Fusarium fujikuroi Species Complex Associated With Rice, Maize, and Soybean From Jiangsu Province, China: Phylogenetic, Pathogenic, and Toxigenic Analysis

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Abstract

Species belonging to the *Fusarium fujikuroi* species complex (FFSC) are of vital importance and are a major concern for food quantity and quality worldwide, as they not only cause serious diseases in crops but also produce various mycotoxins. To characterize the population structure and evaluate the risk of poisonous secondary metabolites, a total of 237 candidate strains were isolated from rice, maize, and soybean samples in Jiangsu Province, China. Species identification of the individual strain was accomplished by sequencing the translation elongation factor 1 α gene (*TEF-1 α*) and the fumonisin (FB) synthetic gene (*FUM1*). The distribution of *Fusarium* species among the different crops was observed. The maize seeds were dominated by *F. proliferatum* (teleomorph, *Gibberella intermedia*) and *F. verticillioides* (teleomorph, *G. moniliformis*), whereas *F. fujikuroi* (teleomorph, *G. fujikuroi*) was the most frequently isolated species from rice and soybean samples. In addition, phylogenetic analyses of these strains were performed, and the results suggested clear groups showing no obvious relationship with the origin source. FFSC

species pathogenicity and toxigenicity were studied. All of the species reduced the rice seed germination rate, with no significant differences. *F. fujikuroi* showed two distinct patterns of influencing the length of rice seedlings, which were correlated with FBs and gibberellic acid synthesis. FBs were mainly produced by *F. verticillioides* and *F. proliferatum*. *F. proliferatum* and *F. fujikuroi* also produced moniliformin and beauvericin. The toxigenicity of *F. andiyazi* (teleomorph, *G. andiyazi*) was extremely low. Further analysis indicated that the sequence variations in *TEF-1 α* and the differences in the expression levels of the toxin synthesis genes were associated with the diversity of secondary metabolites in *F. fujikuroi* strains. These findings provide insight into the population-level characterization of the FFSC and might be helpful in the development of strategies for the management of diseases and mycotoxins.

Keywords: cereal, *Gibberella fujikuroi* species complex, pathogenicity, phylogeny, toxigenicity

Agricultural crops are not only crucial nutrient-rich food sources for human survival, but they are also important industrial and agricultural feed raw materials. Consequently, the quality and safety of agricultural crops and their products is of great significance to human and animal health. As a result of the influence of the agricultural environment and inputs, there are various food security issues associated with agricultural products, such as the presence of heavy metals and pesticide residues (Clemens and Ma 2016; Grewal et al. 2017). According to previous studies, mycotoxins have gradually become the leading threat, especially *Fusarium* toxins produced by *Fusarium* species under favorable environmental conditions (Lee and Ryu 2017).

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The *F. fujikuroi* species complex (FFSC) is an important component of the genus *Fusarium* and consists of more than 50 phylogenetically distinct species (O'Donnell et al. 2015). Among the members of the FFSC, *F. verticillioides* (teleomorph, *Gibberella moniliformis*), *F. fujikuroi* (teleomorph, *G. fujikuroi*), and *F. proliferatum* (teleomorph, *G. intermedia*) are well known for their abilities to cause devastating cereal diseases, such as rice bakanae, maize ear rot, and soybean root rot, leading to considerable reductions in crop yields and economic income.

Another significant challenge is that members of the FFSC can produce a variety of mycotoxins, including fumonisins (FBs) (Rheeder et al. 2002), fusaric acid (Bacon et al. 1996), moniliformin (MON) (Marasas et al. 1986), gibberellic acids (GAs) (Cerdá-Olmedo et al. 1994), beauvericin (BEA) (Logrieco et al. 1998), fusaproliferin (FUP) (Moretti et al. 1996), and enniatins (Nicholson et al. 2004), that contaminate food and cause chronic and acute toxicity to humans and livestock. Among these metabolites, FBs are the most frequently detected, with relatively high concentrations, especially in rice and maize (Ferrigo et al. 2016). Many studies have suggested that FBs inhibit sphingosine-*N*-acyltransferases, which are involved in sphingolipid biosynthesis (Abado-Becognee et al. 1998). Although there is currently no direct evidence that FBs are associated with serious human and animal health problems, the high occurrence of human esophageal cancer is apparently related to the consumption of maize-based products contaminated with FBs (Gelderblom et al. 2001). In addition, FBs exhibit certain degrees of neurotoxicity, cytotoxicity, immunotoxicity, and hepatotoxicity (Stockmann-Juvala and Savolainen 2008).

However, there are significant differences in the levels and types of mycotoxins produced by individual species. *F. verticillioides* and *F. proliferatum* are major FB producers, whereas other species

in the FFSC mainly produce certain amounts of BEA and FUP (Leslie et al. 2004). GA is usually synthesized by *F. fujikuroi* strains. In research studies, two groups of *F. fujikuroi* were distinguished based on the production of GAs and FBs (Niehaus et al. 2017; Suga et al. 2019). These toxic metabolites have been suggested as important tools for species discrimination in the FFSC (Leslie 1995; Moretti et al. 1996).

Some FFSC strains are similar in colony morphology and biology, which makes it difficult to directly differentiate strains. Phylogenetic analysis based on DNA sequence diversity plays a crucial role, and many molecular markers, such as internal transcribed spacer regions, β -tubulin, MAT alleles, and calmodulin are currently used; however, H3 histones and translation elongation factor (*TEF-1 α*) sequences appear to be the most useful in taxonomic studies of fungi, especially for the FFSC as well as other members of the *Fusarium* genus (O'Donnell et al. 2000; Proctor et al. 2013). The latest findings suggested that genes and other sequences involved in the mycotoxin biosynthesis pathway could be good targets in phylogenetic studies, as they have the advantage of possible use in combined approaches for the diagnostics of mycotoxin production abilities (Stepień 2014). Genes from the *FUM* cluster, responsible for the FB biosynthetic pathway, have been successfully utilized as successful markers in phylogenetic research on toxin-producing *Fusarium* species (Baird et al. 2008; Proctor et al. 2013).

In our previous study, we explicitly demonstrated that *F. verticillioides* and *F. proliferatum* were prevalent in maize samples from eastern China (Qiu et al. 2015). Nevertheless, we had little information about toxin production in these species and *Fusarium* occurrence in other crops. The main aims of this study were to determine the FFSC composition in different hosts by analyzing the differences in the sequences of *TEF-1 α* and *FUM1*, a polyketide synthase gene required for FB biosynthesis (Proctor et al. 1999). Moreover, the ability of the identified strains to produce mycotoxins and cause diseases was evaluated. The results will contribute to the development of prevention and control strategies for *Fusarium* species and their metabolites in agricultural crops.

Table 1. List of isolates analyzed in this study

Host	Tissue	Samples (n)	Strains (n)	Location	Year
Maize (<i>Zea mays</i> L.)	Seed	10	11	Xuzhou, Jiangsu	2012
	Seed	10	16	Huaian, Jiangsu	2012
	Seed	10	12	Suqian, Jiangsu	2017
	Seed	10	11	Nantong, Jiangsu	2017
	Seed	10	16	Yancheng, Jiangsu	2017
	Seed	10	12	Huaian, Jiangsu	2017
Rice (<i>Oryza sativa</i> L.)	Seed	10	14	Yangzhou, Jiangsu	2017
	Stem	4	16	Yancheng, Jiangsu	2012
	Stem	4	13	Huaian, Jiangsu	2012
	Seed	4	5	Taizhou, Jiangsu	2017
	Stem	4	2		
	Seed	4	27	Suqian, Jiangsu	2017
	Stem	4	3		
	Seed	4	12	Huaian, Jiangsu	2018
	Stem	4	2		
	Seed	4	5	Changzhou, Jiangsu	2018
	Stem	4	3		
	Seed	4	8	Yancheng, Jiangsu	2018
	Stem	4	4		
	Seed	4	8	Xuzhou, Jiangsu	2018
Soybean [<i>Glycine max</i> (Linn.) Merr.]	Stem	4	8		
	Seed	10	10	Nantong, Jiangsu	2017
	Seed	10	9	Yancheng, Jiangsu	2017
	Seed	10	10	Huaian, Jiangsu	2017

Materials and Methods

Isolation of *Fusarium* species. Fungal strains used in this study were obtained from three hosts: namely, rice, maize, and soybean. A total of 32 rice plants with typical bakanae symptoms in stems were sampled in 2012 (eight samples), 2017 (eight samples), and 2018 (16 samples). In addition, approximately 1 kg of rice seeds from each field was collected at harvest in the latter 2 years. Seeds from a total of 70 freshly harvested maize plants (1 kg each) were collected in 2012 and 2017, and 10 samples were collected from each location. For soybean, seeds from a total of 30 plants (1 kg each) in three locations were sampled during the 2017 growing season.

From each sample, 50 symptomatic/asymptomatic seeds and 10 rice stems with symptoms were chosen randomly, surface-sterilized with 1% sodium hypochlorite for 1 min, rinsed twice in sterile distilled water, and placed in Petri dishes containing potato dextrose agar (PDA); the plates were incubated at 25°C for 3 days. Suspected *Fusarium* strains were transferred to carnation leaf agar plates for conidia production, and each strain was single-spored according to the *Fusarium* laboratory manual (Leslie and Summerell 2006). All strains were grown on PDA as the regular culture medium and stored in 20% glycerol solution at –80°C. Detailed information on all strains is listed in Table 1.

Sequence analysis and phylogenetic reconstruction. Mycelia were harvested from the colonies of *Fusarium* species on PDA, and fungal DNA was extracted with the 2% cetyltrimethylammonium bromide (Leslie and Summerell 2006). Primers Fum1F1 (5'-CACATCTGTGGGCGATCC-3')/Fum1R2 (5'-ATATGGCCCCAGC TGCATA-3') and EF1T (5'-ATGGGTAAGGAGGACAAGAC-3')/EF2T (5'-GGAAGTACCAGTGATCATGTT-3') were used for the amplification of *FUM1* and *TEF-1 α* gene fragments, respectively (O'Donnell et al. 1998a; Proctor et al. 2004, 2013). The primers were synthesized by an oligonucleotide synthesis facility (Shanghai Shengong Biotechnological Ltd., Shanghai, China) and the PCR-amplified DNA fragments were sequenced by the same company.

The phylogenetic relationships were reconstructed with the MEGA software package (Kumar et al. 2016) using the maximum parsimony approach. No gap-containing positions were considered in the phylogenetic analysis. The bootstrap method used a heuristic search with 1,000 replicates.

Pathogenicity tests. Rice germination test. A total of 144 strains representative of four species were selected for the evaluation of their abilities to affect seed germination. After surface sterilization, rice grains were soaked for 1 h in a 10⁶/ml spore suspension, placed on sterile water-moistened filter paper in Petri dishes, and then incubated at 25°C (three plates per strain/10 seeds per plate). The control seeds were soaked in sterile water. The number of germinated seeds was measured after incubation for 7 days.

Rice seedling test. Those strains were also tested for their pathogenicity in two conventional rice varieties (Nangeng 46 and Xinliangyou 6308) with the method described by Cruz et al. (2013). Each strain was grown in shaken potato dextrose broth and culture filtrates were passed through filter paper and a microporous filter. Surface-sterilized rice seeds were transferred to Petri dishes with sterile water-moistened filter paper and incubated at room temperature. Sprouting seeds and culture filtrates were added to a glass test tube and incubated at 25°C under alternating 12-h light/12-h darkness. At 14 days after incubation, elongation or stunting symptoms relative to the untreated control were evaluated by measuring the heights of seedlings.

Chemical analysis. The ability of the above 144 strains to produce mycotoxins was evaluated on autoclaved rice grains. Rice medium was prepared by weighing 30 g of rice grains into 100-ml Erlenmeyer flasks containing 10 ml of distilled water. The flasks were autoclaved for 1 h. Then, rice was stirred with a glass rod to separate rice kernels and the flasks were autoclaved again for 1 h. Each strain was inoculated into the medium with 10 agar plugs of the PDA culture. After 20 days of incubation at 25°C, the rice cultures were harvested, dried, and ground to a fine powder. Five grams of each rice culture was shaken with 20 ml of acetonitrile/water (1:1, vol/vol) treated with 0.1% methanoic acid (ROE Scientific Inc., Newark, DE). After centrifugation at 3,500 rpm

for 5 min, 2 ml of the supernatant was purified with an extraction salt pack (200 mg of MgSO_4 , 100 mg of sodium citrate, 100 mg of C_{18} , and 100 mg of primary secondary amine). After centrifugation, the supernatant was transferred to a new glass tube. The extracts were concentrated under nitrogen, diluted with 0.6 ml of extract solution, and filtered through a nylon filter (13 mm in diameter, 0.22 μm pore size).

The toxins were analyzed with a LC-20ADXR liquid chromatograph (Shimadzu, Kyoto, Japan) coupled to an AB SCIEX Triple Quad mass spectrometer (Applied Biosystems, Foster City, CA). The analytical column was a Kinetex 100A C_{18} column (100 $\times$ 2.3 mm, 2.6 μm) from Phenomenex (Torrance, CA), and the column temperature was maintained at 40°C. The flow rate was 0.5 ml/min, and the injection volume was 2 μl . The mass spectrometric analyses were performed with the following operation parameters: gas temperature, 500°C; gas flow rate, 10 liters/min; nebulizer gas pressure, 50 psi; and capillary voltage, 5,500 V. Nitrogen was used as the ion source and the collision cell. Mycotoxins were analyzed via multiple reaction monitoring.

The mobile phase consisted of 5 mM of ammonium acetate/acetic acid (99.9/0.1, vol/vol) (A) and methanol (B). The separate high-performance liquid chromatography conditions were as follows: 0 to 0.01 min, solvent A linearly increased to 10%; 1.6 to 2 min, solvent A linearly increased from 10 to 35%; 2 to 4.44 min, solvent A linearly increased to 55%; 4.44 to 6.5 min, solvent A linearly increased from 55 to 90%; 6.5 to 12 min, solvent A remained constant at 90%; and 12 to 15 min, solvent A remained constant at 10%.

Quantitative reverse transcription PCR. Conidia ($10^5/\text{ml}$) of five FB-producing and five GA-producing strains were inoculated in liquid ICI media (containing 60 mM of glutamine) and incubated for 3 days. Total RNA was extracted from mycelia with TRIzol reagent (Takara, Dalian, China). Reverse-transcribed complementary DNA was synthesized with the Prime-Script RT reagent kit (Takara). Real-time PCR was performed with SYBR Premix Ex Taq (Takara). All experimental procedures were performed according to the manufacturer's instructions. PCR assays were completed in a LightCycler 96 system (Roche, Basel, Switzerland), and the data were analyzed with sequence detector software. Gene expression profiles were normalized to β -tubulin gene expression, and relative changes in gene expression levels were calculated by the $\Delta\Delta$ cycle threshold method. Relative gene expression levels of *CPS/KS* and *P450* in the GA biosynthetic pathway, *FUM1* and *FUM21* in the FB biosynthetic pathway, and several global regulators (*AreA*, *AreB*, *Lae1*, *Vel*, *SGE1*, and *Csm1*) of each strain were analyzed. The amplification levels of the genes in one GA-producing strain were used as a reference (set to 1.0). The primer sequences for these genes are listed in Supplementary Table S1.

Statistical analysis. Statistical comparisons of mycotoxin production and pathogenicity parameters between species were made with a *t* test. All statistical analyses were performed with the SigmaStat statistical software package (SPSS, version 11). Differences with $P < 0.05$ were considered significant.

Results

Phylogenetic analysis. Two hundred thirty-seven strains were recovered from the rice, maize, and soybean samples, and FFSC species were morphologically identified. Based on the *TEF-1 α* sequences submitted to the NCBI and *Fusarium-ID* databases, these strains were identified as *F. verticillioides* (68 strains), *F. proliferatum* (52 strains), *F. fujikuroi* (106 strains), and *F. andiyazi* (11 strains). The species composition varied among the host crops (Fig. 1). The maize samples were dominated by *F. verticillioides* (56.5%) and *F. proliferatum* (39.1%), whereas soybean samples were dominated by *F. fujikuroi* (75.9%) and *F. proliferatum* (20.7%). Four species were found in rice samples, including seeds and stems, and *F. fujikuroi* was dominant (69.0%). Partial *TEF-1 α* sequences of approximately 600 bp were amplified in all of the strains and the phylogenetic tree consisted of distinct clusters. One cluster was formed by the *F. verticillioides* strains, which were the most frequently detected in maize seeds, and 11 *F. andiyazi* strains

isolated from rice. The other cluster was composed of *F. proliferatum*, the second most common species associated with maize, and *F. fujikuroi*, mainly originating from rice seeds or stems. *F. fujikuroi* was divided into two groups: one consisting of 51 strains with low levels of FB production recovered from rice stem samples with only bakanae symptoms and the other group with stunting and a high FB production isolated from rice, maize, and soybean seeds.

The phylogenetic tree with the partial sequences of *FUM1* of the 133 strains showed three groups: *F. verticillioides*, *F. proliferatum*, and *F. fujikuroi* (Fig. 2). No amplification was detected in *F. andiyazi* strains.

The two trees were generally identical, as all of the strains except for the 11 *F. andiyazi* strains were the same. Both figures illustrated no correlation with the sampling sites, years, and different hosts. In addition, *F. verticillioides* and *F. andiyazi* were phylogenetically closely related, while *F. proliferatum* and *F. fujikuroi* shared a close genetic relationship. All of the obtained *TEF-1 α* sequences were submitted to Genbank as accession numbers from MN695921 to MN696157.

Pathogenicity. The germination rate was significantly ($P < 0.05$) reduced after the incubation of rice seeds for 7 days with the FFSC strains compared with the control (Fig. 3A). The average percentages of germinated seeds were 76.8, 73.3, 75.6, 79.3, and 70.0% for *F. verticillioides*, *F. proliferatum*, *F. fujikuroi*-bakanae, *F. fujikuroi*, and *F. andiyazi*, respectively. There were no significant ($P < 0.05$) differences in the germination rate of rice seeds among the different species.

Either stunting or elongation was observed in rice seedlings inoculated with FFSC strains compared with the controls. *F. fujikuroi* strains from bakanae disease showed elongated seedlings that were typical bakanae symptoms in rice, whereas the FB-producing FFSC strains along with *F. fujikuroi* from other sources caused stunting symptoms in rice seedlings (Fig. 3B). The capacity to influence the development of rice seedlings did not differ between *F. andiyazi* and the untreated control.

Secondary metabolite synthesis. All 144 strains tested in the pathogenicity assay were analyzed for their ability to produce FBs (FB1, FB2, and FB3), hydrolyzed fuminisin B1 (HFB1), BEA, MON, and GA3 (Table 2). HFB1 was detected in 43 strains at low levels (5.1 to 44.7 $\mu\text{g}/\text{kg}$); therefore, further analysis of HFB1 was not conducted.

FBs were the most abundant secondary metabolic products. In particular, FB1 was found in all *F. verticillioides* and *F. proliferatum* strains, ranging in production from 1,371.2 to 24,965.0 and 99.6 to 16,355.5 $\mu\text{g}/\text{kg}$, respectively. Similarly, the production of FB2 and FB3 in the whole *F. verticillioides* population ranged from 457.7 to 15,706.5 and 97.1 to 4,840.9 $\mu\text{g}/\text{kg}$, respectively. The production of FB2 and FB3 in all *F. proliferatum* strains ranged from 86.2 to 9,306.6 and 30.2 to 2,885.0 $\mu\text{g}/\text{kg}$, respectively. Two contrasting types of toxigenicity were associated with *F. fujikuroi*: 25 strains were associated with bakanae disease, among which 24 produced 5.8 to 141.9, 6.2 to 135.2, and 6.2 to 310.4 $\mu\text{g}/\text{kg}$ of FB1, FB2, and FB3, respectively; 37 strains did not cause bakanae disease, all of which synthesized 3,380.2 to 17,496.0, 2,057.2 to 13,575.8, and 146.6 to 2,763.6 $\mu\text{g}/\text{kg}$ of FB1, FB2, and FB3, respectively. FB1, FB2, and FB3 were synthesized by *F. andiyazi* at low levels (ranging from 32.2 to 66.5, 27.9 to 48.8, and 5.1 to 9.9 $\mu\text{g}/\text{kg}$ for FB1, FB2, and FB3, respectively).

MON was produced in 27 of 33 strains of *F. proliferatum* (4.0 to 1,573.6 $\mu\text{g}/\text{kg}$), 21 of 25 strains of *F. fujikuroi*-bakanae (1.5 to 1,688.4 $\mu\text{g}/\text{kg}$), and 37 of 37 strains of *F. fujikuroi* (2.8 to 1,755.6 $\mu\text{g}/\text{kg}$). Low detection rates and concentrations of MON were observed in *F. verticillioides* and *F. andiyazi*.

The capability of BEA synthesis was similar to that of MON synthesis, as BEA production by *F. verticillioides* and *F. andiyazi* was low compared with that by the other two *Fusarium* species. Twenty-eight of the 33 *F. proliferatum* strains, 21 of the 25 *F. fujikuroi*-bakanae strains, and 35 of the 37 *F. fujikuroi* strains were BEA producers, with concentrations ranging from 4.0 to 1,573.6, 1.3 to 3,674.0, and 7.4 to 2,507.4 $\mu\text{g}/\text{kg}$, respectively.

GA was found in only *F. fujikuroi* strains from rice stems with bakanae symptoms; the GA3 content ranged between 27.9 and 3,884.5 µg/kg.

Molecular characterization of *F. fujikuroi*. As the *F. fujikuroi* strains showed a markedly distinct cluster in sequence analysis of the *TEF-1α* gene, the sequences of this marker from GA-producing and FB-producing *F. fujikuroi* strains were further analyzed. Multiple alignments of these sequences revealed common nucleotide substitutions at two sites; all FB producers had cytosine and thymine at nucleotide positions 447 and 618, respectively, and these two nucleotide sites contained adenine and guanine in FB nonproducers, respectively.

Considering the notable differences in the mycotoxin profiles between the two *F. fujikuroi* subgroups, we compared the expression

patterns of two types of randomly selected strains with key GA (*CPS/KS* and *P450*) and FB (*FUM1* and *FUM21*) biosynthetic genes and several vital regulator genes (*AreA*, *AreB*, *Lae1*, *Vel*, *SGE1*, and *Csm1*). However, little change was observed in these regulatory genes between FB- and GA-producing *F. fujikuroi* strains (*unpublished data*), suggesting similar effects of these genes in the field strains. In contrast, the expression characteristics of the GA- and FB-synthetic genes differed dramatically. The *FUM1* and *FUM21* transcript levels of FB producers were significantly ($P < 0.05$) higher than those of GA producers. Furthermore, the bakanae-type strains showed higher expression levels of *CPS/KS* and *P450* than the FB-producing strains (Fig. 4).

In addition, two other genes that encode unknown products (namely, *PKS51* and *NRPS31*) were used to further distinguish the

Fig. 1. Phylogenetic tree inferred from partial translation elongation factor *TEF-1α* gene sequences from members of the *Fusarium fujikuroi* species complex was constructed by a maximum likelihood analysis. Reference sequences were from the latest literature. NRRL, Agricultural Research Service Culture Collection (USA); CBS, Centraalbureau voor Schimmelcultures (the Netherlands). Six NRRL strains (13566, 22172, 22944, 25102, 32157, and 66453) and one CBS strain (134430) were used as references.

(Leslie and Summerell 2006). In our study, most of the *F. fujikuroi* strains were recovered from rice seeds or stems; however, we also recovered 4 and 17 strains of *F. fujikuroi* from maize and soybean seeds, respectively. Despite the prevalence, the *F. fujikuroi* strains we recovered were asymptomatic in the seeds of the three crops. Considering that the prevalent pathogen in soybean is *F. solani* (Jin et al. 1996) and that the occurrence of FFSC species on soybean is rare (Choi et al. 2018; Garcia et al. 2012), further investigation of the pathogenicity of *F. fujikuroi* on soybean is necessary. Moreover, a number of *F. fujikuroi* strains have been isolated from sugarcane and grapes (Bolton et al. 2016). We also detected 11 strains of *F. andiyazi* in rice samples; *F. andiyazi* was first described from sorghum (Marasas et al. 2001) and successively discovered on rice and maize from Africa and Asia (Hsuan et al. 2011; Wulff et al. 2010). To date, the host range of this pathogen is limited. This is the first report of *F. andiyazi* on rice in eastern China.

Fusarium species in the FFSC are difficult to distinguish by only morphological characteristics. In this study, the sequences of *FUM1* and *TEF-1α* were used for phylogenetic analysis, and the strains formed separate and well-supported groups. *F. fujikuroi* and *F. proliferatum* were grouped into one major clade, and *F. andiyazi* and *F. verticillioides* were closely related. However, Proctor et al. (2013) found inconsistent results regarding the evolution of the FFSC species based on 12 primary metabolism genes and nine FB biosynthetic genes. The authors suggested that the ancestor of the phylogenetic housekeeping genes was different from that of the biosynthetic genes. This discrepancy might result from the limited number of molecular markers used in our research.

Several findings showed similar results suggesting that *F. fujikuroi* and *F. proliferatum* are morphologically and biologically very similar and are located in the Asian clade or the neighboring group (Hsuan et al. 2011; O'Donnell et al. 1998b; Wulff et al. 2010). Although the detailed species concept and phylogenetic analysis of *F.*

andiyazi remain to be determined (Kvas et al. 2009), the few relevant publications revealed that this species might be clustered in the main clade with *F. verticillioides*, an African clade member (Hsuan et al. 2011; Wulff et al. 2010). It was obvious that single species were separated into independent groups, and there was no correlation with geographic origin, host, or sampling year. The results of this and most previous studies (Covarelli et al. 2012; de Oliveira Rocha et al. 2011; Jurado et al. 2010) might be explained by the easy dispersal of the pathogens in nature, as they produce a large number of airborne conidia and have high adaptability to different habitats.

We confirmed the pathogenicity of FFSC strains using three different methods, of which the rice seedling test showed stunting or elongation of rice seedlings. Stunting and elongation of rice seedlings are typical symptoms of FFSC species and have been reported in several studies (Amoah et al. 1995; Cruz et al. 2013; Jeon et al. 2013; Wulff et al. 2010). Here, only *F. fujikuroi*-bakanae strains induced the growth of rice roots, and other species significantly ($P < 0.05$) reduced the growth of the rice seedlings, leading to stunted seedlings. It was demonstrated that the elongation of rice seedlings was caused by GA production in *F. fujikuroi* (Bömke and Tudzynski 2009), and the ability of the fungus to synthesize GAs was favorable for efficient colonization in rice roots (Wiemann et al. 2013). *F. verticillioides* and *F. proliferatum* were associated with stunted growth of rice seedlings (Amoah et al. 1995; Desjardins et al. 2000) and were considered potential pathogens of rice (Jeon et al. 2013; Wulff et al. 2010). In a 2017 study, deletion of *FUM1* and *FUB1* (fusaric acid biosynthesis gene) in *F. fujikuroi* strain B14 caused stunting and the production of high amounts of FBs; fusaric acid, the lack of GA accumulation, and additional factors worked together and played crucial roles in causing stunted rice seedlings (Niehaus et al. 2017). The pathogenicity of FFSC strains depends on the balance of secondary metabolites, the environmental conditions, and the nutritional status of hosts (Amoah et al. 1995).

Fig. 3. Pathogenicity assay of 144 strains of the *Fusarium fujikuroi* species complex. **A**, The germination rate of the rice seeds after 7 days of spore treatment with each strain. **B**, Growth of the rice seedlings 7 days after inoculation with each strain. Different letters above bars indicate a significant difference at $P < 0.05$ using a Tukey's student range test.

Table 2. Mycotoxin production of 144 strains of the *Fusarium fujikuroi* species complex

Species (n)	Mycotoxins (μg/kg) ^a						
	FB1	FB2	FB3	HFB1	MON	BEA	GA3
<i>F. verticillioides</i> (38)	13,859.1 ± 6,024.7 (1,317.2–24,965.0)	6,526.7 ± 3,548.3 (457.7–15,706.5)	1,539.4 ± 1,095.6 (99.6–16,355.5)	6.0 ± 10.1 (0–41.2)	0.1 ± 0.8 (0–6.7)	0.8 ± 1.9 (0–12.8)	<0
<i>F. proliferatum</i> (33)	7,538.0 ± 4,361.6 (99.6–16,355.5)	3,138.3 ± 2,422.0 (86.2–9,306.6)	494.5 ± 514.3 (30.2–2,885.0)	2.17 ± 4.5 (5.8–15.7)	115.5 ± 275.0 (4.0–1,573.6)	622.9 ± 537.5 (4.0–1,573.6)	<0
<i>F. fujikuroi</i> -bakanae (25)	21.7 ± 34.0 (5.8–141.9)	15.3 ± 32.0 (6.2–135.2)	7.1 ± 41.5 (6.2–310.4)	<0	246.7 ± 485.7 (1.5–1,688.4)	467.7 ± 838.5 (1.3–3,674.0)	1,062.7 ± 1,023.8 (27.9–3,884.5)
<i>F. fujikuroi</i> (37)	10,076.1 ± 4,420.6 (3,380.2–17,496.0)	8,614.1 ± 3,557.3 (2,057.2–13,575.8)	623.5 ± 474.2 (146.6–2,763.6)	9.6 ± 12.6 (5.1–44.7)	485.0 ± 332.8 (2.8–1,755.6)	930.0 ± 691.1 (7.4–2,507.4)	<0
<i>F. andiyazi</i> (11)	33.8 ± 21.7 (32.2–66.5)	25.6 ± 18.6 (27.9–48.8)	3.7 ± 4.4 (5.1–9.9)	<0	23.8 ± 30.8 (7.5–84.27)	1.2 ± 1.5 (1.71–3.23)	<0

^a FB, fumonisin; HFB, hydrolyzed fumonisin; MON, moniliformin; BEA, beauvericin; GA, gibberellic acid.

Mycotoxin production by the FFSC species was determined with rice culture. In the case of FBs, the majority of the *F. verticillioides* and *F. proliferatum* strains produced high amounts of this type of toxic substance. *F. proliferatum* produced higher amounts of FBs than *F. verticillioides* in maize, soybean, and wheat substrates (Leslie et al. 2004; Palacios et al. 2015; Waskiewicz et al. 2013). However, significant variability was not found in this study or in other relevant studies (Choi et al. 2018; de Oliveira Rocha et al. 2011). FB

production by *F. verticillioides* and *F. proliferatum* might depend on the host source and culture conditions and may vary among different strains of the same species (Darnetty and Salleh 2013). In addition to the most common FBs, the emerging *Fusarium* toxins MON and BEA have been reported to be present in substantial quantities and represent potential health threats that need to be further investigated. The results obtained for MON and BEA production suggested that these two toxins are common metabolites of *F.*

Fig. 4. Relative expression levels for the key genes involved in gibberellic acid and fumonisin biosynthesis of 10 strains of the *Fusarium fujikuroi* species complex. The transcript levels of **A**, *FUM21* and *FUM1* and **B**, *CPS/KS* and *P450* were determined. Different letters above bars indicate a significant difference at $P < 0.05$ using a Tukey's student range test.

proliferatum and *F. fujikuroi* but not of other species. These findings are in accordance with several other reports (Leslie et al. 2004), and only a few strains could synthesize high amounts of both toxins.

GA and FB production seemed to act in opposition to each other in *F. fujikuroi* populations. *F. fujikuroi*-*bakanae* strains were able to produce GAs and low levels of FBs and cause typical *bakanae* symptoms. These results are consistent with previous studies on GA production by FFSC members (Desjardins et al. 2000; Wulff et al. 2010). In contrast, *F. fujikuroi* strains from non-*bakanae* rice seeds, maize, and soybean did not produce GA but produced high levels of FBs. Moreover, recent studies demonstrated that a number of *F. fujikuroi* strains from various sources produced a high amount of FBs (Bolton et al. 2016; Choi et al. 2018; Cruz et al. 2013; Suga et al. 2014, 2019). The defect of some genes in the *FUM* cluster was observed in some non-FB-producing strains of *F. fujikuroi* (Chiara et al. 2015; Suga et al. 2019), implying that a lack of essential genes for FB synthesis could result in a complete loss of FB production. However, the entire FB gene cluster was detected in most *F. fujikuroi* strains producing either no or only very small amounts of FBs (Niehaus et al. 2017), suggesting that other factors may also be involved in FBs production. Low levels of *Fum21* expression might be the cause of the low levels of FBs; the toxin yield increased 100-fold under the enhanced expression of this transcription factor (Rösler et al. 2016), suggesting that a *F. fujikuroi* strain with no FB synthesis can be transformed into a potent FB producer. However, an analysis of the effect of overexpression of *Fum21* on the transcription levels of other elements in the *FUM* cluster had not been performed. It is questionable whether *Fum21* is naturally silent. Several mutations in *FUM21* and *FUM7* were recently found, indicating causative variations leading to the loss of FB production (Sultana et al. 2019). In addition, single nucleotide polymorphisms (SNPs) have been reported in *FUM8* and *FUM18* (Proctor et al. 2013; Suga et al. 2019).

We compared the expression of biosynthetic genes of FBs (*FUM1* and *FUM21*) and GAs (*CPS/KS* and *P450*) between *F. fujikuroi* strains and observed different expression characteristics. The results suggested that these genes might be used to differentiate the GA- and FB-producing *F. fujikuroi* strains. Some strains from the Philippines that were strongly capable of expressing *FUM1* had undetectable levels of FBs, indicating the absence of FB production attributable to the lack of functionality of other synthetic genes (Cruz et al. 2013). In addition to these specific elements, variations in other sequences were observed in the two types of *F. fujikuroi* strains. *TEF-1α* was suggested to contain a SNP (T618G) in Japanese *F. fujikuroi* groups (Suga et al. 2014). Here, another specific variation site (C447A) was identified. Although these mutations correlated with FB synthesis, SNPs were unlikely to be the cause of the variation in FB production because this molecular marker is a housekeeping gene. No connection between T618G and FBs synthesis was elucidated in *F. fujikuroi* from grapes (Bolton et al. 2016). These SNPs could be an effective tool in genetically characterizing GA and FB producers because of the long-term evolution and development of these subsequences.

In conclusion, we found a high degree of contamination of rice, maize, and soybean samples with different *Fusarium* species belonging to the FFSC and examined the pathogenicity and toxigenicity of the strains in this study. This contamination should be considered a potential public health threat with regard to mycotoxins produced at high levels by a large number of the strains.

Literature Cited

- Abado-Becognee, K., Mobio, T. A., Ennamany, R., Fleurat-Lessard, F., Shier, W. T., Badria, F., and Creppy, E. E. 1998. Cytotoxicity of fumonisin B1: Implication of lipid peroxidation and inhibition of protein and DNA syntheses. *Arch. Toxicol.* 72:233-236.
- Amoah, B. K., Rezanoor, H. N., Nicholson, P., and MacDonald, M. V. 1995. Variation in the *Fusarium* section *Liseola*: Pathogenicity and genetic studies of isolates of *Fusarium moniliforme* Sheldon from different hosts in Ghana. *Plant Pathol.* 44:563-572.
- Bacon, C. W., Porter, J. K., Norred, W. P., and Leslie, J. F. 1996. Production of fusaric acid by *Fusarium* species. *Appl. Environ. Microbiol.* 62:4039-4043.
- Baird, R., Abbas, H., Windham, G., Williams, P., Baird, S., Ma, P., Kelley, R., Hawkins, L., and Scruggs, M. 2008. Identification of select fumonisin

- forming *Fusarium* species using PCR applications of the polyketide synthase gene and its relationship to fumonisin production in vitro. *Int. J. Mol. Sci.* 9: 554-570.
- Bolton, S., Brannen, P., and Glenn, A. 2016. A novel population of *Fusarium fujikuroi* isolated from Southeastern US winegrapes reveals the need to re-evaluate the species' fumonisin production. *Toxins (Basel)* 8:254.
- Bömke, C., and Tudzynski, B. 2009. Diversity, regulation, and evolution of the gibberellin biosynthetic pathway in fungi compared to plants and bacteria. *Phytochemistry* 70:1876-1893.
- Cerdá-Olmedo, E., Fernández-Martín, R., and Ávalos, J. 1994. Genetics and gibberellin production in *Gibberella fujikuroi*. *Anton. Leeuw. Int. J. G.* 65: 217-225.
- Chiara, M., Fanelli, F., Mule, G., Logrieco, A. F., Pesole, G., Leslie, J. F., Horner, D. S., and Toomajian, C. 2015. Genome sequencing of multiple isolates highlights subtelomeric genomic diversity within *Fusarium fujikuroi*. *Genome Biol. Evol.* 7:3062-3069.
- Choi, J. H., Lee, S., Nah, J. Y., Kim, H. K., Paek, J. S., Lee, S., Ham, H., Hong, S. H., Yun, S. H., and Lee, T. 2018. Species composition of and fumonisin production by the *Fusarium fujikuroi* species complex isolated from Korean cereals. *Int. J. Food Microbiol.* 267:62-69.
- Clemens, S., and Ma, J. F. 2016. Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annu. Rev. Plant Biol.* 67:489-512.
- Covarelli, L., Stifano, S., Beccari, G., Raggi, L., Lattanzio, V. M. T., and Albertini, E. 2012. Characterization of *Fusarium verticillioides* strains isolated from maize in Italy: Fumonisin production, pathogenicity and genetic variability. *Food Microbiol.* 31:17-24.
- Cruz, A., Marín, P., González-Jaén, M. T., Aguilar, K. G. I., and Cumagun, C. J. R. 2013. Phylogenetic analysis, fumonisin production and pathogenicity of *Fusarium fujikuroi* strains isolated from rice in the Philippines. *J. Sci. Food Agric.* 93:3032-3039.
- Darnetty, T., and Salleh, B. 2013. Toxigenicity of *Fusarium* species in *Gibberella fujikuroi* species complex (GFSC) associated with stalk and ear rot disease of corn. *eSci J. Plant Pathol.* 2:147-154.
- de Oliveira Rocha, L., Reis, G. M., Da Silva, V. N., Braghini, R., Teixeira, M. M. G., and Corrêa, B. 2011. Molecular characterization and fumonisin production by *Fusarium verticillioides* isolated from corn grains of different geographic origins in Brazil. *Int. J. Food Microbiol.* 145:9-21.
- Desjardins, A. E., Manandhar, H. K., Plattner, R. D., Manandhar, G. G., Poling, S. M., and Maragos, C. M. 2000. *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. *Appl. Environ. Microbiol.* 66:1020-1025.
- Ferrigo, D., Raiola, A., and Causin, R. 2016. Fusarium toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. *Molecules* 21:627.
- García, D., Barros, G., Chulze, S., Ramos, A. J., Sanchis, V., and Marín, S. 2012. Impact of cycling temperatures on *Fusarium verticillioides* and *Fusarium graminearum* growth and mycotoxins production in soybean. *J. Sci. Food Agric.* 92:2952-2959.
- Gelderblom, W. C., Abel, S., Smuts, C. M., Marnewick, J., Marasas, W. F., Lemmer, E. R., and Ramljak, D. 2001. Fumonisin-induced hepatocarcinogenesis: Mechanisms related to cancer initiation and promotion. *Environ. Health Perspect.* 109:291-300.
- Grewal, A. S., Grewal, A. S., Singla, A., Kamboj, P., and Dua, J. S. 2017. Pesticide residues in food grains, vegetables and fruits: A hazard to human health. *J. Med. Chem. Toxicol.* 2:40-46.
- Hsuan, H. M., Salleh, B., and Zakaria, L. 2011. Molecular identification of *Fusarium* species in *Gibberella fujikuroi* species complex from rice, sugarcane and maize from Peninsular Malaysia. *Int. J. Mol. Sci.* 12:6722-6732.
- Jeon, Y. A., Yu, S. H., Lee, Y. Y., Park, H. J., Lee, S., Sung, J. S., Kim, Y. G., and Lee, H. S. 2013. Incidence, molecular characteristics and pathogenicity of *Gibberella fujikuroi* species complex associated with rice seeds from Asian countries. *Mycobiology* 41:225-233.
- Jin, H., Hartman, G. L., Nickell, C. D., and Widholm, J. M. 1996. Characterization and purification of a phytotoxin produced by *Fusarium solani*, the causal agent of soybean sudden death syndrome. *Phytopathology* 86:277-282.
- Jurado, M., Marín, P., Callejas, C., Moretti, A., Vázquez, C., and González-Jaén, M. T. 2010. Genetic variability and fumonisin production by *Fusarium proliferatum*. *Food Microbiol.* 27:50-57.
- Jurado, M., Vázquez, C., Callejas, C., and González-Jaén, M. T. 2006. Occurrence and variability of mycotoxigenic *Fusarium* species associated to wheat and maize in the South West of Spain. *Mycotoxin Res.* 22:87-91.
- Kim, J. H., Kang, M. R., Kim, H. K., Lee, S. H., Lee, T., and Yun, S. H. 2012. Population structure of the *Gibberella fujikuroi* species complex associated with rice and corn in Korea. *Plant Pathol. J.* 28:357-363.
- Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33: 1870-1874.
- Kvas, M., Marasas, W. F. O., Wingfield, B. D., Wingfield, M. J., and Steenkamp, E. T. 2009. Diversity and evolution of *Fusarium* species in the *Gibberella fujikuroi* complex. *Fungal Divers.* 34:1-21.
- Lee, H. J., and Ryu, D. 2017. Worldwide occurrence of mycotoxins in cereals and cereal-derived food products: Public health perspectives of their co-occurrence. *J. Agric. Food Chem.* 65:7034-7051.

- Leslie, J. F. 1995. *Gibberella fujikuroi*: Available populations and variable traits. Can. J. Bot. 73 (Suppl 1):282-291.
- Leslie, J. F., and Summerell, B. A., eds. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Oxford, UK.
- Leslie, J. F., Zeller, K. A., Logrieco, A., Mule, G., Moretti, A., and Ritieni, A. 2004. Species diversity of and toxin production by *Gibberella fujikuroi* species complex strains isolated from native prairie grasses in Kansas. Appl. Environ. Microbiol. 70:2254-2262.
- Logrieco, A., Moretti, A., Castella, G., Kostecki, M., Golinski, P., Ritieni, A., and Chelkowski, J. 1998. Beauvericin production by *Fusarium* species. Appl. Environ. Microbiol. 64:3084-3088.
- Marasas, W. F., Rheeder, J. P., Lamprecht, S. C., Zeller, K. A., and Leslie, J. F. 2001. *Fusarium andiyazi* sp. nov., a new species from sorghum. Mycologia 93:1203-1210.
- Marasas, W. F. O., Thiel, P. G., Rabie, C. J., Nelson, P. E., and Toussoun, T. A. 1986. Moniliformin production in *Fusarium* section *Liseola*. Mycologia 78:242-247.
- Moretti, A. A., Logrieco, A., Bottalico, A., Ritieni, A., Fogliano, V., and Randazzo, G. 1996. Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Sydowia 48:44-56.
- Nicholson, P., Simpson, D. R., Wilson, A. H., Chandler, E., and Thomsett, M. 2004. Detection and differentiation of trichothecene and enniatin-producing *Fusarium* species on small-grain cereals. Eur. J. Plant Pathol. 110:503-514.
- Niehaus, E. M., Kim, H. K., Münsterkötter, M., Janevska, S., Arndt, B., Kalinina, S. A., Houterman, P. M., Ahn, I. P., Alberti, I., Tonti, S., Kim, D. W., Sieber, C. M. K., Humpf, H. U., Yun, S. H., Guldener, U., and Tudzynski, B. 2017. Comparative genomics of geographically distant *Fusarium fujikuroi* isolates revealed two distinct pathotypes correlating with secondary metabolite profiles. PLoS Pathog 13:e1006670.
- O'Donnell, K., Cigelnik, E., and Nirenberg, H. I. 1998b. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. Mycologia 90:465-493.
- O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998a. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. USA 95:2044-2049.
- O'Donnell, K., Nirenberg, H. I., Aoki, T., and Cigelnik, E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. Mycoscience 41:61-78.
- O'Donnell, K., Ward, T. J., Robert, V. A., Crous, P. W., Geiser, D. M., and Kang, S. 2015. DNA sequence-based identification of *Fusarium*: Current status and future directions. Phytoparasitica 43:583-595.
- Palacios, S. A., Susca, A., Haidukowski, M., Stea, G., Cendoya, E., Ramirez, M. L., Chulze, S. N., Farnochi, M. C., Moretti, A., and Torres, A. M. 2015. Genetic variability and fumonisin production by *Fusarium proliferatum* isolated from durum wheat grains in Argentina. Int. J. Food Microbiol. 201: 35-41.
- Proctor, R. H., Desjardins, A. E., Plattner, R. D., and Hohn, T. M. 1999. A polyketide synthase gene required for biosynthesis of fumonisin mycotoxins in *Gibberella fujikuroi* mating population A. Fungal Genet. Biol. 27:100-112.
- Proctor, R. H., Plattner, R. D., Brown, D. W., Seo, J. A., and Lee, Y. W. 2004. Discontinuous distribution of fumonisin biosynthetic genes in the *Gibberella fujikuroi* species complex. Mycol. Res. 108:815-822.
- Proctor, R. H., Van Hove, F., Susca, A., Stea, G., Busman, M., van der Lee, T., Waalwijk, C., Moretti, A., and Ward, T. J. 2013. Birth, death and horizontal transfer of the fumonisin biosynthetic gene cluster during the evolutionary diversification of *Fusarium*. Mol. Microbiol. 90:290-306.
- Qiu, J., Xu, J., Dong, F., Yin, X., and Shi, J. 2015. Isolation and characterization of *Fusarium verticillioides* from maize in eastern China. Eur. J. Plant Pathol. 142: 791-800.
- Rheeder, J. P., Marasas, W. F., and Vismer, H. F. 2002. Production of fumonisin analogs by *Fusarium* species. Appl. Environ. Microbiol. 68:2101-2105.
- Rösler, S. M., Sieber, C. M., Humpf, H. U., and Tudzynski, B. 2016. Interplay between pathway-specific and global regulation of the fumonisin gene cluster in the rice pathogen *Fusarium fujikuroi*. Appl. Microbiol. Biotechnol. 100: 5869-5882.
- Stepień, Ł. 2014. The use of *Fusarium* secondary metabolite biosynthetic genes in chemotypic and phylogenetic studies. Crit. Rev. Microbiol. 40:176-185.
- Stockmann-Juvala, H., and Savolainen, K. 2008. A review of the toxic effects and mechanisms of action of fumonisin B1. Hum. Exp. Toxicol. 27:799-809.
- Suga, H., Arai, M., Fukasawa, E., Motohashi, K., Nakagawa, H., Tateishi, H., Fuji, S. I., Shimizu, M., Kageyama, K., and Hyakumachi, M. 2019. Genetic differentiation associated with fumonisin and gibberellin production in Japanese *Fusarium fujikuroi*. Appl. Environ. Microbiol. 85:e02414-e02418.
- Suga, H., Kitajima, M., Nagumo, R., Tsukiboshi, T., Uegaki, R., Nakajima, T., Kushiro, M., Nakagawa, H., Shimizu, M., Kageyama, K., and Hyakumachi, M. 2014. A single nucleotide polymorphism in the translation elongation factor 1 α gene correlates with the ability to produce fumonisin in Japanese *Fusarium fujikuroi*. Fungal Biol-UK 118:402-412.
- Sultana, S., Kitajima, M., Kobayashi, H., Nakagawa, H., Shimizu, M., Kageyama, K., and Suga, H. 2019. A natural variation of fumonisin gene cluster associated with fumonisin production difference in *Fusarium fujikuroi*. Toxins (Basel) 11: 200.
- Waśkiewicz, A., Stepień, Ł., Wilman, K., and Kachlicki, P. 2013. Diversity of pea-associated *F. proliferatum* and *F. verticillioides* populations revealed by *FUM1* sequence analysis and fumonisin biosynthesis. Toxins (Basel) 5:488-503.
- Wiemann, P., Sieber, C. M., Von Barga, K. W., Studt, L., Niehaus, E. M., Espino, J. J., Huß, K., Michielse, C. B., Albermann, S., Wagner, D., Bergner, S. V., Connolly, L. R., Fischer, A., Reuter, G., Kleigrewe, K., Bald, T., Wingfield, B. D., Ophir, R., Freeman, S., Hippler, M., Smith, K. M., Brown, D. W., Proctor, R. H., Munsterkotter, M., Freitag, M., Humpf, H., Guldener, U., and Tudzynski, B. 2013. Deciphering the cryptic genome: Genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveal complex regulation of secondary metabolism and novel metabolites. PLoS Pathog 9:e1003475.
- Wulff, E. G., Sørensen, J. L., Lübeck, M., Nielsen, K. F., Thrane, U., and Torp, J. 2010. *Fusarium* spp. associated with rice bakanae: Ecology, genetic diversity, pathogenicity and toxigenicity. Environ. Microbiol. 12:649-657.