Fusaric Acid-Producing Strains of *Fusarium oxysporum* Alter 2,4-Diacetylphloroglucinol Biosynthetic Gene Expression in *Pseudomonas fluorescens* CHA0 In Vitro and in the Rhizosphere of Wheat

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The phytotoxic pathogenicity factor fusaric acid (FA) represses the production of 2,4-diacetylphloroglucinol (DAPG), a key factor in the antimicrobial activity of the biocontrol strain *Pseudomonas fluorescens* CHA0. FA production by 12 *Fusarium oxysporum* strains varied substantially. We measured the effect of FA production on expression of the *phlABCD* biosynthetic operon of strain CHA0 in culture media and in the wheat rhizosphere by using a translational *phla*-lacZ fusion. Only FA-producing *F. oxysporum* strains could suppress DAPG production in strain CHA0, and the FA concentration was strongly correlated with the degree of *phlA* repression. The repressing effect of FA on *phla*-lacZ expression was abolished in a mutant that lacked the DAPG pathway-specific repressor PhlF. One FA-producing strain (798) and one nonproducing strain (242) of *F. oxysporum* were tested for their influence on *phlA* expression in CHA0 in the rhizosphere of wheat in a gnotobiotic system containing a sand and clay mineral-based artificial soil. *F. oxysporum* strain 798 (FA−) repressed *phlA* expression in CHA0 significantly, whereas strain 242 (FA+) did not. In the *phlF* mutant CHA638, *phlA* expression was not altered by the presence of either *F. oxysporum* strain 242 or 798. *phlA* expression levels were seven to eight times higher in strain CHA638 than in the wild-type CHA0, indicating that PhlF limits *phlA* expression in the wheat rhizosphere.

Antibiosis is an important mechanism used by plant-beneficial microorganisms to overcome the effects of soil-borne fungal pathogens (44). The polyketide metabolite 2,4-diacetylphloroglucinol (DAPG) is one of the most effective antimicrobial metabolites produced by strains of fluorescent pseudomonads (25, 40) and is effective against bacteria, fungi, and helminths (13, 18, 23, 30, 34, 39). In *Pseudomonas fluorescens* Q2-87 and CHA0, the four DAPG biosynthesis genes, *phlABCD*, are organized as an operon and are indispensable for the production of DAPG as well as monoacetylphloroglucinol (MAPG), which is either a precursor to or a degradation product of DAPG. Bacterial metabolites produced by strains of fluorescent pseudomonads are organized as an operon and are indispensable for the promotion of DAPG in that its synthesis is autoinduced and repressed by other bacterial extracellular metabolites of strain CHA0 (38).

The fungal toxin fusaric acid (FA) is also a potent inhibitor of DAPG synthesis in *P. fluorescens* CHA0 (14, 38). FA was first isolated by Yabuta et al. (47) from *Fusarium heterosporum* Nees as a compound that inhibited the growth of rice seedlings. FA is produced by many *Fusarium* spp. and is toxic to various plants, fungi, and bacteria (1, 8, 19, 20, 22, 31). Synergistic interactions of FA with other naturally co-occurring mycotoxins can cause animal toxicity (3, 12, 41, 46).

In the present study, we screened 12 *Fusarium oxysporum* strains for FA production. Our objective was to determine whether the ability of these fungi to produce FA in culture media and on wheat roots correlates with the suppression of DAPG production in CHA0. The *phlF* mutant CHA638, lacking the DAPG pathway-specific repressor PhlF, was used to investigate the mode of action of the microbial interaction.

**MATERIALS AND METHODS**

Microorganisms, plants, and culture conditions. *P. fluorescens* CHA0 is a DAPG-producing strain isolated from the rhizosphere of tobacco grown in a Swiss soil naturally suppressive to *Thielaviopsis basicola* (43). The *phlF* mutant CHA638 was constructed by gene replacement (38). Plasmid pME6259 is a derivative of pME6010 and contains a *phlA*-lacZ translational fusion and a
tetrasycline resistance gene (38). pME6010 is present at approximately six copies per cell. pME6010 is maintained stably in P. fluorescens in the absence of antibiotic selection (21). Earlier work has shown that retention of the reporter plasmid pME6259 by strain CHA0 is 98.8 ± 0.9% after 20 generations of growth in Luria-Bertani (LB) (36) broth (33). All strains were routinely cultivated at 27°C on King’s B agar (KB) (26) and in LB with the addition of tetracycline (125 µg/ml) as required, unless otherwise mentioned.

F. oxysporum strains 233, 234, 235, 236, 237, 238, 240, 241, and 410 were isolated from wheat roots (Triticum aestivum cv. Arina) by the Swiss Federal Research Station for Plant Production, Changins, Switzerland. Strains 798 and 801 (obtained from Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands) were isolated from soil cultivated with wheat in Grignon, France. All F. oxysporum strains were routinely cultured on 1.5% malt agar plates (Difco, Detroit, Mich.) at 24°C.

Seeds of winter wheat (T. aestivum cv. Arina) were surface disinfested for 15 min in 7% sodium hypochlorite (vol/vol), followed by 10 min in 10% H2O2 (vol/vol), and then thoroughly rinsed with sterile double-distilled water. Seeds were pregerminated for 3 days on 0.85% water agar at 24°C in darkness.

Production of FA by F. oxysporum strains. Malt broth (2%), Czapex Dox medium (Oxoid, Hampshire, United Kingdom), and R2 broth (7%) amended or not with 89 mg of ZnSO4·7H2O (Merck, Darmstadt, Germany) (250 ml in 500-ml baffled flasks) per liter were inoculated with one 7-mm agar plug taken from 5- to 7-day-old malt agar cultures. Cultures were incubated for 7 days at 24°C on a rotary shaker (180 rpm). Fungal biomass (mycelia and microconidia) was collected by centrifugation (2,200 g) for 15 min. One part of the supernatant was filtered through a 0.8-µm membrane (Nalgene, Rochester, N.Y.) and stored at 2°C. In addition, 50 ml of the supernatant was acidified to pH 2 with approxi- mately 400 µl of 2 M HCl, mixed with 50 ml of ethylacetate, and shaken vigorously for 1 min. The organic phase was separated from the aqueous phase by filtering through a silicone-coated filter paper (catalog no. 484015; Macherey and Nagel, Duren, Germany) and brought to dryness in vacuo. The residue was dissolved in 1 ml of methanol and analyzed by high-performance liquid chromato- graphy (HPLC) using a Hewlett Packard 1090 liquid chromatograph (Hewlett Packard Co., Palo Alto, Calif.) equipped with a reverse-phase column (4 by 125 mm) packed with Nucleosil 120-5-C18 (Macherey-Nagel, Oensingen, Switzerland) and set at 50°C. The samples (10 µl) eluted with a linear methanol gradient from 25 to 100% in 0.43% phosphoric acid over 12 min. FA was detected by monitoring A270. The retention time was approximately 2 min, with a mobile-phase flow rate of 0.7 ml/min. The samples were quantified against a standard curve of synthetic FA (Acros Organics, Geel, Belgium). The fraction containing the FA standard from the HPLC was collected, and the presence of FA was qualitatively and quantitatively confirmed by mass spectrometry (MS) on a TSQ8000 triple quadrupole mass spectrometer with electrospray ionization (Thermo Finnigan, Bremen, Germany). The experiment was carried out twice with similar results. Data from all trials were analyzed for a trial-by-treatment interaction with analysis of variance using Systat version 9.0 (Systat Inc., Evanston, Ill.) to analyze trial-by-treatment interaction. Data could not be pooled. One and two individual trials are presented separately in Table 3. Means of four replicate flasks were separated using Fisher’s protected (P = 0.05) least significant difference test.

β-Galactosidase assays and plasmid stability. β-Galactosidase assays were conducted by the method of Miller (32). Activities were expressed as units per 106 CFU. Unit values were calculated by the method of Miller (32), following the formula 1,000 × [OD420/time [minutes] × sample volume [milliliters]] = units of β-galactosidase. The stability of plasmid pME6259 was evaluated by replica plating onto KB agar supplemented with tetracycline (125 µg/ml). Plates were incubated at 27°C for 48 h, and the percentage of tetracycline-resistant colonies, indicative of the presence of pME6259, was determined.

RESULTS

Production of FA from 12 strains of F. oxysporum in four different media. We extracted FA from F. oxysporum cultures grown in four different media (malt broth, Czapex Dox medium, and R2 broth with and without zinc). The FA detection limit was 0.2 nmol per ml of medium. F. oxysporum strains 235, 240, and 242 did not produce detectable amounts of FA in any of the four media (data not shown). The other nine strains did produce FA in at least one of the media tested (Table 1). In malt liquid medium, only strain 241 produced significant amounts of FA in both experiments (Table 1). Strains 238, 798, and 801 produced small amounts in only one of the two experiments. In Czapex Dox broth, strains 233, 234, 236, 237, 238, 241, and 410 produced small but detectable amounts of FA. Strains 801 and 798 produced large amounts of FA in this medium, ranging between 0.8 and 3.5 mM. In the saccharose- based medium R2, strain 241 produced large amounts of FA, reaching a concentration up to 550 µM. In this medium, FA production by strains 233, 798, and 801 was also detected (Table 1). The addition of zinc at a concentration of 310 µM to R2 medium abolished production of FA in all strains tested (data not shown).
Repression of a phlA-lacZ fusion in P. fluorescens CHA0 in vitro depends on FA concentration. The bacteria were grown in the presence of 100, 300, 500, and 750 μM FA under the same conditions (e.g., temperature, medium, inoculation, and rpm) as in the experiments with the culture filtrates of F. oxysporum (Table 2). The plasmid-borne translational phlA-lacZ gene fusion was expressed from the mid-exponential to the early stationary growth phase in the absence of added FA (Fig. 1). β-Galactosidase activities at mid-exponential growth (OD_{600} of 0.638) were approximately 120 U per 10^8 CFU and (strain 241) or undetectable (strains 240, 242, and 410) amounts of FA in Czapek Dox medium after 7 days of growth (Table 2). FA concentrations of 500 and 750 μM blocked expression of the phlA-lacZ fusion almost completely (Fig. 1). The addition of FA did not affect bacterial growth (data not shown).

**Table 1. Production of the phytotoxin FA by different strains of F. oxysporum**

| FA production (nmol/m of medium) | Malt | Czapek Dox | R2 without zinc |
|----------------------------------|------|------------|----------------|
|                                  | Expt 1 | Expt 2 | Expt 1 | Expt 2 | Expt 1 | Expt 2 |
| 233                              | 6.8 a   | 5.7 a | 104.6 a |
| 234                              | 0.8 a   | 0.2 a |
| 236                              | 5.9 a   | 0.2 a |
| 237                              | 0.9 a   | 0.2 a |
| 238                              | 0.5 a   | 0.2 a |
| 241                              | 114.7 a | 550.0 b | 308.3 b |
| 410                              | 1.6 a   | 0.2 a |
| 798                              | 3.424 b | 0.9 a | 1.4 a |
| 801                              | 1.9623  | 2.3 a | 7.7 a |

a The F. oxysporum strains were cultivated in 250 ml of liquid malt, Czapek Dox, or R2 without zinc for 7 days at 24°C and 180 rpm. The detection limit for production of FA was 0.2 nmol/ml. Values have been adjusted to reflect the 25% efficiency of extraction. Each value is the mean of three replicate cultures. Means for the same experiment within the same column followed by the same letter were not significantly different at P ≤ 0.05 according to Fisher’s protected least significant difference test.

![Figure 1](image)

**Table 2. Influence of F. oxysporum culture filtrates on phlA expression in P. fluorescens CHA0 and CHA638**

| P. fluorescens strain | F. oxysporum strain | FA concn in bacterial growth medium (μM) | β-Galactosidase activity (U per 10^8 CFU) | OD_{600} = 1.5 | OD_{600} = 2.5 |
|------------------------|---------------------|----------------------------------------|------------------------------------------|----------------|----------------|
| CHA0                   | None                | 0                                       | 1,510 a                                  | 2,100 a         |                |
| 240                    | 0                   | 1,520 a                                 | 2,000 a                                  |                |
| 241                    | 25                  | 1,480 a                                 | 1,910 a                                  |                |
| 242                    | 0                   | 1,460 a                                 | 1,950 a                                  |                |
| 410                    | 0                   | 1,640 a                                 | 1,920 a                                  |                |
| 798                    | 700                 | 220 b                                   | 590 b                                    |                |
| 801                    | 160                 | 200 b                                   | 1,520 c                                  |                |
| CHA638                 | None                | 0                                       | 1,850 a                                  | 1,720 a         |                |
| 240                    | 0                   | 1,660 a                                 | 1,390 a                                  |                |
| 241                    | 25                  | 1,590 a                                 | 1,590 a                                  |                |
| 242                    | 0                   | 1,540 a                                 | 1,560 a                                  |                |
| 410                    | 0                   | 1,740 a                                 | 1,680 a                                  |                |
| 798                    | 700                 | 1,660 a                                 | 1,490 a                                  |                |
| 801                    | 160                 | 1,680 a                                 | 1,470 a                                  |                |

a P. fluorescens CHA0 (wild type) and CHA638 (phlF mutant) carrying a translational phlA-lacZ fusion on plasmid pME6259 were tested. The F. oxysporum strains were cultivated in Czapek Dox medium for 7 days at 24°C and 180 rpm. Filtrates from F. oxysporum cultures were added to bacterial growth medium OSG at a ratio of 20% (vol/vol). The FA content of the culture filtrates was determined, and the final FA concentration in the bacterial growth medium is indicated. β-Galactosidase activities were determined in bacterial cultures grown to an OD_{600} of 1.5 ± 0.1 or 2.5 ± 0.1, as indicated. Data represent means of three replicate cultures. Means for the same P. fluorescens strain within a column followed by a different letter are significantly different at P ≤ 0.05 according to Fisher’s protected least significant difference test.
pME6259 at both cell densities reported (OD<sub>600</sub> of 1.5 and 2.5) compared to those of the control (Table 2). Addition of culture filtrates of strains 798 and 801 repressed <i>phlA</i> expression to different extents. At an OD<sub>600</sub> of 1.5, the <i>β</i>-galactosidase activities of strain CHA0/pME6259 grown in the presence of filtrate from strain 798 or 801 were dramatically repressed and did not exceed 220 U per 10<sup>8</sup> CFU, which represents a sevenfold <i>phlA</i> repression. At an OD<sub>600</sub> of 2.5, the <i>β</i>-galactosidase activity measured in CHA0/pME6259 grown with 801 filtrate was still significantly lower than that of the control, but repression was only 30%, whereas repression in the presence of 798 filtrate was 70% (Table 2). The repressing effects of culture filtrates 798 and 801 on the <i>phlA</i>-<i>lacZ</i> fusion were abolished when the fusion was expressed in the <i>phlF</i> mutant CHA638 (Table 2).

In a follow-up experiment, we tested the effect of culture filtrates of <i>F. oxysporum</i> strain 801 with different FA contents on <i>phlA</i> expression in <i>P. fluorescens</i> CHA0. Filtrates from cultures grown in Czapek Dox (Table 1) were used to obtain final FA concentrations of 400 and 160 μM in the bacterial growth medium. Average <i>β</i>-galactosidase activity in <i>P. fluorescens</i> CHA0/pME6259 remained below 680 U per 10<sup>8</sup> CFU in medium amended with culture filtrate of experiment 1 (400 μM) (Fig. 2). <i>phlA</i> expression in medium with filtrate from experiment 2 (160 μM) was repressed to a smaller extent; the maximal <i>β</i>-galactosidase activity in this treatment was 1,550 U per 10<sup>8</sup> CFU at an OD<sub>600</sub> of 2.7, whereas that of the control was >2,000 U per 10<sup>8</sup> CFU.

**Expression of a phlA<sup>-</sup>-lacZ fusion in strains CHA0 and CHA638 in the rhizosphere of wheat in the presence of two different F. oxysporum strains.** After 2 weeks on wheat roots, retention of plasmid pME6259 was 97.3 ± 2.1% in wild-type strain CHA0 and 65.8 ± 4.3% in <i>phlF</i> mutant CHA638, based on the viable bacteria reisolated from the rhizosphere that grew on tetracycline-amended agar. <i>β</i>-Galactosidase activities were calculated per CFU still carrying reporter plasmid pME6259. Plant fresh weight did not differ significantly between the treatments in both experiments shown in Table 3 and ranged between 2.6 and 3.1 g in experiment 1 and between 2.3 and 2.7 g in experiment 2. No visible lesions were detected on the wheat plants, and no <i>F. oxysporum</i> could be reisolated on Komada's medium from the inside of wheat roots, stems, or leaves, indicating the absence of an endophytic or pathogenic interaction (data not shown). Colonization of the rhizosphere with <i>F. oxysporum</i> was around 1.9 × 10<sup>7</sup> CFU per g of rhizosphere and did not differ significantly between the experiments (data not shown). No statistical differences could be found for bacterial rhizosphere colonization between the treatments in the two experiments (Table 3). Rhizosphere colonization was estimated at from 0.9 × 10<sup>7</sup> to 2.7 × 10<sup>7</sup> CFU per g of rhizosphere in experiment 1 and ranged between 0.8 × 10<sup>7</sup> and 1.5 × 10<sup>7</sup> CFU per g of rhizosphere in experiment 2. No <i>P. fluorescens</i> cells were detected in the treatments without bacteria added (Table 3).

In both experiments, <i>β</i>-galactosidase activity measured in strain CHA0 was lowered significantly in the treatment with <i>F. oxysporum</i> strain 798, i.e., to 60% of that of the control treat-

**TABLE 3. Expression of a phlA<sup>-</sup>-lacZ fusion in P. fluorescens strains CHA0 and CHA638 in the wheat rhizosphere with and without F. oxysporum strain 242 or 798<sup>a</sup>**

| P. fluorescens strain | F. oxysporum strain | β-Galactosidase activity (U per 10<sup>8</sup> CFU) | Plant fresh wt (g) | Bacterial rhizosphere colonization (10<sup>7</sup> CFU per g) |
|------------------------|---------------------|-------------------------------------|-------------------|----------------------------------------|
|                        |                     | Expt 1 | Expt 2 | Expt 1 | Expt 2 | Expt 1 | Expt 2 |
| None                   | None               | nd    | nd    | 2.7  | 2.3  | nd    | nd    |
|                        | 242                | nd    | nd    | 3.1  | 2.4  | nd    | nd    |
|                        | 798                | nd    | nd    | 3.1  | 2.7  | nd    | nd    |
| CHA0/pME6259           | None               | 65 a  | 34 a  | 2.6  | 2.6  | 2.7  | 1.4  |
|                        | 242                | 304 b | 56 b  | 2.8  | 2.6  | 0.9  | 1.1  |
|                        | 798                | 38 c  | 22 c  | 2.6  | 2.6  | 1.9  | 1.1  |
| CHA638/pME6259         | None               | 460 b | 287 d | 2.9  | 2.6  | 2.5  | 1.5  |
|                        | 242                | 514 b | 365 d | 3.0  | 2.7  | 1.7  | 1.0  |
|                        | 798                | 420 b | 252 d | 3.0  | 2.6  | 2.4  | 0.8  |

*P. fluorescens* strains CHA0 (wild type) and CHA638 (<i>phlF</i> mutant) carrying a <i>phlA</i>-<i>lacZ</i> translational fusion on plasmid pME6259 were tested. <i>F. oxysporum</i> strain 242 or 798 was added to the gnotobiotic system at 10<sup>7</sup> microcomidia per g of soil. Plants were grown for 14 days in artificial soil under gnotobiotic conditions. Fresh weights of all plants (always five) in one flask are presented. Values represent the means of four replicate flasks with five plants each. Means within a column followed by a different letter were significantly different at <i>P</i> ≤ 0.05 according to Fisher’s protected least significant difference test. nd, not detected.
DISCUSSION

F. oxysporum is found worldwide. This fungus can persist almost indefinitely in soil due to its ability to survive winter in the mycelial or chlamydospore state, with the result that normal crop rotation is not a practical control measure (6). The species occurs as a soil saprophyte as well as a wilt pathogen of many crop plants, and many of the strains isolated produce FA (2, 4, 10, 35, 42). In this study we addressed in vitro and in the wheat rhizosphere the impact of different F. oxysporum strains on a gene critical for the biocontrol activity of the model organism P. fluorescens CHA0.

FA production by F. oxysporum is strain dependent. Production of FA varied markedly between F. oxysporum strains and media (Table 1). Succrose has been reported to favor FA production (10, 28), and indeed, F. oxysporum strains 798 and 801 did produce large amounts of FA in Czapek Dox broth, which is a sucrose-based medium. Richard’s medium is also supposed to favor FA production (9, 37). In the slightly modified form of this medium (R2) used in our experiments (7), only four strains produced detectable amounts of FA. The addition of zinc to this medium abolished FA production, as has been shown for other Fusarium strains (9, 14, 17).

DAPG biosynthesis in CHA0 is repressed in vitro by the fungal metabolite FA. Synthetic FA represses biosynthesis of DAPG in CHA0, with an almost complete block at 500 μM (Fig. 1), as observed by Schnider-Keel et al. (38). Here we show that an FA concentration of 300 μM gave an intermediate inhibition of phlA expression, and at a concentration of 100 μM, no significant repressive effect could be observed in vitro (Fig. 1).

Strains of the species F. oxysporum produce a wide range of secondary metabolites such as phytotoxins, pigments, and mycotoxins and cause diverse responses in plants, animals, humans, and microorganisms (29). In our fungal filtrate experiments, three lines of evidence show that FA is the secondary metabolite most likely to be responsible for phlA repression. (i) Repression of gene expression increased with increasing concentrations of synthetic FA added to the medium (Table 2, Fig. 1). (ii) Moreover, addition of the same amount of two different fungal filtrates from the same Fusarium strain, 801, with different FA contents to a growing culture of CHA0 repressed phlA expression differentially. Amendment of one culture filtrate (Table 1, experiment 1) brought the FA concentration in the bacterial growth medium to 400 μM and blocked phlA expression almost completely. Addition of a different filtrate, in which FA concentration was only 160 μM, resulted in partial repression only (Fig. 2). These effects correspond well with those obtained with the same concentration of synthetic FA (Fig. 1). (iii) Evidence that an intact phlF gene is needed for repression of phlA by FA has been provided by Schnider-Keel et al. (38). Similarly, in our study, repression of phlA expression through the addition of fungal filtrates was abolished in the phlF mutant CHA638 (Table 2).

Interactions between F. oxysporum strains and wheat. Although the F. oxysporum strains were isolated from wheat roots or from fields cultivated with wheat, the absence of visible lesions, no reduction in plant weight, and lack of detection of the fungus inside the plant material strongly indicate that there are no pathogenic interactions with the wheat cultivar used in our plant experiments. F. oxysporum is a cosmopolitan soil saprophyte, and specialized pathogenic strains are causal agents of vascular wilts and damping-off diseases. They exhibit a high degree of host specificity, and many formae speciales have been described (6). In our case, either the strains are nonpathogenic, or host genotype recognition failed and infection did not take place. However, the strains colonized the wheat rhizosphere, as shown by resolation of the fungi from the rhizosphere.

Interactions between F. oxysporum strains and CHA0 in the wheat rhizosphere. F. oxysporum strains 242 (FA⁺) and 798 (FA⁻) altered phlA expression in P. fluorescens CHA0 differentially compared to the control treatment without Fusarium added. To our knowledge, this is the first report to address the impact of a Fusarium strain on a gene critical for biocontrol in the rhizosphere of wheat without any pathogenic interactions. As expected from in vitro experiments, the FA-producing strain 798 also repressed phlA expression of CHA0 in the wheat rhizosphere. Previously, the tomato-pathogenic forma specialis radicis-lycopersici has been shown to repress DAPG synthesis in CHA0 through production of FA in a hydroponic rockwool system (14). Our results show that a nonpathogenic FA-producing strain of F. oxysporum represses phlA in CHA0 in the wheat rhizosphere. We hypothesize that this suppressing effect is caused by the production of FA in the rhizosphere. The latter is supported by the observation that the repressive effect was abolished in the phlF mutant CHA638. PhlF is proposed to be a pathway-specific repressor of DAPG synthesis in that it binds to the promoter(s) of phlA (5, 11, 38). A PhlF-mediated DAPG repression by FA has been demonstrated in vitro (38). It is conceivable that FA might interact with the repressor, leading to increased binding characteristics of the PhlF-promoter complex. However, it cannot be excluded that other, as yet unidentified, regulatory mechanisms act upstream of phlF and that DAPG repression by FA may not be the result of a higher affinity of an FA-PhlF complex to the phlA promoter region. To our knowledge, this is the first report to show a PhlF-mediated DAPG suppression in the rhizosphere.

The repressing effect of F. oxysporum 798 contrasted even more with an enhancing effect of strain 242 on phlA gene expression in CHA0. Previously, we have shown that Pythium ultimum can stimulate phlA gene expression in CHA0 on both cucumber and maize (33), an effect that might be an indirect consequence of disease due to an increased release of root exudates from damaged roots. By contrast, in the current study, in which no pathogenic interactions were observed, the change in gene expression is interpreted as a direct effect of the fungus. It is
possible that strain 242 produces an inducer that binds to PhIF and thereby prevents the interaction of the repressor protein with the phl promoter. The fact that the presence of strain 242 did not alter phlA expression in the PhIF mutant CHA638 supports this hypothesis.

In vitro, phlA expression of the PhIF mutant CHA638 was not higher than in the wild type (Table 2). However, on wheat roots, phlA was seven-to eightfold overexpressed in strain CHA638 compared with CHA0. This observation suggests that PhIF generally restricts expression of the DAPG operon in the rhizosphere of wheat more profoundly than in liquid cultures.

Implications for biocontrol. Even though the F. oxysporum strains used in this study had no pathogenic impact on the plants, the fungi did influence the production of DAPG, a key factor in biocontrol of beneficial bacteria co-occurring in the rhizosphere. F. oxysporum is nearly universally present in soil, which suggests that at least some FA-producing strains will be found in almost any soil sample taken. The suppressive effect of FA on the production of a secondary metabolite critical in biocontrol may affect the performance of FA-sensitive biocontrol bacteria against any soil-borne pathogen. This fact should be taken into consideration when using biocontrol strains in agricultural systems where FA producers are present. Consistent biocontrol of soil-borne diseases might be achieved by applying beneficial microorganisms, or mutants thereof (e.g., PhIF mutants), that are insensitive to FA. Efficient formulation offers another approach to improving biocontrol performance: coapplication with strains capable of detoxifying FA and amendments with zinc or a substance that neutralizes repressors (e.g., PhIF) could improve the level and reliability of biocontrol (14, 45).

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