Unraveling the Impact of *Frateuria defendens* Colonization on Melon Plants: Insights into Phenotypic, Biochemical, and Microbiota Changes

Abstract

The increasing use of bacteria biocontrol agents in agriculture raises concerns about their potential impact not only on the environment of infected plants but also on that of healthy plants. This study investigated the effects of introducing the endophytic bacterium *Frateuria defendens*, a potential biocontrol agent against phytoplasma diseases, on melon (*Cucumis melo*) as a model plant. Different isolates of *F. defendens* were screened for their antimicrobial activity *in vitro* and their ability to inhabit and impact the plant sap and microbiome.

Only one isolate, designated FD, successfully colonized the melon shoot and sap. The plant sap retained the antimicrobial activity of FD was confirmed in a biological test on *Spiroplasma melliferum*. The presence of FD in the plant tissue induced distinct shifts in the overall structure and diversity of the endophytic microbial populations. These significant findings emphasize the need for a comprehensive understanding of the ecological implications of introducing exogenous microbes into plant systems. This study contributes to the growing body of knowledge on the intricate interactions between plants, and their microbiomes, and introduced biocontrol agents, towards sustainable agricultural practices.

Introduction

The increasing utilization of microbial inoculants and their secreted metabolites as a biological control solution in modern agricultural practices (Santos et al., 2019) emphasizes a significant gap of knowledge concerning the implications of introducing exogenous bacterial species into plants' natural habitats. Given that these agents are applied into environments inhabited by both diseased and healthy plants, questions arise regarding their potential effects on healthy plant systems.

From an environmental perspective, a plant can be seen as an ecological niche for various intrinsic and extrinsic microorganisms that inhabit its tissues. Therefore, inoculation of the plant and its surroundings with exogenous beneficial microorganisms can significantly alter the diversity and structure of the microbial community (Welmillage et al., 2021; Senthilkumar et al., 2011), thereby affecting various plant traits (Yurgel et al., 2022). These changes can be particularly pronounced when the biocontrol agent is an endophyte. Endophytic bacteria or fungi have the unique ability to colonize the internal tissues of their host plant without causing any phytotoxic effects (Pacifico et al., 2019). They can establish a wide range of relationships with their plant hosts, from transient associations to more mutualistic symbioses. These relationships can help mitigate biotic and abiotic stresses that delay plant growth and development (Rosenblueth and Martínez-Romero, 2006; Mercado-Blanco and Lugtenberg, 2014). The mechanisms by which endophytic bacteria reduce biotic stress, caused by phytopathogenic microorganisms, include competition, antibiotic secretion, and the enhancement of the plant's innate immune system (Ryan et al., 2008). These processes can directly or indirectly suppress the growth and virulence of plant pathogens, thereby promoting plant health and productivity.

One significant advantage of utilizing endophytic bacteria as biological control agents is their ability to enter the plant's internal tissues and subsequently spread systemically throughout the host via the vascular system and other transport mechanisms (Rosenblueth and Martínez-Romero, 2006; Hardoim et al., 2008; Vandana et al., 2021). This endophytic colonization enables these bacterial biocontrol agents to combat phytopathogens at the site of infection, offering a more targeted and effective disease control than epiphytic bacteria.

*Frateuria defendens* (Lidor et al., 2019) is a newly discovered endophytic bacterium that was originally isolated from the phloem-feeding planthopper insect *Hyalesthes obsoletus* (Iasur-Kruh et al., 2018). These cells are gram-negative, aerobic, motile, and appear as small rod-shaped structures measuring 0.2–0.3 μm wide and 1.0–1.3 μm long (Lidor et al., 2019). *F. defendens* penetrates plants via roots or leaf stomata, and it can colonize the xylem and phloem of various plant species (such as cotton, melon, orange, etc.) without causing any phytotoxic effects (Lidor et al., 2018). Furthermore, this bacteriumis a potential biocontrol agent against yellowing disease, attributed to *Candidatus* Phytoplasma bacteria of the Mollicutes class (Gonella et al., 2019, Naor et al., 2023). Since phytoplasma cannot be isolated in exogenous culture, it is customary to use the bacterium *Spiroplasma melliferum* (also belongs to the Mollicutes class) as a model bacterium (Iasur-Kruh et al., 2018). Indeed, *F. defendens* showed an ability to suppress *S. melliferum* activity *in vitro* and reduce phytoplasma symptoms *in planta* both in grapevines and periwinkle (Iasur-Kruh et al., 2018, Naor et al., 2019). In addition, when applied in vineyard, *F. defendens* reduced yellowing disease symptoms in infected vines under field conditions (Naor et al. al., 2019). In a previous study we showed that *F. defendens* secrete secondary metabolites that inhibit the growth of mollicutes bacteria, and it was suggested that these secreted metabolites, combined with the bacterium localization *in planta*, are the main mechanism that results in the reduction of disease symptoms of phytoplasma-infected plants (Naama-Amar et al., 2020). However, comparing the efficiency between strains of *F. defendens* has never been studied nor the impact of *F. defendens* on the plant microbiome. In the current study, we utilized several *F. defendens* isolates as a model to investigate their interaction with the entire host plant biosphere, focusing on their effects on plant sap. To facilitate this investigation, we selected healthy melon plants as hosts due to their rich sap production, which is easily extractable and examinable.

Aims

This study aims to compare the inhibitory activity among isolates of *F. defendens* and examine the effects of selected isolates on the antibacterial activity of the plant's sap and its endophytic community composition.

Material and methods

**Bacterial strains**

Different *F. defendens* isolates from the insect *Hyalesthes obsoletus* collected previously and stored in -20OC were used. The strains were denoted as KT14, KT15, KT17, KT24, KT25, KT26, KT27, KT28, KT29, KT30 & LH44 (Table 1).

**Bacteria growth conditions**

Bacterial strains *F. defendens* and *S. melliferum* originated from Professor Shlomo Trachtenberg's laboratory at the University of Jerusalem, Israel. *F. defendens* and S. *melliferum* were grown in LB medium (Lysogeny broth) and S medium (media composition are detailed in Table S1) for 2 and 5 days, respectively. To initiate bacterial starter, 100 microliters from glycerol stock of each isolate of *F.* *defendens* were inoculated into 20 ml of LB culture medium and incubated at 28°C. Subsequently, 5 ml of starter culture of each strain was transmitted separately into 20 ml of S medium for 10-day incubation at 28°C on a shaker.

**Plant material and growth conditions**

The *in-planta* experiments used plants obtained from seeds of the melon variety Nay Yizael, provided by Dr. Amit Gorss's laboratory, Volcani Center-Agricultural Research Center at Newe Yaaer. The seeds were sown in a horticultural soil mixture, Garden Mix Series at 25OC under long-day conditions (16h light/8h dark) for two weeks before application.

***F. defendens* filtrate separation**

In order to obtain a filtrate containing secreted secondary metabolites, the cell culture of each isolate was grown for 10 days. Subsequently, the culture was centrifugated for 10 minutes at 5000 rpm at room temperature and the supernatant was filtered through a 0.22-micrometer filter (FPV, JET BIOFIL, Spain) to obtain cell-free supernatant.

**Filtrate inhibition test**

The filtrate's inhibition level was assessed following a previously described protocol (Iasur-Kruh et al., 2018). Briefly, after incubation at 28°C, 0.5 mL of the cell-free supernatant of each strain was mixed with 0.5 mL of S medium (Table S1) in an Eppendorf tube, adding five microliters of *S. melliferum* cells (approximately 8×10^5 cells) to the mixture. As a positive control, 1 mL of S medium supplemented with five microliters of *S. melliferum* was used, while S medium without bacterial cells served as a negative control. The inhibitory effect was measured using a spectrophotometric analysis after incubation for three days at 28°C. The OD595nm values of each well were measured as an indicator of *S. melliferum* growth. Higher values (red color) indicated no bacterial growth and lower values (yellow color) indicated bacterial growth. The inhibitory activity was measured using a 96-well plate with a volume of 250 microliters: a. at a single time point - post-incubation by adding five microliters of the filtrate to 200 microliters of S liquid medium containing phenol red (5%) to indicate *S. melliferum* growth (Trachtenberg and Gilad, 2001). b. continuous monitoring- using a continuous plate reader system (CLARIOstar). In this test, 0.125% red phenol was added to the medium, and a 430-nanometer wavelength was selected to represent the change in medium color to yellow and the time to color change as a measure of *S. melliferum* growth. In both tests the inhibitory level was calculated as the relative change in yellow color compared to a positive control (%). The inhibition period was calculated as time to complete color change (hours). In order to compare between strains, the time to 75% inhibition was set as a threshold. These two methods enabled us to distinguish between the inhibition levels of different strains over time. The test was performed in 5 replicates for each treatment, with a sterile S medium as negative control.

**Application of *F. defendens* to melon plants**

The three best inhibitory strains, FD, KT15, and KT14, were used to study the strains' ability to enter and reside in vascular plant tissue (Table 1). Cell suspensions containing approximately 2×10^7 cells/mL and supplemented with 0.1% Tween 20 in DDW were sprayed on fifteen-day-old melon plants. Fifteen plants were sprayed with LB liquid medium supplemented with 0.1% Tween 20 as a control, and 15 plants were sprayed with DDW as another control for the entire process. The presence and identity of *F. defendens* isolates in plant tissues were determined using classical microbiological methods and specific PCR. In addition, the bacterium's impact on plant morphology (length and weight) was examined five and ten days after application. The experiment was repeated three times.

**Plant sap extraction**

Each plant was surface sterilized separately by immersing the plant parts in 70% ethanol for 30 seconds, a 2-minute immersion in NaOCl 0.6%, and two 10-minute rinses in DDW. Subsequently, plant sap extraction was performed separately from the stem of each plant. The stem was cut into 2 cm pieces under sterile conditions, and from each piece, plant sap was extracted by inserting the tip of a small pipette into the bottom part of each stem piece, primarily aiming to obtain pure phloem sap. Also, the pressure exerted on the stem by fingers resulted in a mixed sap that contained phloem and xylem sap as well, hence referred to as plant sap). Approximately 500 microliters of mixed sap were collected from a pool of three treated plants, with five biological replicates (a total of 15 plants for each time point). Sap from non-inoculated plants served as a control with three biological repeats (a total of 9 plants for each time point).

**Examination of *F. defendens* presence in melon plants**

Fifty microliters of plant sap were taken to assess the presence of *F. defendens* by live count plating on CV agar (Iasur-Kruh et al., 2018) followed by colony identification by PCR analyses using specific primers: PCR mixture of each tube contained 10 μl of DDW, 12 μl of Kodaq 2x PCR Master Mix, and 1 μl of each primer (5 picomoles): F- CTCTGTGGGTGGCGAGTGGC, R- ACCGTCAGTTCCGCCGGG (Naor et al., 2019). The reaction conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 64°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes.

In addition, the same PCR analysis was applied to DNA extracted from the shoot and root of control and treated melon plants at each time point during the experiment.

**DNA extraction from plant**

Plant tissues were sterilized as detailed above and homogenized with lysis buffer in a 1:2 ratio using a sterilized mortar and pestle. Total DNA was extracted using a Plant/Fungi DNA Isolation kit according to the manufacturer’s instructions (Plant/Fungi DNA Isolation kit, Norogen Biotek Corp., Thorold, Canada). DNA quantity and quality were examined using a NanoDrop spectrophotometer (NanoDrop 2000/2000c; Thermo Fisher Scientific, Waltham, MA).

**Experimental design**

**Evaluation of the inhibitory activity of *F. defendens* strains**

The inhibitory effect of *F. defendens* isolates was assessed *in vitro* by comparing the inhibition activity of the isolates’ filtrate. The working hypothesis assumed that secondary metabolites are secreted into the growth medium and retain the inhibition activity (Naama-Amar et al., 2020). The filtration of 13 strains was tested at a single time point after three days of incubation and by continuous monitoring for six days (As detailed above). Each treatment was performed in five replicates.

**Evaluation of the inhibitory activity of the plant sap**

In order to examine the changes in sap antimicrobial traits, 450 microliters of plant sap were heat-treated by boiling for 5 minutes to prevent contamination while analyzed in a single time point *in vitro* test (See above). The working hypothesis posits that the isolate inhabits the plant's vascular tissues and secretes its metabolites directly into the sap, even in the absence of pathogenic bacteria. Therefore, FD isolate presence can alter the microbiome of a healthy plant.

**The effect of FD on microbiota of melon plants**

In order to examine the effect of FD isolate on melon endophytic communities, the extracted DNA (See above) was used for amplicon sequencing: Genomic DNA was PCR amplified with primers CS1\_520F and CS2\_799R (modified from the primer set employed by the Earth Microbiome Project (EMP; GTGYCAGCMGCCGCGGTAA and GGACTACNVGGGTWTCTAAT) targeting the V4 regions of microbial small subunit ribosomal RNA genes, with reduced affinity to plastid sequence (Moroenyane et al., 2021). Amplicons were generated using a two-stage PCR amplification protocol as described previously (Naqib et al., 2018). The re-pooled libraries, with a 15% phiX spike-in, were loaded onto a Miniseq flow cell, and sequenced (2x153 paired-end reads). Fluidigm sequencing primers, targeting the CS1 and CS2 linker regions, were used to initiate sequencing. De-multiplexing of reads was performed on instrument. Library preparation, pooling, and sequencing were performed at the Genome Research Core (GRC) within the Research Resources Center (RRC) at the University of Illinois at Chicago (UIC). Variances were considered significantly different when P values were <0.05. Alpha diversity parameters were calculated using the R ‘vegan’ package (version 2.5.7). The Shannon diversity index (H’), the Simpson index of evenness, and the number of ASVs were calculated based on a count table rarified to 450 sequences per sample. We further examined similarities in bacterial community composition using NMDS ordination. We used the permutational analysis of variance test implemented in the R package ‘vegan’ to assess differences in composition related to treated and un-treated melons.

**Statistics**

Microsoft Excel calculated descriptive statistics (i.e., means with standard error). To compare growth rates of *F. defendens* and levels of inhibitory activity, one-way analyses of variance (ANOVAs) were performed using SPSS (IBM, USA).

Results and Discussion:

The current study surveyed for isolates of *F. defendens* exhibiting potent inhibitory activity. These isolates were then introduced as exogenous endophytes into melon plants to investigate the impact on the plant phenotype, the antibacterial properties of the plant sap, and the composition of the plant's endophytic community.

***The inhibitory activity of F. defendens strains filtrate***

Given that bacteria secrete inhibitory substances in a strain-specific manner (Hashem et al., 2019; Bertuzzi et al., 2022; Islam et al., 2012; Park & Cooper, 2019), the initial step of the current research was the identification of the optimal isolate for this study; Several isolates were obtained from the same site at the same season where the original *F. defendens* (FD strain) was isolated (Table 1). These strains were isolated in 2020, seven years after FD was isolated. All isolates were isolated from the guts of *Hyalesthes obsoletus* specimens, which is the primary insect vector that transmits *Phytoplasma*, the causal pathogen of yellow disease in grapevine in Israel (Sharon et al., 2015). The insect specimens were captured from plants of *Vitex Agnus cactus* located in a rich vine-growing area during the insect flight period in the northeastregion of Israel. Previous studies have shown that FD produces secondary metabolites that reduce yellow disease symptoms on the one hand and inhibit model Mollicute (*Spiroplasma*) on the other (Naor et al., 2019; Naama-Amar et al., 2022). Hence the rate of secretions of the different isolates was examined for their effect on *Spiroplasma*. After a three-day incubation period, eight out of the 13 isolates displayed maximum inhibitory activity, while the remaining five strains exhibited a lesser degree of inhibition, ranging from 25% to 94%. However, only KT-14, KT-15, and the first isolate- FD, sustained a strong inhibition activity of 75% or higher after a prolonged incubation of six days; these were chosen for further examination *in planta*.

**Table 1.** The inhibitory effect of different isolates of *F. defendens* filtrates on *S. melliferum* growth.

|  |  |  |  |
| --- | --- | --- | --- |
| Isolate | Origin | % Inhibition after three days ($\pm $SD) | No. hours until inhibition decreases (Below 75% inhibition)  |
|  |
| FD | Fall 2013- Kidmat Tsvi region | 75$\pm $6.3% | 144 |  |
| KT14 | Fall 2020- Kidmat Tsvi region | 100$\pm $1% | 144 |  |
| KT15 | Fall 2020- Kidmat Tsvi region | 100$\pm $1.3% | >162  |  |
| KT16 | Fall 2020- Kidmat Tsvi region | 94$\pm $3.8% | 48 |  |
| KT17 | Fall 2020- Kidmat Tsvi region | 78$\pm $3.8% | 48 |  |
| KT24 | Fall 2020- Kidmat Tsvi region | 25$\pm $5.7% | 64 |  |
| KT25 | Fall 2020- Kidmat Tsvi region | 100$\pm $6% | 66 |  |
| KT26 | Fall 2020- Kidmat Tsvi region | 100$\pm $22% | 40 |  |
| KT27 | Fall 2020- Kidmat Tsvi region | 100$\pm $5.5% | 64 |  |
| KT28 | Fall 2020- Kidmat Tsvi region | 100$\pm $6.6% | 68 |  |
| KT29 | Fall 2020- Kidmat Tsvi region | 100$\pm $9.7% | 120 |  |
| KT30 | Fall 2020- Kidmat Tsvi region | 100$\pm $13% | 120 |  |
| LH44 | Fall 2020- Neve Ya'ar region | 100$\pm $19% | 56 |  |

Various techniques exist for employing endophytic bacteria as biocontrol agents, depending upon the plant type and the endophyte strain, including seed (Rana et al., 2019), soil (Munif et al.,2013) and foliage (Fu et al., 2020) application. When applied through leaf spraying, endophytic bacteria enter the phyllosphere via plant stomata or enzymatic breakdown of the plant wall. They form biofilms, creating a conducive microenvironment for endophyte colonization, and distribute by moving within the apoplast or vascular tubes (Liu et al., 2017; Pinski et al., 2019). While some endophytic bacteria remain near their entry point, others spread systemically throughout the plant (Pinski et al., 2019). FD possesses flagella, possibly facilitating its movement and settlement in plant tissues (Lidor et al., 2018). In the current study, out of the three isolates applied via foliar spraying, only FD penetrated and colonized the inner tissues of melons, as confirmed by PCR analysis (Table S1 show as part of results). Furthermore, FD could penetrate the melon's shoot but not the root and inhabit it for at least ten days without causing changes in the plant morphology (Table S2), conforming endophytic interaction.

These findings emphasize the importance of adopting a strain-specific approach in the application of biocontrol agents, as the functional properties of bacteria can vary significantly even within the same species (Medina et al., 2007). The observed isolate-specific differences in inhibitory activities and interactions with the plant host, align with characteristic differences of other bacterium strains. For example, Numerous studies have demonstrated strain-specific production of diverse secondary metabolites by *Bacillus subtilis* bacteria (Hashem et al., 2019; Bertuzzi et al., 2022; Islam et al., 2012). Different strains of *Streptomyces rimosus* exhibited distinct antibiotic production profiles (Park & Cooper, 2019). Furthermore, other endophytes show a strain-specific match between endophyte strains and the plant hosts; Different starins of the same endophyte species can exhibit differential abilities to interact with distinct plant genotypes, depending on their strain-specific capacities for chemotaxis, enzyme secretion, and biofilm formation (Rosenblueth & Martínez-Romero, 2006; Brader et al., 2014; Pinski et al., 2019; Lalzar et al , 2022).

***The presence of FD in melon sap and the effect on its inhibitory activity.***

A previous field trial has shown that FD foliage-spray application reduced *Phytoplasma* symptoms in the vineyard (Naor et al., 2019). The current study found that FD can reach the plant's sap when applied via foliar spraying on melons (Fig. 1) ~~but fails to spread from stem to root (Table 1S)~~. The sap contains two main types of fluids found in plant transport tissues: phloem and xylem. The composition of plant phloem includes various components such as sugars, organic compounds, hormones, and a wide variety of macromolecules, including RNA and proteins, which can act as mobile signals to regulate developmental processes and environmental responses (Serra-Soriano et al., 2015).



Fig 1: FD presence (live count) in melon-sap (lower panel) and effect on the sap inhibition activity against *S. melliferum in vitro* (upper panel) five- and ten-days post-application in FD-treated and control melons.

Fig. 1 demonstrates that the FD-treated sap contained 105 and 103 viable *F. defendence* five- and ten-days post-application, respectively. The sap of FD-treated plants five days post-application retained the capability to inhibit *S. melliferum* *in vitro,* while both the sap of control plants and the sap obtained from treated plants ten days post-application did not display any inhibitory effect. In addition, while in the artificial environment (S medium), FD cells secreted secondary metabolites, which caused a high inhibition level (75% - Table 1), under the plant sap environment, the inhibition level was relatively low (13%) but still significant (*P*<0.01) compared to untreated plants' sap. Two factors can explain the differences in inhibition levels between natural medium (sap) and artificial medium (S-growth medium):

**1**. Change in the composition or concentration of substances in the media: Although melon sap is rich in sugars (382 millimoles) (Ofosu-Anim & Yamaki, 1994), the quantity of sugars added to an artificial substrate is significantly higher (82×103 millimoles). Naama et al. (2022) proposed that elevated osmotic pressure is one of the mechanisms stimulating the secretion of inhibitory secondary metabolites by *F. defendens* (FD). As osmotic pressure increases, the bacterium secretes higher levels of these inhibitory substances, consequently suppressing the growth of *Spiroplasma*. **2**. Bacterial concentration within the system: The concentration of FD in the artificial substrate reaches ~107 cells/ml (Naama-Amar et al., 2022), whereas in the natural substrate, concentrations were ~105 and ~103 cells/ml five- and ten-days post-application, respectively. Concurrently, the inhibition levels exhibited a decreasing trend over this time period. This observation suggests a potential direct relationship between the bacterial population density and the secretion of inhibitory metabolites. The lower FD concentrations in the plant sap could account for the reduced inhibition levels observed in this natural environment compared to the artificial substrate system. Such a relationship between bacterial density and inhibitory metabolite production has also been documented in other bacterial species (Abbasiliasi et alt., 2017; Oliveira et al., 2017).



Fig 2:Bacterial community composition of melon plants five and ten days post-application in FD-treated and control melons: (A) Non-metric multidimensional scaling (NMDS) analysis based on a Bray-Curtis dissimilarity matrix between samples. (B) Relative abundance of specific genera significantly influenced by FD-treatment. Shapes and colors represent treatment and time points in both panels.

***The effect of FD on bacterial endophytic community composition***

The plant microbiome represents a diverse array of microorganisms interacting with and occupying various niches associated with the host plant (Müller et al., 2016). Introducing an exogenous endophyte like *F. defendens* can induce changes in the composition of the native endophytic bacterial communities of the host plant. For example, certain bacterial populations may become more dominant, while others may decline or even disappear, altering the relative abundances of different endophytic species (Yurgel et al., 2022; Ou et al., 2022). Therefore, the bacteriome of control and treated melon plants (5- and 10-days post-spraying with FD isolate) was examined by Next-Generation Sequencing (NGS). β-diversity analysis examined whether the FD spraying treatment, the sampling time, and the interaction between these two factors affected the composition of the microbial populations in the different samples. According to the results of the PERMANOVA (Permutational Multivariate Analysis of Variance) statistical test, both the FD spraying treatment and the sampling time significantly affected the composition of the microbial communities (*P*<0.05) : As a result, certain bacteria, primarily Gammaproteobacteria class, which includes diverse groups with different ecological roles, became more dominant within the system. Notably, Gammaproteobacteria and Betaproteobacteria are known to constitute a significant portion of the melon microbiome (Glassner et al., 2015; Saminathan et al., 2018).

Figure 2A illustrates the non-metric multidimensional scaling (NMDS) analysis of bacterial community composition in melon plants treated with the FD isolate compared to untreated control plants. The bacterial populations significantly affected by FD application are shown in Fig 2B. As anticipated, *F. defendens* was detected in treated melon plants but absent from untreated control plants. Interestingly, while the absolute concentration of *F. defendens* in plants decreased from five to ten days post-application (Fig. 1), its relative abundance within the endophytic bacterial community increased over this timeframe. A potential explanation is that the total population of endophytic bacteria declined in the plants, meaning that although *F. defendens* cell numbers dropped, its successful establishment and persistence allowed it to become more prevalent within the diminished endophytic community over time. Notably, not only the introduced FD isolate was detected in treated melons, but the genus *Herbaspirillum* was also exclusively found in these plants. *Herbaspirillum* was absent from control plants but became the dominant genus in treated plants 5 days after spraying, though its abundance declined by 10 days post-treatment. Thus, the relative abundance of this genus exhibited a time-dependent response to the FD treatment. *Herbaspirillum* species are known to enhance plant growth and productivity through mechanisms like nitrogen fixation (Alves et al., 2015). Hence, introducing FD to melons may foster cooperative interactions with growth-promoting endophytic bacteria in these plants. Moreover, the introduction of exogenous FD led to reductions in certain native bacterial species such as *Dechloromonas* and *Alcaligenes*. *Dechloromonas* displayed a consistent decline at both 5 and 10-day time points, While *Alcaligenes* decreased five days post-application, its levels returned to those of the control group by ten days, suggesting a re-establishment of equilibrium. This "return to equilibrium" trend is also reflected in the NMDS plot (Fig 2A), which shows the variation between treated and untreated populations was greater at five days than ten days post-treatment, though not statistically significant. Further investigation at additional time points is needed to determine if a new equilibrium state will be established or if the divergence between treated and untreated bacterial communities will diminish over time.

The current research explores the endophyte-plant relationship from an ecological perspective, assessing the impact of the endophyte *F. defendens* (FD isolate) on melon plants as hosts. The results suggest that this exogenous endophyte influences even healthy plants in various ways. This includes alterations to the biochemical composition of the plant sap as well as changes to the structure and dynamics of the native plant microbiome. The significant effects of both the treatment and the sampling time on β-diversity highlight the importance of considering both spatial (treatment) and temporal (time) factors when studying the dynamics of microbial communities in response to introducing a biocontrol agent like *F. defendens*. The current study supports the assumption of Yurgel et al., (2022) that since the initial introduction of exogenous endophytes may alter the structure and composition of plants’ microbiome, these introduced bacteria have the potential to enhance the collaboration among existing plant growth-promoting endophytes, leading to sustained beneficial effects for the host plant over an extended period.

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Top of Form

Bottom of Form

**Table S1.** Different media used in the current reaserch

|  |  |
| --- | --- |
| Components | Medium  |
| 1 g/L Fructose, 1 g/L Glucose, 2.5 g/L Heart Infusion Broth Powder (Sigma, USA), 20 g/L Lysogeny Broth Powder (LB Miller, Sigma, USA), 4.5 g/L NaCl, 70 g/L Sorbitol, 10 g/L Sucrose and after sterilization 100 ml/L Inactivated Horse Serum | S |
| 1 g/L Fructose, 1 g/L Glucose, 2.5 g/L Heart Infusion Broth Powder (Sigma, USA), 20 g/L Lysogeny Broth Powder (LB Miller, Sigma, USA), 4.5 g/L NaCl, 70 g/L Sorbitol, 10 g/L Sucrose, 5 ml/L Phenol Red 5%, and after sterilization 100 ml/L Inactivated Horse Serum, 2 ml/L Penicillin and 2.5 ml/L Thallium Acetate 5% |  Phenol red |
| 2 g/L Dipotassium phosphate, 0.6 g/L Monopotassium phosphate and 6 g/L Lysogeny Broth Powder. | LB |
| 66.66 g/L Sucrose, 10 g/L Sorbitol, 2 g/L Lysogeny Broth Powder and 8 g/L Agar Powder.  | CV |

**Table S2.** Presence and effect of FD on treated and control melon plants. (DPI=days post inoculation)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Control melon**5 DPI | **Control melon**10 DPI | **FD Spraying melon**5DPI | **FD Spraying melon**10DPI |
| Length SE [cm] | 10.1± 1.06 | 20±1.04 | 12.8 ±1.75 | 15.5 ±3.41 |
| Weight SE [gr] | 1.04±0.21 | 2.35±0.08 | 1.48±0.25 | 2.25±0.39 |
|  | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root |
| FD specific PCR | ND | ND | ND | ND | + | ND | + | ND |