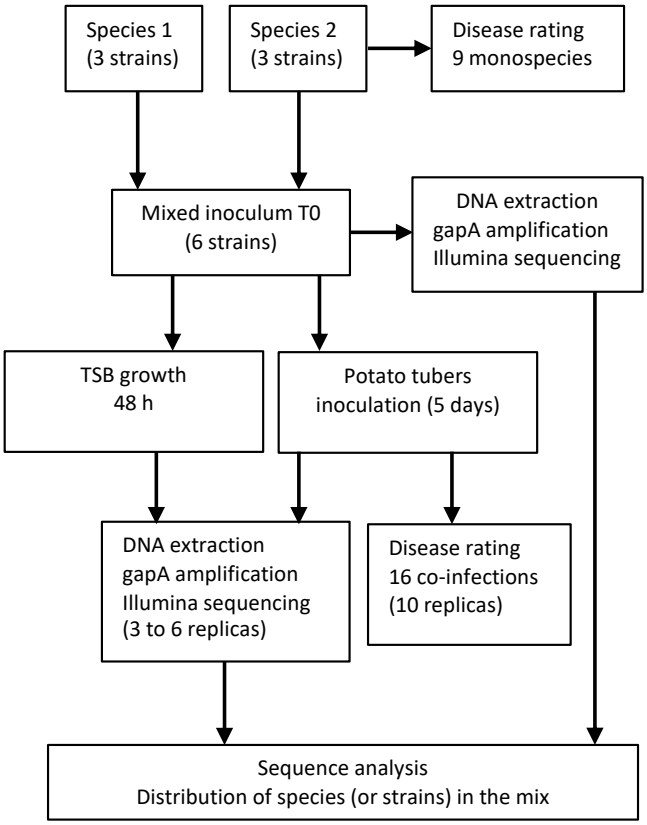


Figure 2: Symptoms and bacterial growth of the SRP synthetic communities on potato tubers.

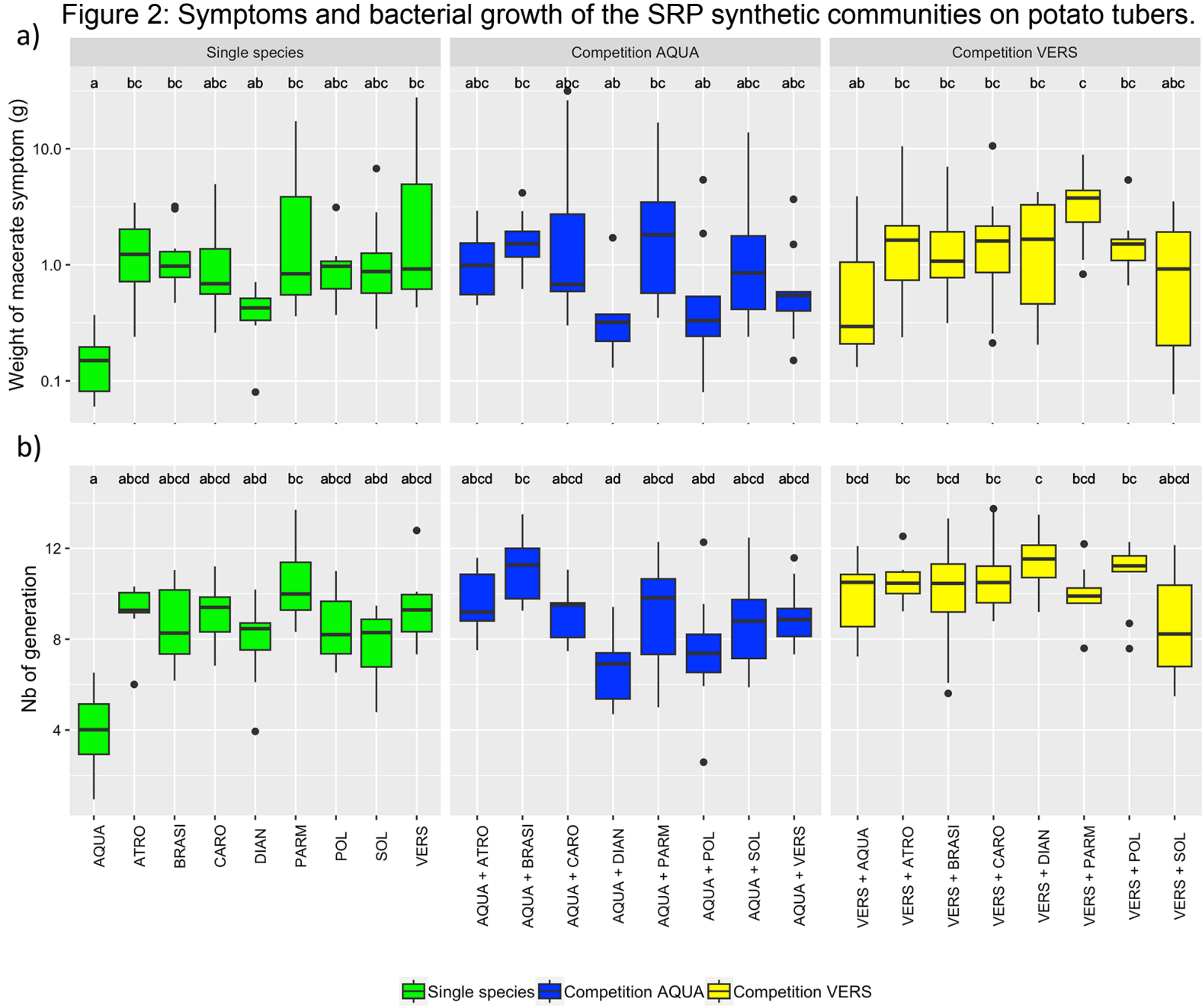


Figure 3: Species outcome within SRP communities

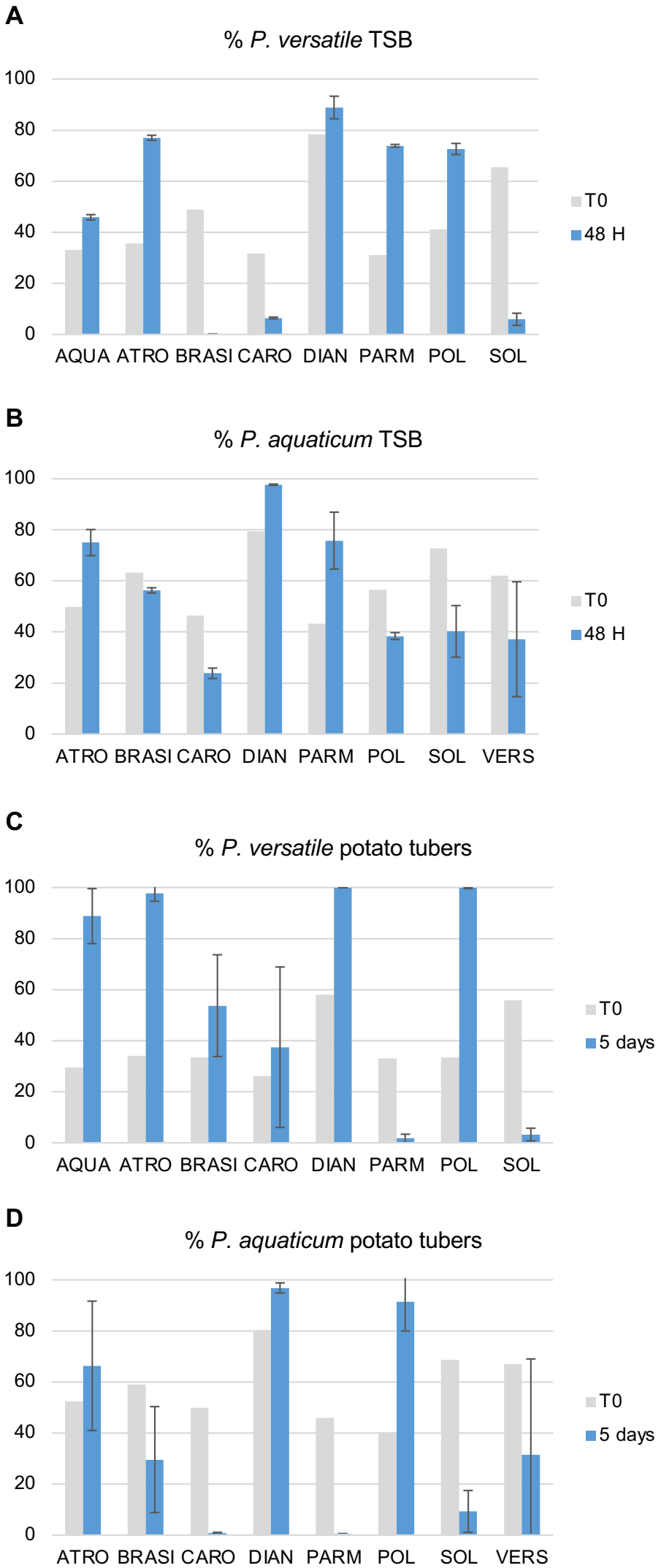


Figure 4: Modeling of cheater abundance

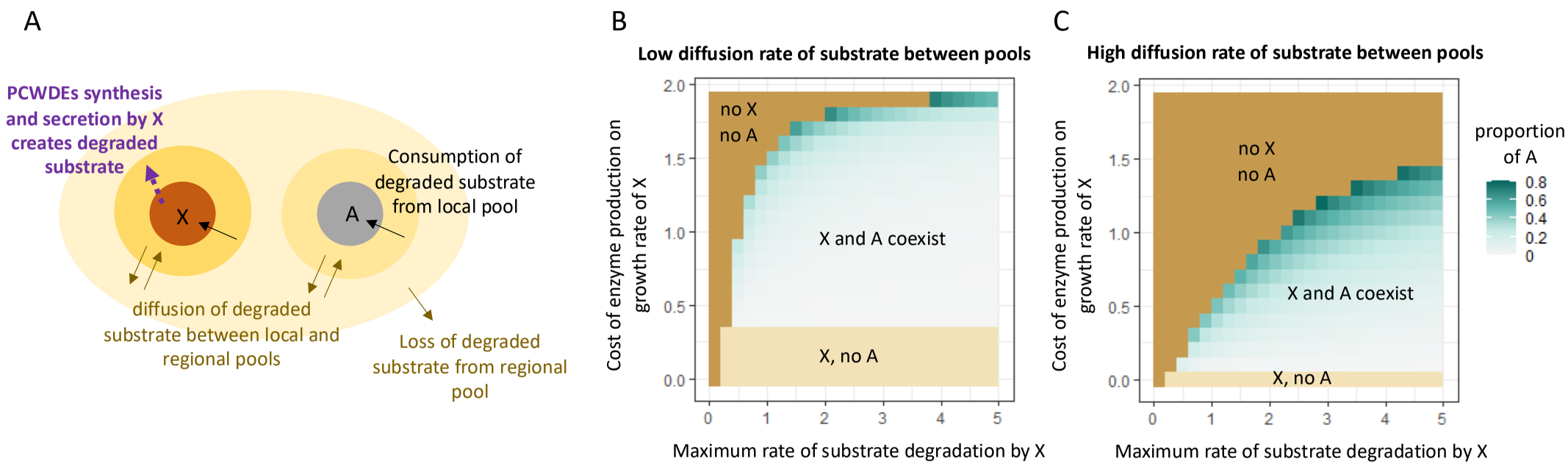
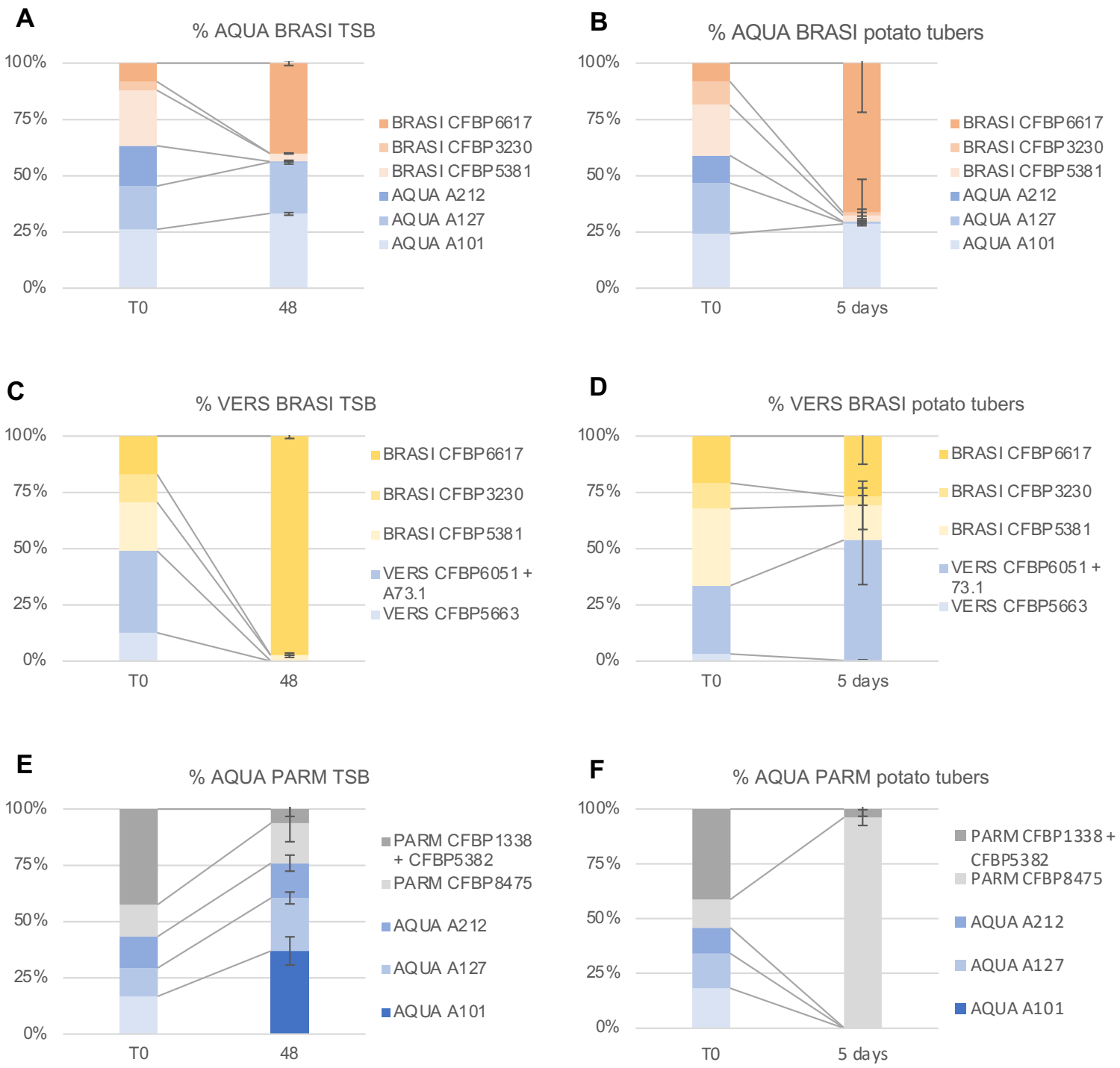
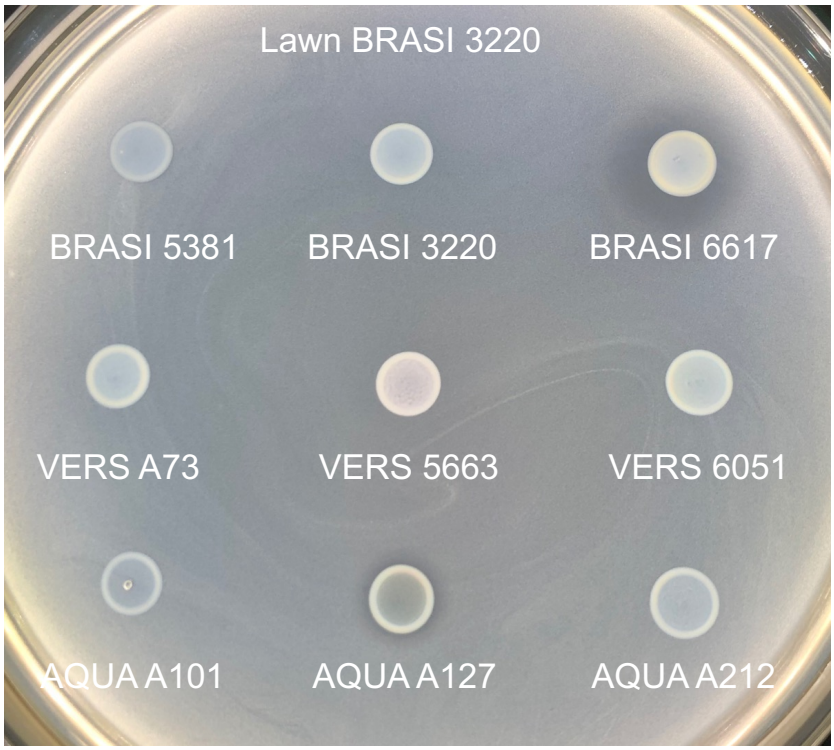


Figure 5: Example of strains outcome within SRP communities



A

Figure 6: Pairwise competition assays.



B

		Spot	
	strain	BRASI 6617	AQUA A127
Lawn	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

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

Blue: coexistence. Green: *P. versatile or P. aquaticum* dominates. Red: *P. versatile* or *P. aquaticum* was outcompeted. The number indicated the % mean of *P. aquaticum* or *P. versatile* of analyzed potato tubers

Table 2: Outcome of individual strains discriminated with gapA barcoding within the 16 tested SRP communities

Single strain discriminated with the gapA barcode	nb of tested combination	nb of potato tubers			nb of 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	8	1	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	2	0	1	0	1
CARO CFBP2046	2	0	2	0	0	1	1
PARM CFBP8475	2	1	0	1	0	0	2
Total	48	1	36	11	1	16	31
%		2%	72%	22%	2%	32%	62%

VERS: *P. versatile*; AQUA: *P. aquaticum*; ATRO: *P. atrospecticum*; BRASI: *P. brasiliense*; CARO: *P. carotovorum*; PARM: *P. parmentieri*.