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Fusarium proliferatum strains change fumonisin biosynthesis and accumulation when exposed to host plant extracts

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ABSTRACT

Fumonisin concentrations in mycelia and media were studied in liquid *Fusarium proliferatum* cultures supplemented with host plant extracts. Furthermore, the kinetics of fumonisin accumulation in media and mycelia collected before and after extract addition was analysed as well as the changes in the expression of the *FUM1* gene. Fumonisin content in culture media increased in almost all *F. proliferatum* strains shortly after plant extracts were added. The asparagus extract induced the highest FB level increase and the garlic extract was the second most effective inducer. Fumonisin level decreased constantly until 14th day of culturing, though for some strains also at day 8th an elevated FB level was observed. Pineapple extract induced the highest increase of *fum1* transcript levels as well as fumonisin synthesis in many strains, and the peas extract inhibited fungal growth and fumonisin biosynthesis. Moreover, fumonisins were accumulated in mycelia of studied strains and in the respective media.

Introduction

Fusarium proliferatum is a polyphagous fungal species able to infect a broad range of agriculturally important crops (Kerényi *et al.* 2002; von Bargen *et al.* 2009; Jurado *et al.* 2010; Stępień *et al.* 2011; Waśkiewicz *et al.* 2013). Moreover, it has been isolated from uncultivated plants, such as reed, sorrel, prairie grasses or pine (Bhale *et al.* 2012). Worldwide geographical distribution and a wide range of hosts serve as proof of the extraordinary adaptation ability of the species to colonize new environments in diverse climatic conditions. Recently, evidence of considerable genetic and phenotypic variance among the species genotypes in relation to its plant origin was described, exhibiting mainly biomass and fumonisin synthesis changes (Stępień *et al.* 2011, 2015). Fumonisins B (FBs) are the prevailing mycotoxins synthesized by *F. proliferatum* (Desjardins 2006). When consumed by animals, FBs were able to cause liver cancer, pulmonary oedema and equine

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leucoencephalomalacia, whereas in human FBs have been correlated to elevated incidence of oesophageal cancer in South Africa (Van Rensburg *et al.* 1985; Wang *et al.* 2005; Odhav *et al.* 2008). In laboratory conditions, the *F. proliferatum* strains originating from asparagus, garlic, and pineapple produced more fumonisins than the strains derived from maize or peas (Stępień *et al.* 2011; Waśkiewicz *et al.* 2013), however, in liquid cultures supplemented with host plant extracts, no similar correlation was observed, as the reaction of individual strains to the plant extract addition was genotype-dependent (Stępień *et al.* 2015).

Intra-specific differences in fumonisin biosynthesis for both *Fusarium verticillioides* and *F. proliferatum* strains were proven in earlier studies, particularly when temperature, humidity, media composition, or even light wavelength, were altered (López-Errasquín *et al.* 2007; Fanelli *et al.* 2012a, 2012b). Similarly, the strains' response to the addition of plant extracts from common hosts of *F. proliferatum*, was variable (Stępień *et al.* 2015). It was hypothesized that distinct metabolic relationships may exist between *F. proliferatum* genotypes inhabiting a particular host and the host. Consequently, the extracts can contain metabolites that act as elicitors inducing specific metabolic reaction of the pathogen – mycotoxin biosynthesis or activation of stress response pathways, like reactive oxygen species or redox pathways (authors' studies, unpubl.). The addition of host plant extracts influenced the overall fumonisin content, presumably also as a consequence of the elevated expression levels of genes from the *FUM* cluster. Transcriptional changes are usually momentary and the secondary metabolites tend to be accumulated, while usually may not be metabolized by the fungus itself (Waśkiewicz *et al.* 2013). Active transport removing the toxin from the organism is one of the possible ways of avoiding their toxic effects (Proctor *et al.* 2003), which makes even more difficult to answer the question why the toxigenic potential is unevenly used by the strains and which factors related to the habitat of the fungus can influence the mycotoxin biosynthesis. In the case of maize, amylopectin could be pointed out as it has already been proven to stimulate the biosynthesis of fumonisins (Bluhm & Woloshuk 2005). For other crop plants hosting *F. proliferatum* (and other pathogens as well), these molecular signals are still to be determined.

The main objectives of the present study were: (i) to reveal the kinetics of fumonisin biosynthesis by *F. proliferatum* strains of different origin in culture media, collected before and after aqueous host plant extracts were added, (ii) to analyse the changes in fumonisin contents in mycelia and media from respective cultures, and (iii) to search for patterns in the changes of the *fum1* transcript levels and fumonisin concentrations in *F. proliferatum* cultures exposed to asparagus, maize, garlic, peas, and pineapple extracts.

Materials and methods

Fungal strains and media

Sixteen *Fusarium proliferatum* genotypes originating from different host plant species were used in the study (Table 1). The strains were characterized genetically and phenotypically during previous studies (Stępień *et al.* 2011, 2013; Waśkiewicz *et al.* 2013; Wilman *et al.* 2014) and they are all stored in the KF pathogenic fungi collection at the Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland. Five main groups of strains, isolated from maize (*Zea mays*), garlic (*Allium sativum*), asparagus (*Asparagus officinalis*), peas (*Pisum sativum*), and pineapple (*Ananas comosus*) were included in the experiments. Additionally, genotypes originating from three different host species (*i.e. Oryza sativa*, *Saccharum officinarum*, and *Phoenix dactylifera*) were used.

Table 1 – *Fusarium proliferatum* strains used in the study along with their host plant species, year of isolation, geographical origin and external collection number, if available.

Isolate	Host plant	Year	Origin
KF 391	*Saccharum officinarum*		India (NRRL 13286)
KF 422	*Oryza sativa*	1973	Taiwan (NRRL 13620)
KF 496	*Zea mays*	1983	Italy (ITEM 382)
KF 925	*Zea mays*	1986	Poland
KF 3301	*Ananas comosus*	2008	Costa Rica
KF 3360	*Asparagus officinalis*	2009	Poland
KF 3362	*Asparagus officinalis*	2009	Poland
KF 3369	*Allium sativum*	2009	Poland
KF 3372	*Allium sativum*	2009	Poland
KF 3385	*Ananas comosus*	2009	Vietnam
KF 3404	*Ananas comosus*	2010	Costa Rica
KF 3416	*Phoenix dactylifera*	2010	Tunisia
KF 3654	*Zea mays*	2011	Poland
KF 3732	*Pisum sativum*	2012	Poland
KF 3738	*Pisum sativum*	2012	Poland
KF 3758	*Pisum sativum*	2012	Poland

Strains of *F. proliferatum* were grown in 100 ml flasks containing 40 ml of slightly modified fumonisin-inducing liquid medium developed for *Fusarium verticillioides* and described by López-Errasquín *et al.* (2007). The medium contained: malt extract 0.5 g l^{-1}, yeast extract 1 g l^{-1}, mycological peptone 1 g l^{-1}, KH_2PO_4 1 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.3 g l^{-1}, KCl 0.3 g l^{-1}, $ZnSO_4 \cdot 7H_2O$ 0.05 g l^{-1}, $CuSO_4 \cdot 5H_2O$ 0.01 g l^{-1}, and D-fructose 20 g l^{-1}. The only modification was lowering the water amount by 20 %. About 4 cm^2 of mycelium harvested of the 7-day-old potato dextrose agar (PDA) plate cultures were used for the inoculation.

Plant extracts preparation

Plant material used for extract preparation included: pineapple fruit, white asparagus spears, garlic bulbs, six-week-old peas plants and young maize cobs. An equivalent amount (50 g of fresh plant tissue per 50 ml of liquid culture) was used for extract preparation procedure (Stępień *et al.* 2015). After freezing overnight at −80 °C and complete thawing plant tissues were homogenized using a blender. Obtained pulp was centrifuged at 6000 ×*g* for 15 min, extracts were filtered through 0.45 μm membrane filters and stored at −20 °C.

Culture conditions and sample collection

Liquid *Fusarium proliferatum* cultures were incubated at 25 °C without shaking at 12 h photoperiod. The extracts were