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Research Article

# Plipastatin is a shared good by *Bacillus subtilis* during combating *Fusarium* spp.

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## Abstract

*Bacillus subtilis* a Gram-positive soil-dwelling bacterium known for its wide range of bioactive secondary metabolites. The lipopeptide plipastatin produced by most *B. subtilis* isolates have been shown to exhibit potent antifungal activity against plant pathogenic fungi. While the effect of these antifungal compounds are well studied in the context of biocontrol, much less is known of their role in the environment, which also harbor nonproducing strains of these compounds. *Fusarium* species produce multiple antibacterial compounds resulting in dysbiosis of the plant-associated microbiome and inhibition of plant beneficial bacteria like *B. subtilis*. While plipastatin is expected to be important for survival of *B. subtilis*, not all isolates carry the biosynthetic gene cluster for plipastatin suggesting that the protective effect of plipastatin might be shared. In this study, we investigated the protective effect of plipastatin against *Fusarium oxysporum* in a coculture using a producer and a nonproducer isolate of plipastatin. We tested the survival of single and cocultured strains under *Fusarium* challenge in liquid media and solid agar plates to dissect the influence of spatial structure. Our results highlights that plipastatin protects the nonproducer strain in a density-dependent manner.

**Keywords:** *Bacillus subtilis*; *Fusarium oxysporum*; plipastatin; public goods; secondary metabolite

## Introduction

Microbial interactions are the drivers of host health and disease, from individual microbe–microbe interactions to the whole network that make up the host microbiome. The many interactions that take place in the microbiome around and in a plant is one of the main factors contributing to host fitness (Mendes et al. 2013, Compant et al. 2019). Numerous bacteria have been shown to possess plant beneficial effects, by promotion of growth, nutrient utilization, or protection against plant pathogens either by induction of systemic resistance or direct inhibition of the plant disease-causing agent (Lugtenberg and Kamilova 2009, Raaijmakers et al. 2009, Blake et al. 2021).

*Fusarium oxysporum* is a filamentous fungus and causative agent of *Fusarium* wilt in several economically important crops including banana, tobacco, and tomato (Summerell 2019). The disease is cause of significant yield losses due to clogging the vascular vessels of the plant leading to wilting of the leaves and death of the plant. Among fungal plant pathogens, *F. oxysporum* is placed as the fifth most scientifically and economically important fungal pathogen due to the breadth of its host range and severe impact on several large sectors of the agricultural market (Dean et al. 2012). Historically, chemical fungicides have been employed in combating Fusarium wilt, which has led to widespread ecological consequences and risk of resistance developing in *F. oxysporum* (Bawa 2016, Hudson et al. 2021, El-Aswad et al. 2023) underlining the need for more environmentally friendly biocontrol agents.

*Bacillus subtilis* group species are known to produce a suite of bioactive secondary metabolites, including lipopeptides such as surfactin, fengycins, and iturin (Ongena and Jacques 2008), including plipastatin that belongs to fengycins (Honma et al. 2012, Gong et al. 2015), which together with surfactin elicits systemic resistance in plants (Ongena et al. 2007, Farace et al. 2015). Plipastatin variants exhibit species-specific inhibition of *Fusarium*, while some plipastatin was reported to only affect *F. graminearum* and not *F. oxysporum* (Zhao et al. 2014, Gong et al. 2015), others exhibit activity against both (Kiesewalter et al. 2021) making *Bacillus* a possible candidate for future biocontrol of the fungal pathogen. *Fusarium* produces several antibacterial compounds, which negatively affect the plant-associated microbial community (Sondergaard et al. 2016, Venkatesh and Keller 2019), leading to dysbiosis and death of plant beneficial bacteria underlining the importance of effective countermeasures. Therefore, it is also expected that production of *Fusarium*-inhibiting metabolites, like plipastatin, could benefit the bacterial community by combating the invading fungi.

Interestingly, plipastatin production capability is not present in all *B. subtilis* isolates, and even in the same soil samples some isolates display plipastatin production while others do not (Kiesewalter et al. 2021), which raises the question whether the protective effect of plipastatin is shared with the species and broadly the microbial community. While sharing of plipastatin could protect the community, it might also suffer from cheating by other nonproducing *Bacilli*, which could potentially out-compete the producer strain due to fitness costs leading to reduced level of producers and a loss of plipastatin protection in the community. The lack of plipastatin and fengycin production has been broadly proposed in wide variety of isolates of *B. subtilis* and *Bacillus velezensis*, respec-

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tively, as either frameshift mutations or partial deletion have been identified in the respective biosynthetic gene clusters of these secondary metabolites (Kiesewalter et al. 2021, Steinke et al. 2021).

In this study, we evaluate the role of plipastatin in a coculture system of two *B. subtilis* isolates when challenged by *F. oxysporum*. The two *B. subtilis* isolates originate from the same sample site, but one, MB9_B1, is a producer of plipastatin and thus able to inhibit *Fusarium* while the other isolate, MB9_B6, is unable to produce this secondary metabolite (Kiesewalter et al. 2021). In our coculture setup, we demonstrate that the plipastatin producer can provide protection to the nonproducer in a density-dependent manner.

## Materials and methods

### Strains, culturing, and genetic modification

Strains used in this study can be found in Table 1. *Bacillus subtilis* was routinely cultured in lysogeny broth (Lenox, Carl Roth) for overnight cultures while *F. oxysporum* was cultured in potato dextrose broth (PDB; Carl Roth). Strains were genetically engineered using the protocol below which is based on Kunst and Rapoport (1995).

Bacterial inoculants were prepared from 1 ml overnight cultures, which were spun down and resuspended in 100 µl Milli-Q (MQ) water. 10 µl of the inoculum was transferred into 2 ml of competence medium (80 mmol/l $K_2HPO_4$, 38.2 mmol/l $KH_2PO_4$, 20 g/l glucose, 3 mmol/l $Na_3$–citrate, 45 µmol/l ferric $NH_4$–citrate, 1 g/l casein hydrolysate, 2 g/l K-glutamate, and 0.335 µmol/l $MgSO_4 \cdot 7H_2O$) and incubated at 37°C with shaking for 3.5 h. Donor DNA was prepared from 1 ml of an overnight culture of the donor strain using Bacterial and Yeast Genomic DNA Purification Kit from EURx, yielding a typical DNA concentration in the range of 50–150 ng/µl. After the initial growth phase, 2 µl donor DNA was mixed with 400 µl competent cells and incubated further for 2 h before plating on selective agar plates which were incubated overnight at 37°C.

For interaction experiments on solid media, potato dextrose agar (PDA; Carl Roth) plates were prepared following the supplier's instruction and plates were dried in a laminar flow cabinet for 1 h to avoid excessive expansion of *B. subtilis* colonies.

### Population dynamics

Overnight cultures of MB9_B1 mKate2, MB9_B6 GFP, and MB9_B1 mKate2 Δ*ppsC* were adjusted to 0.1 at $OD_{600}$, and mixed in ratios of 100:1, 1:1, and 1:100 (MB9_B1/MB9_B1 Δ*ppsC* and MB9_B6, respectively), and used in the experimental setups outlined below.

Population dynamics in liquid culture: spores of *F. oxysporum* were harvested from a 5-day grown culture cultivated in 50 ml PDB with shaking at room temperature and filtered through miracloth to remove remaining mycelia fragments before washing in sterile MQ water. The spore solution was diluted 500× followed by 2-fold serial dilution in PDB in a 96-well microtiter plate and 11 µl of the adjusted single strains and mixes were subsequently added into the microtiter plate to a final volume of 111 µl.

Growth of the bacterial populations in the presence or absence of *Fusarium* spores were followed using a microplate reader (BioTek Synergy HTX Multi-mode Microplate Reader). The plate was incubated at 28°C with shaking and measurements of $OD_{600}$, green fluorescence for MB9_B6, and red fluorescence for MB9_B1 were taken every 15 min for 70 h (Optics position: Bottom, GFP: ex: 485/20 em: 528/20, Gain: 50, mKate2: Ex: 590/20, Em: 635/20, Gain 50).

Population dynamics on solid media: 5 µl of the single strains and mixed cocultures (100:1, 1:1, and 1:100) were spotted ∼3 cm from the center of PDA medium containing plates and allowed to dry before spotting of 5 µl of *F. oxysporum* spore suspension in the center of the plates. The samples were incubated at room temperature. Growth and inhibition patterns were imaged every hour for 7 days using a ReShape imaging system (ReShape Biotech, Denmark) and annotated for when *Fusarium* reached the edge and outer center of the bacterial colonies. For fluorescence imaging, long pass emission filters at 530 and 630 nm and excitation at 467/5 nm and 527/5 nm for green and red fluorescence, respectively. Fluorescence images of mono and co-cultures without *Fusarium* were processed using ImageJ, briefly, image stacks were converted to 8-bit and colony region of interest (ROI) were selected from the normal light image by autothresholding using Otsu's algorithm. Each fluorescence channel was measured for total area of the colony and the raw integrated density, which is a sum of pixel values in the selected area.

## Results

### Plipastatin is protective in a well-mixed liquid culture

The inhibitory effect of the *B. subtilis* and its secondary metabolite plipastatin on *Fusarium* is well documented using single bacterial cultures on agar medium (Kiesewalter et al. 2021, Kjeldgaard et al. 2022); however, its role within multistrain systems is less well understood. To dissect how plipastatin influences the dynamics between different *B. subtilis* strains, we established a two-species coculture system, in the same setup displaying inhibition of *Fusarium*, consisting of a plipastatin producer (MB9_B1) and a coisolated nonproducer isolate (MB9_B6) in the presence of germinating *F. oxysporum* spores. MB9_B1 and MB9_B6 have earlier been studied and shown to be close relatives (ANI of 99.95 for the four housekeeping genes *gyrA*, *recA*, *dnaJ*, and *rpoB*) and nonantagonistic toward each other, with MB9_B6 featuring a point-nonsense mutation in *ppsB* resulting in inability to produce plipastatin (Kiesewalter et al. 2021). As a control, a plipastatin deficient mutant of MB9_B1 (MB9_B1 Δ*ppsC*) was also cocultivated in the presence of MB9_B6 to detect the influence of any other genetic differences between the two isolates.

Our liquid culture approach assayed survival and growth of the mono- and co-cultures of MB9_B1 or MB9_B1 Δ*ppsC* and MB9_B6 against serial 2-fold dilutions of a freshly prepared *F. oxysporum* spore suspension (see Materials and Methods) ranging from 2-fold dilution to a 128-fold dilution. Coculture populations were further split into ratios of 100:1, 1:1, and 1:100 of MB9_B1 and MB9_B6, respectively, to assay possible density dependent protection.

In liquid media, the *Fusarium* challenge often resulted in a collapse of the bacterial culture inferred from the loss of fluorescence signal (Fig. 1, gray lines) or sharply downward trajectory before the end-point compared with the control (Fig. 1, orange lines). The isolate in lower concentration was undetectable from the background in 100:1 and 1:100 ratios, and these were excluded from further analysis (Figs S1 and S2).

All three monocultures exhibited similar population crashes at high *Fusarium* loads of 2-, 4-, and 8-fold (2- and 4-fold included in Figs S3–S5), but differentiated at the 16-fold dilution and lower spore concentrations (Fig 1, green lines). MB9_B1 populations survived with one-third populations at 16-fold, two-thirds at 32- and 64-fold, and all three-thirds at 128-fold (Fig. 1, left panel) whereas

**Table 1.** Strains used in the study.

| Strain | Characteristics | Reference |
|---|---|---|
| *B. subtilis* MB9_B1 | Natural isolate, plipastatin producer | Thérien et al. (2020) |
| *B. subtilis* MB9_B6 | Natural isolate, plipastatin nonproducer | Kiesewalter et al. (2021) |
| *B. subtilis* MB9_B1 mKate2 | MB9_B1 transformed with gDNA from TB501 *amyE*::$P_{hyperspank}$-*mKate2* (spec$^R$) (Dragoš et al. 2018) | This study |
| *B. subtilis* MB9_B1 mKate2 Δ*ppsC* | MB9_B1 mKate2 transformed with gDNA from DS4114 Δ*ppsC*::*tet*$^R$ (Müller et al. 2014) | This study |
| *B. subtilis* MB9_B6 GFP | MB9_B6 transformed with gDNA from TB500 *amyE*::$P_{hyperspank}$-*gfp* (spec$^R$) (Mhatre et al. 2017). | This study |
| *F. oxysporum* IBT 40 872 | From surface of an equipment in a Danish factory, 2005, by Anne Svendsen | IBT Culture Collection of Fungi, DTU |

*gfp*: *gfpmut2*, spec$^R$: spectinomycin resistance, and tet$^R$: tetracycline resistance.

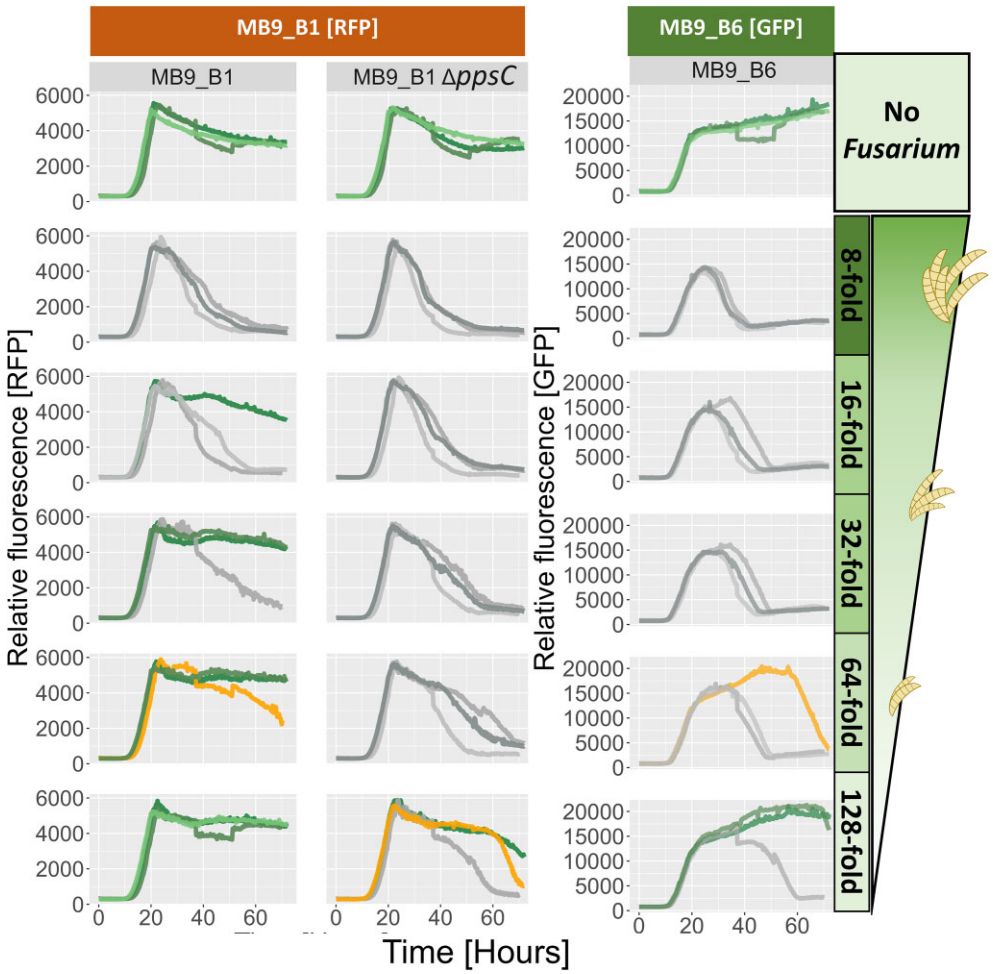


**Figure 1.** Survival of monocultures when challenged with different concentrations of *F. oxysporum* spores in liquid cultures at 28°C over the course of 70 h. MB9_B1 and Δ*ppsC* mutant was followed by red fluorescence while MB9_B6 was followed by green fluorescence. The bar to the right denotes the *Fusarium* concentration, with no-spores at the top, followed by the lowest spore dilution and increasing dilutions and thus lower concentration of spores toward the bottom. Surviving populations are colored in green, whereas populations that crashed judging by fluorescence signal are gray, and populations with sharp declines in the late stage are orange. Three replicates of each monoculture were followed.

MB9_B6 only featured two-third survivors at 128-fold (Fig. 1, right panel) and MB9_B1 Δ*ppsC* featured two sharply decreasing populations even at 128-fold dilution of *Fusarium* spores (Fig. 1, middle panel).

The differences in survival indicated certain degree of plipastatin-mediated protection against germinating *F. oxysporum* spores in our setup. Next, we determined whether this effect was shared or privatized by the producing strain MB9_B1.

Cocultures were generally less robust against *Fusarium* invasion, likely due to halving of the producer density. In the 1:1 mixes, MB9_B1 did not survive at the 16-fold dilution or higher spore concentrations, and featured one-sixth, three-sixth, and five-sixth survivors at 32-, 64-, and 128-fold, respectively (Fig. 2, left panel) while MB9_B1 Δ*ppsC* only showed one-third surviving populations at 128-fold spore dilution (Fig. 2, second panel from the left). Plipastatin producers were beneficial towards nonproducers,

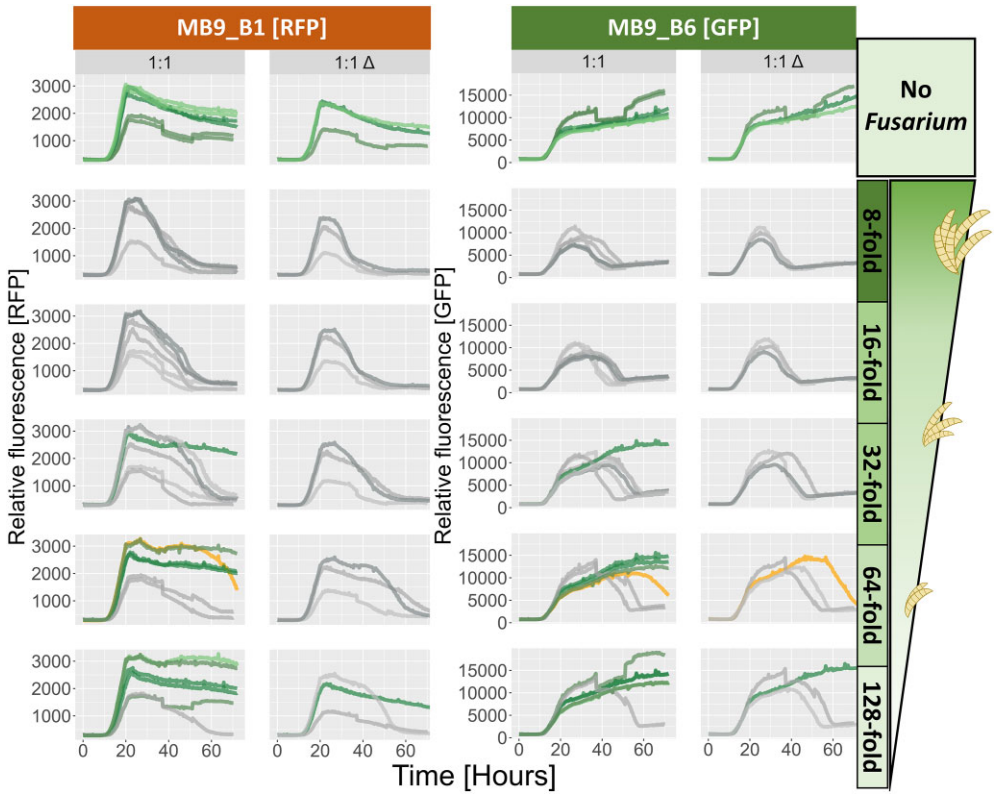


**Figure 2.** Survival of cocultures when challenged with different concentrations of *F. oxysporum* spores in liquid cultures at 28°C over the course of 70 h. Cocultures with wild-type MB9_B1 are marked 1:1, while cocultures using the nonproducer mutant MB9_B1 Δ*ppsC* are marked 1:1 Δ. MB9_B1 and Δ*ppsC* mutant in the coculture was followed by red fluorescence while MB9_B6 was followed by green fluorescence. The bar to the right denotes the *Fusarium* concentration, with no-spores at the top, followed by the lowest spore dilution and increasing dilutions and thus lower concentration of spores toward the bottom. Surviving populations are colored in green, whereas populations that crashed judging by fluorescence signal are gray, and populations with sharp declines in the late stage are orange. 1:1 culture was performed in six replicates while 1:1 Δ was performed in three replicates.

as MB9_B6 featured increased survival in the 1:1 coculture with MB9_B1 with one-sixth, three-sixth, and five-sixth surviving populations respectively at 32-, 64-, and 128-fold dilutions (Fig. 2, third panel from the left) versus one-third at the 128-fold dilution when MB9_B6 was cocultured 1:1 with MB9_B1 Δ*ppsC* (Fig. 2, right panel).

As cocultures proved somewhat beneficial to the nonproducer, we further inspected the growth of each species without *Fusarium* challenge to determine whether the nonproducer had a fitness benefit over the producer. Here, we noted only minor differences in growth characteristics of the monoculture with MB9_B6 featuring a slightly higher carrying capacity followed by MB9_B1 Δ*ppsC* and close after MB9_B1 (Fig. 3A) although these differences were not significant when comparing at 20 h (Fig. S6). Likewise in the 1:1 cocultures, only minor nonsignificant differences at 20 and 70 h (Fig. S7) were observed. However, MB9_B1 wild-type featured slightly higher fluorescence signal compared to the plipastatin nonproducing MB9_B1 Δ*ppsC* in coculture (Fig. 3B, middle panel), while MB9_B6 performed slightly better in coculture with the plipastatin nonproducer (Fig. 3B, right panel). Overall, we were unable to observe a significant fitness burden associated with plipastatin production and, on the contrary, MB9_B1 featured slightly higher growth compared to MB9_B1 Δ*ppsC* in coculture with MB9_B6.

## Protection by the shared plipastatin depends on a structured environment

In nature, bacteria are often found in spatially structured environments, including biofilms, which lacks the homogeneous milieu of a well-mixed liquid culture, which might favor cheaters due to constant mixing. Therefore, we next tested whether plipastatin was also protective toward a nonproducer on solid agar plates. On solid agar medium, bacterial colonies and *Fusarium* spores were spaced 3 cm apart and left at room temperature to grow, expand, and interact with each other and timed for when *Fusarium* hyphae reached the edge of the colony (Fig. 4A) and the colony center (Fig. 4B).

Generally, given enough time, *F. oxysporum* was able to overgrow *B. subtilis* colonies regardless of plipastatin production, but with considerable difference in the time points when hyphae reached the edge and center of the colony. For monoculture colonies, a clear inhibitory zone was observed for plipastatin producers with accompanying delay in *Fusarium* invasion when compared to nonproducers, taking 25 h longer before *Fusarium* reached the edge and 51 h before reaching the center of the colony (Fig. 4C) at which time nonproducers have already been fully overgrown by *Fusarium*. Representative images at days 5 and 7 and colony morphology of mono- and cocultures are displayed in Fig. S8.

Plipastatin production was beneficial in cocultures with invasion delay depending on the relative abundance of the producer strain, *Fusarium* reached the colony edge of the 1:100 culture first at 90 h, followed by 1:1 at 100 and finally the 100:1 colony at a 108 h delay (Fig. 5A). In all cases, the delay observed was smaller than that of the producer monoculture, which was likely due to the lower producer density compared with monoculture producer strain, MB9_B1. The importance of plipastatin was further underlined by cocultures replacing MB9_B1 with its plipastatin nonpro-

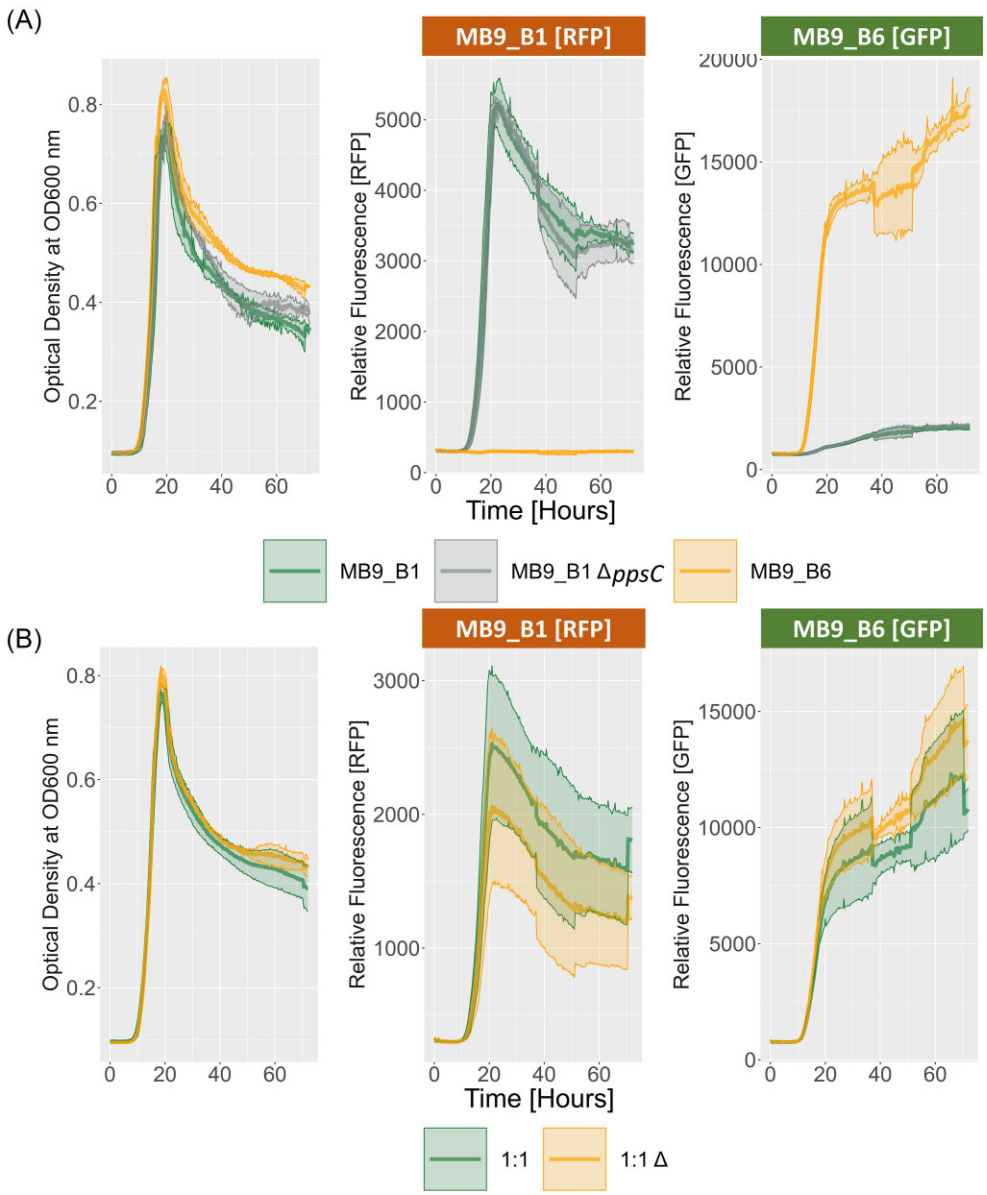


**Figure 3.** Growth of mono- and cocultures in the absence of *Fusarium* challenge, incubated at 28°C for 70 h and measured for optical density at 600 nm and fluorescence signal from RFP and GFP. (A) Monocultures of MB9_B1 (green), MB9_B1 Δ*ppsC* (gray), and MB9_B6 (orange), left panel showing $OD_{600}$, middle panel showing red fluorescence, and right panel showing green fluorescence. Line shows the mean of three replicates with ribbon denoting standard deviation. (B) Cocultures of MB9_B1 and MB9_B6 (green) and MB9_B1 Δ*ppsC* and MB9_B6 (orange). 1:1 culture was performed in six replicates while 1:1 Δ was performed in three replicates, line shows the mean of these replicates and ribbon represents the standard deviation.

ducer derivative, MB9_B1 Δ*ppsC*, in which no inhibitory zone or delay was observed for *F. oxysporum* (Fig. 5B). The time point when *F. oxysporum* reached the center of the bacterial colonies followed a similar trend as detected for the colony edge.

Fluorescence labeling of the strains allowed us to roughly estimate the abundance of producers and nonproducer populations in the coculture colonies. Using the summed pixel values of the colony adjusted by its area for both fluorophores, we could assay the differences between cocultures without *Fusarium* challenge at day 5 of the experiment as a control. We noted little difference between cocultures featuring MB9_B1 or MB9_B1 Δ*ppsC* for both red and green fluorescence, suggesting that plipastatin production had little effect on the composition of the cocultured colonies alone (Fig. 6).

## Discussion

The efficacy of biological control agents are highly dependent on the context of the environment as the resident microbiome members can interact with the biocontrol strain and its effector molecules. Here, we focused on the population dynamics of bioactive secondary metabolite sharing. Beneficial extracellular metabolite producing organisms are at risk of cheating, which could compromise the long-term survival of a producer strain. By cheating or exploitation, nonproducers might gain a fitness advantage over producers through saving metabolic resources and benefiting from the secreted compounds (so called public goods). Evolutionarily, the producer strain is out-competed, leading to the loss of the beneficial trait in the population (i.e. tragedy of commons). Therefore, producers must ensure cooperation or di-

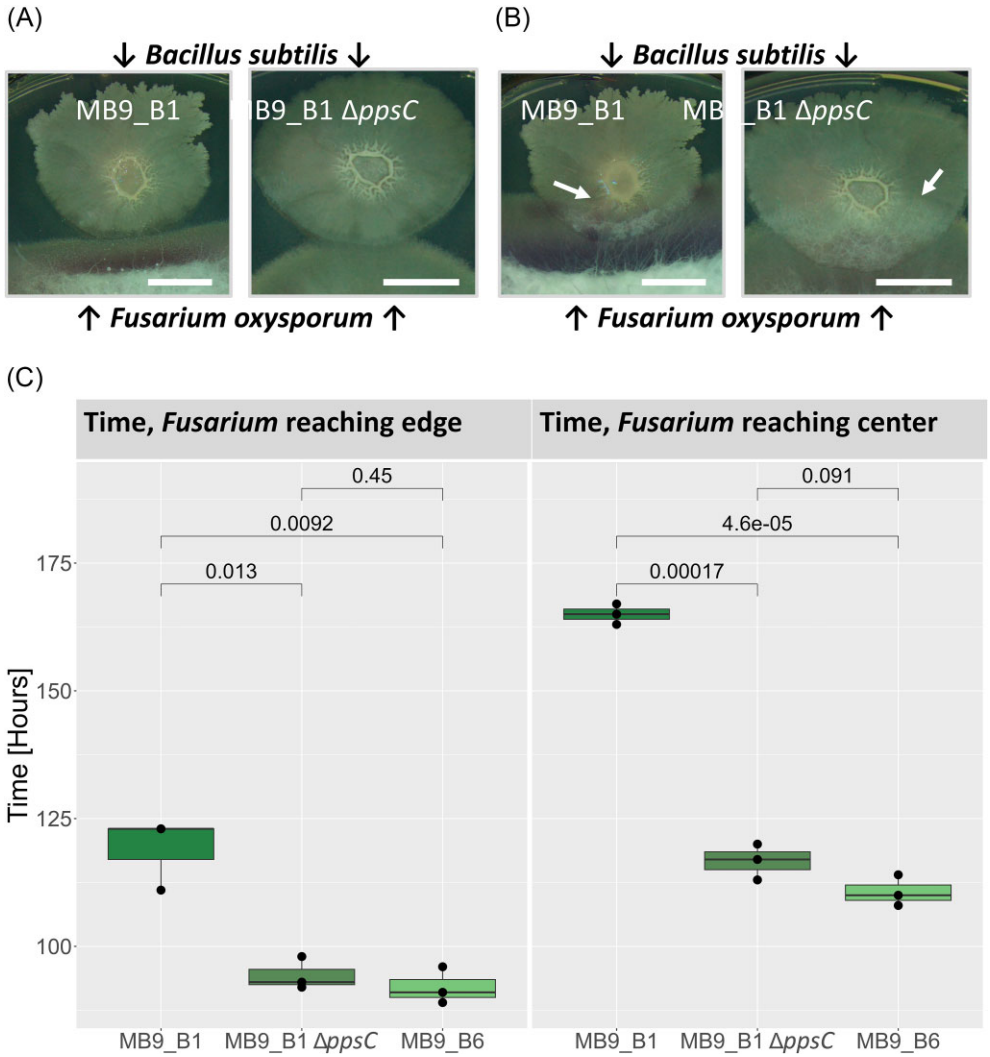


**Figure 4.** Interactions between *B. subtilis* monocultures and *Fusarium* on solid media PDA, grown for 7 days at room temperature. Representative images of when *F. oxysporum* was determined to meet *B. subtilis* colonies at the edge (A) and center (B) with white arrows pointing out the less visible *Fusarium* front when invading into the center. Scale bars indicate 1 cm. (C) Boxplot displaying the timing of *F. oxysporum* encountering edge (left panel) and center (right panel) of monoculture colonies of MB9_B1, MB9_B1 Δ*ppsC*, and MB9_B6 with three replicates of each.

rect beneficial behavior toward kins that are more likely to reciprocate. Understanding how producers of active metabolites such as plipastatin function in the context of other strains is immensely important for formulating effective biosolutions against plant pathogens like *F. oxysporum*.

Here, we describe that protection against *F. oxysporum* by plipastatin is shared among *B. subtilis* isolates with variable production of this antifungal secondary metabolite. Our experiments indicate that protection by plipastatin is dependent on the density of the *B. subtilis* producer strain and also influenced by the level of *Fusarium* spores. Under planktonic conditions, we observed either normal growth or collapse of the bacterial coculture containing the mixture of plipastatin producer and nonproducer strains. The fate of the untagged *Fusarium* strain is difficult to determine in the platereader setup. Therefore, it is possible that *Fusarium* might be present in the surviving *B. subtilis* cultures, thus additional culturing or inclusion of a tagged *Fusarium* strain could provide additional insights into the dynamics between *Bacillus* and *Fusarium* and the role of plipastatin. In the solid agar media setup, *Fusarium* and *B. subtilis* were spatially separated at the beginning and allowed to meet during growth. Here, presence of plipastatin delayed the fungal invasion, but since *Fusarium* was not completely eradicated, *B. subtilis* colonies were eventually invaded by the fungus regardless of plipastatin producer strain proportion. The mechanism by which *Fusarium* invades and inhibits *Bacillus* has not yet been elucidated, but determination of those could provide further context to the role of plipastatin, which in our setup and experimental conditions would seem more efficient as a preventative measure against *Fusarium* establishment rather than during direct competition.

In the planktonic cocultures, efficient inhibition of *Fusarium* protected the cohabiting nonproducing strain, which raises the question of whether the plipastatin producing strains have fitness a disadvantage compared with the cohabiting nonproducers. However, our results show that the plipastatin producer MB9_B1 featured similar growth compared with nonproducer mutant derivative when cocultured with MB9_B6 under the planktonic conditions, thus we conclude that plipastatin production does not carry a fitness cost in these conditions. Although not significant, MB9_B1 displayed a trend of increased growth compared to the nonproducer, which could implicate plipastatin in intraspecific competition or could otherwise be caused by epistatic effects

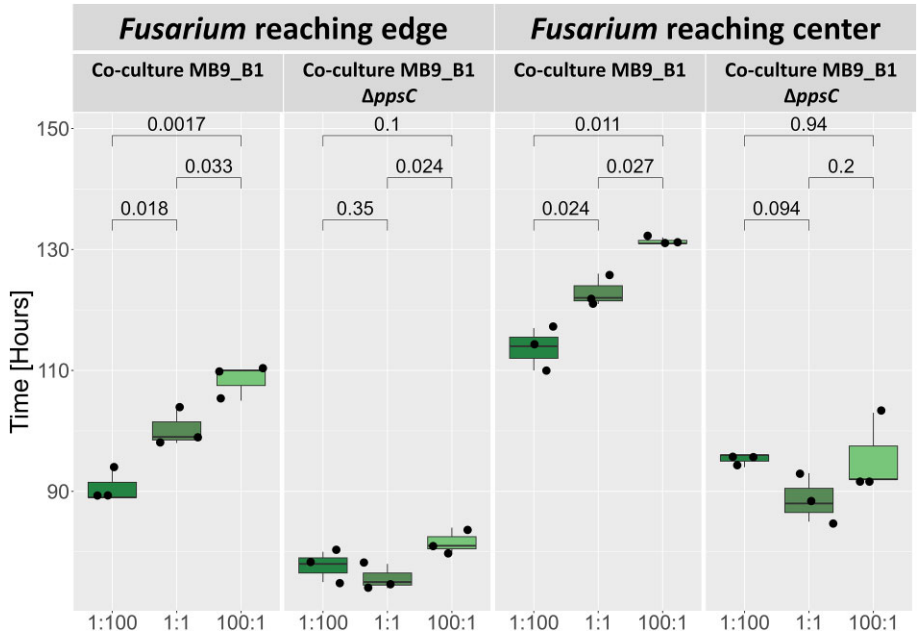


**Figure 5.** Interactions between *B. subtilis* cocultures and *Fusarium* on solid media PDA, grown for 7 days at room temperature. Boxplot showing the timing of *F. oxysporum* encountering edge (left top panel) and center (right top panel) of cocultures of MB9_B1 and MB9_B6 (left subpanels) and MB9_B1 Δ*ppsC* and MB9_B6 (right subpanels) at ratios of 1:100, 1:1, and 100:1 of MB9_B1 and MB9_B6, respectively with three replicates of each.

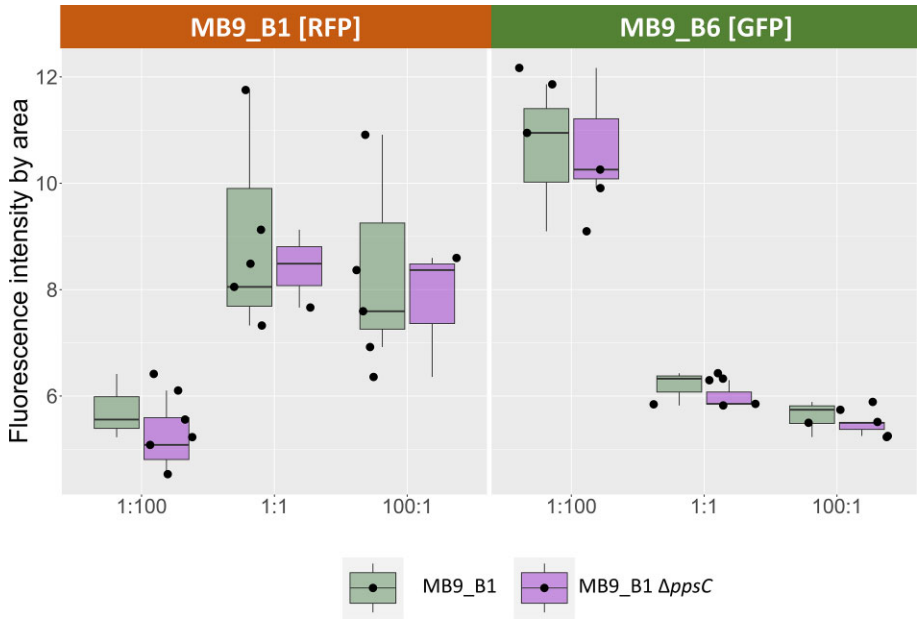


**Figure 6.** Summed fluorescence intensity divided by the area of colonies for each coculture without *Fusarium* challenge at day 5 with MB9_B6 at ratios of 1:100, 1:1, and 100:1 of MB9_B1 and MB9_B6, respectively. Red fluorescence of MB9_B1 shown in left panel and green fluorescence of MB9_B6 shown in right panel. Cocultures with MB9_B1 shown in light green and cocultures with MB9_B1 Δ*ppsC* shown in purple, boxplots are the result of three replicates.

of plipastatin deletion in the *B. subtilis* isolate. To exclude other factors between MB9_B1 and MB9_B6 that might have played a role in the lack of fitness difference, a coculture using MB9_B1 and MB9_B1 Δ*ppsC* should be tested in future studies with the addition CFU enumeration or flow cytometry to more accurately determine changes in the coculture population. Further, the timeframe of our experiments were all only one iteration lasting up to 3 days in liquid and 7 days on solid media, which might simply be too short period to detect potential fitness differences or changes in the population dynamics. Potentially, a successive experimental setup, in which the coculture is repeatedly transferred and challenged, could potentially allow to determine the evolutionary stability of plipastatin production in the mixture of plipastatin producer and nonproducer strains.

Additionally, other secondary metabolites might influence the stability of microbial consortia, offering another stabilizing mechanism, like division of labor, whereby each partner organism specialize in production of one or a subset of metabolites or ma-

trix components, which has been documented in *B. subtilis* in earlier literature (Gestel et al. 2015, Liu et al. 2015, Dragoš et al. 2018, Jautzus et al. 2022, Yannarell et al. 2023). Following expression or directly the concentration of plipastatin and other SMs in each isolate during coculture and when being challenged by *Fusarium* could shed light on potential task distribution, prudent metabolism, and subpopulations that the coculture might develop and determine whether the presence of *Fusarium* affects production of plipastatin. Regulation of the biosynthetic gene clusters in *B. subtilis* has been demonstrated to be influenced by intra- and interspecies signals (Dinesen et al. 2025, Lozano-Andrade et al. 2025).

While we have demonstrated that plipastatin protects kin nonproducers in liquid cultures and on solid agar medium, there are still many questions left unanswered regarding the stability of the behavior, plipastatin expression patterns in the producer population, and the role of other secondary metabolites in inhibiting *F. oxysporum*. However, we hope this study will aid in future understanding of how *B. subtilis* functions within a consortium.

## Author contributions

Rune Overlund Stannius (Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft), and Ákos T. Kovács (Conceptualization, Funding acquisition, Supervision, Writing – original draft)

## Supplementary data

Supplementary data is available at *FEMSEC Journal* online.

*Conflict of interest*: None declared.

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