Microbial basis of Fusarium wilt suppression by Allium cultivation

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Crop rotation and intercropping with Allium plants suppresses Fusarium wilt in various crops. However, the mechanisms underlying this phenomenon have not been fully elucidated. This study was designed to assess the role of microorganisms inhabiting Allium rhizospheres and antifungal compounds produced by Allium roots in Fusarium wilt suppression by Allium cultivation. Suppression of cucumber Fusarium wilt and the pathogen multiplication by Allium (Welsh onion and/or onion)-cultivated soils were eliminated by heat treatment at 60 °C, whereas those by Welsh onion-root extract were lost at 40 °C. The addition of antibacterial antibiotics eliminated the suppressive effect of Welsh onion-cultivated soil on pathogen multiplication, suggesting the contribution of antagonistic gram-negative bacteria to the soil suppressiveness. The Illumina MiSeq sequencing of 16S rRNA gene amplicons revealed that genus Flavobacterium was the predominant group that preferentially accumulated in Allium rhizospheres. Flavobacterium species recovered from the rhizosphere soils of these Allium plants suppressed Fusarium wilt on cucumber seedlings. Furthermore, confocal laser scanning microscopy revealed that Flavobacterium isolates inhibited the multiplication of the pathogen in soil. Taken together, we infer that the accumulation of antagonistic Flavobacterium species plays a key role in Fusarium wilt suppression by Allium cultivation.

Fusarium oxysporum has an extremely broad range of hosts and is one of the most devastating soil-borne pathogens, causing symptoms such as damping-off, root rot, and vascular wilt in crop plants12,14. It can saprophytically survive on soil and plant debris in the absence of a host5 and remain viable for a long time by producing chlamydospores, thereby making Fusarium wilt very difficult to control. Although effective control measures for Fusarium wilt include the use of resistant cultivars or rootstock14,15, this resistance is often overcome by new races of pathogens; moreover, the development of new resistant cultivars is time-consuming5. Another strategy for control includes the fumigation of soil using chemicals, such as chloropicrin2; however, this approach often negatively affects the environment as well as human health5.

The consecutive monocultures of agricultural crops lead to the accumulation of soil-borne fungal pathogens, including F. oxysporum5. Therefore, crop rotation and intercropping have received increasing attention in recent years because they have potential for managing soil-borne diseases10-13. In Japan and China, crop rotation and intercropping with Allium plants, such as Welsh onion (Allium fistulosum), onion (A. cepa), and Chinese chive (A. tuberosum), reportedly prevent the Fusarium wilt of bottle gourds (Lagenaria siceraria), spinach (Spinacia oleracea), tomato (Solanum lycopersicum), and banana (Musa spp.)10,11,14,15.

Two hypotheses that explain the mechanisms responsible for the suppression of Fusarium wilt by Allium cultivation have been proposed. The first hypothesis implicates the involvement of antimicrobial compounds released from roots of Allium plants16. However, there is limited evidence that the antimicrobial compounds released from the roots of Allium plants indeed reduce the incidence of Fusarium wilt. The second hypothesis, based on the known importance of the soil microbiome in the suppression of soil-borne diseases17,18, implicates microorganisms associated with Allium plants in the suppression of Fusarium wilt. However, although intercropping with Allium plants, such as onion and garlic (A. sativum), changes the bacterial diversity and structure of the soil19,20, no explicit evidence indicates that these changes play a role in the suppression of Fusarium wilt. Rhizosphere microbial communities are directly influenced by the root exudates of host plants and differ across plant species11,21. Therefore, we hypothesized that rhizospheres of Allium plants harbor unique microbial communities.

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and that some of the predominant microorganisms are involved in the suppression of Fusarium wilt induced by Allium cultivation.

In this study, we first investigated whether microorganisms inhabiting Allium rhizospheres and antifungal compounds produced by Allium roots contribute to the suppression of cucumber Fusarium wilt caused by F. oxysporum f. sp. cucumerinum (Focu) isolate GUS77, which was used as a representative pathogenic isolate of F. oxysporum. We further identified the predominant rhizobacterial groups of Allium plants by the Illumina MiSeq sequencing of 16S rRNA gene amplicons. The identified bacteria were then isolated and assessed for their ability to suppress cucumber Fusarium wilt by culture-dependent measures to elucidate the importance of the predominant rhizobacteria of Allium plants in Fusarium wilt suppression.

**Results**

**Fusarium wilt suppressiveness of plant-cultivated soils and of soil amended with Welsh onion root-extract.** The severity of cucumber Fusarium wilt was significantly reduced in Allium (Welsh onion and onion)-cultivated soils compared with non-cultivated soil and cucumber-cultivated soil (P < 0.01) (Fig. 1a), indicating that Allium cultivation conferred cucumber Fusarium wilt suppressiveness to soil. Similarly, cucumber Fusarium wilt severity in soil amended with an aqueous extract of Welsh onion roots was also significantly lower than that in unamended soil (P < 0.01) (Fig. 1b). The suppressive effect of Allium-cultivated soils on cucumber Fusarium wilt was not diminished after heat treatment at 40 °C. However, when Allium-cultivated soils were treated at 60 °C, 80 °C, or 121 °C, they lost their ability to suppress cucumber Fusarium wilt (Fig. 1a). In contrast, the suppressiveness of soil supplemented with Welsh onion root extract was reduced almost completely after heat treatment at 40 °C (Fig. 1b).

**Impact of heat treatment on culturable bacterial and fungal populations in Allium-cultivated soils.** The population densities of culturable gram-negative bacteria in non-heat-treated Welsh onion-cultivated soil and non-heat-treated onion-cultivated soil were 0.7–1.3 × 10^8 and 3.4–6.0 × 10^7 cfu/g dry soil, respectively (Table 1). Heat treatment at 40 °C did not influence the population densities of culturable gram-negative bacteria. However, the population densities of culturable gram-negative bacteria in Allium-cultivated soils treated at a temperature of 60 °C or higher were reduced more than 10-fold compared to the non-heat-treated cultivated soils. Similarly, the population densities of cultivable fungi in both cultivated soils decreased sharply when the soil was treated at a temperature ≥ 60 °C. In contrast, the population densities of cultivable gram-positive bacteria in both cultivated soils did not change after heat treatment at a temperature between 40 °C and 80 °C.
of soils was added into the liquid medium. cThe concentrations of Welsh onion- and cucumber-root extract cultivated soil was 1000-fold diluted with sterile distilled water, and then a 0.3 ml of each 1000-fold dilution chloramphenicol (300 µg ml⁻¹) were estimated using rose bengal-streptomycin agar. The experiment was repeated three times. ND: not detected.

Table 1. Effect of heat treatment on population densities of culturable bacteria and fungi in Allium plant-cultivated soils (cfu gram⁻¹ dry soil). The culturable bacterial densities were estimated using 1/10 strength tryptic soy agar, and Gram reaction was determined using the KOH method. The culturable fungal densities were estimated using rose bengal-streptomycin agar. The experiment was repeated three times. ND: not detected.

| Experiment | Type of amendment in the liquid medium¹ | Heat/Antibiotics treatment² | Focu density (log spores ml⁻¹)³ |
|------------|----------------------------------------|-----------------------------|---------------------------------|
| Control (SDW) | No treatment                                     |                              | 6.58 ± 0.03 c                  |
| Experiment 1 | Welsh onion-cultivated soil³                       | No treatment                             | 5.76 ± 0.10 a                  |
|              | Welsh onion-cultivated soil³                       | 40 °C                                    | 5.82 ± 0.09 a                  |
|              | Welsh onion-cultivated soil³                       | 60 °C                                    | 6.13 ± 0.02 b                  |
|              | Cucumber-cultivated soil³                        | No treatment                             | 6.11 ± 0.04 b                  |
|              | Non-cultivated soil³                             | No treatment                             | 6.21 ± 0.05 b                  |
|              | Control (SDW)                                     | No treatment                             | 6.58 ± 0.03 c                  |
| Experiment 2 | Welsh onion-cultivated soil³                       | No treatment                             | 5.78 ± 0.23 a                  |
|              | Welsh onion-cultivated soil³                       | Antibiotics⁴                             | 6.30 ± 0.08 b                  |
|              | Control (SDW)                                     | No treatment                             | 6.77 ± 0.09 b                  |
|              | Control (SDW)                                     | Antibiotics⁴                             | 6.63 ± 0.12 b                  |
| Experiment 3 | Welsh onion-root extract⁵                        | No treatment                             | 6.01 ± 0.37 a                  |
|              | Welsh onion-root extract⁵                        | 40 °C                                    | 6.78 ± 0.16 b                  |
|              | Welsh onion-root extract⁵                        | 60 °C                                    | 6.75 ± 0.16 b                  |
|              | Cucumber-root extract⁵                           | No treatment                             | 6.86 ± 0.09 b                  |
|              | Control (SDW)                                     | No treatment                             | 6.81 ± 0.03 b                  |

Table 2. Inhibitory effect of Welsh onion-cultivated soil and root extracts on multiplication of Fusarium oxysporum f. sp. cucumerinum in liquid medium. ¹Liquid medium: potato sucrose broth including the spores of Fusarium oxysporum f. sp. cucumerinum. ²Welsh onion-cultivated soil, cucumber-cultivated soil, or non-cultivated soil was 1000-fold diluted with sterile distilled water, and then a 0.3 ml of each 1000-fold dilution of soils was added into the liquid medium. ³The concentrations of Welsh onion- and cucumber-root extract were 50 mg root material per ml. A 1.5 ml of each root extract was added into the liquid medium. ⁴Antibiotics means a mixture of antibacterial antibiotics comprising ampicillin (300 µg ml⁻¹), imipenem (300 µg ml⁻¹), and chloramphenicol (300 µg ml⁻¹). ⁵Mean ± SD shown (n = 3). For each experiment, figures followed by different letters indicate significant differences (P < 0.01, Tukey’s test).

Inhibitory effect of plant-cultivated soils and root extracts on multiplication of Fusarium oxysporum. In Experiment 1, multiplication of FocuGFP-10, a green fluorescent protein (GFP)-tagged isolate of GUS77, in liquid medium was significantly inhibited by the supplementation of soil suspensions, regardless of soil type, when compared with the control (P < 0.01) (Table 2). When comparing Welsh onion-cultivated soil with the other two soils (i.e., non-cultivated soil and cucumber-cultivated soil), the inhibitory effect of the former was significantly higher than that of the latter two types of soil (P < 0.01). This inhibitory effect of Welsh onion-cultivated soil was not affected by heat treatment at 40 °C. However, when the suspension of Welsh onion-cultivated soil was treated at 60 °C, the inhibitory effect on FocuGFP-10 multiplication was diminished. Additionally, in Experiment 2, treatment with antibacterial antibiotics completely abolished the inhibitory effect of Welsh onion-cultivated soil. In Experiment 3, amendment with Welsh onion root extract also significantly inhibited FocuGFP-10 multiplication in liquid medium compared with the control (P < 0.01). In contrast, amendment with cucumber root extract did not affect FocuGFP-10 multiplication. The inhibitory effect of Welsh onion-root extract was abolished after heat treatment at both 40 °C and 60 °C.

Microbial community analysis based on 16S rRNA gene amplicons obtained by Illumina MiSeq sequencing. The sequencing resulted in a total of 1,466,567 raw reads detected from 12 soil DNA samples (See Supplementary Table S1). After merging forward and reverse reads using dada2, the number of merged reads
Inhibitory effect of Flavobacterium isolates on hyphal growth of Focu in soil. In autoclaved non-bacterized soils, FocuGFP-10 grew hyphae vigorously and the average total hyphal length/camera field reached 2015 μm (Fig. 4). In contrast, hyphal growth was significantly suppressed in soils treated with bacterial isolates, regardless of the bacterial genus ($P < 0.01$) (Fig. 4). However, when comparing Flavobacterium and...
**Chryseobacterium** treatments, the former displayed a significantly stronger inhibitory effect (with the average total hyphal length/camera field ranging from 312 to 411 μm) than the latter (mean = 1195 μm) (P < 0.01).

**Discussion**

Our study demonstrated that the cultivation of Welsh onion and onion conferred suppressiveness to soil with respect to Fusarium wilt in cucumber plants (Fig. 1a). This result indicates that **Allium** cultivation alters the microbial or chemical properties of soil in a manner that suppresses pathogenic **F. oxysporum**. Generally, the role of microorganisms in soil suppressiveness to soil-borne diseases has been made apparent by the fact that the disease suppressive effects of soils are lost upon pasteurization\(^\text{1-24}\). We found that the Fusarium wilt suppressiveness of **Allium**-cultivated soils disappeared after heat treatment at ≥ 60 °C (Fig. 1a). Similarly, the inhibitory effect of Welsh onion-cultivated soil on the multiplication of Focu in liquid medium was lost after heat treatment at 60 °C but not at 40 °C (Table 2). Heat treatment at 60 °C reduced the population density of culturable gram-negative bacteria and fungi in **Allium**-cultivated soils but not that of culturable gram-positive bacteria, whereas treatment...
at 40°C did not affect the populations of those microorganisms (Table 1). In addition, supplementation with antibacterial antibiotics abolished the inhibitory effect of Welsh onion-cultivated soil on Focu multiplication. Taken together, these results suggest that gram-negative antagonistic bacteria accumulated in Allium-cultivated soils may be a major factor in cucumber Fusarium wilt suppressiveness.

Cluster analysis based on the data from the Illumina MiSeq sequencing of 16S rRNA gene amplicons demonstrated that the bacterial community structures of Allium rhizosphere soils were similar to each other and different from those of cucumber rhizosphere soil and non-cultivated soil (Fig. 2). Interestingly, the gram-negative genus Flavobacterium was predominant in (relative abundance >1.0%) and characteristic of the bacterial communities of Welsh onion and onion rhizosphere soil (Table 3). Shen, et al.25 recently reported that Flavobacterium was one of the most abundant bacterial genera present in the soil of banana fields in which Fusarium wilt decline had been occurring. Therefore, we hypothesized that the accumulation of Flavobacterium species is a key component of Fusarium wilt suppressiveness of Allium-cultivated soils. Indeed, Flavobacterium isolates recovered from rhizospheres of Welsh onion and onion exhibited significant suppressive effects against Fusarium wilt on cucumber seedlings (Fig. 3). In addition, isolates having a strong disease-suppressing effect significantly inhibited the growth of Focu hyphae in soil (Fig. 4), suggesting that the accumulation of antagonistic Flavobacterium species in soil is an important mechanism of Fusarium wilt suppression by Allium cultivation. Although little is known about the suppressive ability of Flavobacterium species against soil-borne fungal pathogens, some species of Flavobacterium isolated from rhizosphere soil and soil have been reported to produce antimicrobial substances such as hydrogen cyanide, chitinase, and siderophore26–28. We are currently investigating the mode of action of our Flavobacterium isolates against Fusarium wilt pathogen.

Shen, et al.18 reported that bacterial diversity might be an important factor in soil suppressiveness because bacterial diversities were significantly higher in suppressive soils than in conducive soils. However, our data revealed that there were no significant differences in the α-diversity of bacterial communities (Shannon indices) from rhizospheres of Allium (i.e., Welsh onion and onion) and cucumber (See Supplementary Table S1), indicating that bacterial diversity is not a crucial factor in the suppression of Fusarium wilt by Allium cultivation.

Antifungal compounds released from Allium roots are recognized to play a role in Fusarium wilt suppression conferred by intercropping or rotation with Allium plants16,29. In accordance with these reports, the aqueous extract of Welsh onion roots significantly suppressed Focu multiplication in liquid medium and Fusarium wilt on cucumber seedlings (Fig. 1b and Table 2). These suppressive effects of Welsh onion root extract were almost completely lost after heat treatment at 40°C. These results demonstrated that the suppression of cucumber Fusarium wilt in sterilized soil by supplementation with Welsh onion root extract may be attributed to the direct inhibition of Focu multiplication by heat-sensitive compounds, possibly volatiles, such as 2-methyl-2-pentenal and organosulfur compounds (dimethyl trisulfide, dimethyl disulfide, dipropyl disulfide, and dipropyl trisulfide)16,26–28. Zhang, et al.39 suggested that these antifungal volatiles potentially play a role in the suppression of banana Fusarium wilt when Chinese chive is used as a rotating or intercropping plant. Similarly, Li, et al.12 found that suppression of Fusarium root rot of peanut by intercropping with a medicinal herb (Atractylodes lancea) was mainly due to antifungal volatiles released by the below-ground parts of A. lancea. However, given that the suppressive effects of Welsh onion and onion-cultivated soils were retained even after heat treatment at 40°C (Fig. 1a), antifungal volatiles in root exudate of Allium plants may be partially involved but are not a major factor responsible for the suppression of cucumber Fusarium wilt by Allium cultivation.

In conclusion, the findings of this study clearly demonstrate that the suppression of Fusarium wilt by Allium cultivation is mainly due to the accumulation of antagonistic Flavobacterium species. We believe that our results provide important insights into multitrophic interactions among plants, soil-borne pathogens, and natural antagonistic bacteria in crop rotation/intercropping systems. Moreover, we also believe that the elucidation of mechanisms underlying the recruitment and accumulation of antagonistic bacteria by Allium plants may lead to the development of novel eco-friendly Fusarium wilt management strategies.

Materials and Methods
Preparation of pathogen inoculum.  Fusarium oxysporum f. sp. cucumerinum (Focu) isolate GUS77 used in this study as the challenging pathogen was previously recovered from cucumber fields and was chosen based on its performance on aggressiveness tests. FocuGFP-10, a green fluorescent protein (GFP)-tagged isolate of GUS77, was used for fluorescent microscopy and confocal laser scanning microscopy. Full description is reported in the supplementary information.

Preparation of soils cultivated with Allium and cucumber plants.  In order to confirm whether Allium cultivation induces soil suppressiveness against pathogenic F. oxysporum, we evaluated soils cultivated with Allium plants or cucumber and a non-cultivated soil. For this, we prepared soils cultivated with Welsh onion, onion, and cucumber (Cucumis sativus). Full description is reported in the supplementary information.

Preparation of root extracts from Welsh onion and cucumber.  Root extracts of Welsh onion and cucumber were prepared. Full description is reported in the supplementary information.

Impact of heat treatment on Fusarium wilt suppressiveness by plant-cultivated soils and soil amended with Welsh onion root-extract.  Each type of plant-cultivated soil and soil not-cultivated root, prepared as described above, was mixed with double-autoclaved potting soil (Ikubyou-baido: Takii seed and seedling, Kyoto, Japan) and with double-autoclaved river sand in a ratio of 4:1:1 (w/w/w). Six grams of each type of soil mixture were then placed in flat-bottom glass tubes (3 cm in diameter and 12 cm high, Iwaki Glass, Chiba, Japan) and heat-treated in one of the following ways: 1) no treatment, 2) incubation in a water bath at 40°C for
Impact of heat treatment on culturable bacterial and fungal populations in *Allium*-cultivated soils. In parallel with the assessment of an effect of heat treatment on soil suppressiveness, we investigated possible changes in the bacterial and fungal populations in *Allium*-cultivated soil. Full description is reported in the supplementary information.

**Inhibitory effect of plant-cultivated soils and of the root extract on multiplication of *Fusarium oxysporum*.** To clarify whether the accumulation of antagonistic bacteria and/or antifungal compounds produced by *Allium* plants play a role in soil suppressiveness against cucumber *Fusarium* wilt, the following three experiments (Experiment 1–3) were performed. In Experiment 1, the inhibitory effect of Welsh onion-cultivated soil against *Focu* multiplication was compared with those of cucumber-cultivated soil and non-cultivated soil. Additionally, the impact of heat treatment on the inhibitory effect of Welsh onion-cultivated soil was also investigated. For heat treatment, 10 g of Welsh onion-cultivated soil, prepared as described before, were suspended in 90 ml of SDW in a 200-ml Erlenmeyer flask and treated at 40 °C and 60 °C, as described above. To evaluate the inhibitory effect of soils against *Focu* multiplication, a 0.3 ml of each 1000-fold dilution of soils was added into test tubes containing 0.2 ml of potato sucrose broth and 1.5 ml of SDW. Each tube was then inoculated with 1 ml of spore suspension of *FocuGFP-10* (3 × 10⁵ spores ml⁻¹) and shaken for 16 h at 25 °C on a rotary shaker at 150 rpm. After incubation, the concentration of spores was determined using a haemocytometer under a fluorescence microscope. The spores were counted in five fields of view per sample. There was one tube per treatment and the experiment was repeated three times.

In Experiment 2, the impact of an antibacterial treatment on the inhibitory effect of Welsh onion-cultivated soil was investigated. For the antibacterial treatment, a 100 µl of 100-fold diluted suspension of Welsh onion-cultivated soil was mixed with 900 µl of a mixture of antibacterial antibiotics comprising ampicillin (300 µg ml⁻¹), imipenem (300 µg ml⁻¹), and chloramphenicol (300 µg ml⁻¹), and then incubated for 1 h on a rotary shaker at 25 °C. The inhibitory effect of the soils against *Focu* multiplication was tested by the same procedures in Experiment 1. There was one tube per treatment and the experiment was repeated three times.

In Experiment 3, the inhibitory effect of Welsh onion-root extract against *Focu* multiplication was compared with that of cucumber-root extract. Simultaneously, the impact of heat treatment on the inhibitory effect of Welsh onion-root extract was also investigated. For heat treatment, Welsh onion-root extract was treated at 40 °C and 60 °C as described above. Either 1.5 ml of each root extract (Welsh onion or cucumber) or SDW was added to a test tube containing 1 ml of potato sucrose broth, and this was then inoculated with 500 µl of *Focu* suspension (6 × 10⁴ spores ml⁻¹) and shaken at 150 rpm for 30 h at 25 °C. After the incubation, the spores were counted using a haemocytometer. There was one tube per treatment and the experiment was repeated three times.

**Microbial community analysis based on 16S rRNA gene amplicons obtained by Illumina MiSeq sequencing.** This analysis was carried out through the Illumina MiSeq sequencing of 16S rRNA gene amplicons, using soil DNA extracted from non-cultivated soil and from rhizosphere soils of Welsh onion, onion, and cucumber plants. Non-cultivated soil was prepared as described before. Rhizosphere soils were collected from each of three plants of Welsh onion, onion, and cucumber, which were grown in vinyl pots containing field soil for 70 days as described before. The rhizosphere soils and non-cultivated soil were stored at −80 °C until DNA extraction. The DNA extraction was conducted using a FastDNA SPIN Kit (MP Biomedicals, CA, USA) following the manufacturer’s protocol with little modifications for the first step. Briefly, 0.4 g of either each rhizosphere soil or non-cultivated soil was added to lysing matrix tubes containing 878 µl of sodium phosphate buffer, 122 µl of MT buffer, and 100 µl of 20% skim milk solution. The following processes were conducted according to the manufacturer’s protocol. The DNA extraction was repeated three times. The V3–V4 region of each DNA sample was amplified with specific primers³³ and paired-end sequenced following the manufacturer’s protocol (https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) on an Illumina MiSeq (Illumina, CA, USA).

Sequence processing was conducted using QIIME2 (version 2018.2) with demux-summarize, dada2⁴⁰, and feature-table. A pre-trained Naive Bayes classifier based on the Greengenes 13.8 99% operational taxonomic units (OTUs) database (http://greengenes.secondgenome.com), which had been trimmed to include V3–V4
regions of 16S rRNA gene, bound by the 465 F/805 R primer pair, was applied to paired-end sequence reads to make taxonomy tables.

Community dissimilarity according to the Bray–Curtis index was calculated based on the OTUs data processed by coverage-based rarefaction using the pvclust package in R 3.3.1 software. Predominant bacterial groups of rhizosphere soils of Allium plants and cucumber, and non-cultivated soil, with relative abundance of more than 1.0% at the genus level were selected. Furthermore, rhizobacterial groups predominant only in Allium plants were selected as potential antagonistic bacteria. Rhizobacterial sequence data were deposited in the Sequence Read Archive database under accession numbers DRX121065–DRX121076 (BioProject: PRJDB6419).

Isolation of Flavobacterium species from Allium rhizosphere. It was postulated that Flavobacterium species were involved in Fusarium wilt suppression by Allium-cultivation. To test this hypothesis, Flavobacterium species were isolated from the rhizosphere soils of Welsh onion and onion. Both plants were grown in vinyl pots as described above. Rhizosphere soils of these plants were collected, diluted with SDW, and then spread on the surface of a semi-selective medium (namely PSR2A-C/T agar) for species of Flavobacterium and Chryseobacterium. Simultaneously, Chryseobacterium species were also recovered from Welsh onion rhizosphere soil. Flavobacterium and Chryseobacterium species belong to the same family, i.e., Flavobacteriaceae.

Suppressive effect of Flavobacterium isolates on cucumber Fusarium wilt. Flavobacterium isolates obtained from Allium rhizosphere were evaluated for their ability to suppress cucumber Fusarium wilt in the cucumber seedling assay. For comparison, suppressive effect of Chryseobacterium isolates obtained from Welsh onion were also tested (designated as "bacterized control"). Full description is reported in the supplementary information.

Inhibitory effect of Flavobacterium isolates on hyphal growth of Focu in soil. To determine whether the Flavobacterium isolates could antagonize Focu in soil, hyphal growth in soil bacterized with Flavobacterium isolates was examined by confocal laser scanning microscopy (CLSM) using FocuGFP-10 as the challenging pathogen. The soil was bacterized with Flavobacterium isolates GUAF6005 (from Welsh onion), GUAF6009 (from Welsh onion), or GUAC6072 (from onion), or with Chryseobacterium isolate GUAF6006 (from Welsh onion). The Flavobacterium isolates used here exhibited a strong suppressive effect against cucumber Fusarium wilt (RDI < 50%) in the cucumber seedling assay, whereas the GUAF6006 Chryseobacterium isolate slightly reduced cucumber Fusarium wilt severity (RDI = 88.9%). As illustrated in Supplementary Fig. S2, a chamber was filled with seven grams of the double-autoclaved soil mixture (field soil: river sand: potting soil 5:2:8). A small hole was drilled into one of the shorter sides of the chamber. Four milliliters of FocuGFP-10 spore suspension (1.8 × 10^5 spores ml^-1) was inoculated into the soil mixture. Subsequently, a 3-ml aliquot of each bacterial isolate (ca. 0.5–1.0 × 10^5 cells ml^-1) or SDW was separately applied to the FocuGFP-10-inoculated soil mixtures. Surface-sterilized and germinated cucumber seeds were then planted into the holes (one seed per chamber), and soil surfaces were covered with a small amount of sterile vermiculite. The sides of the coverglasses were covered with aluminum foil and the chambers were incubated in a controlled environmental chamber (25 °C, 12 h of daylight) for 1 week. After incubation, images of FocuGFP-10 hyphae in the soil mixtures were captured at three different locations by CLSM (LSM710, Carl Zeiss, Jena, Germany). The mean of total length of FocuGFP-10 hyphae in each camera field-of-view was then measured using LIA32 software (https://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/). There were three replicates for each treatment.

Statistical analyses. Statistical analyses were performed using BellCurve for Excel (version 2.13) (Social Survey Research Information, Tokyo, Japan). Full description is reported in the supplementary information.

Data Availability
The datasets supporting the conclusions of this study are included within this manuscript and its supplementary files.

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Author Contributions
T.N., H.S. and M.S. designed the study. T.N. and M.M. contributed to experiments. T.N., I.K., Y.K. and H.T. contributed to data analysis. T.N. and M.S. wrote the manuscript and all authors reviewed the manuscript.

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