International Journal of Food Microbiology 225 (2016) 27–37

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Four-locus phylogeny of *Fusarium avenaceum* and related species and their species-specific identification based on partial phosphate permease gene sequences

Alexander A. Stakheev *, Dina R. Khairulina, Sergey K. Zavriev

M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Miklukho-Maklaya st., 16/10, Moscow 117997, Russia

ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 1 February 2016
Accepted 15 February 2016
Available online 22 February 2016

Keywords:
Fusarium spp.
PHO
Phylogenetic analysis
Species-specific identification
Quantitative PCR

ABSTRACT

The fungus *Fusarium avenaceum* and its closest relatives are responsible for contamination of agricultural plants and their products by mycotoxins such as enniatins and moniliformin. Precise identification of mycotoxin producers is necessary for estimation of the accumulation risk of those compounds and for preventing the consumption of highly contaminated products. Nucleic acids amplification-based techniques proved to be the most rapid and reliable approach for pathogen diagnostics and identification. In this study partial phosphate permease gene (*PHO*) sequences were determined for *Fusarium avenaceum* (including one isolate identified as *F. arthrosporioides*), *F. tricinctum*, *F. acuminatum* and *F. torulosum*. Phylogenetic analysis of 40 isolates of those species from different climates and geographical regions of Russia and some neighboring countries based on sequences of *PHO*, translation elongation factor 1 alpha (*TEF1α*), beta-tubulin (*β-TUB*), enniatin synthetase (*Esyn1*) genes and combined data set demonstrated that the *PHO* gene possesses the highest rate of variability among them and can be considered as an informative marker for phylogenetic studies of these species. According to the combined data set phylogeny, the isolates of each species formed clusters with a high bootstrap support. Analysis of *PHO* sequences revealed a high intraspecific variability of *F. avenaceum*: there were 5 independent clusters on the dendrogram, including one cluster which was closer to *F. torulosum* than to other *F. avenaceum* isolates. Variable sites in *PHO* sequences have been used for the design of species-specific primers and a fluorescent hydrolysis probe. The specificity of the assay was shown for DNA samples extracted from 68 isolates of 23 *Fusarium* species. Quantitative PCR approach was applied to estimate the contamination rate of 17 naturally infected oat and barley samples, previously characterized by microbiological procedures.

1. Introduction

Fusarium fungi are dangerous plant pathogens affecting agricultural plants all around the world. In addition to decrease in the quality and quantity of harvest, *Fusarium* infection can lead to accumulation of wide range of mycotoxins — fungal secondary metabolites which are hazardous for humans and livestock. *Fusarium avenaceum*, together with *F. graminearum*, *F. culmorum*, *F. sporotrichioides*, and *F. poae* is one of the main pathogens causing head blight, crown and root rot of most cereals (Logrieco et al., 2003; Parry et al., 1995; Somma et al., 2010; Yli-Mattila et al., 2002a). *F. avenaceum* has a worldwide distribution: in particular, it is dominant in the complex of pathogens that cause *Fusarium* head blight (FHB) throughout the regions with cool and wet climate, such as Northern, Central Europe and Canada (Bottalico and Perrone, 2002; Jestoi et al., 2004; Uhlig et al., 2007; Yli-Mattila et al., 2004b). The species also has been reported as a common soil saprophyte and plant pathogen from temperate and warm areas (Joffe and Palti, 1977; Spanic et al., 2010). The closest relatives of *F. avenaceum* are usually less widespread and relatively weak pathogens *F. arthrosporioides*, *F. tricinctum*, *F. acuminatum* and *F. torulosum* — species which are found in regions with cool or temperate climate (Andersen et al., 1996; Booth, 1971; Kosiak et al., 2003; Marín et al., 2012). In Russia *F. avenaceum* is the most common *Fusarium* species in northwestern regions (together with *F. arthrosporioides*), western and eastern Siberia (together with *F. acuminatum*) and Far East (Ivaschenko et al., 2000; Yli-Mattila et al., 2004b; Yli-Mattila, 2010). *F. tricinctum* is distributed throughout the country, but the rate of grain contamination with this fungus is usually relatively low. *F. torulosum* had not been found in Russia until 2006, when the first isolate (90604, see Table 1) was identified in the Leningrad region (Gagkaeva et al., 2012). These five species are characterized by full absence or low frequency of sexual stage occurrence as well as by generally slow growth on potato dextrose agar (PDA) (Gerlach and Nirenberg, 1982; Leslie and Summerell, 2006). They are known as producers of mycotoxins such as enniatins (ENNs) and moniliformin (MON) (Covarelli et al., 2015; Jestoi et al., 2004;

* Corresponding author.
E-mail address: stakheev.aa@gmail.com (A.A. Stakheev).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.02.012
0168-1605/

Table 1
Single-spore *Fusarium* isolates used.

№	Collection number	*Fusarium* spp.	Origin	Host plant
1	74005*	*F. avenaceum*	Krasnodar region	Wheat
2	42301*	*F. avenaceum*	North Ossetia	Wheat
3	91205*	*F. avenaceum*	Leningrad region	Barley
4	K-0238**	*F. avenaceum*	Kirov region	Wheat
5	103100*	*F. avenaceum*	Kaliningrad region	Oat
6	114003*	*F. avenaceum*	Novgorod region	Oat
7	116504*	*F. avenaceum*	Kirov region	Barley
8	70585*	*F. avenaceum*	Leningrad region	Cock's-foot
9	80212*	*F. avenaceum*	Khabarovsk region	Oat
10	80310*	*F. avenaceum*	Khabarovsk region	Oat
11	93401*	*F. avenaceum*	Leningrad region	Barley
12	108802*	*F. avenaceum*	Pskov region	Barley
13	109902*	*F. avenaceum*	Vologda region	Wheat
14	108701*	*F. avenaceum*	Pskov region	Barley
15	114605*	*F. avenaceum*	Kaliningrad region	Barley
16	119913*	*F. avenaceum*	Kirov region	Oat
17	120015*	*F. avenaceum*	Kirov region	Oat
18	58901*	*F. avenaceum*	Moscow region	Wheat
19	59402*	*F. avenaceum*	Moscow region	Wheat
20	111502*	*F. avenaceum*	Vologda region	Barley
21	112804*	*F. avenaceum*	Novgorod region	Barley
22	115014*	*F. avenaceum*	Kaliningrad region	Barley
23	118902*	*F. avenaceum*	Kirov region	Oat
24	F-132***	*F. avenaceum*	Moscow region	Wheat
25	F-825***	*F. avenaceum*	Unknown	Unknown
26	F-843***	*F. avenaceum*	Sakhalin island	Pine
27	F-1242***	*F. avenaceum*	Moscow region	Wheat
28	F-2307***	*F. avenaceum*	Germany	Wheat
29	F-2302***	*F. arthrosporioides*	Unknown	Unknown
30	30141*	*F. tricinctum*	Finland	Barley
31	50010*	*F. tricinctum*	Finland	Wheat
32	70524*	*F. tricinctum*	Leningrad region	Timothy-grass
33	F-2318***	*F. tricinctum*	Unknown	Unknown
34	F-2319***	*F. tricinctum*	Unknown	Unknown
35	80550*	*F. acuminatum*	Buryatia	Chamomile
36	96800*	*F. acuminatum*	North Ossetia	Wheat
37	131802*	*F. acuminatum*	Irkutsk region	Oat
38	90604*	*F. torulosum*	Leningrad region	Barley
39	MM-0035**	*F. torulosum*	Moscow region	Wheat
40	F-1178***	*F. torulosum*	Portugal	*Pseudotsuga* sp.
41	41806*	*F. graminearum*	North Ossetia	Wheat
42	58033*	*F. graminearum*	Leningrad region	Barley
43	58212*	*F. graminearum*	Far-East region	Wheat
44	58801*	*F. culmorum*	Moscow region	Wheat
45	70505*	*F. culmorum*	Belarus	Wheat
46	74007*	*F. culmorum*	Arkhangelsk region	Potato
47	64722*	*F. cerealis*	Khabarovsk region	Wheat
48	37032*	*F. cerealis*	Harbin, China	Wheat
49	39142	*F. cerealis*	Harbin, China	Wheat
50	33100*	*F. sporotrichioides*	Far-East region	Wheat
51	74006*	*F. sporotrichioides*	Leningrad region	Barley
52	MM-7**	*F. sambucinum*	Moscow region	Wheat
53	55201*	*F. langsethiae*	Kaliningrad region	Oat
54	47401*	*F. poae*	Moscow region	Wheat
55	61701*	*F. poae*	Saratov region	Wheat
56	64414*	*F. equiseti*	Kaliningrad region	Barley
57	HTiMi**	*F. solani*	Unknown	Unknown
58	F-847***	*F. solani*	Kherson region, Ukraine	Cotton
59	F-3260***	*F. concolor*	Montevideo, Uruguay	Barley
60	F-832***	*F. decemcellulare*	Germany	Unknown
61	F-136***	*F. fujikuroi*	Japan	Asian rice
62	F-133***	*F. heterosporum*	Unknown	Unknown
63	F-2811***	*F. lateritium*	Moscow region	Unknown
64	F-845***	*F. oxysporum*	Kherson region, Ukraine	Maize
65	F-3481***	*F. redolens*	Moscow region	Lucerne
66	F-673***	*F. verticillioides*	Hungary	Unknown
67	F-674***	*F. verticillioides*	USA	Unknown
68	58514*	*F. venenatum*	Leningrad region	Oat

Jestoi, 2008). Some authors reported the ability of these species to produce beauvericin (Logrieco et al., 2002; Morrison et al., 2002) but more recent studies did not confirm this fact (Kokkonen et al., 2010; Yli-Mattila et al., 2006). Enniatins (ENNs) are cyclic hexadepsipeptides acting as ion channels which are able to cause ionic imbalance in cells, leading to DNA fragmentation and apoptosis (Kamyar et al., 2004; Kouri et al., 2003; Macchia et al., 2002). Toxic effects of these compounds to different cell lines as well as their insecticidal, antibiotic and phytotoxic activities have also been shown (Ferrer et al., 2009; Fornelli et al., 2004; Ivanova et al., 2006; Logrieco et al., 2002). Moniliformin is an inhibitor of pyruvate dehydrogenase and other thiamine pyrophosphate depending enzymes (Uhlig et al., 2007). It is reported to reduce immune function and cause molecular weakness and acidosis in poultry (Harvey et al., 2002). All of these species do not produce trichothecenes due to the absence of genes essential for biosynthesis of these compounds (Tan and Niessen, 2003).

Non-ambiguous taxonomic identification of *Fusarium* species using morphological and physiological characters is often very complicated because of their high similarity. For instance, it is almost impossible to distinguish *F. avenaceum* and *F. arthrosporioides* using morphological characters and these two species are often considered to be single species *F. avenaceum* (Leslie and Summerell, 2006; Nelson et al., 1983). Moreover, *F. avenaceum* is often confused with *F. acuminatum* (Harrow et al., 2010; Leslie and Summerell, 2006), *F. tricinctum* can be misidentified as *F. sporotrichioides*, *F. poae*, or *F. chlamydosporum* (Leslie and Summerell, 2006; Stakheev et al., 2011), and *F. torulosum* is similar to *F. culmorum* and *F. sambucinum* (Gagkaeva et al., 2012). Taxonomic systems based on morphology also are contradictory. Gerlach and Nirenberg (1982) placed *F. avenaceum* in the section *Roseum*, whereas *F. tricinctum*, *F. torulosum*, and *F. acuminatum* belong to the sections *Sporotrichiella*, *Discolor*, and *Gibbosum*, respectively. At the same time the *Sporotrichiella* section contains such species as *F. sporotrichioides* and *F. langsethiae*, which are trichothecene producers and do not produce enniatins. According to the profiles of the produced mycotoxins, *F. tricinctum* is closer to *F. avenaceum* and related species (Burmeister and Plattner, 1987; Chelkowski et al., 1990; Jestoi, 2008; Kokkonen et al., 2010).

Modern *Fusarium* taxonomy increasingly uses data of crossing experiments (biological species concept) and genetic polymorphism analysis (phylogenetic species concept). The use of DNA-based techniques for the taxonomic studies has led to establishment of a number of new *Fusarium* species which are difficult or impossible to identify morphologically (Nirenberg and O'Donnell, 1998; O'Donnell et al., 2004; Yli-Mattila et al., 2011). It is noteworthy that application of DNA markers makes it possible to reveal intraspecific relationships. Several DNA loci, such as translation elongation factor 1 alpha (Geiser et al., 2004; Knutsen et al., 2004; Kristensen et al., 2005), beta-tubulin (Yli-Mattila et al., 2002b, 2004a), intergenic spacer region of the ribosomal RNA gene (*IGS*, Llorens et al., 2006), sterol 14 α-demethylase (*CYP51C*, Fernández-Ortuño et al., 2010), and aminoadipate reductase (Watanabe et al., 2011) proved to be highly polymorphic and suitable for evolutionary studies of the genus and describing species boundaries. Another promising target used for phylogenetic analysis of enniatin-producing *Fusarium* species is enniatin synthetase gene (Stępień and Waśkiewicz, 2013). There were several studies of intraspecific diversity of *F. avenaceum* (Kulik et al., 2011b; Nalim et al., 2009) and its relationship with other species (Harrow et al., 2010; Yli-Mattila et al., 2002b, 2004b). At the same time, there is little information on genetic variation between isolates of *F. avenaceum* and closest relatives from Russia, especially Siberia and Far East. Moreover, current phylogenetic studies usually rely on sequences of relatively few genes and accuracy of revealed relationships can be challenged.

Polymorphism of the marker genes has been used successfully to design primers for the identification of FHB agents and mycotoxin producers (Jurado et al., 2006; Niessen and Vogel, 1998; Nitschke et al., 2009; Ryazantsev et al., 2008) and quantification of fungal biomass by qPCR (Fernández-Ortuño et al., 2013; Fredlund et al., 2008; Nicolaisen et al., 2009; Waalwijk et al., 2004). However, there were difficulties in developing a system to differentiate *F. avenaceum* from related species.