Transmission of synthetic seed bacterial communities to radish seedlings: impact on microbiota assembly and plant phenotype

Authors: Marie Simonin[1], Anne Préveaux[1], Coralie Marais[1], Tiffany Garin[1], Gontran Arnault[1], Alain Sarniguet[1], Matthieu Barret[1]

Affiliation: [1]Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

ABSTRACT

Seed-borne microorganisms can be pioneer taxa during germination and seedling emergence. Still, the identity and phenotypic effects of these taxa that constitute a primary inoculum of plant microbiota is mostly unknown. Here, we studied the transmission of bacteria from radish seeds to seedlings using the inoculation of individual seed-borne strains and synthetic communities (SynComs) under *in vitro* conditions. The SynComs were composed of highly abundant and prevalent, sub-dominant or rare bacterial seed taxa. We monitored the transmission of each strain alone or in communities using *gyrB* gene amplicon sequencing and assessed their impacts on germination and seedling phenotype.

All strains and SynComs successfully colonized seedlings and we were able to reconstruct a richness gradient (6, 8 and 12 strains) on both seeds and seedlings. *Stenotrophomonas rhizophila* became dominant on seedlings of the three SynComs but most strains had variable transmission success (i.e increasing, stable or decreasing during seed to seedling transition) that also depended on the SynCom richness.

Some seed-borne strains (*Pseudomonas viridiflava, Paenibacillus sp)* had detrimental effects on germination and seedling development. Abnormal seedling morphologies were also observed with SynComs but their proportions decreased at the highest richness level. Interestingly, the three bacterial strains identified as core taxa of radish seeds (*Pseudomonas viridiflava*, *Pantoea agglomerans*, *Erwinia persicina)* were associated with detrimental effects on seedling phenotypes either in isolation or in SynComs. These results confirm that the plant core microbiome includes pathogenic and not only commensal or mutualistic taxa.

Altogether, these results show that SynCom inoculation can effectively manipulate seed and seedling microbiota diversity and highlight strong fitness differences between native seed-borne taxa in the colonization of these habitats.

Key-words: Plant microbiota, Seed-borne bacteria, Core microbiota, Synthetic community, Phytobiome, Pathobiome

INTRODUCTION

The impact of seed-borne pathogens on plant fitness has been extensively studied but the influence of all the other commensal or mutualistic microorganisms living on/in seeds is mostly unknown. Seed microbiota harbor diverse microbial communities that constitute a primary inoculum for plant microbiota assembly that could originate from maternal transmission or environmental sources during seed maturation (Chesneau *et al.* 2020, 2022). Still, the influence of this primary inoculum on germination and seedling emergence have not been established (Lamichhane *et al.* 2018). Moreover, many knowledge gaps remain regarding the fraction of seed-borne taxa that can be transmitted to seedlings and the influence of the initial seed microbiota composition on their transmission success.

Few studies investigated the transmission of seed microbiota to seedlings and they reported a high variability in the contribution of seed taxa to plant microbiota depending on the soil tested (Rochefort *et al.* 2021; Walsh *et al.* 2021). These studies had the advantage to work directly on native seed communities but this comes with the limitation that the seed microbiota had to be characterized on pools of seeds due to the insufficient microbial biomass and DNA present on individual seeds. As a consequence, it is not possible to truly determine which microbial taxa were transmitted from one seed to one seedling. Few studies attempted to characterize the microbiota of individual seeds using culture-dependent (Mundt and Hinkle 1976; Newcombe *et al.* 2018) and culture-independent techniques (Bintarti *et al.* 2021; Chesneau *et al.* 2022). These studies demonstrated a very high natural variability in microbiota composition between individual seeds and a low bacterial richness. Hence, studying the transmission of natural microbiota from seed to seedling and assessing their impacts on seedling phenotype is extremely challenging. Controlled conditions with the inoculation of known microbial assemblages are required to characterize the seed to seedling transmission and establish causal links between microbiota and seedling phenotypes.

Recent studies using synthetic microbial communities (SynComs) to assess the role of the microbiota on plant nutrition or resistance to pathogens have been performed on the rhizosphere and phyllosphere compartments (e.g. Kwak *et al.* 2018; Carlström *et al.* 2019; Finkel *et al.* 2020). But as for the rest of plant microbiome research, the seed compartment has been neglected. Despite the crucial role of seeds for food production and maintenance of plant biodiversity, microbiome studies on the seed compartment are still a minority (Shade, Jacques and Barret 2017). One correlative study between seed microbiota structure and seed germination of different rapeseed genotypes offers promising results about the key role of microbiota in seed vigor (Rochefort *et al.* 2019). Recent studies are starting to be published on the influence of seed microbiota composition on plant phenotypes using SynComs to infer causal relationships. For instance, Figueiredo dos Santos *et al.* (2021) showed that seed disinfection reduced maize germination rates that could be recovered after inoculation of a SynCom on seeds. These reports encourage further investigations of the role of seed microbiota in seedling microbiota assembly and fitness.

In this context, we set up seed inoculation experiments to address the following objectives:

- Characterize the transmission of individual seed-borne bacteria and synthetic bacterial communities from seed to seedlings (here on radish plants)
- Determine whether individual seed-borne bacteria or synthetic bacterial communities can impact seedling phenotype.

We selected 12 bacterial strains that are representative of radish seed microbiota that we studied individually or in communities (mix of 6, 8, or 12 strains) to monitor their transmission from seed to seedling and their impact on seedling phenotypes (germination, emergence) in *in vitro* conditions.

MATERIAL AND METHODS

Seed material and bacterial strains

The radish seeds (*Raphanus sativus* var. Flamboyant5) used for strain isolation and the inoculation experiments were obtained from a field trial conducted in 2013 and 2014 at the experimental station of the National Federation of Seed Multipliers (FNAMS, 47°28'012.42"N - 0°23'44.30"W, Brain-sur-l'Authion, France).

A bacterial culture collection was obtained from a seed sample composed of approximately 1000 mature seeds (Torres-Cortés et al. 2019). Seed samples were soaked in 25 ml of phosphate-buffered saline (PBS, Sigma-Aldrich) with Tween® 20 (0.05 % v/v, Sigma-Aldrich) at 6°C under agitation (150 rpm) for 2h30. One hundred microliters of the suspension was plated on tryptic soy agar 1/10 strength (TSA 10%) supplemented with cycloheximide (50 µg.ml-1, Sigma-Aldrich, Saint-Louis, Missouri, USA). Plates were incubated at 20°C during a minimum 5 days. Isolated colonies were picked and grown on TSA 10% plates for 24-48 hours to obtain pure cultures. The taxonomy of the isolated strains was determined using Sanger sequencing of the *gyrB* gene. A total of 528 strains were obtained that represented the 4 main phyla (Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria) found in radish seeds, for a total of 11 assigned bacterial families and 17 genera.

To select strains representative of the radish seed bacterial community, we used the Seed Microbiota Database (Simonin *et al.* 2022) originating from a meta-analysis that gathers re-processed amplicon sequencing datasets from 50 plant species. We extracted from the database all the radish seed datasets using the *gyrB* marker gene (studies all conducted in our team). We obtained data from 7 independent studies on the Flamboyant5 genotype (n=295 seed samples). After filtering the ASVs with a low read number (<100 reads), we calculated the prevalence and relative abundance of the ASVs across all samples. We then compared the *gyrB* sequences of the ASVs of the database to the ones of the strains in our culture collection to select a diverse set of strains in terms of phylogenetic diversity, abundance and prevalence (see Result section and Figure 1).

Seed inoculation experiment

Subsamples of seeds (1g ~100 seeds) were surface-sterilized using the following protocol : 1 min sonication (40 Hertz), soaking for 1 min in 96° ethanol, 5 min in 2.6% sodium hypochlorite, 30 sec in 96° ethanol and rinsed 3 times with sterile water. A subsample of 30 seeds was used to verify the efficacy of the surface-sterilization by soaking the seeds under agitation (150 rpm) for 2h30, plating on TSA 10% and incubating a minimum of 2 days at 20°C. The seeds were dried on sterile paper before the inoculation.

Twelve bacterial strains of our culture collection conserved at the CIRM-CFBP were selected to build a simplified seed radish bacterial community (more details in the Result section, Figure 1). The strains were either inoculated alone on seeds or as SynComs of 6, 8, or 12 strains. The inoculation of the strains or SynComs were performed on subsamples of 30 seeds by placing them for 30 minutes under agitation (70 rpm) at 20°C in a suspension at a final concentration around 10^7 CFU/mL (Optical density 600 nm = 0.01) from fresh 24/48-hour cultures. The inoculated seeds were then placed individually at the surface of a sterile cotton pad moistened with 4 mL of sterile water in sterile glass tubes. A total of 30 seeds were sown by condition (control, single strains or SynComs, total of 16 conditions) that were incubated for 4 days in a growth chamber (photoperiod: 16h/8h, temperature 25/22°C).

The bacterial cell density of the inocula and inoculated seeds (pool of 30 seeds) were assessed by plating on TSA 10% (CFU/mL or /seed). In the SynCom experiment, an amplicon sequencing approach was used to measure the relative abundance of the different strains in the inocula, inoculated seeds and seedlings. Thus, 3 repetitions of 500 µL of the inocula and of the macerate of inoculated seeds were stored at -80˚C in a 96-well plate before DNA extraction and library preparation for the *gyrB* gene amplicon sequencing.

After 4 days of growth, the phenotypes of the seedlings were determined and then the seedlings were individually processed to assess bacterial cell density by plating and bacterial community composition by amplicon sequencing. The effects on seedling phenotypes were assessed using the protocol established by the International Seed Testing Association (https://www.seedtest.org/en/home.html). Three types of phenotypes could be observed: non-germinated seeds, normal seedling or abnormal seedling. A seedling was considered abnormal if at least 50% of the cotyledons or leaves were necrotic or rotten, if the hypocotyl or epicotyl were deformed, or if the root system was absent, stunted or rotten.

To measure the bacterial cell density of seedlings, each plant was crushed in a sterile plastic bag (n=30 seedlings by condition), resuspended in 2 mL of sterile water and homogenized. The seedling suspension was then serial-diluted and plated on TSA 10% to determine the CFU by seedling and to assess the capacity of strains and SynComs to colonize seedlings. The remaining seedling suspensions were stored at -80˚C in a 96-well plate before DNA extraction and library preparation for the *gyrB* gene amplicon sequencing.

Amplicon sequencing of seed and seedling bacterial community

DNA extraction was performed on the inocula, inoculated seeds and seedlings of the SynCom conditions (control, 6-strain, 8-strain, 12-strain SynComs) with the NucleoSpin® 96 Food kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions.

The first PCR was performed with the primers gyrB_aF64/gyrB_aR553 (Barret *et al.* 2015), which target a portion of *gyrB* gene in bacteria. PCR reactions were performed with a high-fidelity Taq DNA polymerase (AccuPrimeTM Taq DNA Polymerase High Fidelity, Invitrogen, Carlsbad, California, USA) using 5µL of 10X Buffer, 1µL of forward and reverse primers (100µM), 0.2µL of Taq and 5 µl of DNA. PCR cycling conditions were done with an initial denaturation step at 94°C for 3 min, followed by 35 cycles of amplification at 94°C (30 s), 55°C (45 s) and 68°C (90 s), and a final elongation at 68°C for 10 min. Amplicons were purified with magnetic beads (Sera-MagTM, Merck, Kenilworth, New Jersey). The second PCR was conducted to incorporate Illumina adapters and barcodes. The PCR cycling conditions were: denaturation at 94°C (1 min), 12 cycles at 94°C (1 min), 55°C (1 min) and 68°C (1 min), and a final elongation at 68°C for 10 min. Amplicons were purified with magnetic beads and pooled. Concentration of the pool was measured with quantitative PCR (KAPA Library Quantification Kit, Roche, Basel, Switzerland). Amplicon libraries were mixed with 5% PhiX and sequenced with MiSeq reagent kits v2 500 cycles (Illumina, San Diego, California, USA). A blank extraction kit control, a PCR-negative control and PCR-positive control (*Lactococcus piscium*, a fish pathogen that is not plant-associated) were included in each PCR plate. The raw amplicon sequencing data are available on the European Nucleotide Archive (ENA) with the accession number PRJEB58635.

The bioinformatic processing of the amplicons was performed in R. In brief, primer sequences were removed with cutadapt 2.7 (Martin 2011) and trimmed fastq files were processed with DADA2 version 1.10 (Callahan *et al.* 2016). Chimeric sequences were identified and removed with the removeBimeraDenovo function of DADA2. Amplicon Sequence Variant (ASV) taxonomic affiliations were performed with a naive Bayesian classifier (Wang *et al.* 2007) with

our in-house *gyrB* database (train_set_gyrB_v4.fa.gz) available upon request. Unassigned sequences at the phylum level and *parE* sequences (a *gyrB* paralog) were filtered. After these filtering steps, samples with less than 1000 reads were excluded from the study.

Statistics and microbial community analyses

All the scripts and datasets used to conduct the study are available on GitHub (https://github.com/marie-simonin/Radish_SynCom). We assessed the effect of the inoculation and sample type on the various univariate variables (e.g. seedling bacterial cell density, strain relative abundance, ASV richness) using generalized mixed models (*glm* function in lme4 package) and post hoc comparisons were performed using the Tukey method (*warp.emm* function in package *emmeans*).

The analyses on bacterial community structure based on the amplicon sequencing data were done after rarefaction at 14116 reads per sample to conserve sufficient replicates while having a good sampling depth. Diversity and community structure analyses were performed in R 3.6.2 using the *phyloseq* (v1.28.0), *vegan* (v2.5-7) and *microbiome* (v1.7.21) packages (Oksanen *et al.* 2007; McMurdie and Holmes 2013; Lahti, Shetty and Blake 2017). The diversity of the inocula, inoculated seeds and seedlings was characterized using ASV richness. The effects of inoculation and sample types on bacterial community structure were assessed using Bray-Curtis dissimilarity associated with a permutational multivariate analysis of variance (*adonis and pairwise.adonis* functions, 999 permutations). Non-Metric Multidimensional Scaling (NMDS) were used to plot the ordinations. The influence of the inoculation of community beta-dispersion was assessed using the *betadisper* function in *vegan* based on Bray-Curtis distance matrix and distance to centroid to each condition. The statistical effects were evaluated using a permutation-based test of multivariate homogeneity of group dispersions followed by a Tukey's honest significant differences test.

All figures were prepared using the *ggplot2* (v3.3.3) package and the data management was done using the *dplyr* (v1.0.4) and *tidyverse* (v1.3.0) packages in R.

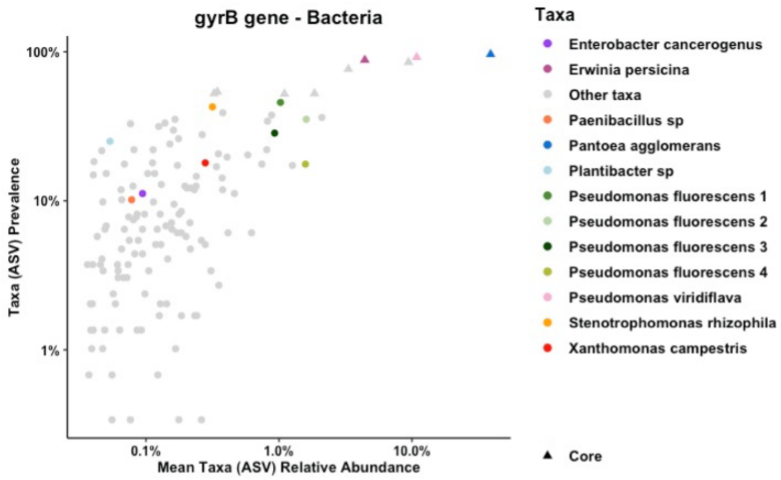
RESULTS

1. Bacterial strain selection based on a meta-analysis of radish seed microbiota

We have selected twelve bacterial strains based on an original selection design based on a meta-analysis on seed microbiota studies (Simonin et al. 2022). The twelve strains were representative of the diversity and prevalence-abundance profiles of radish seed microbiota (Figure 1A). The selection included three extremely abundant and prevalent taxa (i.e core taxa, Figure 1A and 1B), but also five sub-dominant (relative abundance < 1.5% and prevalence < 20%) and four rare taxa (prevalence < 20%). Ten strains belonged to Gammaproteobacteria (Enterobacteriaceae, Erwiniaceae, Pseudomonadaceae, Xanthomonadaceae) and the two remaining strains were from the Actinobacteria and Bacilli classes (Figure 1B). In addition to selecting strains that were phylogenetically diverse, we also included intra-species diversity with four strains of the *Pseudomonas fluorescens* subgroup (Hesse et al., 2018) with contrasted prevalence and abundance profiles. The twelve strains presented different *gyrB* gene sequences (subspecies level detection) to enable their individual tracking using *gyrB* amplicon sequencing. The strains were studied in isolation and in SynComs of 6, 8 and 12 strains (Figure 1B) to match the bacterial diversity observed on individual radish seeds (median = 8 ASVs; Figure S1; Chesneau et al. 2022). We performed a nested design for the three diversity levels, with the 6-strain SynCom composed of two high

prevalence strains, two intermediate and two low prevalence strains. The 8-strain and 12-strain SynComs were built on this initial community with an increasing number of high-, intermediate- and low-prevalence strains.

A. Prevalence-Abundance of selected taxa in radish seed microbiota



B. Identity of selected strains for SynCom experiments

Strain	Class	Species	Type	Prevalence	Relative Abundance	SynCom 6 strains	SynCom 8 strains	SynCom 12 strains
CFBP13505	Gammaproteobacteria	Pantoea agglomerans	Core	96.3%	39.04%	●	●	●
CFBP13507	Gammaproteobacteria	Pseudomonas viridiflava	Core	91.9%	10.86%	●	●	●
CFBP13511	Gammaproteobacteria	Erwinia persicina	Core	88.1%	4.42%			●
CFBP13509	Gammaproteobacteria	Pseudomonas fluorescens subgroup 1		45.8%	1.03%			●
CFBP13503	Gammaproteobacteria	Stenotrophomonas rhizophila		42.7%	0.32%	●	●	●
CFBP13528	Gammaproteobacteria	Pseudomonas fluorescens subgroup 2		35.3%	1.60%			●
CFBP13506	Gammaproteobacteria	Pseudomonas fluorescens subgroup 3		28.5%	0.93%	●	●	●
CFBP13513	Actinobacteria	Plantibacter sp		25.1%	0.05%		●	●
CFBP6650	Gammaproteobacteria	Xanthomonas campestris		18.0%	0.28%	●	●	●
CFBP13502	Gammaproteobacteria	Pseudomonas fluorescens subgroup 4		17.6%	1.58%			●
CFBP13530	Gammaproteobacteria	Enterobacter cancerogenus		11.2%	0.09%		●	●
CFBP13512	Bacilli	Paenibacillus sp		10.2%	0.08%	●	●	●

Figure 1: Selection of bacterial strains representative of the radish seed microbiota. A. Prevalence and relative abundance of bacterial ASVs (*gyrB* amplicon sequencing) in radish seed microbiota (*R. sativus* Flamboyant5) from seven independent studies (n=295 seed samples, 139 ASVs with minimum of 100 reads in dataset). The colored points represent the taxa selected in this study and the triangle shapes represent the taxa identified as members of the radish core seed microbiota. Data extracted from the Seed Microbiota Database (Simonin et al. 2022). B. Identity of the strains isolated from radish seeds and used in the SynCom experiments.

2. Transmission of single strains and synthetic communities from seed to seedling

a. Bacterial colonization of seedlings by all strains and SynComs

Transmission of the twelve individual strains and the three SynComs to radish seedlings was first assessed. When individual strains were seed-inoculated, they were all successfully transmitted to seedlings with a median of 6.8 log CFU per seedling. The seedlings originating from seeds inoculated with SynComs were colonized at similar levels of bacterial cell density (median = 7.4 log CFU/seedling). Single strains and SynComs all reached around 7 log CFU/seedling that appear to be the bacterial carrying capacity of a radish seedling in our system. The control seedlings that originated from surface-sterilized seeds were below detection limit or close to 2 log CFU/seedling, validating that the bacterial populations observed on seedlings in the different conditions originated from our inocula.

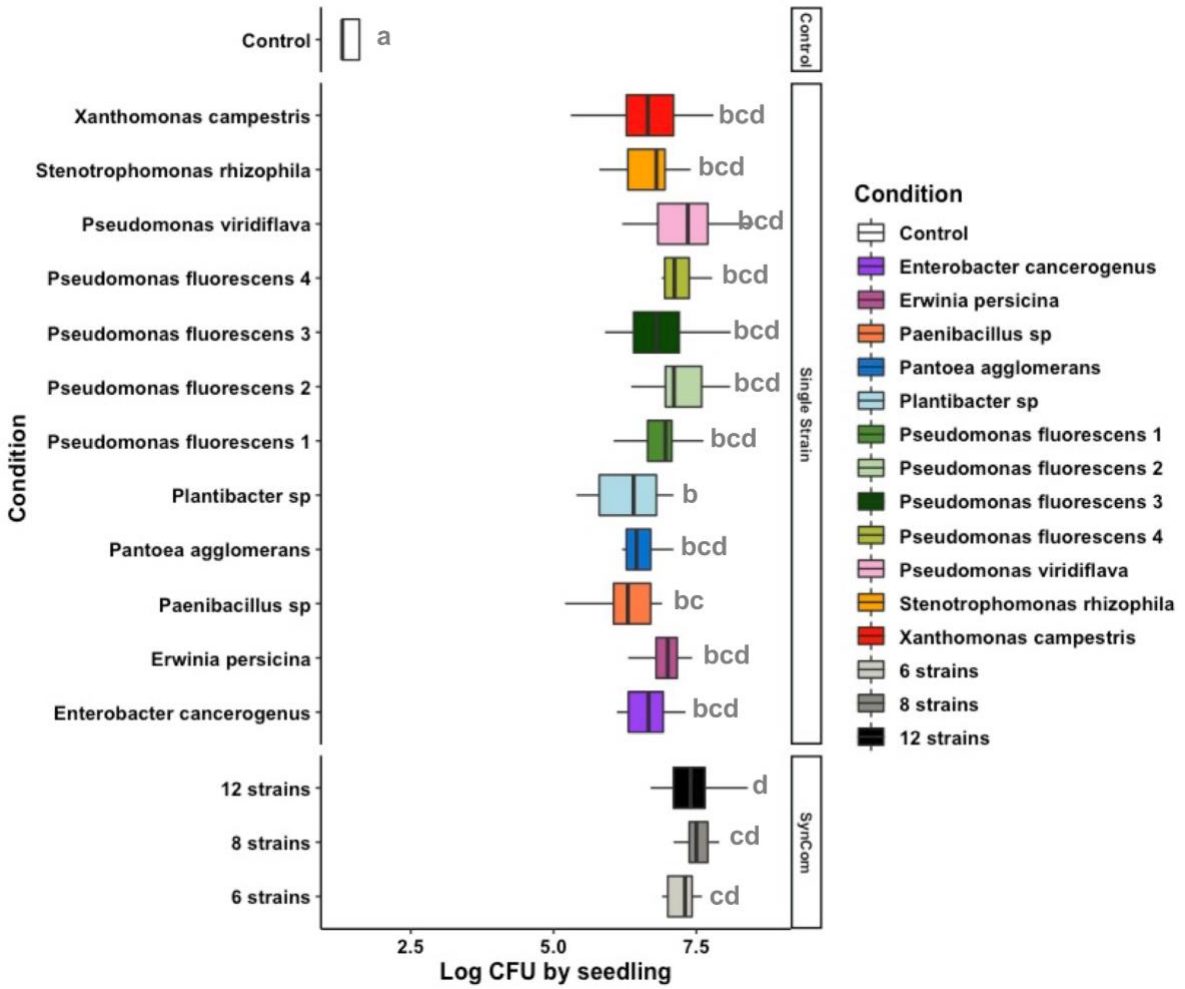


Figure 2: Seedling bacterial colonization for the different inoculation treatments (n=30 seedlings per condition). CFU: Colony Forming Unit. The different letters represent the results of a post-hoc Tukey HSD test.

b. Bacterial community structure of seeds and seedlings inoculated with SynComs

We wanted to assess our ability to manipulate the seed and seedling bacterial community using SynCom inoculation compared to non-inoculated control seeds. Using *gyrB* gene amplicon sequencing, we validated that the SynCom inoculation led to a significant modification of seed and seedling bacterial community compared to the control condition (pairwise adonis: P<0.007; Figure 3A). On the NMDS ordination, the three sample types (inoculum, seed and seedling) clustered closely by SynCom indicating a high degree of similarity, whereas the control seeds and seedlings were very dispersed. This observation was further confirmed by a beta-dispersion analysis (distance to centroid) indicating that the SynCom inoculation strongly reduced the variability in seed and seedling community structure compared to the control condition (Figure 3B, 3C). We also confirmed that the three SynComs were significantly distinct in the three types of samples (inocula, seeds, seedlings,Figure 3D), with an expected structuration showing the 6 and 12-strain SynComs as the most different and the 8-strain SynCom with an intermediate composition.

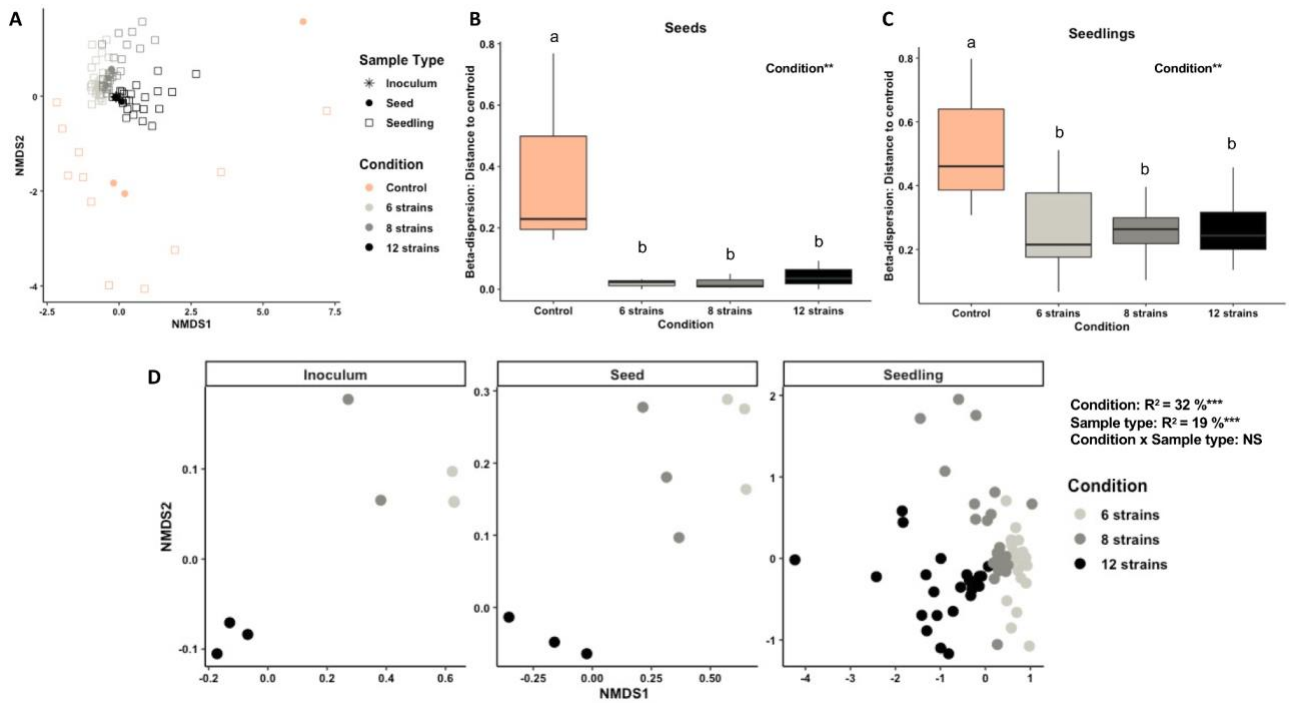


Figure 3: A) Influence of SynCom inoculation and sample type on bacterial community structure visualized through an NMDS ordination based on Bray-Curtis distances (stress = 0.155). Beta-dispersion assessed using distance to centroid of the B) seed and C) seedling community structure in the control and SynCom conditions. D) Bacterial community structure of the three SynComs in the inocula, seeds and seedlings visualized through an NMDS ordination based on Bray-Curtis distances (stress = 0.165). The asterisks represent the significativity of the Permanova test:****P*<0.001, NS=Non-Significant. The different letters represent the results of a post-hoc Tukey HSD test; two conditions with no letters in common are statistically different.**

c. Effect of SynCom inoculation on seed and seedling richness

We assessed the effect of SynCom inoculation on the bacterial richness of seeds and seedlings. We verified that the SynCom inoculation enabled us to reconstruct a diversity gradient on both seeds and seedlings (Figure 4A). The bacterial richness measured on seeds and seedlings corresponded to the expected taxa richness present in the inocula (6, 8 or 12 ASVs). The control seeds were surface-disinfected but they still harbored a low bacterial diversity likely of endophytic bacteria, including ASVs of strains included in the SynComs because the strains selected have been isolated from the same radish genotype. Altogether, these results show the efficacy of the SynCom inoculation on seeds to drive the bacterial diversity of both seeds and seedlings.

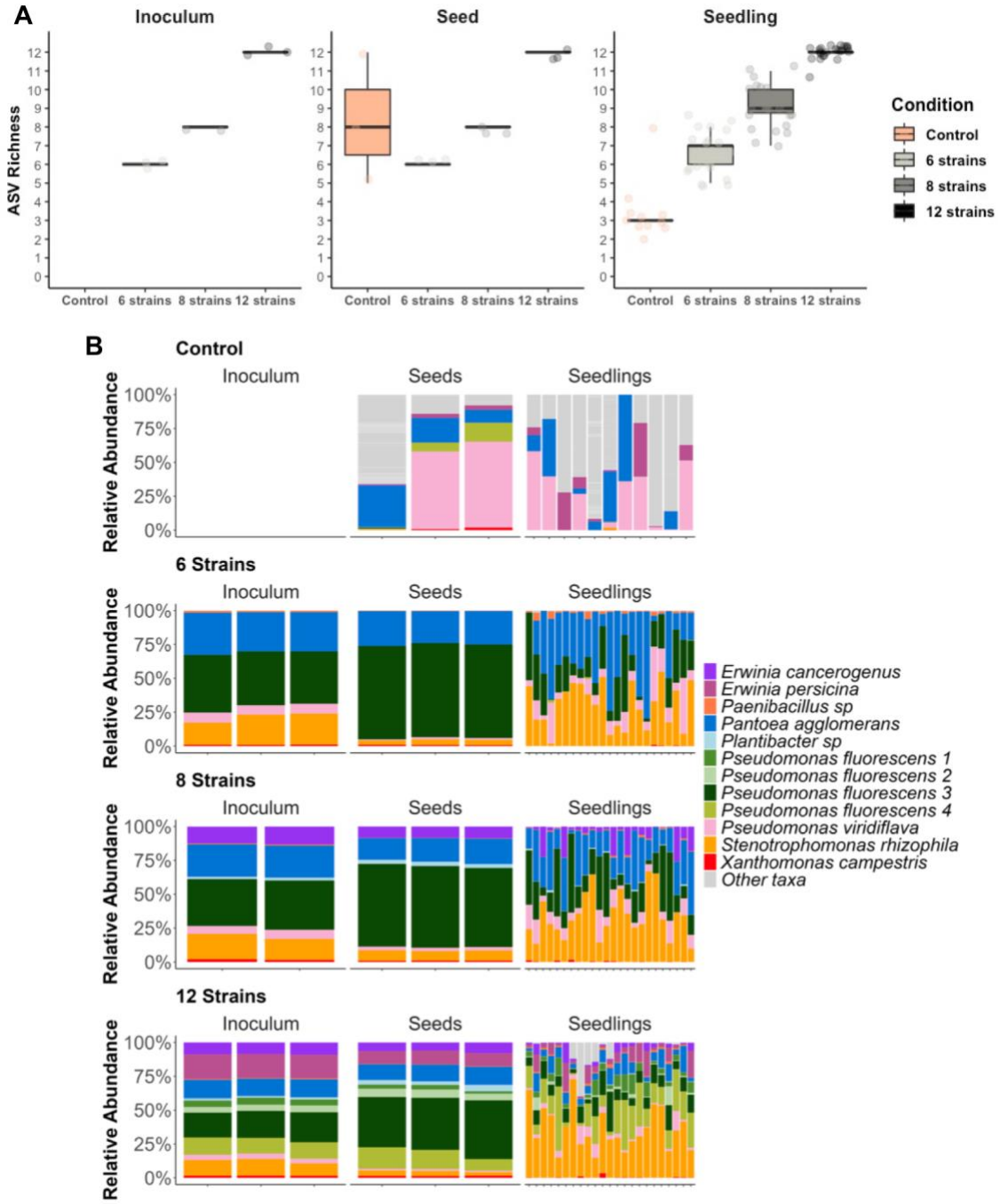


Figure 4: A) Bacterial taxa richness (ASV richness) of the inocula, inoculated seeds and seedlings in the different conditions. B) Taxonomic profile of the inoculum, seeds and seedlings in the four different conditions (control, 6 strains, 8 strains and 12 strains). Each stacked bar represents a sample.

d. Tracking of the transmission of the SynCom strains between the inoculum, seeds and seedlings

Based on the unique *gyrB* ASV of the 12 strains, we were able to track their transmission patterns from the inoculum to the seedling. First, we characterized the natural abundance of the strain ASVs in the control seeds and seedlings. The 12 ASVs were naturally found in control seeds and seedlings, especially the ASVs associated with the *Pseudomonas viridiflava*, *Pantoea agglomerans*, *Pseudomonas fluorescens* 4 and *Erwinia persicina* strains (Figure 4B). As already seen in Figure 3A, the SynCom inoculations led to completely different

seed and seedling bacterial community compositions than in the control condition (Figure 4B). Interestingly, we observed some differences in the abundance of strains between the inoculum and the seed community profile (Figure 5A), indicating differences between strains for their capacity to colonize the seed during inoculation (Figure 5B). For instance, the strain *Pseudomonas fluorescens* 3 had a higher relative abundance on seeds (40-70%) compared to the inocula (20-40%), while the strains *Pseudomonas viridiflava* and *Stenotrophomonas rhizophila* had the opposite pattern (Figure 5B).

During the phenological transition from seed to seedling, we observed an important restructuration of the bacterial community (Figure 4B). All the strains were able to transmit from seeds to seedlings but with variable success depending on the SynCom richness and the seedling individual (Figure 4B, 5). Across the three SynComs, *Stenotrophomonas rhizophila* was the most successful seedling colonizer (mean relative abundance = 30 to 36%), followed by *Pantoea agglomerans* but only in the 6- and 8-strain SynComs (mean relative abundance = 34 and 25%). In contrast, the strain *Pseudomonas fluorescens* 3 that was dominant on seeds strongly decreased in abundance on seedlings (11 to 24%).

We analyzed the fate of each strain during the seed to seedling transmission across the three SynComs and we grouped the strains in three categories: increasers, stable, decreasers (Figure 5C). We identified four 'increaser' strains and three of them presented this positive transmission pattern across the three SynComs (*Stenotrophomonas rhizophila*, *Pseudomonas viridiflava*, *Paenibacillus sp*). In contrast, four 'decreaser' strains were identified with three of them with a consistent negative transmission pattern (*Pseudomonas fluorescens* 3, *Xanthomonas campestris*, *Plantibacter sp*.). Finally, four strains presented a stable transmission or a variable profile depending on the inoculated SynCom. For instance, *Pantoea agglomerans* and *Enterobacter cancerogenus* had a stable transmission in the 6- or 8-strain SynComs, but decreased in the 12-strain SynCom. Interestingly, the 4 strains of *Pseudomonas fluorescens* tested presented contrasted transmission patterns or abundance profiles and were found in the three categories (increaser, stable or decreaser). Another interesting result was that the four strains identified as "increasers" were not the best seed colonizers after inoculation (Figure 5A, 5B). Inversely, some of the best seed colonizers (*Pseudomonas fluorescens* 3) were identified as "decreasers" on seedlings, indicating contrasted strain fitness depending on the plant phenological stage.

We also analyzed the influence of the SynCom composition on the ability of each strain to colonize seedlings compared to their initial relative abundance on seeds (i.e ratio of relative abundance seedling / seed, Figure 5D). We observed that the SynCom richness strongly influenced the abundance patterns of the strains. Most strains had a lower seedling colonization in the 12-strain SynCom (e.g. *Pantoea agglomerans*, *Enterobacter cancerogenus*), with the exception of *Stenotrophomonas rhizophila* (Figure 5D). Some strains had a higher seedling colonization in the 6-strain SynCom than the 8-strain SynCom (*Pseudomonas viridiflava*, *Paenibacillus sp*, *Stenotrophomonas rhizophila*). Altogether, these results indicate strong fitness differences between strains in the seed and seedling habitat, that is highly dependent on the surrounding community.

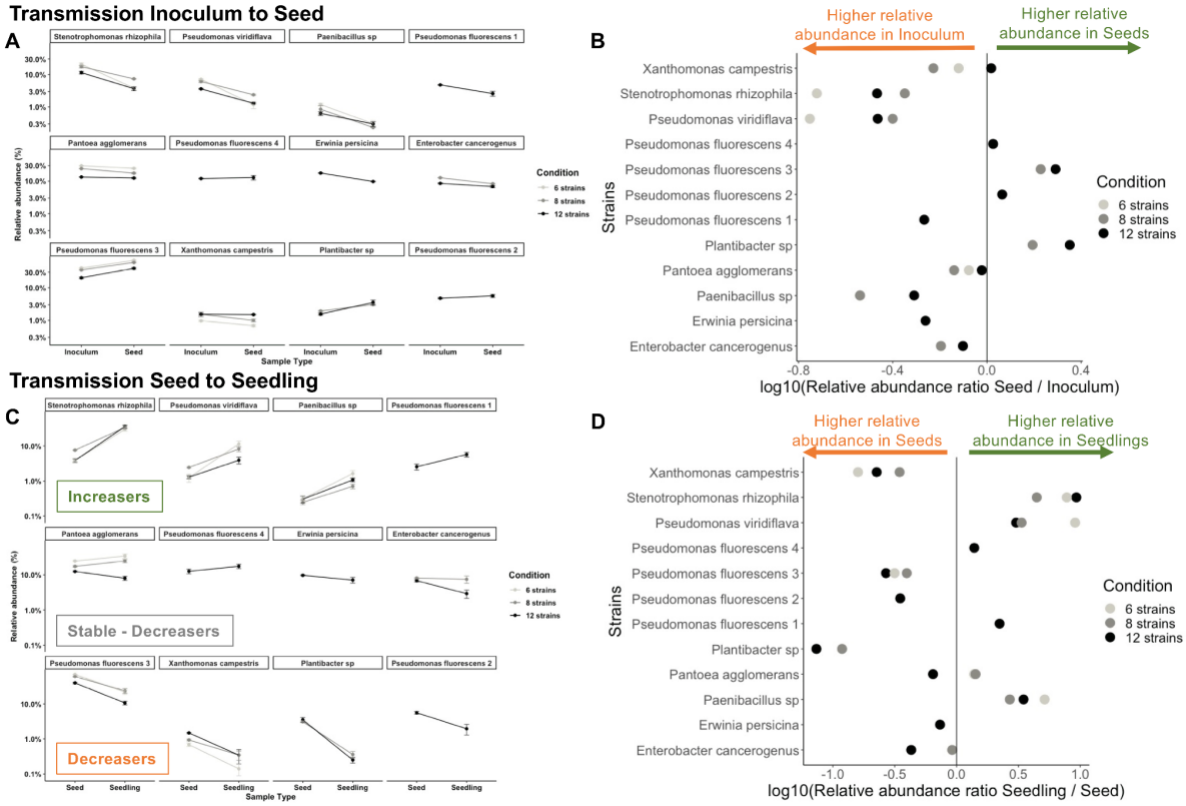


Figure 5: A. Relative abundance patterns of each strain during the transmission from inoculum to seed (A) and from seed to seedling (C) in synthetic bacterial communities. B. Ability of each strain to colonize: (B) seeds compared to their initial relative abundance in the inocula: ratio of relative abundance seed / inoculum, and (D) seedlings compared to their initial relative abundance on seeds: ratio of relative abundance seedling / seed.

3. Effect of single bacterial strains or synthetic communities on seedling phenotype

a. Some strains and SynComs increase the proportion of abnormal seedlings and non-germinated seeds

The impact of the inoculation of single strains or SynComs on seedling phenotypes was assessed based on the proportion of non-germinated seeds, normal and abnormal seedlings observed (Figure 6). In the control condition, we observed a low proportion of non-germinated (7%) and abnormal seedlings (7%). Several individual strains and the three SynComs caused detrimental effects on germination and seedling phenotypes. In particular, *Pseudomonas viridiflava*, *Paenibacillus sp* and two of the SynComs (6 and 8 strains) presented large proportions of abnormal seedlings (60 to 73%). The conditions that had the highest proportion of non-germinated seeds were the strains *Enterobacter cancerogenus* (21%), *Xanthomonas campestris* (20%), *Pantoea agglomerans* (20%) and the three SynComs (17 to 20%). The conditions that presented the highest proportion of normal seedlings were *Stenotrophomonas rhizophila* (90%), *Plantibacter sp* (87%) and the four *Pseudomonas fluorescens* strains (73 to 90%).

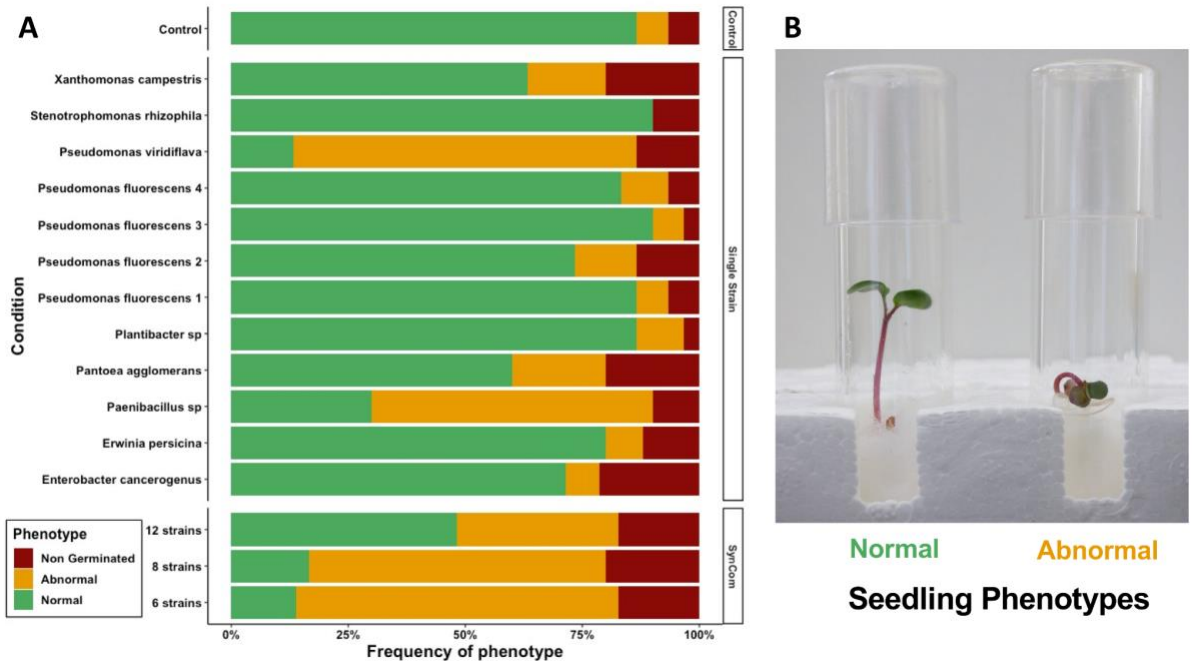


Figure 6: **A) Effect of the inoculation of single bacterial strains or synthetic bacterial community on germination and seedling phenotypes. B) Photography of the typical phenotypes observed in the experiment (Credit: Guillaume Chesneau).**

b. Modifications in the composition of seedling microbiota between normal and abnormal seedlings

Next, we analyzed if the seedling bacterial community structure was different depending on the seedling phenotype (Figure 7A). The seedling phenotype was a significant driver of seedling microbiota, especially in the 12-strain SynCom (interaction Condition x Phenotype, *P*=0.006), for which the number of normal and abnormal seedlings was more balanced (14 normal vs 10 abnormal). In the 12-strain SynCom, we analyzed if some strains had a significantly higher or lower relative abundance in abnormal seedlings (Figure 7B). The strains *Enterobacter cancerogenus* and *Erwinia persicina* were more abundant in abnormal seedlings, while *Pseudomonas viridiflava* and *Xanthomonas campestris* were less abundant.

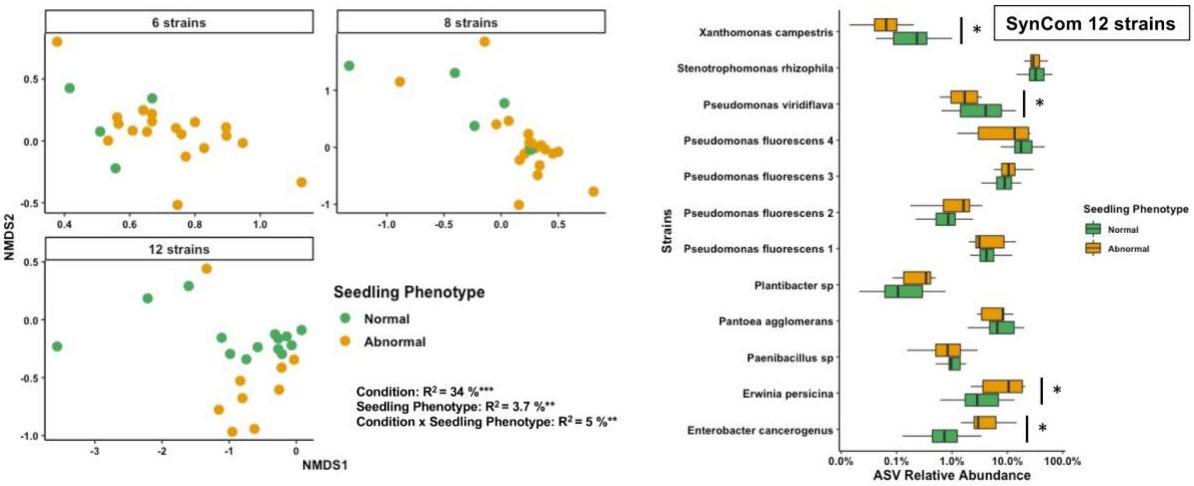


Figure 7: Influence of seedling phenotype (normal or abnormal) on A) bacterial community structure (stress=0.17) and B) on the strain relative abundance on the 12-strain SynCom. The asterisks represent the significativity of the Permanova test or post-hoc Tukey test: **P*<0.05; ***P*<0.01; ****P*<0.001.

DISCUSSION

Community- and strain-specific patterns of seedling transmission of seed-borne bacteria

Using inoculation experiments of bacterial SynComs, we studied the relative ability of communities composed of diverse strains naturally present in radish seed microbiota to colonize seedlings. All SynComs and the individual strains were able to colonize seedlings at high cell density levels, indicating their capacity to survive the major environmental changes that represent germination and seedling emergence compared to the seed habitat. The SynComs inoculations enabled to successfully reconstruct a richness gradient (6, 8 and 12 strains) on both seeds and seedlings and to significantly modulate plant microbiota composition. An important result was that SynCom inoculation allowed to strongly reduce the natural variability of seed and seedling microbiota structure (beta-dispersion) and obtain more homogeneous microbial community compositions between replicates compare to native communities (Moroenyane *et al.* 2021; Walsh *et al.* 2021). This is a prerequisite to study the transmission of microorganisms from seed to seedling and establish causal relationship between seed microbiota and plant microbiota or phenotype. The inoculation of the three different SynComs on seeds led to the assembly of significantly distinct seedling bacterial communities at the expected richness levels. However, the relative abundance of the strains changed drastically from the inocula to seeds and then to seedlings. Some strains like *Pseudomonas fluorescens* 3 were excellent seed colonizers, while others had a reduced abundance compared to the inocula (ex: *Stenotrophomonas rhizophila*). These differences in seed colonization can be due to contrasted adhesion capacity of strains related to the secretion of large adhesins (i.e LapA, LapF) and the presence of flagella (DeFlaun *et al.* 1994; Yousef-Coronado, Travieso and Espinosa-Urgel 2008; Duque *et al.* 2013).

Interestingly, the best seed colonizers were not the best seedling colonizers. On the opposite, all the strains that declined during the inocula-seed transition phase increased in the seed-seedling transition (i.e "increasers"). In particular, *Stenotrophomonas rhizophila* became dominant on seedlings of the three SynComs. These results indicate that the bacterial traits required to colonize seeds or seedlings are different. Moreover, these findings show that being dominant on seeds does not provide an advantage to become dominant on seedlings. These findings are in line with Rochefort et al. (2021) and Chesneau et al. (2022) demonstrating a low transmission success of abundant seed taxa to seedlings in natural microbial communities.

Additionally, we observed that SynCom richness influenced the seedling transmission of the strains, indicating an important role of biotic interactions in seedling microbiota assembly. The ability of each strain to colonize seedlings compared to their initial relative abundance on seeds (i.e ratio of relative abundance seedling / seed) was strongly modified depending on the SynCom considered. For instance, *Pantoea agglomerans* had a positive seedling colonization success in the 6- and 8-strain SynComs and a negative one in the 12-strain SynCom. These SynCom-specific patterns of each strain are likely driven by exploitative and interference competition between individuals but the specific mechanisms involved here are difficult to identify in a community context *in planta* (Hibbing *et al.* 2010; Granato, Meiller-Legrand and Foster 2019).

Our experiments included four different strains of *Pseudomonas fluorescens* to compare the intra-specific patterns of seedling transmission. Both seed and seedling colonization patterns were highly strain-specific. We observed all possible outcomes depending on the *Pseudomonas fluorescens* strain considered (increasers, stable or decreasers). This result

suggests important differences between strains in traits involved in habitat colonization and competitive interactions, that prevent any generalization of these observations at the species level.

Altogether, these results show that SynCom inoculation can effectively manipulate seed and seedling microbiota diversity and highlight strong fitness differences between native seed-borne taxa in the colonization of these habitats.

Detrimental effects of seed-borne bacteria on seedling phenotype

We observed that individual seed-borne bacteria or synthetic bacterial communities can impact germination and seedling morphology. Some seed-borne strains and SynComs had detrimental effects on germination and seedling development. In isolation *Pseudomonas viridiflava* and *Paenibacillus sp* strongly increased the proportion of abnormal seedlings. *Pseudomonas viridiflava* is a very common plant-associated bacteria with various life-styles (endophytes, epiphytes, saprotrophs) that can also be pathogenic to a large diversity of hosts (Lipps and Samac 2022). *Pseudomonas viridiflava* is frequently seed-transmitted and can cause germination arrest or symptoms on seedlings, including in radish (Shakya and Vinther 1989; Samad *et al.* 2017). *Paenibacillus* strains are mainly recognised for their biocontrol capacities against various bacterial or fungal pathogens (Ryu *et al.* 2006; Ling *et al.* 2011), but one report indicate root damages caused by this bacteria under gnotobiotic conditions like in our study (Rybakova *et al.* 2016). Another important observation was that the three bacterial strains identified as seed core taxa (*Pseudomonas viridiflava*, *Pantoea agglomerans*, *Erwinia persicina)* were associated with detrimental effects on seedling phenotypes either in isolation or in SynComs. These results confirm that the plant core microbiome includes pathogens and opportunistic pathogens and not only commensal or mutualistic taxa (Simonin *et al.* 2020).

Abnormal seedling phenotypes were also observed with SynComs but their proportions decreased at the highest richness level (12 strains). This observation could be explained by a "dilution" of the negative effects of the detrimental strains as the diversity increased and their relative abundances decreased. Still surprisingly, the strains enriched in abnormal seedlings in the 12-strain SynCom had not been identified as detrimental in isolation (*Enterobacter cancerogenus* and *Erwinia persicina*). Furthermore, we found that *Pseudomonas viridiflava and* the seed-borne pathogen *Xanthomonas campestris* were even reduced in abundance in abnormal seedlings in this condition. We can propose different hypotheses to explain these results. One is that the strains responsible for the abnormal phenotypes are different when inoculated in a community (*Enterobacter cancerogenus* and *Erwinia persicina)* or in isolation (*Pseudomonas viridiflava* and *Paenibacillus)*. Another hypothesis is that *Enterobacter cancerogenus* and *Erwinia persicina* are opportunistic taxa that are enriched in abnormal seedlings (e.g necrotrophy or “cry for help” of the plant) but are not responsible for the altered phenotype.

Additionally, we show that the inoculation of multiple strains with potential pathogenic effects (i.e pathobiome) do not have additive effects that could have led to an absence of germination or 100% of abnormal seedlings. The reduction of detrimental effects in the 12-strain SynCom experimentally confirms the importance of bacterial interactions in plant disease development in a pathobiome context (Bass *et al.* 2019). Increasing microbial interactions with higher SynCom diversity is a promising leverage for reducing the impact of deleterious bacterial strains.

Further research is needed to determine the functional role of these seed core taxa especially during seed development and the conditions of expression of their detrimental effects under natural conditions.

Altogether, these results indicate that this synthetic ecology approach is valuable to better understand the role of seed microbiota for plant health and the context dependency of disease expression in a community setting. This approach permitted identifying several seed core bacteria with detrimental effects on germination and seedlings but also to characterize the transmission success of diverse bacterial strains (i.e increasers, stable, decreasers) to seedlings in three community contexts.

REFERENCES

Barret M, Briand M, Bonneau S *et al.* Emergence Shapes the Structure of the Seed Microbiota. *Appl Environ Microbiol* 2015;**81**:1257–66.

Bass D, Stentiford GD, Wang H-C *et al.* The Pathobiome in Animal and Plant Diseases. *Trends Ecol Evol* 2019;**34**:996–1008.

Bintarti AF, Sulesky-Grieb A, Stopnisek N *et al.* Endophytic Microbiome Variation Among Single Plant Seeds. *Phytobiomes J* 2021:PBIOMES-04-21-0030-R.

Callahan BJ, McMurdie PJ, Rosen MJ *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3.

Carlström CI, Field CM, Bortfeld-Miller M *et al.* Synthetic microbiota reveal priority effects and keystone strains in the *Arabidopsis* phyllosphere. *Nat Ecol Evol* 2019;**3**:1445–54.

Chesneau G, Laroche B, Préveaux A *et al.* Single Seed Microbiota: Assembly and Transmission from Parent Plant to Seedling. *mBio* 2022;**0**:e01648-22.

Chesneau G, Torres-Cortes G, Briand M *et al.* Temporal dynamics of bacterial communities during seed development and maturation. *FEMS Microbiol Ecol* 2020;**96**, DOI: 10.1093/femsec/fiaa190.

DeFlaun MF, Marshall BM, Kulle E-P *et al.* Tn 5 insertion mutants of *Pseudomonas fluorescens* defective in adhesion to soil and seeds. *Appl Environ Microbiol* 1994;**60**:2637–42.

Duque E, de la Torre J, Bernal P *et al.* Identification of reciprocal adhesion genes in pathogenic and non-pathogenic Pseudomonas. *Environ Microbiol* 2013;**15**:36–48.

Figueiredo dos Santos L, Fernandes Souta J, de Paula Soares C *et al.* Insights into the structure and role of seed-borne bacteriome during maize germination. *FEMS Microbiol Ecol* 2021;**97**:fiab024.

Finkel OM, Salas-González I, Castrillo G *et al.* A single bacterial genus maintains root growth in a complex microbiome. *Nature* 2020;**587**:103–8.

Granato ET, Meiller-Legrand TA, Foster KR. The Evolution and Ecology of Bacterial Warfare. *Curr Biol* 2019;**29**:R521–37.

Hibbing ME, Fuqua C, Parsek MR *et al.* Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* 2010;**8**:15–25.

Kwak M-J, Kong HG, Choi K *et al.* Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nat Biotechnol* 2018;**36**:1100–9.

Lahti L, Shetty S, Blake T. Tools for microbiome analysis in R. *Microbiome Package Version 099* 2017;**88**:2012–7.

Lamichhane JR, Debaeke P, Steinberg C *et al.* Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. *Plant Soil* 2018;**432**:1–28.

Ling N, Huang Q, Guo S *et al.* Paenibacillus polymyxa SQR-21 systemically affects root exudates of watermelon to decrease the conidial germination of *Fusarium oxysporum f.sp. niveum*. *Plant Soil* 2011;**341**:485–93.

Lipps SM, Samac DA. Pseudomonas viridiflava: An internal outsider of the Pseudomonas syringae species complex. *Mol Plant Pathol* 2022;**23**:3–15.

Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* 2011;**17**:10–2.

McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One* 2013;**8**:e61217.

Moroenyane I, Tremblay J, Yergeau É. Soybean Microbiome Recovery After Disruption is Modulated by the Seed and Not the Soil Microbiome. *Phytobiomes J* 2021;**5**:418–31.

Mundt JO, Hinkle NF. Bacteria within ovules and seeds. *Appl Environ Microbiol* 1976;**32**:694–8.

Newcombe G, Harding A, Ridout M *et al.* A Hypothetical Bottleneck in the Plant Microbiome. *Front Microbiol* 2018;**9**, DOI: 10.3389/fmicb.2018.01645.

Oksanen J, Kindt R, Legendre P *et al.* The vegan package. *Community Ecol Package* 2007.

Rochefort A, Briand M, Marais C *et al.* Influence of Environment and Host Plant Genotype on the Structure and Diversity of the *Brassica napus* Seed Microbiota. *Phytobiomes J* 2019;**3**:326–36.

Rochefort A, Simonin M, Marais C *et al.* Transmission of Seed and Soil Microbiota to Seedling. *mSystems* 2021;**6**:e00446-21.

Rybakova D, Schmuck M, Wetzlinger U *et al.* Kill or cure? The interaction between endophytic *Paenibacillus* and *Serratia* strains and the host plant is shaped by plant growth conditions. *Plant Soil* 2016;**405**:65–79.

Ryu C-M, Kim J, Choi O *et al.* Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. *Biol Control* 2006;**39**:282–9.

Samad A, Antonielli L, Sessitsch A *et al.* Comparative genome analysis of the vineyard weed endophyte *Pseudomonas viridiflava* CDRTc14 showing selective herbicidal activity. *Sci Rep* 2017;**7**:17336.

Shade A, Jacques M-A, Barret M. Ecological patterns of seed microbiome diversity, transmission, and assembly. *Curr Opin Microbiol* 2017;**37**:15–22.

Shakya DD, Vinther F. Occurrence of *Pseudomonas viridiflava* in Seedlings of Radish. *J Phytopathol* 1989;**124**:123–7.

Simonin M, Briand M, Chesneau G *et al.* Seed microbiota revealed by a large-scale meta-analysis including 50 plant species. *New Phytol* 2022;**234**:1448–63.

Simonin M, Dasilva C, Terzi V *et al.* Influence of plant genotype and soil on the wheat rhizosphere microbiome: evidences for a core microbiome across eight African and European soils. *FEMS Microbiol Ecol* 2020;**96**, DOI: 10.1093/femsec/fiaa067.

Torres-Cortés G, Garcia BJ, Compant S, Rezki S, Jones P, Préveaux A *et al*. Differences in resource use lead to coexistence of seed-transmitted microbial populations. *Sci Rep* 2019; **9**(1), 6648.

Walsh CM, Becker-Uncapher I, Carlson M *et al.* Variable influences of soil and seed-associated bacterial communities on the assembly of seedling microbiomes. *ISME J* 2021;**15**:2748–62.

Wang Q, Garrity GM, Tiedje JM *et al.* Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl Environ Microbiol* 2007;**73**:5261–7.

Yousef-Coronado F, Travieso ML, Espinosa-Urgel M. Different, overlapping mechanisms for colonization of abiotic and plant surfaces by *Pseudomonas putida*. *FEMS Microbiol Lett* 2008;**288**:118–24.

ACKNOWLEDGEMENTS

The authors are grateful to Guillaume Chesneau, Gloria Torres-Cortes and Samir Rezki that produced several radish seed microbiota datasets used in the meta-analysis performed. We thank Emmanuelle Laurent and Vincent Odeau (FNAMS) for field crop management and the production of the initial seed samples used in this study. We thank Muriel Bahut (ANAN platform, SFR Quasav) for amplicon sequencing.

The authors declare that they have no conflicts of interest.

FUNDING

This work was supported by the French National Research Agency [ANR-17-CE20-0009-01].

DATA AVAILABILITY

The raw amplicon sequencing data are available on the European Nucleotide Archive (ENA) with the accession number PRJEB58635.

All the scripts (bioinformatics, analyses, figures) and datasets used to conduct the study are available on GitHub (https://github.com/marie-simonin/Radish_SynCom).

SUPPLEMENTARY INFORMATION

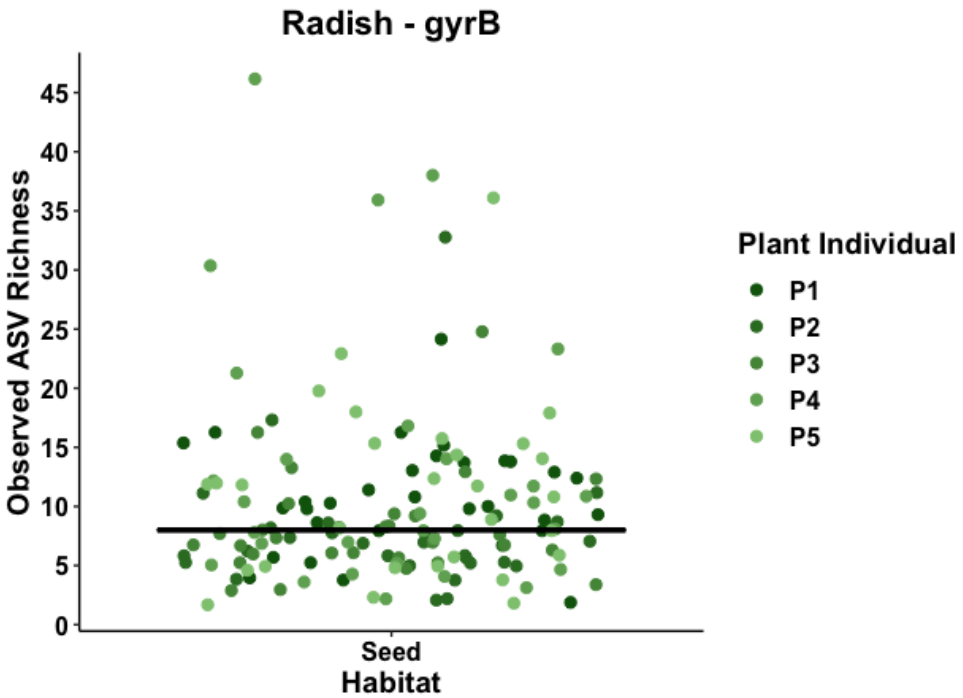


Figure S1: Observed ASV richness of individual mature seeds of radish (Flamboyant5). The data were obtained from Chesneau et al. (2022) based on 149 seeds analyzed. The horizontal line represents the median value at 8 ASVs per seed.