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Morphological, chemical and molecular differentiation of *Fusarium equiseti* isolated from Norwegian cereals

Elzbieta Barbara Kosiak[a,*], Arne Holst-Jensen[a], Thomas Rundberget[a], Maria Teresa Gonzalez Jaen[b], Mona Torp[a]

[a]*National Veterinary Institute, Department of Feed and Food Hygiene, P.O. Box 8156 Dep., N-0033 Oslo, Norway*
[b]*Departamento de Genetica Facultad de Biologia Universidad Complutense de Madrid Jose Antonio Novais, 2, 28040-Madrid, Spain*

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Abstract

The morphological variation, secondary metabolite profiles and restriction fragment length polymorphisms (RFLPs) of PCR amplified intergenic spacer (IGS) ribosomal DNA (rDNA) were studied in 27 isolates of *Fusarium equiseti*, 25 isolated from Norwegian cereals and 2 from soil obtained from the IBT culture collection (BioCentrum, Technical University of Denmark). All 27 isolates were tested for production of fusarochromanone (FUSCHR), zearalenone (ZEA) and the trichothecenes: 15-monoacetoxy-scirpentriol (MAS), diacetoxy-scirpenol (DAS), T-2 and HT-2 toxins, T2-triol, neosolaniol (NEO), deoxynivalenol (DON), nivalenol (NIV) and 4-acetylnivalenol (Fus-X). The trichothecenes were analysed by GC-MS in a selected ion monitoring mode, while FUSCHR was determined by ion pair HPLC with fluorometric detection and production of ZEA by TLC. For amplification of IGS rDNA primers CNL12 and CNS1 were applied. IGS rDNA was digested with the four restriction enzymes: *Ava*II, *Cfo*I, *Eco*RI and *Sau*3A. In addition, we sequenced the IGS rDNA region of three of the Norwegian isolates. There were two morphological types among the Norwegian strains of *F. equiseti*, type I with short apical cells (dominating) and type II with long apical cells, with four haplotypes identified based on the RFLP data. Variation in secondary metabolite profiles within and between the morphological groups was observed and the levels of produced toxins were: FUSCHR 3000–42,500 and 25–30 ng/g, NIV 20–2500 and 120–700 ng/g, FUS-X 20–15,000 and 0 ng/g, DAS 30–7500 and 0–600 ng/g, and MAS 10–600 and 0–500 ng/g, for strains with short and long apical cells, respectively. NEO was detected in 16/27 strains tested (all morphotype I). All but four strains of type I (these four lacked a restriction site for *Eco*RI) had identical RFLP profiles. The isolates of type II had two haplotypes. The IGS sequence similarity data indicated differences between these morphotypes corresponding to two separate lineages apparently at the species level.

Keywords: *Fusarium equiseti*; Mycotoxins; Norwegian cereals

* Corresponding author. Tel.: +47 22 21 02 52; fax: +47 23 21 62 43.
E-mail address: barbara.kosiak@vetinst.no (E.B. Kosiak).

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1. Introduction

Fusarium equiseti is an ubiquitous soil saprophyte associated with fruit rots and dead and dying plant tissues that also may be a pathogen on a wide range of agricultural plants (Booth, 1971; Bosch and Mirocha, 1992). This species is particularly common in subtropical and tropical areas, but also has been isolated from overwintered cereals in the former USSR, and cereal grains in temperate regions of Europe and North America (Marasas et al., 1984). In surveys of Norwegian grains for toxigenic field fungi 1994–1998, *F. equiseti* was frequently isolated and a few samples were found to be highly infected with this species (Kosiak et al., 2003, 2004).

Three morphologically distinct types were provisionally included in *F. equiseti* by Nelson et al. (1983); one with short apical cells, corresponding to the description of *F. equiseti* var. *bullatum* Wollenw. A second with long apical cells corresponded to the description of *F. scirpi* var. *filliferum* Wollenw, but was later renamed *F. scirpi* since it produced microconidia on polyphialides (Burgess et al., 1985). A third type, *F. scirpi* var. *compactum*, also is very similar to *F. equiseti*, but has more robust conidia and red pigment in freshly isolated cultures, and was recognized as *F. compactum* (Wollenw.) Raillo by Gerlach and Nirenberg (1982). Isolates with long apical cells that do not produce polyphialides are still included in *F. equiseti* (Corda) Sacc. (Samson et al., 2000).

Secondary metabolites produced by *F. equiseti* vary in amount and toxicity. When grown on rice, strains of this species can produce the trichothecenes: 4-acetylnivalenol (FUS-X), nivalenol (NIV), scirpentriol or its mono- and diacetyl derivatives (MAS and DAS). *F. equiseti* can also produce important non-trichothecene compounds such as zearalenone (ZEA), equisetin (EQ), butenolide and fusarohromanone (FUSCHR) (Thrane, 1989; Wu et al., 1990; Langseth et al., 1999; Thrane, 2001). Langseth et al. (1999) reported that *F. equiseti* isolates had cytotoxicity similar to the most toxic *F. culmorum* isolates, when tested on swine kidney cells, using a methylthiazoltetrazolium (MTT)-cell culture assay.

Many taxonomic problems related to the morphological and physiological variation within the species have recently been resolved by using biological, chemical and molecular or polyphasic approaches (Nirenberg, 1995; Thrane and Hansen, 1995; O'Donnell et al., 1998; Leslie et al., 2001). Both internal transcribed spacer (ITS) and the more rapidly evolving intergenic spacer (IGS) regions of the nuclear ribosomal DNA (rDNA) have been applied to study closely related taxa (Appel and Gordon, 1996; Bateman et al., 1996; Yli-Mattila et al., 2002). Variation obtained by PCR amplification of the IGS region combined with restriction fragment length polymorphism (RFLP)-analysis have been frequently applied to test intraspecific phenotypic-based taxonomic hypotheses in the genus *Fusarium* (Appel and Gordon, 1995; Alves-Santos et al., 1999; Carter et al., 2000) and was therefore the choice also for the present study.

The results of previous studies revealed frequent occurrence of *F. equiseti* in Norwegian cereals and indicated qualitative and quantitative intraspecific variation in the secondary metabolite profiles, toxicity and morphological features in Norwegian strains of *F. equiseti* (Langseth et al., 1999; Morrison et al., 2002; Kosiak et al., 2003). At present, limited information on the species and its uncertain taxonomic integrity unable proper quality assessment of grain samples highly contaminated with *F. equiseti*.

The aim of this study was to describe the variation within the Norwegian population of *F. equiseti* applying a polyphasic approach comprising selected morphological, chemical and a molecular marker.

2. Materials and methods

2.1. Strains

Twenty-seven isolates of *F. equiseti* were included in the study, 25 of which were from Norway and two of which were obtained from the IBT culture collection (BioCentrum, Technical University of Denmark) (Table 1). *F. equiseti* strains were isolated on Czapek-Dox Iprodione Dichlorane agar (CZID) (Abildgren et al., 1987) from grain samples collected during studies in 1996, 1997 and 1998. All isolates were maintained on sterile soil/Synthetisher Nährstoffarmer Agar (SNA) (Nirenberg, 1976) slants and are available from the culture collection at the National Veterinary Institute, Oslo.