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Scientific paper

MYCOTOXINS BIOSYNTHESIZED BY PLANT-DERIVED *FUSARIUM* ISOLATES*

Agnieszka WAŚKIEWICZ*, and Łukasz STĘPIEŃ[2]

Department of Chemistry, Poznan University of Life Sciences[1], Department of Metabolomics, Institute of Plant Genetics, Polish Academy of Sciences[2], Poznań, Poland

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There is little information on secondary metabolites produced by *Fusaria* infecting crop plants other than cereals. Many members of *Fusarium* genus have the ability to colonise perennial crops with only scarce infection or disease symptoms or with no symptoms at all while still being detectable. Even in case of such asymptomatic infection, significant mycotoxin contamination of the plant tissues is possible. The aim of this study was to characterise the spectrum of *Fusarium* species isolates obtained from different plant hosts (like asparagus, garlic, pineapple, banana, rhubarb, peppers, rice, maize, wheat, and oncidium) and evaluate their ability to biosynthesize the most common mycotoxins *in vitro*. Among the *F. proliferatum* isolates, up to 57 % of them biosynthesized fumonisins at very high mass fractions, amounting to above 1000 μg g^{-1}, while other *Fusarium* species such as *F. verticillioides*, *F. lactis, F. polyphialydicum*, *F. concentricum*, *F. temperatum,* and *F. fujikuroi* formed fumonisins mostly at much lower level. Only *F. ananatum* and *F. oxysporum* did not produce these toxins. Co-occurrence of FBs with other mycotoxins [moniliformin (MON) and beauvericin (BEA)] was often observed and it was mainly *F. proliferatum* species that formed both mycotoxins (0.4 μg g^{-1} to 41.1 μg g^{-1} BEA and 0.1 μg g^{-1} to 158.5 μg g^{-1} MON).

KEY WORDS: *beauvericin, fumonisins, moniliformin, phylogeny*

Mycotoxins are toxic metabolites produced by certain species of fungi capable of infecting and colonising numerous agricultural crops in most of growth stages in the field as well as during storage. Environmental factors such as temperature and humidity significantly influence the occurrence of these toxins on various plants susceptible to mould infestation (1-3).

Most of the research concerning *Fusarium* species diversity and toxigenicity has focused on cereals for many years (2, 4-6). However, there is little information on secondary metabolites produced by *Fusaria* infecting crop plants other than cereals (7-12). Due to systemic dispersal inside the host plant, many members of the *Fusarium* genus have the ability to colonise perennial crops with only scarce infection or disease symptoms or with no symptoms at all while still being detectable (11, 13). Moreover, even in case of such asymptomatic infection, significant mycotoxin contamination of the plant tissues is possible (7, 14, 15). Different *Fusarium* species can produce a wide range of secondary metabolites, many of which are toxic to humans and animals. Trichothecenes, zearalenone, and fumonisins have been recognized as the most harmful (4, 16, 17). Fumonisin analogues can be classified into four main groups, series FA, FB,

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FC, and FP (18). Among these, toxicologically the most important are FB analogues, which are mainly produced by *Fusarium verticillioides* and *F. proliferatum*. More new fumonisins (19-21) have been detected using liquid chromatography/electrospray ionisation ion-trap multistage mass spectrometry (MS) but only three of them [fumonisin B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3)] seem to occur more frequently (22). Fumonisins (FBs) are structurally similar to sphinganine and they disrupt sphingolipid metabolism by inhibiting ceramide synthase - a process underlying the mechanism of toxicity and the pathogenesis of fumonisin-related diseases (23). They cause different toxicological effects in both humans and animals (24). Feed contaminated with fumonisins has been shown to cause a number of mycotoxicoses, including leukoencephalomalacia in horses, pulmonary oedema in pigs, altered hepatic and immune function in cattle as well as liver cancer and neural tube defects in experimental rodents (25, 26). Fumonisin B_1 is toxic to the liver and kidneys in many species, causing an apoptosis followed by mitosis in the affected tissues. It is also toxic to the cardiovascular system of pigs and horses (27). Epidemiological studies also suggest that fumonisins could be associated with human esophageal cancer in regions where fumonisin-contaminated maize is consumed (28).

Beauvericin (BEA) is a bioactive cyclohexadepsipeptide containing an alternating sequence of three N-methyl L-phenylalanyl and three D-a-hydroxyisovaleryl residues. It has been found to be the main metabolite produced by *Fusarium subglutinans* strains isolated from maize ears worldwide (29). The role of BEA as an important secondary metabolite of phytopathogenic *Fusarium* species was suggested when its production by several strains of *F. proliferatum* isolated from different diseased host plants, including maize, asparagus, and date palm was detected (30-32). This toxin is a specific cholesterol acylotransferase inhibitor and it is toxic to several human cell lines, as it induces apoptosis and DNA fragmentation (4,33). Furthermore, BEA was shown to exert a negative inotropic effect (a decrease in cardiac contraction strength) as well as a negative chronotropic effect (a decrease in the frequency of cardiac spontaneous beating activity) (34). Investigation of the *Fusarium* genus showed that various species produced BEA, including some strains of *F. oxysporum* isolated from maize and melon (35, 36), *F. subglutinans* isolated from maize ears (37), and *F. proliferatum* isolated from maize and asparagus (29).

Moniliformin (MON) is a toxin occurring naturally as the sodium or potassium salt of 3-hydroxycyclobut-3-ene-1,2-dione, which is toxic to plant and animal species (38). MON is produced mostly by *Fusarium subglutinans* but also by other *Fusarium* species: *F. acuminatum*, *F. anthophilum F. avenaceum*, *F. concolor*, *F. denticulatum, F. equiseti*, *F. fujikuroi, F. fusarioides*, *F. moniliforme* (now called *F. verticilioides)*, *F. oxysporum*, *F. proliferatum, F. ramigenum, F. sambucinum, F. semitectum, F. succisae, F. tricinctum,* and *F. thapsinum* (13, 39). The metabolite is also suspected of causing the Kesha's disease, a human myocardial impairment reported in rural areas of China and South Africa (40, 41). The mechanism of acute toxicity of MON is believed to be the inhibition of pyruvate dehydrogenase, i.e. the blockage of entry of pyruvate into Krebs's cycle and a decrease in mitochondrial respiration (42). The occurrence of mycotoxins in plant tissue is affected by several factors, including fungus strain, genetic background of the host, and environmental conditions.

The aim of this study was to characterise the spectrum of *Fusarium* species isolates obtained from different plant hosts (like asparagus, garlic, pineapple, banana, rhubarb, peppers, rice, maize, wheat, and oncidium) and evaluate their ability to biosynthesize the most common mycotoxins *in vitro*.

MATERIALS AND METHODS

Fungal strains

Thirty-four *Fusarium* strains were isolated and purified from ten host species during this study. Pieces of infected plant tissues were plated on potato dextrose agar (PDA) medium. After few days of incubation at room temperature, tips of single hyphae of all *Fusarium* fungi were transferred onto new plates. Obtained fungal strains were passaged onto fresh PDA plates in the same way, at least three times to assure their purity. All isolates are stored in the KF *Fusarium* collection at the Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland. For toxin biosynthesis analyses, rice cultures were used (29, 43).

Genomic DNA extraction, species identification, tef-1α gene sequence analysis and phylogeny reconstruction

Genomic DNA of all isolates was extracted using a hexadecyltrimethylammonium bromide (CTAB) method described in detail previously (44). Species identification was based on the partial sequences analysis of the translation elongation factor 1α (*tef*-1α) gene. PCRs were carried out in 25 μL aliquots in PTC-200 and C-1000 thermal cyclers [Bio-Rad, Hercules, CA, USA] using previously validated EF-728M/TEF-1R primers (10). Each reaction tube consisted of one unit of Platinum HotStart Taq DNA polymerase [Invitrogen, Carlsbad, CA, USA], 2.5 μL of 10x PCR buffer, 12.5 pmol of forward/reverse primers, 2.5 mmol L^{-1} of each dNTP, and about 10 ng to 20 ng of fungal genomic DNA. PCR conditions were as follows: 15 min at 95 °C, 35 cycles of (45 s at 94 °C, 45 s at 63 °C, 1 min at 72 °C) and 5 min at 72 °C. Amplicons were electrophoresed in 1.5 % agarose gels [Invitrogen] with ethidium bromide.

As regards the sequence analysis, PCR-amplified DNA fragments were purified with exonuclease I [Epicentre, Madison, WI, USA] and shrimp alkaline phosphatase [Promega, Madison, WI, USA] using the following programme: 30 min at 37 °C and 15 min at 80 °C. Both strands were labelled using BigDyeTerminator 3.1 kit [Applied Biosystems, Foster City, CA, USA], in line with Stępień et al. (10) and precipitated with ethanol. Sequence reading was carried out using Applied Biosystems equipment. BLASTn algorithm was used for species identification confirmation. For phylogenetic analysis, the sequences of the PCR products were initially aligned with ClustalW algorithm. The following GenBank sequences were included as references: HE802668.1 (*F. ananatum*), AF333935.1 (*F. concentricum*), JN675675.1 (*F. fujikuroi*), JF715934.1 (*F. incarnatum*), FR870283.1 (*F. lactis*), NRRL34936 (*F. oxysporum* f.sp. *lycopersic*i), GQ425229.1 (*F. polyphialidicum*), EU220408.1 (*F. proliferatum*), HM067690.1 (*F. temperatum*), and FGSC7600 (*F. verticillioides*). *A.niger* CBS513.88 was used as an outgroup. Phylogenetic relationships were reconstructed with MEGA5 software package (45) using maximum parsimony approach (Closest Neighbor Interchange heuristics) and tested by bootstrapping with 1000 replicates.

Chemicals and reagents

Standards of pure MON, BEA, FB_1, FB_2, and FB_3, were purchased from Sigma (St. Louis, MO, USA). Acetonitrile, methanol (HPLC grade), disodium tetraborate, 2-mercaptoethanol, and *t*-butyl-ammonium hydroxide were purchased from Sigma-Aldrich. Potassium hydroxide, sodium dihydrogen phosphate, acetic acid, *n*-hexane, *o*-phosphoric acid, and dichloromethane were purchased from POCh (Poland). Water for the HPLC mobile phase was purified using a Milli-Q system (Milipore, Bedford, MA, USA).

Mycotoxin analyses

Fumonisins

Samples (5 g) of dry two-week-old cultures were homogenised for 3 min in 10 mL of methanol:water (3:1) and filtered through Whatman No. 4 filter paper. The extract was adjusted to pH 5.8 to 6.3 using 0.1 mol L^{-1} KOH. A SAX cartridge was attached to the solid-phase extraction (SPE) manifold unit (Supelco, Bellefonte, PA, USA) following the method described by Waśkiewicz et al. (13). The *o*-phosphoric acid (OPA) reagent (20 mg per 0.5 mL of methanol) was prepared and diluted with 2.5 mL of 0.1 mol L^{-1} disodium tetraborate ($Na_2B_4O_7$ x 10 H_2O). It was then combined with 25 mL 2-mercaptoethanol, which was added to the solution. The FBs standards (5 mL) or extracts (20 mL) were derivatised with 20 mL or 80 mL of the OPA reagent. The reaction mixture (10 mL) was injected onto an HPLC column 3 min later. After filtration through a 0.45 mm Waters HV membrane, methanol–sodium dihydrogen phosphate (0.1 mol L^{-1} in water) solution (77:23), adjusted to pH 3.35 with *o*-phosphoric acid, was used as the mobile phase with a flow rate of 0.6 mL min^{-1}. A Waters 2695 apparatus (Waters Division of Millipore, Milford, MA, USA), with an X-Bridge column (3.9 mmx100 mm) and a Waters 2475 fluorescence detector (l_{ex}=335 nm, l_{em}=440 nm) were used for determining the quantity of metabolites. The detection limit was 10 ng g^{-1} for FBs. HPLC analysis and the comparison with the relevant calibration curve (correlation coefficients for FB_1, FB_2, and FB_3 were 0.9967, 0.9991, and 0.9956, respectively) confirmed positive results (on the basis of retention time). Recoveries for fumonisins were 93 %, 97 %, and 87 %, respectively, which were measured in triplicate by extracting the mycotoxins from blank samples spiked with 10 ng g^{-1} to 100 ng g^{-1} of the compound. The relative standard deviations (RSD) were below 8 %.

Beauvericin and moniliformin

Culture samples (15 g) of each strain were homogenised with 75 mL of acetonitrile:methanol: water (16:3:1) and filtered (Whatman No. 4 filter

paper). The filtrate was defatted twice with 25 mL of heptane and the bottom layer was evaporated to dryness. Then, the residue was dissolved in 50 mL of methanol:water (55:45) and extracted twice with 25 mL of dichloromethane. The aqueous, bottom phase (containing MON) was concentrated to 1 mL, while the CH_2Cl_2 phase (containing BEA) was evaporated to dryness and purified according to the method described by Kostecki et al. (43). MON was quantified using a Waters 2695 apparatus (Waters Division of Millipore, Milford, MA, USA), with a C_{18} Nova Pak column (3000 mm x 3.9 mm) and a Waters 2996 Photodiode Array Detector (λ_{max}=229 nm). Acetonitrile: water (15:85) buffered with 10 mL 0.1 mol L^{-1} K_2HPO_4 in 40 % *t*-buthyl-ammonium hydroxide in 1 L of solvent was used as the mobile phase (flow rate - 0.6 mL min^{-1}). The MON detection limit was 25 ng g^{-1}. BEA was quantified using a Waters 2695 apparatus with a C_{18} Nova Pak column (150 mm x 3.9 mm) and a Waters 2996 Photodiode Array Detector (Waters) set at 205 nm. HPLC conditions included a constant flow rate of 0.6 mL min^{-1} and the mobile phase consisted of acetonitrile:water (85:15). The detection limit for BEA was 10 ng g^{-1}. HPLC analysis and the comparison with the relevant calibration curve (correlation coefficients for MON and BEA were 0.9990 and 0.9997, respectively) confirmed positive results (on the basis of retention time). Recoveries for MON and BEA were 90 % and 92 %, respectively, which were measured in triplicate by extracting the mycotoxins from blank samples spiked with 10 ng g^{-1} to 100 ng g^{-1} of the compounds. The RSD was below 7 % for MON and below 6 % for BEA.

RESULTS

Thirty-four isolates were purified from plant tissues of ten species of plants: *Ananas comosus, Asparagus officinalis, Allium sativum, Capsicum annuum, Musa sapientum, Oncidium* sp., *Oryza sativa, Rheum rhabarbarum, Triticum aestivum,* and *Zea mays* (Table 1). Most of the host species represent agriculturally important crops cultivated worldwide regardless of the climatic conditions. This fact confirms once again the cosmopolitism of *Fusarium* pathogens and their ability to colonise wide range of hosts. Using genomic DNA extracted from the purified mycelia, molecular identification of the pathogenic species was performed, based on the BLASTn analysis of the *tef*-1α gene sequences and their comparison with sequences deposited in NCBI GenBank database. *F. proliferatum* isolates were isolated with the highest frequency (Table 1). Based on those sequences a maximum parsimony dendrogram was calculated in order to show the most possible phylogenetic relationships among the genotypes. The analysis was supplemented with type strains GenBank sequences of the ten species identified in the study. *Aspergillus niger* CBS513.88 strain was included in the analysis as an outgroup showing the divergence of the sequence region analysed. Also, KF 497 wheat-derived isolate

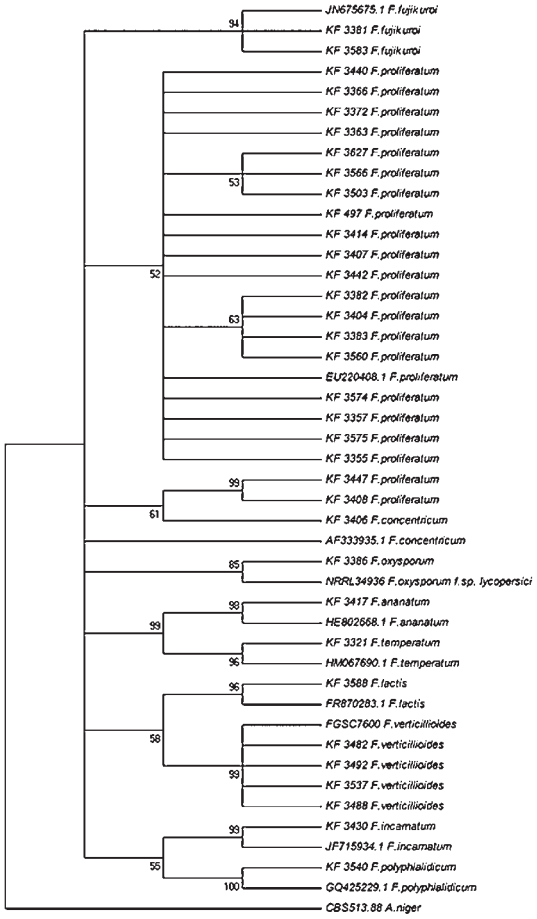

Figure 1 *The most parsimonious tree for 34 isolates of ten* Fusarium *species, based on the translation elongation factor 1α (tef-1α) sequences. GenBank sequences were included for reference: HE802668.1* (F. ananatum)*, AF333935.1 (*F. concentricum*), JN675675.1* (F. fujikuroi)*, JF715934.1* (F. incarnatum)*, FR870283.1* (F. lactis)*, NRRL34936 (*F. oxysporum *f.sp. lycopersici), GQ425229.1* (F. polyphialidicum)*, EU220408.1 (F. proliferatum), HM067690.1* (F. temperatum)*, and FGSC7600* (F. verticillioides). A.niger *CBS513.88 was used as an outgroup. The maximum parsimony approach and bootstrapping (1000 replicates) test were used and the cut-off value was set at 50 %. Of 376 bases analysed, 124 were parsimony informative. Consistency and retention indices for the most parsimonious tree were CI = 0.713499 and RI = 0.765766, respectively.*

Table 1 *Isolates used in this study with their host plant species and year of acquisition*

Isolate	*Fusarium* species	Host	Origin	Year of isolation
KF 3417	*F. ananatum*	pineapple	Ecuador	2010
KF 3406	*F. concentricum*	pineapple	Costa Rica	2009
KF 3381	*F. fujikuroi*	pineapple	Hawaii	2009
KF 3583	*F. fujikuroi*	rice	Italy	2011
KF 3430	*F. incarnatum*	banana	Ecuador	2010
KF 3588	*F. lactis*	peppers	Poland	2011
KF 3386	*F. oxysporum*	pineapple	Vietnam	2009
KF 3540	*F. polyphialidicum*	pineapple	Costa Rica	2010
KF 3355*	*F. proliferatum*	asparagus	Poland	2009
KF 3357*	*F. proliferatum*	asparagus	Poland	2009
KF 3363*	*F. proliferatum*	garlic	Poland	2009
KF 3366*	*F. proliferatum*	garlic	Poland	2009
KF 3372*	*F. proliferatum*	garlic	Poland	2009
KF 3382	*F. proliferatum*	pineapple	Hawaii	2009
KF 3383*	*F. proliferatum*	pineapple	Hawaii	2009
KF 3404*	*F. proliferatum*	pineapple	Costa Rica	2009
KF 3407*	*F. proliferatum*	pineapple	Costa Rica	2009
KF 3408	*F. proliferatum*	pineapple	Costa Rica	2009
KF 3414	*F. proliferatum*	pineapple	Honduras	2010
KF 3440*	*F. proliferatum*	maize	Poland	2006
KF 3442*	*F. proliferatum*	maize	Poland	2006
KF 3447	*F. proliferatum*	oncidium	n/a	2010
KF 3503*	*F. proliferatum*	garlic	Poland	2011
KF 3560	*F. proliferatum*	rhubarb	Poland	2011
KF 3566	*F. proliferatum*	rice	Thailand	2011
KF 3574	*F. proliferatum*	asparagus	Poland	2011
KF 3575	*F. proliferatum*	asparagus	Poland	2011
KF 3627	*F. proliferatum*	rice	Thailand	2011
KF 497	*F. proliferatum*	wheat	Portugal	1987
KF 3321	*F. temperatum*	pineapple	Costa Rica	2008
KF 3537	*F. verticillioides*	pineapple	Costa Rica	2010
KF 3492	*F. verticillioides*	maize	Poland	2010
KF 3482	*F. verticillioides*	maize	Poland	2010
KF 3488	*F. verticillioides*	maize	Poland	2010

** strains already included in Stępień et al. (11)*

of *F. proliferatum* was analysed as an example of collection strain after long term storage and passaging (Figure 1 and Table 1). Twenty-one isolates of *F. proliferatum* were purified from eight plant host species. Only *F. oxysporum* could be regarded as the species infecting such a wide host species range (authors' results, unpublished).

Individual isolate cultures were used for mycotoxin biosynthesis evaluation. Two-week-old rice cultures were used for mycotoxin extraction procedure (see Materials and Methods section). The comparison of the isolates tested showed a great variation in the amount of mycotoxins produced (fumonisins, beauvericin, and moniliformin). Rice cultures were successfully used to show the potential of the *Fusarium* genotypes to synthesize FBs (Table 2). However, the efficacy of BEA and MON synthesis on rice medium was significantly lower (Table 3).

Among the *F. proliferatum* isolates from different hosts (maize, rice, wheat, garlic, asparagus, pineapple, rhubarb, and oncidium), up to 57 % of them biosynthesized fumonisins at very high mass fractions, amounting to above 1000 $\mu g\ g^{-1}$ (in the range of 1186.9 $\mu g\ g^{-1}$ to 3299.0 $\mu g\ g^{-1}$). Other isolates of *F.*

Table 2 *Mass fractions of fumonisins B_1-B_3 (μg g^{-1}) produced* in vitro *by* Fusarium *isolates of ten species*

	***Fusarium* species**	**FB_1**	**FB_2**	**FB_3**
KF 3417	*F. ananatum*	0.0	0.0	0.0
KF 3406	*F. concentricum*	10.4	0.0	0.3
KF 3381	*F. fujikuroi*	1559.0	466.0	81.6
KF 3583	*F. fujikuroi*	6.97	3,98	0.78
KF 3430	*F. incarnatum*	4.8	1.5	0.4
KF 3588	*F. lactis*	11.74	0,26	ND
KF 3386	*F. oxysporum*	0.0	0.0	0.0
KF 3540	*F. polyphialydicum*	18.9	4.3	1.2
KF 3407	*F. proliferatum*	2419.3	379.9	139.8
KF 3404	*F. proliferatum*	856.4	330.0	109.1
KF 3560	*F. proliferatum*	797.9	103.4	60.8
KF 3408	*F. proliferatum*	2686.2	757.9	367.8
KF 3447	*F. proliferatum*	393.4	124.2	57.0
KF 3503	*F. proliferatum*	1186.9	185.5	66.2
KF 3382	*F. proliferatum*	1785.8	450.5	132.9
KF 3414	*F. proliferatum*	3299.0	855.5	593.1
KF 3442	*F. proliferatum*	1414.73	288.37	75.72
KF 497	*F. proliferatum*	3.3	0.8	0.1
KF 3355	*F. proliferatum*	1235.0	501.2	119.1
KF 3357	*F. proliferatum*	1536.0	657.1	123.7
KF 3363	*F. proliferatum*	1732.0	789.0	139.0
KF 3366	*F. proliferatum*	1810.0	188.0	90.0
KF 3372	*F. proliferatum*	408.8	108.1	76.1
KF 3440	*F. proliferatum*	840.5	402.0	84.9
KF 3383	*F. proliferatum*	930. 0	204.0	79. 0
KF 3566	*F. proliferatum*	995.54	148.37	41.33
KF 3575	*F. proliferatum*	2604.67	1412.44	273.63
KF 3574	*F. proliferatum*	1720.36	862.21	212.51
KF 3627	*F. proliferatum*	118.95	10.32	1.76
KF 3321	*F. temperatum*	2.23	0.4	0.1
KF 3537	*F. verticillioides*	59.7	19.4	5.9
KF 3492	*F. verticillioides*	712.93	198.47	15.89
KF 3482	*F. verticillioides*	273.38	60.35	0.88
KF 3488	*F. verticillioides*	39.77	0.96	0,06

proliferatum formed FBs at lower levels (between 118.9 μg g^{-1} and 995.5 μg g^{-1} of FB_1). The collection strain (KF 497) displayed the lowest ability to biosynthesize FBs (3.3 μg g^{-1} of FB_1). Other *Fusarium* species such as *F. verticillioides*, *F. lactis, F. polyphialydicum*, *F. concentricum*, *F. temperatum,* and *F. fujikuroi* formed fumonisins mostly at much lower level concentrations. Only *F. ananatum* and *F. oxysporum* did not produce these toxins.

Co-occurrence of FBs with other mycotoxins (moniliformin and beauvericin) was often observed. In the present study, 15 isolates showed to possess the ability of simultaneous biosynthesis of moniliformin and beauvericin (Table 3). *F. proliferatum* species frequently formed both mycotoxins (0.4 μg g^{-1} to 41.1 μg g^{-1} - BEA and 0.1 μg g^{-1} to 158.5 μg g^{-1} - MON). In other species such as *F. atanatum*, *F. concentricum*, *F. fujikuroi*, *F. oxysporum*, *F. polyphialydicum,* and *F. verticillioides,* mycotoxin levels were lower than for *F. proliferatum* isolates.

DISCUSSION

Thirty-four isolates of ten *Fusarium* species were obtained from ten various host species (Table 1). In many cases, there were no visible symptoms of

Table 3 *Mass fractions of beauvericin (BEA) and moniliformin (MON) produced* in vitro *by* Fusarium *isolates of eight species.*

Isolate	*Fusarium* species	BEA / µg g^{-1}	MON / µg g^{-1}
KF 3417	*F. ananatum*	4.7	0.0
KF 3406	*F. concentricum*	0.5	30.0
KF 3381	*F. fujikuroi*	1.7	27.6
KF 3386	*F. oxysporum*	91.5	0.0
KF 3540	*F. polyphialydicum*	6.9	0.0
KF 3407	*F. proliferatum*	4.2	81.7
KF 3404	*F. proliferatum*	4.0	18.4
KF 3408	*F. proliferatum*	0.4	27.7
KF 3447	*F. proliferatum*	1.1	54.2
KF 3503	*F. proliferatum*	0.9	0.1
KF 3382	*F. proliferatum*	3.4	18.4
KF 3414	*F. proliferatum*	41.1	16.0
KF 3383	*F. proliferatum*	1.1	158.5
KF 3321	*F. temperatum*	12.5	7.5
KF 3537	*F. verticillioides*	0.1	0.1

infection, which constricted the recognition of pathogenesis. Thus, some of the strains isolated during this study could be regarded as endophyte-like. Nevertheless, even when they displayed moderate or low aggressiveness, they were still able to synthesize toxic metabolites inside plant tissues (3, 13, 46). Several reports concerning pathogenicity of *Fusarium* species associated with onion were presented by Bayraktar et al. (47), Cramer (48), Dissanayake et al. (49-51) Galvan et al. (52), and Stankovic et al. (9); the most prevalent species were *F. oxysporum* and *F. proliferatum,* followed by *F. avenaceum*, *F. culmorum*, *F. verticillioides*, and *F. solani*. In most cases *F. proliferatum* biosynthesized mycotoxins such as fumonisins, moniliformin, beauvericin, and fusaproliferin (9). In turn, Ducan et al. (53), Stankovic et al. (9), and Sankar et al. (54) investigated *F. proliferatum* and mycotoxins biosynthesis in garlic. In other studies, *F. subglutinans* (55) and *F. ananatum* (56) were identified as the causes of fusariosis in pineapple, *F. oxysporum* and *F. proliferatum* (3, 13, 47, 57) in asparagus, and *F. oxysporum* (58) in banana. Therefore, it is important to recognize the fungal species present inside the plant being investigated and also to evaluate the toxigenic potential of the potential plant pathogens. Molecular identification of fungal pathogens has been successfully used as a complementary method to morphological identification using optical microscope (6, 44). Among many others, the translation elongation factor 1α (*tef*-1α) has been proven to be so far the most versatile and useful phylogenetic marker (8, 10, 59). The BLASTn analysis of this divergent region of 34 isolates studied helped to identify ten *Fusarium* species (results not shown). Multiple alignment of *tef*-1α amplified fragments using CLUSTAL W algorithm revealed to some extent the interspecific divergence of the *Fusarium* genus members (Figure 1). It was also possible to detect some intraspecific polymorphism among *F. proliferatum* isolates, as it has been already postulated previously (11). Similarly, it was described that the genotypes belonging to two or more isolated populations of the same species can be distinguished from each other on the basis of *tef*-1α gene analysis (8,12). It is possible that such a high level of polymorphism among genotypes is not unusual and could also have been found elsewhere, e.g. if larger populations of *F. verticillioides* or *F. oxysporum* had been tested.

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Sažetak

TVORBA MIKOTOKSINA U VRSTAMA RODA *FUSARIUM* IZOLIRANIM IZ BILJAKA

Podaci o sekundarnim metabolitima, proizvedenim od plijesni roda *Fusarium* koje inficiraju druge usjeve osim žitarica, vrlo su oskudni. Premda mnogi članovi roda *Fusarium* koloniziraju višegodišnje usjeve izazivajući tek oskudnu infekciju, slabe simptome bolesti, a katkad vidljivi simptomi bolesti sasvim izostanu, čak se i u slučaju takve asimptomatske infekcije može otkriti značajna kontaminacija biljnih tkiva mikotoksinima. Cilj ovog istraživanja bio je karakterizirati spektar vrsta roda *Fusarium* izoliranih iz različitih biljnih domaćina (poput šparoga, češnjaka, ananasa, banana, rabarbare, paprike, riže, kukuruza, pšenice i orhideje) te ocijeniti njihovu sposobnost tvorbe najčešćih mikotoksina u uvjetima *in vitro*. Utvrdili smo da 57 % izolata *F. proliferatum* može tvoriti fumonizine s vrlo visokim masenim udjelima, višim od 1000 μg g^{-1}, dok druge vrste roda *Fusarium*, poput *F. verticillioides*, *F. lactis, F. polyphialydicum*, *F. concentricum*, *F. temperatum* i *F. fujikuroi* stvaraju fumonizine s nižim masenim udjelima. Jedino *F. ananatum* i *F. oxysporum* ne proizvode spomenute toksine. Nerijetko je zamijećena istodobna pojava fumonizina s ostalim mikotoksinima [moniliformin (MON) i beauvericin (BEA)], a najčešći proizvođač obaju mikotoksina bila je vrsta *F. proliferatum* (0,4 μg g^{-1} do 41,1 μg g^{-1} BEA i 0,1 μg g^{-1} do 158,5 μg g^{-1} MON).

KLJUČNE RIJEČI: *beauvericin, filogenija, fumonizini, moniliformin*

CORRESPONDING AUTHOR:

Agnieszka Waśkiewicz
Department of Chemistry
Poznan University of Life Sciences
Wojska Polskiego 75, 60-625 Poznań, Poland
E-mail: *agat@up.poznan.pl*