Differential modulation of the bacterial endophytic microbiota of Festuca arundinaceae (tall fescue) cultivars by the plant-growth promoting strain Streptomyces albidoflavus UYFA156

Federico Battistoni (fbattistoni@iibce.edu.uy)
IIBCE https://orcid.org/0000-0002-5648-1304
Patricia Vaz Jauri
Cecilia de los Santos
Belén Fernández
José Sotelo

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Abstract

Purpose

This work explores the effect of inoculation of the plant growth-promoting endophyte *Streptomyces albidoflavus* UYFA156 on the endophytic microbiota of its host, *Festuca arundinaceae* SFRO Don Tomás, and of another cultivar, Tacuabé, in which no growth promotion is observed.

Methods

In vitro growth inhibition of fourteen bacterial endophytes from both cultivars was tested in dual cultures. Then, bacterial endophytic DNA was extracted from seeds and 7 and 30 days old plants of both cultivars with and without inoculation, with three replicates of each treatment. The V4 region of the 16S rRNA gene was amplified and sequenced through Ion Torrent and sequences were analyzed with R packages.

Results

Diversity and composition in the seeds, along plant development, and in response to inoculation varied significantly. In 7 days old uninoculated plants, endophytic bacterial diversity was lower in cultivar SFRO Don Tomás than Tacuabé. However, upon inoculation, diversity in the cultivar SFRO Don Tomás increased significantly, approximately two-fold, with respect to uninoculated plants. On the other hand, no differences in diversity were observed in cultivar Tacuabé. Furthermore, microbiota composition varied significantly in plants of the cultivar SFRO Don Tomás upon inoculation, whereas this did not occur in Tacuabé plants.

Conclusion

Growth promotion by the endophyte *S. albidoflavus* UYFA156 is accompanied by an early effect in bacterial microbiota diversity and composition associated with *F. arundinaceae* plants. These results encourage further explorations about the relationship between bacterial community modulation and plant growth promotion upon bacterial endophyte inoculation.

Introduction

In recent years, the scientific community has broadly accepted that macroorganisms are not isolated. Instead, we are considered what is now called a holobiont (Rosenberg *et al.*, 2007), which is a being composed of several organisms, both macro and micro, coexisting together and giving shape to the phenotypes that are macroscopically observed (Partida-Martínez and Heil, 2011; Mitter *et al.*, 2017; Shade *et al.*, 2017; Khare *et al.*, 2018). In fact, plant-associated microbiomes are so large that they possess a higher number of genes than their host, giving way to the term “second genome” of the plants (Berendsen *et al.*, 2012). This concept changed dramatically the way in which science develops, and even more, the way in which scientific concepts are applied.

It has long been known that microbes may play crucial roles in plant health, initially in pathogen suppression, but also in plant growth, and thus their utilization has been introduced in agronomic practices worldwide in diverse fashions (Weller *et al.*, 2002; Xiao *et al.*, 2002; Mazzola, 2007; Meng *et al.*, 2012; Weerakoon *et al.*, 2012; van Bruggen *et al.*, 2015). These alternative biotechnologies in agriculture are promising tools for the care of the environment, aiming at the minimization of the impact of agriculture on ecosystems. With this goal, microorganisms have been isolated from soils, water and plants, and their plant-associated traits evaluated.

The mechanisms that underlie the beneficial effects of microorganisms on plants are many. In the case of disease control, mechanisms may be as diverse as antibiosis through secreted metabolites (Liu *et al.* 1995; Patil *et al.* 2010; Kobayashi *et al.* 2015; Cha *et al.* 2016), competition for nutrients or space (Cao *et al.*, 2005), induction of systemic acquired resistance (ISR; van Loon *et al.*, 1998) and disarming the pathogens quorum sensing mechanisms (Helman and Chernin, 2014). The mechanisms for plant growth promotion (PGP) are also diverse, ranging from direct facilitation of nutrients, such as nitrogen fixation (Tlusty *et al.*, 2004) or phosphate solubilization in the rhizosphere (Rodríguez *et al.*, 2006), to the induction of growth through plant hormones synthesis (Passari *et al.*, 2016; Myo *et al.*, 2019). Several groups of fungi and bacteria have been typically associated with these activities, and with rare exceptions (e.g. *Streptomyces* are excellent antibiotic producers, and thus likely to control disease through antibiotic production), it is not possible to predict activities on the basis of phylogeny.

Research on *Festuca arundinaceae* cv SFRO Don Tomás (DT) was originally motivated by the observation that this cultivar showed higher resistance to cold and drought than other commercial cultivars, like Tacuabé (Ta). There are several reports that plant microbiome can play a role in conferring resistance to stressing abiotic factors to their hosts (Caddell *et al.*, 2019; Zhang *et al.*, 2019; Dasgupta *et al.*, 2020). The strain *Streptomyces albidoflavus* UYFA156 was originally isolated from surface-sterilized seeds of DT (de los Santos *et al.*, 2015). In the context of a culture collection screening, it was not found to produce indoleacetic acid (IAA), solubilize P or K or fix nitrogen. However, it did promote growth of the tall fescue cultivar DT), and interestingly, it did not promote growth on another tall fescue cultivar, Ta. Microscopy and colonization tests were carried out, which confirmed the ability of the strain UYFA156 to colonize equally well the internal tissues of both cultivars (de los Santos *et al.*, 2015; Vaz Jauri, *et al.*, 2019b). Thus, specificity of growth promotion is not given by differences in the first stages of the plant-microbe interaction (Vaz Jauri *et al.*, 2019b).

Bacteria of the genus *Streptomyces* are gram positive, high GC-content organisms, with characteristic traits such as spore formation and a mycelial growth habit. They are found ubiquitously in soil, water and associated with other organisms (Vaz Jauri et al., 2016). Although there are a number of reports of bacteria of the genus *Streptomyces* as being plant growth promoters (Nozari et al., 2021; Akbari et al., 2020; Olanreawaju and Babalola, 2018), they have been mostly studied for their ability to produce bioactive metabolites, such as antibiotics and anti-tumor compounds. The mechanism(s) by which *Streptomyces albidoflavus* UYFA156 promotes plant growth in *F. arundinaceae* cv. DT has been elusive. The genome of *S. albidoflavus* UYFA156 has been fully sequenced, and its analysis revealed a high

potential for secondary metabolites production (Vaz Jauri et al., 2019a). Some of these secondary metabolites are likely to signal other microorganisms, thus altering growth and activity of coexisting endophytes. This work is based on the hypothesis that the mechanism of PGP by the strain UYFA156 on the cv. DT does not rely solely on these two partners, but also on the rest of the endophytic microbiota. The aim of this work was to explore the effect of inoculation of *S. albidoflavus* UYFA156 on the bacterial endophytic microbiota of plants of the cv. DT and Ta. The two cultivars show different bacterial endophytic communities and evolve differently in terms of diversity and composition. Upon inoculation, the bacterial endophytic communities of the two cultivars also react differently. These differences in their microbiota encourage further experiments to explore the ways in which they could be associated with the observed specificity in plant growth promotion.

Materials And Methods

Plant material and microbial strains used in this work

All the biological materials used in work are listed in Table 1. Seeds of *F. arundinaceae* cv. Ta and cv. DT were kindly shared by the Instituto Nacional de Semillas (INASE), Uruguay and Santiago Larghero of the Sociedad de Fomento Rural Ortiz (SFRO), Uruguay, respectively. Endophytic bacterial strains from cv. DT were isolated in our laboratory (de los Santos *et al.*, 2015), as well as those isolated from cv. Ta (this work). The *Botrytis cinerea* isolate used was kindly shared by Inés Ponce de León of the Departamento de Biología Molecular, Instituto de Investigaciones Biológicas Clemente Estable (IIBCE), Uruguay.

In vitro inhibition experiments

For bacterial growth inhibition assays, petri dishes containing 20 mL TSA were inoculated with 20 uL of a spore suspension of UYFA156 (1x 10^7 spores/mL) in straight lines. At that time, liquid cultures of each of the test bacterial strains were made to be used as inoculum for the assays (Table 2). All cultures were incubated at 30ºC. After 72 h, 4 uL of culture of the test strains were spotted on the plate, such that 2 spots of each strain were made at each side of the plate. Inhibition was observed as compared to control plates containing 4 uL spots of the test strains growing in plates without the strain UYFA156 and recorded at 48 h of incubation at 30ºC. Three replicates per isolate (of two spots each) were carried out. Inhibition was rated on a qualitative basis, considering inhibition as positive when at least one colony completely inhibited.

Inhibition of *Botrytis cinerea* and *Alternaria brassicicola* was tested in dual cultures on TSA medium as a proxy for general antagonism toward fungi. Petri dishes were inoculated with two 4 uL drops of a spore suspension of UYFA156 (1x 107 spores/mL) located opposite from each other. In the center of the plate, an agar plug from a freshly grown test organism was added. Growth control plates of *B. cinerea* contained only the agar plugs used as inoculum. Plates were incubated at 25ºC until the test microorganism on the control plates (without UYFA156) reached the borders of the plate.

Experimental design of bacterial microbiota study

For endophytic microbiota evaluation, plants were grown in gnotobiotic conditions as previously described (de los Santos *et al.* 2015). Briefly, seeds were surface sterilized: 5 min in 95% ethanol, 40 min in 5% NaClO and washed five times with sterile deionized water. They were immediately transferred to water agar plates for germination and incubated for 1 week at 20ºC in the dark. Upon germination, seedlings were transferred to plant tubes containing 20 mL of Jensen medium and polypropylene beads (3 seedlings per tube). After 24 h in the growth tubes, plants were inoculated with 100 µL of a spore suspension containing 1x 10^7 spores of strain UYFA156 in sterile water and controls with 100 µL of sterile water.

The evaluated treatments were: 1- uninoculated seeds of DT and Ta cultivars; 2- plants of DT and Ta cultivars at 7 days post-inoculation (dpi) and uninoculated control plants of the same age; 3- plants of DT and Ta cultivars at 30 dpi and uninoculated control plants of the same age (Supplementary Table 1). All treatments had three biological replicates.

Bacterial microbiome DNA extraction and 16S rRNA sequencing

For each 16S rRNA sequencing, 7-8 tubes with 3 plants were pooled as one biological sample ($n \geq 21$) for root DNA extraction and amplification, with 3 replicates per treatment (n = 30). Plant material was surface-disinfected (de los Santos *et al.* 2015), roots were separated from the stems, cut into small pieces (1-2 mm) using a sterile scalpel and DNA extracted using Power Soil DNA extraction kit (Qiagen). Seeds were surface disinfected (de los Santos *et al.* 2015), macerated with sterile mortar, and DNA extracted from 0.4-0.5 g of material as mentioned above. DNA samples were PCR amplified in the 16S rRNA gene V4 regions using primers (512F and 810R) containing barcodes in the forward primer (512F) and GAT adapters between the barcodes and the primer sequence (Claesson *et al.*, 2009). The cycle used for amplification had an initial denaturation step of 5' at 95ºC, 35 cycles of 30" at 94ºC, 30" at 54ºC and 1:30' at 72ºC, and a final extension time of 10' at 72ºC. Products approximately 300 bp long were purified using Qiagen Gel Purification Kit. At all stages, DNA samples were quantified by Nanodrop. Ion Torrent PGM sequencing was carried out at the IIBCE Sequencing Facility (http://iibce.edu.uy/SECUENCIACION/index.html) on the purified samples.

Bioinformatic analyses

The obtained fastq files were selected by barcodes and quality, and primer sequences removed using FASTX-Toolkit (RRID:SCR_005534). The resulting sequences were further treated using DADA2 (Callahan *et al.*, 2016) as described in the tutorial https://benjjneb.github.io/dada2/tutorial.html. All downstream work with the sequences was carried out using the Phyloseq and Vegan packages in R (Dixon, 2003; McMurdie and Holmes, 2013). For community composition analyses only amplicon sequence variants (ASVs) with a number of reads > 0.05 % were considered. For core microbiota determinations, an ASV was considered present in a cultivar when it was found in > 50% of its samples.

Statistical analyses

Alpha diversity of samples was evaluated using Shannon's diversity indices and compared using one-way analysis of variance (ANOVA) followed by pairwise t-tests using Holms correction for multiple tests. Beta diversity, or variation among endophytic bacterial communities, was visualized using unconstrained principal coordinate analyses (PCoA) and supported statistically using permutation analyses of variance (PERMANOVA) using the function ADONIS from Vegan, based on Bray-Curtis dissimilarity indices. Upon significant PERMANOVA results ($p > 0.05$), differences among variables were validated by testing for homogeneity of dispersion using the *betadisper* function from the R package vegan, using a cutoff value of 0.05. Identification of shifts in specific microbial groups were carried out using the R function SIMPER, of the package vegan.

Results

In vitro and *in vivo* inhibition of other microorganisms by strain UYFA156

All bacteria tested in *in vitro* antagonism assays showed some level of inhibition (Table 2). Although sensibly less than in the control conditions, most isolates were still able to grow when inoculated more distantly from UYFA156. The strains less affected in their growth were UYFA61, UYFA249, UYFA343 and UYFA346, belonging to the genera *Microbacterium*, *Pseudomonas*, *Kosakonia* and *Escherichia,* respectively, all of which were isolated from cv. DT. These results show that either one broad-range bacterial growth inhibiting compound, or several with more narrow activity are being produced, thus inhibiting both Gram positive and negative bacteria. In addition, the broad range fungal phytopathogens *B. cinerea* and *A. brassicicola* showed growth inhibition in co-culture experiments (Table 2).

Assessment and evaluation of overall sequencing results

Rarefaction curves showed saturation in all samples, indicating most ASVs from the sample that were identifiable with this technique were found (Supplementary Figure 1). Out of 2,956,034 reads, a total of 292 ASVs were found among all samples. Principal Coordinates Analysis (PCoA) plots allowed visualization of differences among treatments, which were found statistically significant between cultivars, dpi, and inoculated vs. uninoculated samples (Figure 1, PERMANOVA, $p = 0.001$; betadisper, $p < 0.05$). Although dispersion within treatments was not found to be significant, seed treatments were localized as a condensed group both for DT and Ta treatments, while the rest of the treatments were more dispersed, indicating higher variability in the plant bacterial microbiota than in the seeds.

Richness and alpha diversity

Species richness did not differ significantly among the different treatments. However, diversity among treatments, as determined by Simpson's diversity index, did vary significantly both in DT and Ta cultivars (ANOVA, $p= 0.0002$, $p = 0.023$, respectively; Figure 2). In DT endophytic bacterial communities, diversity was significantly lower in 7d uninoculated plants with respect to the seeds (t-test, $p < 0.05$). Interestingly, Simpson's diversity index in UYFA156-inoculated DT plants at 7 dpi was similar to that found in the seeds and significantly higher than that of 7d uninoculated plants. However, at 30 dpi DT plants showed similar

levels of diversity in inoculated and uninoculated treatments. In Ta endophytic bacterial communities, diversity at 7dpi was similar to that of seeds, both in UYFA156-inoculated and uninoculated plants. At 30d, uninoculated plants had a mild decrease in diversity with respect to seeds, but this difference failed to be significant. Otherwise, diversity levels similar in Ta were maintained in all treatments (Figure 2B). Thus, 7d DT plants show increased diversity upon inoculation, while no differences were detected in Ta. In other words, in the DT cultivar, plants inoculated with UYFA156 maintain the level of diversity found within seeds.

Composition of endophytic microbiota in the DT and Ta cultivars

As was visualized initially (Figure 1) and tested with PERMANOVA, differences in bacterial endophytic communities in plants between cultivars were significant ($p = 0.001$). However, at the seed stage, microbiota of DT and Ta did not differ in richness, diversity and composition (ANOVA $p > 0.05$, PERMANOVA $p = 0.1$). Moreover, differences in bacterial endophytic community composition among different developmental stages were significant when comparing all treatments, but not when only plant microbiota (7 and 30 dpi) were considered. Thus, further comparisons between the two cultivars were made based on the plant endophytic bacterial communities (7 and 30 d).

Differences in bacterial community composition between plants of the two cultivars were found at all taxonomic levels tested: phylum, order, family and genus. Although bacteria belonging to the Phylum Proteobacteria were overwhelmingly more abundant in all treatments, the proportion of this group in the Ta cultivar was significantly higher than in the DT cultivar ($p < 0.001$) and accounted for approximately 46% of the differences between cultivars (Figure 3A). On the other hand, DT bacterial endophytic communities were richer in Firmicutes and Synergistetes ($p = 0.002$ and 0.001, respectively), accounting for 10 and 9% of the differences between cultivars (Figure 3A). Other phyla were also found at significantly different proportions between the two cultivars, but their proportional abundances were below 1%.

In accordance with the results obtained at higher taxonomic levels, among all families in both cultivars, the Pseudomonadaceae (order Pseudomonadales, phylum Proteobacteria) and Xanthomonadaceae (order Xanthomonadales, phylum Proteobacteria), which accounted for 35 and 3% of the differences, respectively, were more abundant in the Ta cultivar (Figure 3B). Alongside these results, the DT cultivar had higher proportion of ASVs belonging to the families Synergistaceae and Lactobacillaceae, and ASVs of the families Rhizobiaceae and Paenibacilliaceae were also in higher proportion in DT although less abundant overall ($p < 0.05$; Supplementary Table 2). ASVs of the family Enterobacteriaceae, which were also abundant in both cultivars, were mildly significantly higher in DT than in Ta ($p = 0.079$).

Observing the lowest taxonomic level, genus, there were seven groups that differed significantly in proportion between cultivars within the top 90% most influential genera. The only genus that was significantly more abundant in cultivar DT was Syner-01 (Supplementary Figure 2). Of the six genera that were significantly more abundant in cultivar Ta, the genus *Pseudomonas* stood out, with a proportion of ASVs belonging to this group almost nine-fold higher in Ta than in DT, and accounted for almost 50% of

the differences. The remaining genera that were significantly more abundant in Ta than in DT were *Stenotrophomonas, Siccibacter, Saccharibacillus, Clostridium sensu stricto 1* and *Longilinea*, the last two not found in cultivar DT. However, many ASVs were not considered in this comparison, given that they had not been assigned to a specific genus. This was particularly relevant in the cultivar DT.

Approaching the opposite perspective, 11 ASVs were shared among the two cultivars. They belonged to the genera *Streptomyces*, *Pantoea*, *Defulvitoga*, *Neorhizobium*, *Syner-01*, *Xanthomonas*, *Klebsiella* and *Pseudomonas*, along with 3 ASVs with unassigned genus, which belong to the families Enterobacteriaceae, Synergistaceae and Anaerolinaceae. Upon comparison with the bacterial collection from which UYFA156 was selected, only two of these ASVs could correspond to isolates. One of these is ASV5, which could correspond to *Pantoea* sp. UYFA190, and the other is ASV3, which corresponds to *Streptomyces albidoflavus* UYFA156, the subject of this study.

Evolution of the microbiota of DT and Ta plants without inoculation

In uninoculated treatments, the evolution of the endophytic communities within cultivars over time differed between DT and Ta. Bacteria of the phylum Synergistetes in DT plants at 7 days of growth (7d) had significantly higher abundances of ASVs than seeds ($p < 0.01$; Figure 4A), and although the proportion of these bacteria seems to decrease at 30 days of growth (30d), the difference with the proportion at 7d was not significant. Interestingly, the opposite pattern was observed in the Ta cultivar, although the differences did not reach statistical significance.

The phylum Thermotogae also showed opposite trends in the two cultivars. The proportion of these ASVs increased from seeds to 30d plants in DT, although not significantly. On the other hand, in Ta the proportion decreased from seeds to 7d and 30d plants. These decreases were significant both from seeds to 7 d plants ($p = 0.05$) and to 30d plants ($p < 0.01$).

In addition, the proportion of ASVs within the phylum Bacteroidetes decreased from seeds to 7d plants in the DT cultivar ($p < 0.01$), and it kept decreasing significantly to the point of having 0 reads at 30 days ($p < 0.01$). In the Ta cultivar, no significant differences were found between proportion of ASVs of this phylum in seeds and plants at any time point. The phylum Actinobacteria, although abundant in the DT cultivar, did not shift significantly over time (Figure 4A).

ASVs of the phylum Proteobacteria were the most abundant in both cultivars, and as other phyla, their proportion on the total bacterial microbiota showed opposite trends. Nonetheless, although the proportion of Proteobacteria declined from seeds to 7d plants and 30d plants of the DT cultivar, differences between timepoints failed to be significant. Instead, the Ta cultivar showed the opposite trend: Proteobacterial proportions increased with time. Although 7d Ta plants were only mildly significantly different in Proteobacterial proportion to seeds ($p = 0.07$), 30d plants did differ with seeds ($p = 0.02$), alongside with an increase of > 50% in proportion.

Since the phylum Proteobacteria was overwhelmingly predominant in both cultivars at all timepoints, significantly more abundant in Ta than in DT, and increased significantly in Ta along time, we explored further into this group. Although the proportion of Proteobacteria had increased in the Ta plants with respect to the seeds, the proportion of the different families that composed the phylum was barely altered, being the only significant change an increase in the proportion of ASVs belonging to the Xanthomonadeacea in 30 d plants with respect to seeds ($p = 0.039$, Figure 4B). This family, along with the Pseudomonadaceae, were found on larger proportions in 30d plants of the Ta cultivar than on the DT cultivar ($p < 0.05$). On the other hand, although in the DT cultivar the proportion of endophytic bacteria of the phylum Proteobacteria remained unchanged along the three tested timepoints, the composition of the phylum at the family taxonomic level had several significant changes ($p < 0.05$). DT seeds had a number of proteobacterial families with reduced proportions in 7 d plants, such as Burkholderiaceae, Beijerinokaceae, Rhizobiaceae, Xanthomonadaceae, Sphingomonadaceae and Caulobacteraceae, while ASVs belonging to the Enterobacteriacea family were significantly increased. Endophytic microbiota of DT 30d plants were similar to those of 7d plants, with the addition of Rhodanobacteraceae to the list of significantly decreased families. Overall, within the DT cultivar, the most abundant proteobacterial genus at all timepoints was *Pantoea*.

Evolution of the microbiota of DT and Ta plants with inoculation

Sequences assigned to the genus *Streptomyces* with a 100% identity with UYFA156 were found in all inoculated treatments, however their proportion within each community did not differ significantly between inoculated and uninoculated plants. Overall differences in endophytic bacterial composition between UYFA156-inoculated and uninoculated plants were significant only in the DT cultivar (PERMANOVA, $p = 0.011$). In the DT cultivar, although dpi was not a significant factor for explaining the differences among treatments, the interaction factor between inoculation and dpi was significant (PERMANOVA, $p = 0.02$). In the Ta cultivar, neither inoculation nor dpi were significant on their own, however their interaction was also significant (PERMANOVA, $p = 0.025$). The significance of the interaction of the factors, inoculation and dpi, which suggests that effects of inoculation vary with time, required the following analyses to take this account.

Cv. SFRO Don Tomás (DT) microbiota upon inoculation

Differences in the proportion of specific groups were observed between inoculated and uninoculated DT plants at all taxonomic levels. At the phylum taxonomic level differences between inoculated and uninoculated plants were only detectable at 7 dpi, with the phyla Firmicutes, Chlorflexi and Lentisphaerae being in significantly higher proportions in the inoculated DT plants and explaining 11, 3 and 0.4% of the variation between the two conditions, respectively (Supplementary Table 2). On the other hand, ASVs of the phylum Proteobacteria were more abundant in uninoculated plants, accounting for 48% of the variation (Figure 5A). Community profiles at 30 dpi were similar to those at 7 dpi, with an overwhelming majority of Proteobacteria, followed by Actinobacteria and Firmicutes. However, no differences in

proportions were found between inoculated and uninoculated plants in these or other phyla at this time point.

The pattern of shifts in community compositions is maintained at lower taxonomic levels, with the proportion of ASVs of the families Enterobacteriaceae, Lactobacilliaceae, Anaerolineaceae, Moraxellaceae and Acetobacteraceae being significantly different between inoculated and uninoculated plants at 7dpi (Figure 5A). Of these families, only the proportion of Enterobacteriaceae was lower in inoculated than in uninoculated plants, and it explained 45.5% of the differences between samples. The other four families were more abundant in inoculated plants and accounted for 4.4, 1.9, 0.7 and 0.3% of the differences between samples, respectively (Supplementary Table 2). At 30dpi differences between inoculated and uninoculated plants were observed only in the family Xanthomonadaceae, with a higher proportion in inoculated than in uninoculated plants ($p < 0.01$).

Communities from 7dpi DT plants presented several genera with significant differences in proportion between inoculated and uninoculated treatments, and all of them were higher in inoculated plants (Supplementary Table 2). Most notably, *Lactobacillus*, *Klebsiella*, *Syntrophomonas*, *Methanospirillum* and *Acinetobacter*, were all significantly increased in the inoculated plants ($p < 0.03$ in all cases). The genus *Lactobacillus* accounted for > 9% of the variation between treatments. A blast search of the most abundant *Lactobacillus* ASV found in DT, indicates that it belongs to the *L. casei* group, with 100% identity. In 30 dpi plants, bacterial endophytic communities of inoculated plants had significantly higher proportions of ASVs of the genus *Stenotrophomonas* ($p < 0.01$; > 8% of the variation) than uninoculated plants.

Overall, significant differences in the composition of the DT endophytic bacterial communities between UYFA156-inoculated and uninoculated plants were more numerous at 7 dpi than at 30 dpi at all taxonomic levels.

Cv. Tacuabé (Ta) microbiota upon inoculation

The influence of inoculation with the strain UYFA156 on the bacterial endophytic communities of the Ta cultivar was analyzed in a similar fashion as for the DT cultivar. At the phylum level, no differences were significant at 7 dpi between inoculated and uninoculated plants, but at 30 dpi several phyla were altered (Figure 5B, Supplementary Table 2). The proportion of ASVs of the phylum Proteobacteria in Ta at 30 dpi was significantly lower in inoculated than in uninoculated plants ($p < 0.02$), comprising most of the observed variability. In turn, ASVs of the phyla Firmicutes, Synergystetes, Chloroflexi and Spirochaetes were significantly more abundant in inoculated plants ($p < 0.05$). These results reflect that the direction of the changes in the endophytic bacterial community in both cultivars may occur in similar directions, i.e. reduced Proteobacteria, increased Firmicutes and Chloroflexi, but the timing of these changes differed, being observed in the DT cultivar at 7dpi, and in the Ta cultivar at 30dpi.

The ASVs of the Tacuabé cultivar grouped at the family taxonomic level again showed more significant changes between inoculated and uninoculated plants at 30 than 7dpi. At 7dpi the proportion of ASVs of

the family Paenibacilliaceae within the whole bacterial community was significantly lower in inoculated plants than in the uninoculated ($p < 0.03$). However, the variance for which this family accounted was 1.2%. A higher number of families shifted in proportions in inoculated plants with respect to uninoculated plants at 30dpi. Synergistaceae, Anaerolineaceae, Clostridiaceae_1, and Spirochaetaceae had significantly higher proportions in inoculated plants ($p < 0.03$, Figure 5B).

Lastly, genera in the Ta cultivar were also shifted between inoculated and uninoculated plants. The genera that were significantly altered at 7 and 30 dpi were different (Supplementary Table 2). At 7 dpi, *Klebsiella, Saccharibacillus*, *Bacillus* and *Duganella* were significantly altered ($p < 0.05$), with *Bacillus* being more abundant in inoculated plants and *Klebsiella*, *Saccharibacillus* and *Duganella* more abundant in uninoculated. At 30 dpi, the only genus with a significantly altered proportion was *Syner-01*. The proportion of ASVs belonging to this genus was 10-fold higher in inoculated plants with respect to uninoculated plants ($p < 0.05$).

Discussion

This work reports that the strain UYFA156 has a trait that is common to most *Streptomyces* species, namely, broad-range growth inhibitory activity, which is often associated to plant protection or microbiota modulation, rather than to PGP (Palaniyandi *et al.*, 2011; Ruanpanun *et al.*, 2011). Specifically, inhibition of the plant pathogen *Botrytis cinerea* has been previously reported on other *Streptomyces* sp. (Castillo *et al.*, 2002; Zhao *et al.*, 2011; Alam *et al.*, 2012; Liu *et al.*, 2012, among others). In addition, the ability of the strain to inhibit growth on other endophytic bacteria isolated from both tall fescue cultivars suggests that the inoculation of strain UYFA156 may impact the endophytic microbial communities associated with the host plant also in *in vivo* conditions.

Deep sequencing of 16S rRNA genes of seed material in both cultivars rendered a low number of reads (after removing eukaryotic plastids and singletons), and thus of ASVs. In a work with different approaches for sample collection, Eyre et al (2019), obtained 37 ASVs from inside rice grains, which is comparable to the numbers we obtained. Thus this result is expected, since in addition, seeds had not been hydrated before DNA extraction and bacteria in these conditions would be present to be in extremely low abundances, not captured by the experiment. As opposed to what was observed in other plant hosts (Rybakova *et al.*, 2017; Eyre et al., 2019), our results did not capture significant differences in bacterial diversity between DT and Ta seeds communities.

Endophytic bacterial microbiota along plant development

High-throughput sequencing of the endophytic microbiota associated with tall fescue plants revealed previously unknown details about the holobionts known as *F. arundinaceae* cv. DT and cv. Ta. Holobionts of both cultivars varied significantly in the diversity of their bacterial microbiota overall and also along development. The significant differences between cultivars observed in our work are in accordance with those observed in similar work in barley and other crops (Winston *et al.*, 2014; Yang *et al.*, 2017). Differences in endophytic communities of the two cultivars are visible as early as 7 days of growth. Alpha

diversity in both cultivars was not higher in plants than in seeds, contrasting with what can be detected in culture-dependent approaches, in which very few bacterial isolates are commonly obtained from seeds and many more are obtained from roots or aerial tissues (de los Santos et al, 2015). This result suggests that the ability of isolating endophytic bacteria is highly dependent on the density of the population within plant tissues, and also highlights the importance of using multiple approaches for studying microbial communities, both culture dependent and independent. It would be interesting to evaluate if the pattern of diversity observed in this experiment, which was carried out in gnotobiotic conditions, is maintained when plants are grown in soil, where a plethora of other factors such as the effect of the root exudates of each cultivar on the rhizosphere and the permeability of each cultivar to new rhizobacteria, play major roles.

The endophytic bacterial communities of the holobionts studied in this work differed not only in diversity, but also in composition. Although microbiota compositions did not differ significantly between cultivars at the seed stage, as individuals of each cultivar developed, compositions were shifted, diverging from that of the seeds and from each other. Overall, bacterial endophytic composition in each cultivar did not vary between plants grown 7 and 30 days, being consistent with previous reports of small changes in endophytic composition along plant development (Lundberg *et al.*, 2012). Once again, this may not hold true when plants are grown in soil conditions, as colonization abilities of soil microbiota add another level of complexity. As observed previously on the culturable bacterial collection of DT endophytes as well as other endophytic communities, bacteria of the phylum Proteobacteria were the most abundant in all conditions (De los Santos et al., 2015; Martiny *et al.*, 2015; Eyre *et al.*, 2019). However, bacteria of this phylum were more abundant in Ta than in DT. Within proteobacterial populations, bacteria of the family Pseudomonadaceae, which are well known for participating in beneficial plant-bacteria interactions, were found at significantly higher proportions at 30 days of growth in Ta than in DT. Plants of the cv. Ta are characterized for having high dry weight yields (Formoso, 2010), and grow faster than plants of the cv. DT, reaching significantly higher dry weight values at 30 days of gnotobiotic growth (unpublished data). On the other hand, DT showed overall higher abundances than Ta of bacteria belonging to other phyla, such as Firmicutes and Synergistetes, and 7 day old plants showed increased proportions of both Syntergistetes and Thermotogae. Interestingly, these phyla showed opposite patterns in the Ta cultivar, declining in proportion with time, especially the phylum Thermotoga. Thermotoga and Syntergistetes use mainly amino acids as nutrient sources (Marchandin *et al.*, 2010; Eyre *et al.*, 2019) and were at undetectable levels in DT seeds. On the other hand, bacteria of the phylum Bacteroidetes, which are characterized for degrading high molecular weight molecules (Thomas *et al.*, 2011; Fernández-Gómez *et al.*, 2013), such as those found in high abundance in seeds, were significantly more abundant in DT seeds than in 7d plants. Thus, these observed shifts in proportion could reflect changes in nutrient availability within the host. Although these nutrient shifts most likely also happened in Ta plants, growth of bacteria of the phyla Thermotoga and Synergistetes seems to have been overridden by the high proliferation of Proteobacteria in this cultivar. In addition, most of the detected changes in families and genera within DT are reductions in proportions in plants with respect to seeds, reflecting the success of the Enterobacteriaceae. Further research looking into how the Pseudomonadaceae and

Enterobacteraceae may affect their hosts could help us understand differences between holobionts. Furthermore, as mentioned above, differences in microbiota between cultivars has been previously reported in other crops (Winston *et al.*, 2014; Edwards *et al.*, 2015).

Together, the slower development of the Thermotoga and Synergistetes, along with the high proportions of Proteobacteria in the Ta cultivar with respect to DT could be related to the phenotypic differences between the two cultivars in their growth traits.

In addition to significant differences, some ASVs were found in most samples of all treatments. Those ASVs are likely to be part of the core microbiome of *Festuca arundinaceae*. Further analysis directed towards describing this core microbiome in the host species and in the different cultivars may also shed light onto the relationship between PGP bacteria and the host microbiota.

Changes in microbiota upon inoculation with UYFA156

The results mentioned previously support our hypothesis that the endophytic microbiomes of the two tall fescue cultivars differ. However, to anticipate specific effects of inoculation with *S. albidoflavus* UYFA156 on tall fescue microbiomes is, at this point, a difficult task. With the study of the inoculated holobionts, we show that inoculation alters such a broad measure as the Simpson's diversity index in both cultivars, although at different time points. Interestingly, at 7 days post inoculation, inoculated DT plants had significantly higher diversity in their bacterial microbiota than the uninoculated plants. This diversity does not significantly differ at 30 days post inoculation, time at which growth promotion in this cultivar had been reported (de los Santos *et al.* 2015). In addition, inoculation of the strain UYFA156 in cultivar Ta does not drive significant differences in bacterial microbiota diversity between inoculated and uninoculated plants at any time point, but rather produces a slight increase in diversity such that the differences with the seed diversity index are not significant. Considering that the strain UYFA156 does not promote or reduce growth in this host (de los Santos *et al.* 2015), these results draw us to the question if the increase of endophytic bacterial diversity itself may be the driver of PGP in the DT cultivar. It could be argued that Ta plants, already armed with a high diversity of bacteria in their endophytic bacteria at 7d growth, are not substantially altered by inoculation with the strain UYFA156, while DT plants increase the diversity of their endophytic bacteria upon inoculation, which in turn triggers a PGP response in the host.

Exploring variations in microbiota composition given by inoculation through focusing on different taxonomic levels reveals again differences between cv DT and Ta. In the DT holobiont, bacteria of the phyla Firmicutes, Chloroflexi and Lentisphaerae and of the families Lactobacilliaceae, Anaerolineaceae, Moraxellaceae and Acetobacteraceae were present in significantly higher proportions in plants inoculated with the strain UYFA156 as early as 7dpi. The opposite trend was observed for ASVs belonging to the phylum Proteobacteria, and specifically, to the family Enterobacteraceae in that cultivar. On the other hand, holobionts of Ta showed no significant differences in proportions of groups at 7dpi, and showed similar trends in phyla shifts at 30dpi as the ones observed in DT. Nonetheless, the families that had been altered in both cultivars were not consistent. Overall, these results show a stronger influence of the inoculation with the strain UYFA156 in plants of the DT cultivar, which when uninoculated, at 7dpi

showed lower bacterial diversity and richness than Ta plants. Previous research (Zegeye *et al.*, 2019) using a simplified matrix to study community stability found evidence of the influence of initial species richness on community stability. In addition, there is also evidence that bacterial inoculants with higher numbers of species lead to higher biomass, regardless of the identity of the inoculated species (Hu *et al.*, 2017). Taking all this information, we suggest that modulation of the bacterial endophytic microbiota could be one of the factors acting in the observed PGP of *F. arundinacea* cv. SFRO Don Tomás by *S. albidoflavus* UYFA156. If this were the case, the specificity of the plant-growth promotion effect could be related to its low richness and diversity. Correlation analyses between PGP and richness and diversity increases in endophytic microbiota upon inoculation may shed some light on this hypothesis. In addition, the exploration of molecules produced by the strain UYFA156 involved in these interactions may give way to further, more specific models.

Previous work on the PGP activity of *Streptomyces* focused on traits like IAA, siderophore or antibiotic production, P solubilization, ACC deaminase activity, or even affecting directly plant metabolism (Verma *et al.*, 2011; Jog *et al.*, 2014; Palaniyandi *et al.*, 2014; Gopalakrishnan *et al.*, 2015; Vurukonda *et al.*, 2018). A report by Le et al (2016) showed the interaction of an endophytic strain of *Streptomyces* with other bacteria as influencing on PGP. These authors noted that coinoculation of the *Streptomyces* with the corresponding rhizobium to *Medicago sativa* plants increased nodulation and N_2 fixation. Our work focused on the interaction of a PGP *Streptomyces* with other endophytes, comparing a sympatric community (inoculation in the original cultivar) with an allopatric community (inoculation in another cultivar of the same plant species). We provide evidence that inoculation with a PGP strain into highly similar plant hosts produces differential shifts in endophytic bacterial communities. Metatranscriptomic analysis to visualize not the taxonomic, but the metabolic activity shifts within these communities will follow.

Declarations

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Statements and Declarations

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References

1. Alam, M., Dharni, S., Abdul-Khaliq, Srivastava, S.K., Samad, A., and Gupta, M.K. (2012) A promising strain of *Streptomyces* sp. with agricultural traits for growth promotion and disease management. *Indian J Exp Biol* **50**: 559–568.

2. Akbari A, Gharanjik S, Koobaz P, Sadeghi A (2020) Plant growth promoting Streptomyces strains are selectively interacting with the wheat cultivars especially in saline conditions. Heliyon 6:e03445. https://doi.org/10.1016/j.heliyon.2020.e03445
3. Berendsen, R.L., Pieterse, C.M.J., and Bakker, P.A.H.M. (2012) The rhizosphere microbiome and plant health. *Trends Plant Sci* **17**: 478–486.
4. van Bruggen, A.H.C., Gamliel, A., and Finckh, M.R. (2015) Plant disease management in organic farming systems. *Pest Manag Sci*.
5. Caddell, D.F., Deng, S., and Coleman-Derr, D. (2019) Role of the plant root microbiome in abiotic stress tolerance.
6. Callahan, B.J., McMurdie, Paul, J., Rosen, Michael, J., Han, Andrew, W., Johnson, A.J., and Holmes, S.P. (2016) DADA2: High resolution sample inference from Illumina amplicon data. *Nat Methods* **13**: 4–5.
7. Cao, L., Qiu, Z., You, J., Tan, H., and Zhou, S. (2005) Isolation and characterization of endophytic streptomycete antagonists of fusarium wilt pathogen from surface-sterilized banana roots. *FEMS Microbiol Lett* **247**: 147–152.
8. Castillo, U.F., Strobel, G.A., Ford, E.J., Hess, W.M., Porter, H., Jensen, J.B., *et al.* (2002) Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on *Kennedia nigriscans*. *Microbiology* **148**: 2675–2685.
9. Cha, J.Y., Han, S., Hong, H.J., Cho, H., Kim, D., Kwon, Y., *et al.* (2016) Microbial and biochemical basis of a Fusarium wilt-suppressive soil. *ISME J* **10**: 119–129.
10. Claesson, M.J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J.R., Smidt, H., *et al.* (2009) Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. *PLoS One* **4**: e6669.
11. Dasgupta MG, Burragoni S, Amrutha S, Muthupandi M, Parveen ABM, Sivakumar V, Ulaganathan K. 2020. Diversity of bacterial endophyte in *Eucalyptus* clones and their implications in water stress tolerance. *Microbiol Res* 241:126579.
12. Dixon, P. (2003) Computer program review VEGAN , a package of R functions for community ecology. *J Veg Sci* **14**: 927–930.
13. Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., *et al.* (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc Natl Acad Sci U S A* **112**: E911–E920.
14. Eyre, A.W., Wang, M., Oh, Y., and Dean, R.A. (2019) Identification and characterization of the core rice seed microbiome. *Phytobiomes J* **3**: 148–157.
15. Fernández-Gómez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S.G., González, J.M., and Pedrós-Alió, C. (2013) Ecology of marine bacteroidetes: A comparative genomics approach. *ISME J* **7**: 1026–1037.
16. Formoso, F. (2010). Festuca arundinacea, manejo para producción de forraje y semillas. Serie Técnica INIA **182**: 1-183

17. Gopalakrishnan, S., Srinivas, V., Alekhya, G., Prakash, B., Kudapa, H., Rathore, A., and Varshney, R.K. (2015) The extent of grain yield and plant growth enhancement by plant growth-promoting broad-spectrum *Streptomyces* sp. in chickpea. *Springerplus* **4**:.
18. Helman, Y. and Chernin, L. (2014) Silencing the mob: disrupting quorum sensing as a means to fight plant disease. *Mol Plant Pathol* **16**: 316–329.
19. Hu, J., Wei, Z., Weidner, S., Friman, V.P., Xu, Y.C., Shen, Q.R., and Jousset, A. (2017) Probiotic *Pseudomonas* communities enhance plant growth and nutrient assimilation via diversity-mediated ecosystem functioning. *Soil Biol Biochem* **113**: 122–129.
20. Jog, R., Pandya, M., Nareshkumar, G., and Rajkumar, S. (2014) Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth. *Microbiol (United Kingdom)* **160**: 778–788.
21. Khare, E., Mishra, J., and Arora, N.K. (2018) Multifaceted interactions between endophytes and plant: developments and prospects. *Front Microbiol* **9**: 1–12.
22. Kobayashi, A., Kobayashi, Y.O., Someya, N., and Ikeda, S. (2015) Community analysis of root- and tuber-associated bacteria in field-grown potato plants harboring different resistance levels against common scab. *Microbes Environ* **30**: 301–309.
23. Le, X.H., Ballard, R.A., and Franco, C.M.M. (2016) Effects of endophytic *Streptomyces* and mineral nitrogen on *Lucerne* (Medicago sativa L.) growth and its symbiosis with rhizobia. *Plant Soil* **405**: 25–34.
24. Liu, D., Anderson, N.A., and Kinkel, L.L. (1995) Biological control of potato scab in the field with antagonistic *Streptomyces scabies*. *Phytopathology* **85**: 827–831.
25. Liu, L., Li, Y., Li, S., Hu, N., He, Y., Pong, R., *et al.* (2012) Comparison of next-generation sequencing systems. *J Biomed Biotechnol* **2012**:.
26. van Loon, L.C., Bakker, P. a, and Pieterse, C.M. (1998) Systemic resistance induced by rhizosphere bacteria. *Annu Rev Phytopathol* **36**: 453–83.
27. de los Santos, M.C., Taulé, C., Mareque, C., Beracochea, M., and Battistoni, F. (2015) Identification and characterization of the part of the bacterial community associated with field-grown tall fescue (*Festuca arundinacea*) cv. SFRO Don Tomás in Uruguay. *Ann Microbiol* **66**: 329–342.
28. Lundberg, D.S., Lebeis, S.L., Paredes, S.H., Yourstone, S., Gehring, J., Malfatti, S., *et al.* (2012) Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**: 86–90.
29. Marchandin, H., Damay, A., Roudière, L., Teyssier, C., Zorgniotti, I., Dechaud, H., *et al.* (2010) Phylogeny, diversity and host specialization in the phylum Synergistetes with emphasis on strains and clones of human origin. *Res Microbiol* **161**: 91–100.
30. Martiny, J.B.H., Jones, S.E., Lennon, J.T., and Martiny, A.C. (2015) Microbiomes in light of traits: A phylogenetic perspective. *Science (80-)* **350**: aac9323–aac9323.
31. Mazzola, M. (2007) Manipulation of rhizosphere bacterial communities to induce suppressive soils. *J Nematol* **39**: 213–220.

32. McMurdie, P.J. and Holmes, S. (2013) Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**:.
33. Meng, Q., Yin, J., Rosenzweig, N., Pathology, P., and Douches, D. (2012) Culture-based assessment of microbial communities in soil suppressive to potato common scab. *Plant Dis* **96**: 712–717.
34. Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., *et al.* (2017) A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Front Microbiol* **8**: 1–10.
35. Myo, E.M., Ge, B., Ma, J., Cui, H., Liu, B., Shi, L., *et al.* (2019) Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. *BMC Microbiol* **19**: 1–14.
36. Nozari RM, Ortolan F, Astarita LV, Santarém ER (2021) Streptomyces spp. enhance vegetative growth of maize plants under saline stress. Brazilian J Microbiol 52:1371–1383.
37. Olanrewaju OS, Babalola OO (2019) Streptomyces: implications and interactions in plant growth promotion. Appl Microbiol Biotechnol 103:1179–1188.
38. Palaniyandi, S. a., Damodharan, K., Yang, S.H., and Suh, J.W. (2014) *Streptomyces* sp. strain PGPA39 alleviates salt stress and promotes growth of “Micro Tom” tomato plants. *J Appl Microbiol* **117**: 766–773.
39. Palaniyandi, S. a., Yang, S.H., Cheng, J.H., Meng, L., and Suh, J.W. (2011) Biological control of anthracnose (Colletotrichum gloeosporioides) in yam by Streptomyces sp.MJM5763. *J Appl Microbiol* **111**: 443–455.
40. Partida-Martínez, L.P. and Heil, M. (2011) The microbe-free plant: fact or artifact? *Front Plant Sci* **2**:100.
41. Passari, A.K., Chandra, P., Zothanpuia, Mishra, V.K., Leo, V.V., Gupta, V.K., *et al.* (2016) Detection of biosynthetic gene and phytohormone production by endophytic actinobacteria associated with *Solanum lycopersicum* and their plant-growth-promoting effect. *Res Microbiol*.
42. Patil, H.J., Srivastava, A.K., Kumar, S., Chaudhari, B.L., and Arora, D.K. (2010) Selective isolation, evaluation and characterization of antagonistic actinomycetes against *Rhizoctonia solani*. *World J Microbiol Biotechnol* **26**: 2163–2170.
43. Rodríguez, H., Fraga, R., Gonzalez, T., and Bashan, Y. (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant Soil* **287**: 15–21.
44. Rosenberg, E., Koren, O., Reshef, L., and Efrony, R. (2007) The role of microorganisms in coral health, disease and evolution. **5**: 355–362.
45. Ruanpanun, P., Laatsch, H., Tangchitsomkid, N., and Lumyong, S. (2011) Nematicidal activity of fervenulin isolated from a nematicidal actinomycete, *Streptomyces* sp. CMU-MH021, on *Melidogyne incognita*. *World J Microbiol Biotechnol* **27**: 1373–1380.
46. Rybakova, D., Mancinelli, R., Wikström, M., Birch-Jensen, A.S., Postma, J., Ehlers, R.U., *et al.* (2017) The structure of the *Brassica napus* seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. *Microbiome* **5**: 104.

47. Shade, A., Jacques, M.A., and Barret, M. (2017) Ecological patterns of seed microbiome diversity, transmission, and assembly. *Curr Opin Microbiol* **37**: 15–22.
48. Thomas, F., Hehemann, J.H., Rebuffet, E., Czjzek, M., and Michel, G. (2011) Environmental and gut Bacteroidetes: The food connection. *Front Microbiol* **2**: 1–16.
49. Tlusty, B., Grossman, J.M., and Graham, P.H. (2004) Selection of rhizobia for prairie legumes used in restoration and reconstruction programs in Minnesota. *Can J Microbiol* **50**: 977–983.
50. Vaz Jauri P, Altier N, Kinkel LL (2016) Streptomyces for Sustainability. In: Castro-Sowinski S (ed) Microbial Models: From Environmental to Industrial Sustainability. Springer Singapore, Singapore, pp 251–276
51. Vaz Jauri, P., Beracochea, M., Fernández, B., and Battistoni, F. (2019) Whole-genome sequencing of *Streptomyces* sp. strain UYFA156, a cultivar-specific plant growth-promoting endophyte of *Festuca arundinacea*. *Microbiol Resour Announc* **8**: 1–2.
52. Vaz Jauri, P., Taulé, C., de los Santos, M.C., Fernández, B., Di Paolo, A., Sotelo-Silveira, J.R., and Battistoni, F.J. (2019) Interactions between putatively endophytic bacteria and tall fescue (*Festuca arundinacea*): plant growth promotion and colonization in host and non-host cultivars. *Plant Soil* 1–22.
53. Verma, V.C., Singh, S.K., and Prakash, S. (2011) Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *J Basic Microbiol* **51**: 550–556.
54. Vurukonda, S.S.K.P., Giovanardi, D., and Stefani, E. (2018) Plant growth promoting and biocontrol activity of *Streptomyces* spp. As endophytes. *Int J Mol Sci* **19**:.
55. Weerakoon, D.M.N., Reardon, C.L., Paulitz, T.C., Izzo, A.D., and Mazzola, M. (2012) Long-term suppression of *Pythium abappressorium* induced by *Brassica juncea* seed meal amendment is biologically mediated. *Soil Biol Biochem* **51**: 44–52.
56. Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M., and Thomashow, L.S. (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu Rev Phytopathol* **40**: 309–48.
57. Winston, M.E., Hampton-Marcell, J., Zarraonaindia, I., Owens, S.M., Moreau, C.S., Gilbert, J.A., *et al.* (2014) Understanding cultivar-specificity and soil determinants of the *Cannabis* microbiome. *PLoS One* **9**:.
58. Xiao, K., Kinkel, L.L., and Samac, D. a. (2002) Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. *Biol Control* **23**: 285–295.
59. Yang, L., Danzberger, J., Schöler, A., Schröder, P., Schloter, M., and Radl, V. (2017) Dominant groups of potentially active bacteria shared by barley seeds become less abundant in root associated microbiome. *Front Plant Sci* **8**: 1–12.
60. Zegeye, E.K., Brislawn, C.J., Farris, Y., Fansler, S.J., Hofmockel, K.S., Jansson, J.K., *et al.* (2019) Selection, succession, and stabilization of soil microbial consortia. *mSystems* **4**: 1–13.
61. Zhang, Y., Yu, X., Zhang, W., Lang, D., Zhang, Xiaojia, Cui, G., and Zhang, Xinhui (2019) Interactions between endophytes and plants: beneficial effect of endophytes to ameliorate biotic and abiotic

stresses in plants. *J Plant Biol* **62**: 1–13.

62. Zhao, K., Penttinen, P., Guan, T., Xiao, J., Chen, Q., Xu, J., *et al.* (2011) The diversity and antimicrobial activity of endophytic Actinomycetes isolated from medicinal plants in Panxi Plateau, China. *Curr Microbiol* **62**: 182–190.

Tables

Table 1

List of all organisms (plants, bacteria and fungi) used in this study.

ORGANISM	REFERENCE
Festuca arundinaceae cv. SFRO Don Tomás	de los Santos et al., 2015
Festuca arundinaceae cv. Tacuabé	de los Santos et al., 2015
Streptomyces albidoflavus UYFA156	de los Santos et al., 2015
Microbacterium sp. UYFA61	de los Santos et al., 2015
Microbacterium sp. UYFA68	de los Santos et al., 2015
Pseudomonas sp. UYFA249	de los Santos et al., 2015
Kosakonia sp. UYFA343	de los Santos et al., 2015
Pantoea sp. UYFA344	de los Santos et al., 2015
Escherichia sp. UYFA346	de los Santos et al., 2015
Bacillus sp. UYFA348	de los Santos et al., 2015
Pantoea sp. UYFA349	this work
Kosakonia sp. UYFA350	this work
Pseudomonas sp. UYFA352	this work
Erwinia sp. UYFA354	this work
Erwinia sp. UYFA355	this work
Kosakonia sp. UYFA357	this work
Kosakonia sp. UYFA358	this work
Alternaria brassissicola	this work
Botrytis cinerea	this work

Table 2

Agar diffusion inhibition tests of strain UYFA156 on other tall fescue endophytes from Don Tomás (DT) and Tacuabé (Ta) cultivars and phytopathogenic fungi. Inhibition was rated on a qualitative bases, on a scale from 0-3: 0, no inhibition; 1, barely visible inhibition; 2, notorious inhibition; 3, total inhibition (no growth).

TARGET MICROORGANISM	HOST	INH
Microbacterium sp. UYFA61	DT	2
Microbacterium sp. UYFA68	DT	3
Pseudomonas sp. UYFA249	DT	2
Kosakonia sp. UYFA343	DT	2
Pantoea sp. UYFA344	DT	3
Escherichia sp. UYFA346	DT	2
Bacillus sp. UYFA348	DT	3
Pantoea sp. UYFA349	Ta	3
Kosakonia sp. UYFA350	Ta	3
Pseudomonas sp. UYFA352	Ta	3
Erwinia sp. UYFA354	Ta	3
Erwinia sp. UYFA355	Ta	3
Kosakonia sp. UYFA357	Ta	3
Kosakonia sp. UYFA358	Ta	3
Alternaria brassissicola	-	2
Botrytis cinerea	-	2

Figures

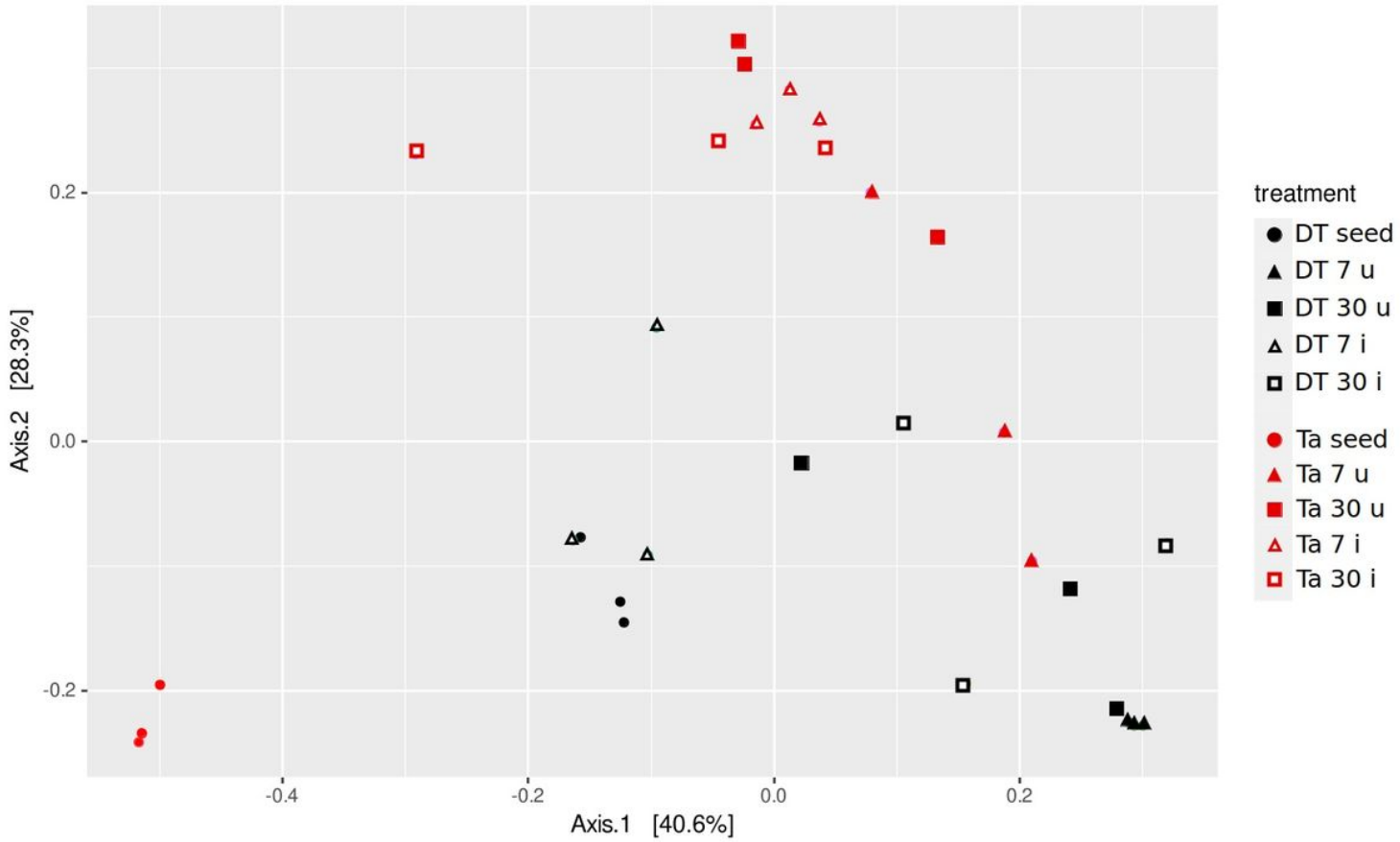

Figure 1. PCoA based on UniFrac distances of all samples. u: uninoculated; i: inoculated. Significant differences were found between cultivars, dpi, and inoculated vs. uninoculated treatments (PERMANOVA, p = 0.001).

Figure 1

See image above for figure legend.

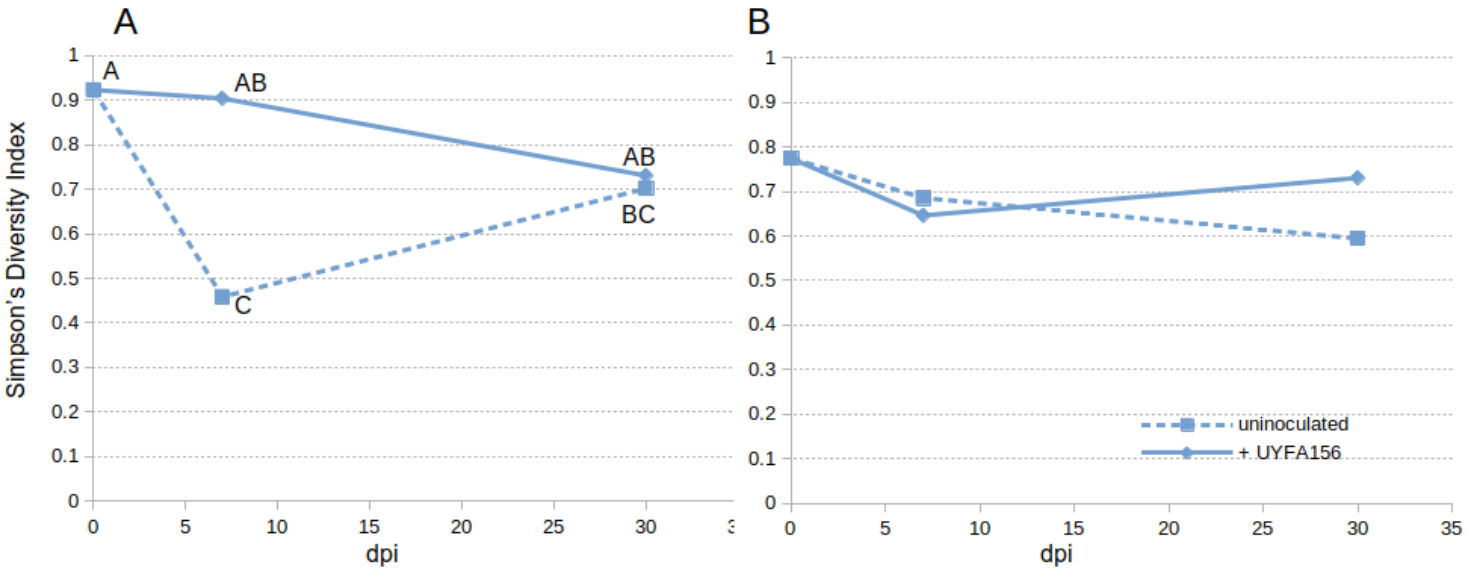

Figure 2. Simpson's diversity indices of bacterial endophytic microbiomes in all treatments over time (dpi: days post inoculation). A: DT cultivar; B: Ta cultivar. Different letters indicate significant differences ($p < 0.05$). No differences were found among treatments in the Ta cultivar.

Figure 2

See image above for figure legend.

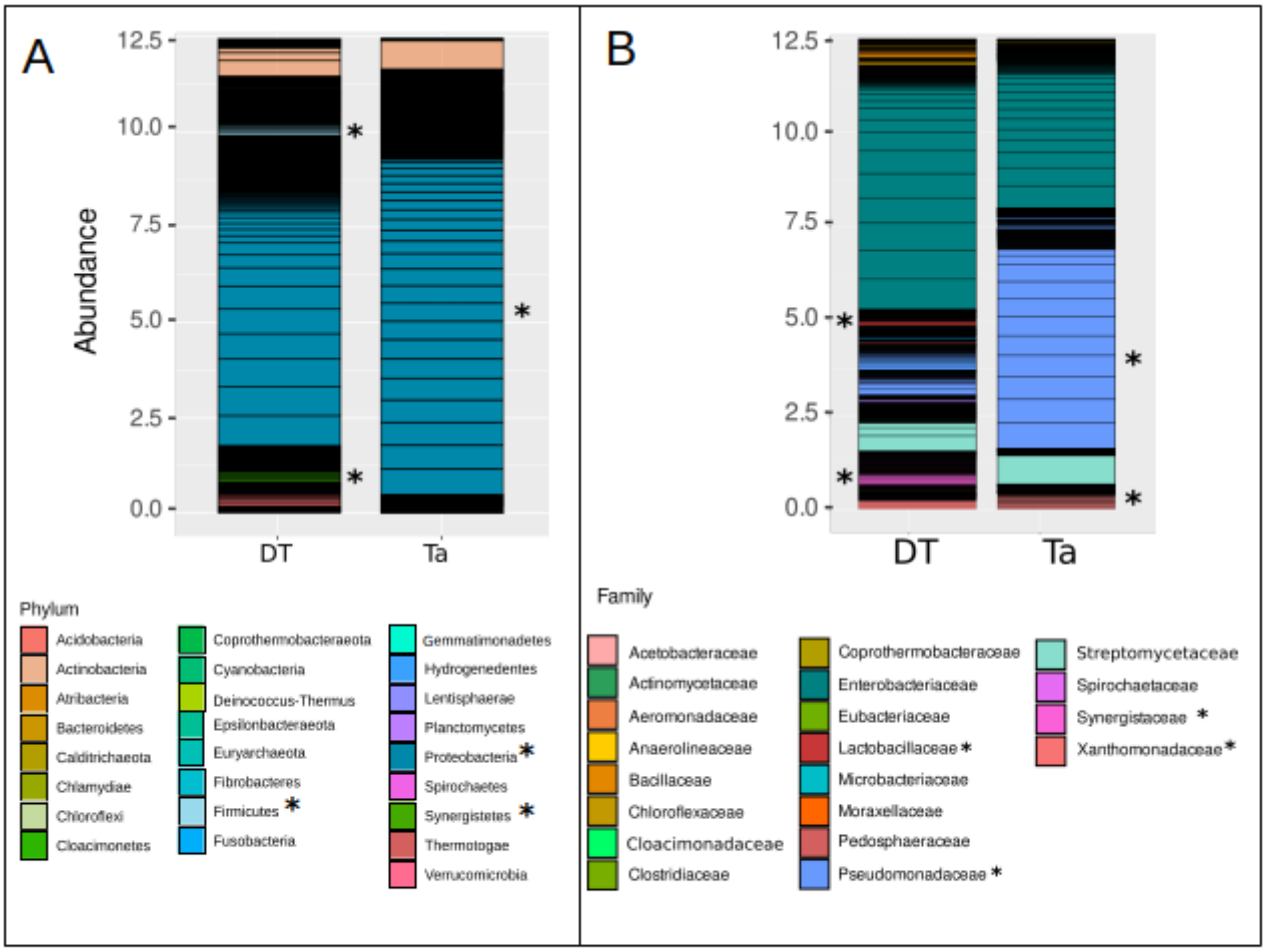

Figure 3. Bar plots of community structure within bacterial endophytic microbiomes of cultivars DT and Ta at different taxonomic levels. A. phyla; B: families.

Figure 3

See image above for figure legend.

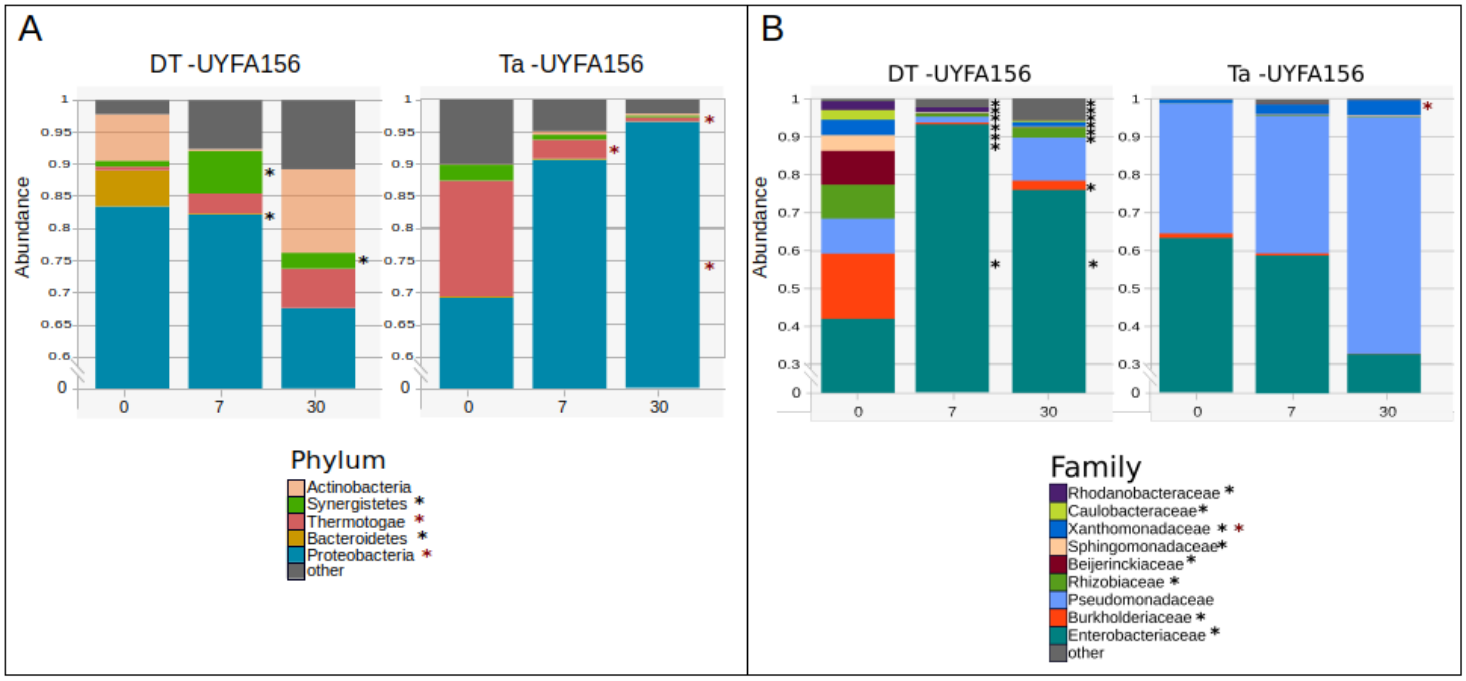

Figure 4. Evolution of the abundance of most abundant groups in the endophytic microbiomes of seeds, 7d and 30d plants in DT and Ta cultivars without inoculation. A: phyla; B: families within the phylum Proteobacteria. Differences in groups with respect to proportion in seeds is indicated with an asterisk.

Figure 4

See image above for figure legend.

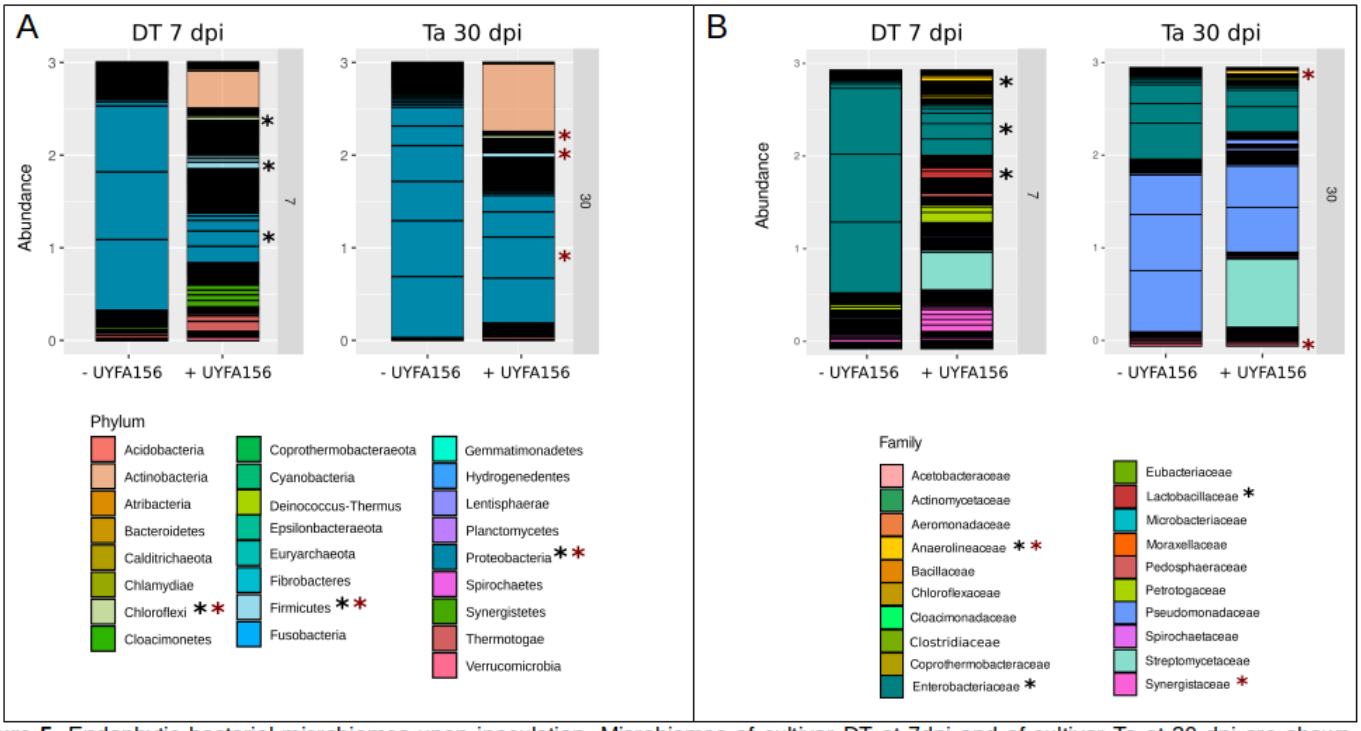

Figure 5. Endophytic bacterial microbiomes upon inoculation. Microbiomes of cultivar DT at 7dpi and of cultivar Ta at 30 dpi are shown, which were those with significant differences between inoculated (+UYFA156) and uninoculated (-UYFA156) plants. Asterisks indicate particular groups which abundance differed significantly. A: phyla in 7 dpi DT and 30 dpi Ta; B: families in 7 dpi DT and 30 dpi Ta. The legend in (B) has a selection of the most abundant families.

Figure 5

See image above for figure legend.

Supplementary Files

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- SupplementalMaterialVazetal.docx