

Fluorescent Pseudomonads in the Phyllosphere of Wheat: Potential Antagonists Against Fungal Phytopathogens

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Abstract Fluorescent pseudomonads isolated from wheat leaves were characterized regarding their antagonistic potential and taxonomy in relation to protect crop plants from infestation by *Fusarium* and *Alternaria* fungi causing diseases in wheat. Using a dual culture assay, inhibition of fungal growth was found for 40 isolates of 175 fluorescent pseudomonads. Twenty-two of the antagonists were able to suppress strains of *Fusarium* as well as *Alternaria*. By means of real-time qPCR, the *phlD* gene encoding the antibiotic 2,4-diacetylphloroglucinol was detected in 20 isolates. On the basis of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry spectral patterns, the isolates with antagonistic activity were assigned to the phylogenetic subgroup *Pseudomonas fluorescens* and the closely related *Pseudomonas gessardii* subgroup. The results of the study suggest that pseudomonads in the phyllosphere of crop plants may possibly contribute to natural plant protection.

Introduction

The yield and quality of wheat (*Triticum* spp.)—a staple food consumed by humans in many countries—are threatened by one of the most devastating fungal diseases worldwide: Fusarium head blight (FHB) [14]. The main causal agents in the temperate climates of Central Europe are *Fusarium graminearum* [teleomorph *Gibberella zeae* (Schwein) Petch] and *Fusarium culmorum* [(W.G. Smith) Sacc (teleomorph unknown)] [1]. So-called “black point” is another fungal disease in wheat, which is caused by *Alternaria alternata* (Fr.) Keissler and *Alternaria tenuissima* [(Kunze ex Nees et T. Nees: Fries) Wiltshire]. These fungi occur ubiquitously on wheat leaves and ears, and the disease is distributed worldwide in both humid and semi-arid regions [31]. In addition to yield losses and reduced grain quality, these *Fusarium* as well as *Alternaria* species are known to produce mycotoxins, which are harmful to both humans and livestock [13, 14].

Bacteria of the genus *Pseudomonas* are commonly found among the predominant genera in the phyllosphere, independent of the host plant species, and are also found on and in the above-ground parts of wheat plants [12, 33]. The relationship between these bacteria and their host plant can be saprophytic, pathogenic or plant-growth promoting [14, 27], the last of which is attributed to a group of non-phytopathogenic, non-necrogenic species of pseudomonads secreting a yellow-green fluorescent pigment: the siderophore pyoverdine [16]. The genus *Pseudomonas* comprises mostly fluorescent as well as a few non-fluorescent species in two lineages of intrageneric groups: *P. aeruginosa* and *P. fluorescens*. The latter is divided into six groups, with that of *P. fluorescens* being the most complex and includes nine subgroups [17]. For rapid and efficient species identification, the matrix-assisted laser desorption/

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ionization time-of-flight mass spectrometry (MALDI-TOF MS) is also suitable to the genus *Pseudomonas* [7].

The ecology and function of plant-associated fluorescent pseudomonads have been most extensively studied in the rhizosphere. High densities of these bacteria in field soils can achieve effective natural protection of crop plants against invading pathogens [11, 32]. The suppression of phytopathogens is mainly due to the production of antimicrobial secondary metabolites. Six classes of antibiotic compounds experimentally proven to inhibit fungal and partly bacterial growth are produced by fluorescent pseudomonads: phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, and hydrogen cyanide [6]. The phenolic derivative 2,4-diacetylphloroglucinol (DAPG) has antifungal, antibacterial and antiviral properties, and is therefore, of special relevance in agricultural systems [15].

The antagonistic activity of fluorescent pseudomonads has also been investigated in a few studies in the phyllosphere, e.g. of apple and pears [10, 29]. The potential of fluorescent pseudomonads to control phytopathogenic fusaria is known *per se* [8, 32, 33]. However, Naik et al. [21] showed that spray inoculation of *Pseudomonas fluorescens* could also significantly reduce the extent of *Alternaria* blight of sesame in India. This capability deserves to be investigated thoroughly also under the local conditions of Central Europe, where winter wheat is exposed to both *Fusarium* and *Alternaria* fungi and may be contaminated with their mycotoxins [1, 18].

The aim of this study was to examine the potential of a natural population of fluorescent pseudomonads in the phyllosphere of winter wheat to protect crop plants from infestation by *Fusarium* and *Alternaria* fungi causing *Fusarium* head blight and black point diseases. To this end, we tested a representative number of isolates from the phyllosphere of wheat using an *in vitro* antagonistic dual culture assay and characterized the antagonistic isolates taxonomically by means of MALDI-TOF MS and in part genetically.

Materials and Methods

Strains

Fusarium culmorum 13 (*Fc*13), *Fusarium graminearum* 23 (*Fg*23), *Alternaria alternata* 219 (*Aa*219) and *Alternaria tenuissima* 220 (*At*220) originating from wheat plants [19] were used as indicators in the dual culture assay. They are stored in the culture collection of microorganisms of the Institute of Landscape Biogeochemistry at the Leibniz-Centre for Agricultural Landscape Research Müncheberg/Germany. The determination of necrotic lesions on leaves,

seedling growth inhibition and *in vitro* mycotoxin production indicated the highly aggressive potential of all four fungal strains [9, 19]. Root-colonizing *Pseudomonas protegens* CHA0 [25] (formerly *Pseudomonas fluorescens* CHA0) produces several antagonistic secondary metabolites [22]. It was used as a reference strain for the production of DAPG and its detection in the present study.

Sampling

Leaves of winter wheat (cultivar Meister) were sampled in a field in the Northeast Lowlands of Germany, the Uckermark region, in 2013. At several points in the field, the third leaf on the main tiller of 20 neighbouring plants were removed aseptically and transported in sterile bags on ice. These bulked batches were stored at $-20\text{ }^{\circ}\text{C}$ until processed as one sample.

Isolation of Bacteria

Leaves (5 g) were immersed in 100 mL of $1\times$ phosphate-buffered saline, treated for 7 min in an ultrasonic cleaning bath, and subsequently shaken using a linear motion shaker for 20 min at 350 motions per min. The suspensions were diluted in quarter-strength Ringer's solution and spread-plated on King's B agar (ROTH, Karlsruhe/Germany). After incubating at $25\text{ }^{\circ}\text{C}$ for 5 days, colonies fluorescing at 366 nm were selected from the agar surface; the isolates were purified twice by streaking on the same agar and then stored in standard I nutrient broth (MERCK, Darmstadt/Germany) and glycerine (1:2, v/v) at $-20\text{ }^{\circ}\text{C}$.

Taxonomic Characterization of *Pseudomonas* Isolates by MALDI-TOF MS

The isolates were cultured on NAI1 (SIFIN, Berlin/Germany) for 24 h in preparation for a protein analysis using MALDI-TOF MS according the method of Toth et al. [30]. Briefly, approximately 10 mg of cell mass was suspended in 300 μL water and vortexed to generate a homogeneous suspension. The suspension was mixed with 900 μL ethanol and centrifuged. The pellet was resuspended in 50 μL 70 % formic acid and subsequently carefully mixed with 50 μL acetonitrile. After centrifugation, 1- μL aliquots of the supernatant were placed immediately on spots of a MALDI target. Each spot was allowed to dry and subsequently overlaid with 1 μL of matrix (α -cyano-4-hydroxycinnamic acid in 50 % aqueous acetonitrile containing 2.5 % trifluoroacetic acid). Mass spectra were acquired using a MALDI-TOF MS spectrometer in a linear positive mode (microflexTM LT, Bruker Daltonics, Bremen/Germany) in a mass range of 2–20 kDa. A bacterial test standard (BTS, Bruker Daltonics, Bremen/Germany) was

used for instrument calibration. The raw spectra were imported into MALDI Biotyper™ (3.1) software and then processed and analysed using standard pattern matching against the spectra in the MALDI Biotyper™ reference database (version 3.0, Bruker Daltonics, Bremen/Germany). A calculated matching score (LogScore value) provides a measure of the probability of a correct classification.

In Vitro Antagonism Tests

The antagonistic effect of fluorescent pseudomonads against *Fusarium* and *Alternaria* indicator strains were tested by dual culturing of the bacteria and fungi on agar as described by Fakhouri and Buchenauer [2]. Tests with the indicators *Fc13*, *Fg23* and *At220* were performed using S1 agar [4], and those with *Aa219* were performed using Potato Dextrose Agar (ROTH, Karlsruhe/Germany) because this strain is not able to grow on S1 medium. The criterion for antagonistic activity was a distance from the edge of the fungal colony to the bacterial streak (the inhibition zone) of more than 5 mm. The tests were repeated twice.

Screening for the Gene Encoding 2,4-diacetylphloroglucinol (DAPG)

All isolates with antagonistic activity detected in the dual culture assays were tested for the *phlD* gene, which encodes the antibiotic DAPG. A 1-mL sample of bacteria grown in standard I nutrient broth (MERCK, Darmstadt/Germany) on a rotary shaker for 24 h was centrifuged (8000×g for 20 min). The supernatant was discarded, and DNA was extracted from the bacterial pellet using UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA/USA). The presence of the *phlD* gene was assessed using real-time qPCR with the primers B2BF and BPR4 described by Shizard et al. [28]. The PCR product size and purity were checked by a melting profile and agarose gel electrophoresis.

Extraction of 2,4-diacetylphloroglucinol (DAPG) from Bacterial Cultures and Detection by HPLC

Pseudomonas isolates containing the *phlD* gene were tested for the production of DAPG on solid medium. The isolates were streaked on S1 agar as described in “In vitro antagonism tests”, but without co-inoculation of a fungal indicator. After incubating at 25 °C for 3 days, the agar was cut into pieces of approximately 5 × 5 mm, packed into 50-mL centrifugation tubes and extracted with 18 mL ethyl acetate (acidified with HCl to pH 1.2) on a linear motion shaker (KS-10, EB Echingen/Germany) for 2.5 h at 350

motions per min. The agar pieces that failed to dissolve were removed from the extract by centrifugation at 7000×g for 12 min. To remove the solvent, the extracts were subjected to a vacuum rotary evaporation at 35 °C. A JASCO HPLC system (Gross-Umstadt/Germany) with a UV-2075 detector, pump PU-1580, autosampler AS 1555-10, degasser and quaternary gradient mixer was used for the qualitative detection of DAPG. Dried extracts were resolved in methanol: water (65:35, v/v). DAPG was separated on a LiChrospher 100 RP 18 column (150 × 4 mm i.d., 5 µm particle size, VDS OptiLab, Berlin/Germany) by isocratic elution using methanol: 0.43 % phosphoric acid (70:30, v/v) at a flow rate of 1 mL min⁻¹ and 25 °C. The DAPG peak was detected by measuring the UV absorbance at 270 nm; the retention time was 6.7 min.

Results

Antagonistic Activity of *Pseudomonas* Isolates

A total of 40 isolates (23 %) of 175 fluorescent pseudomonads produced sizable inhibition zones against at least one of four fungal phytopathogens (Table 1). Growth inhibition was more pronounced against *Alternaria* (21 % of 175 bacterial isolates) than against *Fusarium* (15 %). However, 58 % of the 40 antagonistic isolates were able to suppress at least one strain of both *Fusarium* and *Alternaria*. The isolates are listed in Table 1 according to their clusters resulting from the MALDI-TOF MS analyses. There is a notable relationship between these clusters and the patterns of antagonism in the dual culture tests: the isolates of clusters A and B mainly inhibited the growth of the fungal indicator *Aa219*, whereas most isolates of cluster E and all isolates of cluster F were mainly active against *Fc13*, *Fg23* and *At220*. The antagonistic activity of the isolates in cluster C was directed against all fungal indicators, whereas that in cluster E was variable.

Detection of the *phlD* Gene in Antagonistic Isolates and of DAPG in Their Cultures

The gene encoding the antibiotic DAPG was detected in 20 isolates (Table 1), i.e. in 50 % of all antagonists and in 11 % of the total fluorescent *Pseudomonas* isolates in this study. However, relationships between the patterns of antagonism and the existence of the *phlD* gene were not evident: isolates 97 and 98 contained the gene and inhibited the growth of all four fungal indicators. In contrast, isolates 8 and 9 did not harbour this gene but were nonetheless antagonistically active. Additionally, several isolates (10, 25, 26, 49, 85, 138, 149, 174, and 179) were found to carry the DAPG-encoding gene, but they showed antagonism

Table 1 Isolates of fluorescent pseudomonads in clusters according to their MALDI-TOF MS analyses, patterns of their antagonistic activities in the dual culture assay (means of two tests) against the fungal indicators *Fusarium culmorum* 13 (*Fc13*), *Fusarium*

graminearum 23 (*Fg23*), *Alternaria tenuissima* 220 (*At220*), *Alternaria alternata* 219 (*Aa219*), detection of the *phlD* gene encoding the antibiotic 2,4-diacetylphloroglucinol (DAPG), and detection of this compound in cultures of the isolates

Isolate	Cluster of MALDI-TOF MS analyses	Dual culture assay				PCR detection of <i>phlD</i> gene	HPLC detection of DAPG
		Inhibition zones in mm of					
		<i>Fc13</i>	<i>Fg23</i>	<i>At220</i>	<i>Aa219</i>		
49	A	4	1	2	6	+	+
88	A	1	4	2	10	-	
122	A	2	0	1	9	-	
138	A	5	0	0	8	+	-
149	A	4	0	2	7	+	-
174	A	2	0	1	11	+	+
179	A	2	0	2	10	+	-
188	A	7	2	7	11	-	
189	A	0	7	2	11	-	
197	A	0	0	2	10	-	
10	B	3	0	0	7	+	+
25	B	2	0	0	9	+	-
26	B	0	0	0	8	+	-
115	B	7	2	2	4	-	
120	B	5	0	0	7	-	-
140	B	7	0	2	5	+	-
3	C	4	8	6	9	-	
5	C	6	0	6	10	+	-
8	C	6	7	6	8	-	
9	C	10	6	6	8	-	
32	D	2	2	6	2	-	
CHA0	D	20	6	12	6	+	+
1	E	8	3	5	9	+	-
7	E	9	0	3	2	-	
85	E	1	1	1	8	+	-
107	E	7	2	7	0	-	
108	E	7	1	7	0	+	-
109	E	7	3	8	2	-	
110	E	6	0	2	0	+	-
111	E	9	3	6	1	+	-
119	E	7	1	6	1	-	
95	F	7	10	10	3	+	-
96	F	8	10	10	5	-	
97	F	8	10	9	7	+	-
98	F	7	10	10	9	+	-
99	F	10	7	10	4	-	
102	F	7	9	10	2	+	+
103	F	7	9	11	3	-	
104	F	9	6	10	3	-	
106	F	10	9	10	3	-	
144	F	7	7	10	4	+	-

Cluster D includes the reference strain *Pseudomonas protegens* CHA0 indicated as CHA0. Italics marked fields indicate growth inhibition

only towards the indicator *Aa219*. Furthermore, the gene was present as well as absent in all the MALDI-TOF MS clusters, yet the HPLC detection of DAPG in the cultures of the isolates carrying the *phlD* gene was successful for only four of the isolates.

Taxonomic Characterization of Antagonistic *Pseudomonas* Isolates

A comparison of MALDI-TOF MS spectra obtained for the isolates with reference spectra in the database demonstrated that all isolates belong to the genus *Pseudomonas*. A proportion of 40 % of the total 40 isolates and one reference strain analysed reached LogScore values between 2.0 and 2.3 for matches with reference spectra, which could be considered as a probable classification at the species level.

The cluster analysis of the spectral patterns of the investigated isolates revealed grouping into several clusters, which clearly indicated taxonomic differences among the isolates (Fig. 1). Reference spectra of species displaying the closest matches are shown in the dendrogram in Fig. 1. The strains of clusters E and F revealed the highest similarity to one species, whereas the strains of clusters A to C are most likely taxonomically more heterogeneous, as different species were assigned.

Discussion

Regulating the entire plant-associated microbiome for capitalization on beneficial microbial traits to the hosts should be a goal of future research to develop a more sustainable next-generation agriculture [26]. *Pseudomonas* bacteria appear to play an important role as native

biocontrol agents in soils, in the spermosphere and in the rhizosphere [5, 15, 24] with particular relevance of the antifungal compound DAPG in this context [5, 15].

There is, however, an urgent need to improve our knowledge to manage phyllosphere microbiota in terms of plant health [20]. The present paper provides a complementary thought-provoking step in direction of suppressing FHB and black point in wheat.

The potential antagonistic capacity of the *Pseudomonas* population investigated in this study with 23 % antagonists and 11 % of the total isolates possessing the *phlD* gene was comparable with results available from investigations of pseudomonads isolated from the rhizosphere of crop plants [21, 23, 28].

Although the taxonomic affiliation of the isolates based on MALDI-TOF MS has been proven to be an effective identification tool, it does have limitations. In particular, the discrimination of closely related species is difficult, as the analysis of spectral patterns is mainly based on ribosomal proteins. Therefore, this method mirrors the restrictions of identification by 16S rRNA gene analyses, which are due to the high sequence similarity between most of the species in the genus *Pseudomonas* [17]. Hotta et al. [7] demonstrated that the analysis of special biomarkers based on the ribosomal subunit proteins encoded by the *S10-spc-alpha* operon can improve the discrimination of *Pseudomonas* species by MALDI-TOF MS. However, the results obtained in our study enabled the classification of the isolates into phylogenetic groups, as determined by a multi-locus sequence analysis on the basis of four house-keeping genes (16S rRNA gene, *gyrB*, *rpoB* and *rpoD*) by Mulet et al. [17]. The species with highly similar spectra belong to the *P. fluorescens* subgroup and the closely related *P. gessardii* subgroup, and therefore, these isolates are also related to these phylogenetic groups. Furthermore,

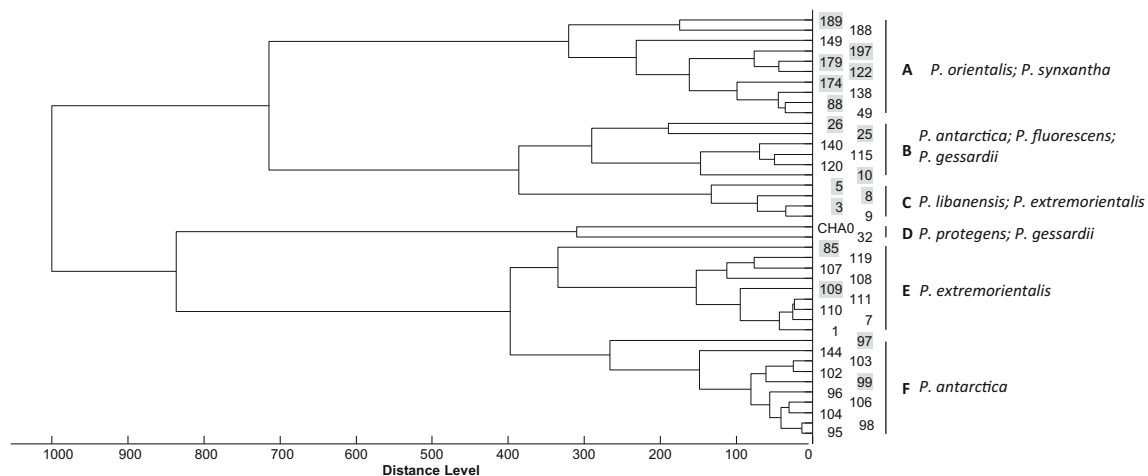


Fig. 1 Dendrogram, generated by the BioTyper program (version 3.1; Bruker Daltonics, Bremen/Germany), showing the similarities of MALDI-TOF mass spectra of investigated strains; species with most

similar spectra of their type strains are given for the formed cluster. Strains highlighted in grey revealed scores considering probable classification at species level

cluster analyses of the spectral patterns allowed classification of the isolates into several groups that differed also in their antagonistic activities (Table 1).

A broad spectrum of antagonistic activity against all the fungal indicators used in this study was especially pronounced in the isolates of clusters C and D, which showed the highest spectral similarity to both *P. libanensis* and *P. extremorientalis* and to *P. gessardii*, respectively (Fig. 1). Conversely, the antagonism of the isolates in clusters A and B, which were most similar to *P. orientalis* and *P. synxantha* and to *P. antarctica*, *P. fluorescens* and *P. gessardii*, respectively, was found to be limited to a narrow spectrum of fungi. Cluster D also contains the reference strain *Pseudomonas protegens* CHA0, which was inhibitory against all the fungal indicators tested and caused the widest inhibition zones (Table 1). This strain is characterized by extraordinarily diverse traits responsible for antagonistic activity [6].

In this study, antagonistic activity was demonstrated in isolates containing the *phlD* gene but also in others that did not possess this gene. Hence, DAPG is obviously not the only antagonistic compound produced by fluorescent pseudomonads isolated from the wheat phyllosphere. This is in accordance with the results of a study by Shirzard et al. [28] on *Pseudomonas* isolates from the rhizosphere of various crop plants. The authors detected strains possessing the genes encoding phenazine-1-carboxylic acid, pyoluteorin and DAPG.

DAPG could be detected in only four single cultures of 20 isolates containing the *phlD* gene spread on S1 agar. The reason for this controversial result is possibly the instability of this substance. The DAPG standard spread alone onto S1 agar was decreased by approximately 95 % in only 4 days at 25 °C. Hence, it appears that the detection of the corresponding gene is more reliable than the biochemical analysis of the substance as performed in this study.

Most of the antagonists in the present study were able to inhibit both *Fusarium* and *Alternaria* in vitro. The effects of antagonistic compounds and traits of fluorescent pseudomonads perhaps generally address a broad spectrum of fungi as also indicated in a recent study by Funnell-Harries et al. [3].

The results of the present study suggest the antagonistic potential against fungi causing FHB and black point diseases by fluorescent pseudomonads in the phyllosphere. Further studies are necessary to clarify other antibiotics than DAPG involved in the antagonism, to detect these antibiotics in above-ground plant parts, and to check the antagonistic activity of pseudomonads in vivo. Furthermore, the consequences of the co-occurrence of phytopathogenic fungi and fluorescent pseudomonads on above-

ground plant parts in wheat fields with regard to plant health and crop quality should be addressed.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Bottalico A, Perrone G (2002) Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *Eur J Plant Pathol* 108:124–611
2. Fakhouri W, Buchenauer H (2003) Characteristics of fluorescent pseudomonad isolates towards controlling of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. *J Plant Dis Protect* 110:143–156
3. Funnell-Harris DL, Sattler SE, Pedersen JF (2013) Characterization of fluorescent *Pseudomonas* spp. associated with roots and soil of two sorghum genotypes. *Eur J Plant Pathol* 136:469–481
4. Gould WD, Hagedorn C, Bardinelli TR, Zablutowicz RM (1985) New selective media for enumeration and recovery of fluorescent pseudomonads from various habitats. *Appl Environ Microbiol* 49:28–32
5. Haas D, Défago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* 3:307–319
6. Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu Rev Phytopathol* 41:117–153
7. Hotta Y, Teramoto K, Sato H, Yoshikawa H, Hosoda A, Tamura H (2010) Classification of genus *Pseudomonas* by MALDI-TOF MS based on ribosomal protein coding in *S10-spc-alpha* operon at strain level. *J Proteome Res* 9:6722–6728
8. Khan MR, Fischer S, Egan D, Doohan FM (2006) Biological control of *Fusarium* seedling blight disease of wheat and barley. *Phytopathol* 96:386–394
9. Korn U, Müller T, Ulrich A, Müller MEH (2011) Impact of aggressiveness of *Fusarium graminearum* and *F. culmorum* isolates on yield parameters and mycotoxin production in wheat. *Mycotox Res* 27:195–206
10. Kucheryava N, Fiss M, Auling G, Kroppenstedt RM (1999) Isolation and characterization of epiphytic bacteria from the phyllosphere of apple, antagonistic in vitro to *Venturia inaequalis*, the causal agent of apple scab. *Syst Appl Microbiol* 22:472–478
11. Landa BB, Mavrodi OV, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS, Weller DM (2002) Differential ability of genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* strains to colonize the roots of pea plants. *Appl Environ Microbiol* 68:3226–3237
12. Legard DE, McQuilken MP, Whipps JM, Fenlon JS, Fermor TR, Thompson IP et al (1994) Studies of seasonal changes in the microbial populations on the phyllosphere of spring wheat as a prelude to the release of a genetically modified microorganism. *Agric Ecosyst Environ* 50:87–101

13. Logrieco A, Moretti A, Solfrizzo M (2009) *Alternaria* toxins and plant diseases: an overview of origin, occurrence and risks. *World Mycotoxin J* 2:129–140
14. McMullen M, Bergstrom G, de Wolf E, Dill-Macky R, Hershman D, Shaner G et al (2012) A united effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant Dis* 96:1712–1728
15. McSpadden Gardener BB (2007) Diversity and Ecology of biocontrol *Pseudomonas* spp. in agricultural systems. *Phytopathol* 97:220–226
16. Meyer JM, Geoffroy VA, Baida N, Gardan L, Izard D, Lemanceau P et al (2002) Siderophore typing, a powerful tool for the identification of fluorescent and nonfluorescent pseudomonads. *Appl Environ Microbiol* 68:2745–2753
17. Mulet M, Lalucat J, García-Valdés E (2010) DNA sequence-based analysis of the *Pseudomonas* species. *Environ Microbiol* 12:1513–1530
18. Müller MEH, Korn U (2013) *Alternaria* mycotoxins in wheat—a 10 years survey in the Northeast of Germany. *Food Control* 34:191–197
19. Müller MEH, Steier I, Köppen R, Siegel D, Proske M, Korn U et al (2012) Cocultivation of phytopathogenic *Fusarium* and *Alternaria* strains affects fungal growth and mycotoxin production. *J Appl Microbiol* 113:874–887
20. Müller T, Ruppel S (2014) Progress in cultivation-independent phyllosphere microbiology. *FEMS Microbiol Ecol* 87:2–14
21. Naik MK, Rajalaxmi K, Rangeshwaran R, Amaresh YS, Reddy MS (2013) Search for 2,4 DAPG positive gene in fluorescent *Pseudomonas* and their exploitation for sustainable disease management. In: Reddy MS, Ilao RI, Faylon PS, Dar WD, Sayed R, Sudini H et al (eds) Recent advances in biofertilizers and biofungicides (PGPR) for sustainable agriculture. Proceedings of 3rd Asian conference on plant growth-promoting rhizobacteria (PGPR) and other microbials. Manila, Philippines, 21–24 Apr, 2013, pp 152–163
22. Natsch A, Keel C, Pfirter HA, Haas D, Défago G (1994) Contribution of the global regulator gene *gacA* to persistence and dissemination of *Pseudomonas fluorescens* biocontrol strain CHA0 introduced into soil microcosms. *Appl Environ Microbiol* 60:2553–2560
23. Picard C, Di Cello F, Ventura M, Fani R, Guckert A (2000) Frequency and biodiversity of 2,4-diacetylphloroglucinol-producing bacteria isolated from the maize rhizosphere at different stages of plant growth. *Appl Environ Microbiol* 66:948–955
24. Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. *Antonie Van Leeuwenhoek* 81:537–547
25. Ramettea A, Frapolli M, Fischer-Le Saux M, Gruffaz C, Meyer J-M, Défago G et al (2011) *Pseudomonas protegens* sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Syst Appl Microbiol* 34:180–188
26. Schlaeppli K, Bulgarelli D (2015) The plant microbiome at work. *Mol Plant Microbe Interact* 28:212–217
27. Schroth MN, Hildebrand DC, Panopoulos N (1992) Phytopathogenic pseudomonads and related plant-associated pseudomonads. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds) *The procaryotes*, 2nd edn. Springer, New York, pp 3104–3131
28. Shirzad A, Fallahzadeh-Mamaghani V, Pazhouhandeh M (2012) Antagonistic potential of fluorescent pseudomonads and control of crown and root rot of cucumber caused by *Phytophthora drechleri*. *Plant Pathol J* 28:1–9
29. Stockwell VO, Jonhson KB, Loper JE (2002) Biological control of fire blight: understanding interactions among introduced and indigenous microbial communities. In: Lindow SE, Hecht-Poinar EI, Elliot VJ (eds) *Phyllosphere microbiology*. APS, St. Paul, pp 225–239
30. Tóth EM, Schumann P, Borsodi AK, Kéki Z, Kovács AL, Márialigeti K (2008) *Wohlfahrtiimonas chitiniclastica* gen. nov., sp. nov., a new γ -proteobacterium isolated from *Wohlfahrtia magnifica* (Diptera: *Sarcophagidae*). *Int J Syst Evol Microbiol* 58:976–998
31. Vučković JN, Brkljača JS, Bodroža-Solarov MI, Bagi FF, Stojšin VB, Čulafić JN et al (2012) *Alternaria* spp. on small grains. *Food Feed Res* 39:79–88
32. Weller DM, Landa BB, Mavrodi OV, Schroeder KL, De La Fuente L, Blouin Bankhead S et al (2007) Role of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in the defense of plant roots. *Plant Biol* 9:4–20
33. Yoshida S, Ohba A, Liang Y-M, Koitabashi M, Tsushima S (2012) Specificity of *Pseudomonas* isolates on healthy and Fusarium head blight-infected spikelets of wheat heads. *Microb Ecol* 64:214–225