International Journal of Food Microbiology 189 (2014) 164–182

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Review

Genetic approaches to chemotype determination in type B-trichothecene producing *Fusaria*

Matias Pasquali [a,*], Quirico Migheli [b]

[a] Centre de Recherche - Gabriel Lippmann, 41, rue du Brill, L-4422 Belvaux, Luxembourg
[b] Dipartimento di Agraria - Sezione di Patologia Vegetale ed Entomologia and Unità di ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi di Sassari, Viale Italia 39, I-07100 Sassari, Italy

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 30 July 2014
Accepted 5 August 2014
Available online 10 August 2014

Keywords:
TRI genes
Deoxynivalenol
Nivalenol
Acetylated deoxynivalenol
Fusarium culmorum
Fusarium graminearum species complex

ABSTRACT

This review summarises the genetic methods used for chemotype determination of the main *Fusarium* type B-trichothecene producing species. Literature on *Fusarium* chemotype epidemiology over the last 15 years is reviewed in order to describe temporal and spatial chemotype distribution of these fungi worldwide. Genetic approaches used for chemotype determination are also reviewed and discussed, highlighting successes and potential pitfalls of the technique. Results from both genetic and chemical approaches are summarised to compare reliability, advantages and limitations of the two methods. Potential applications of genetic chemotyping to toxigenic *Fusarium* species are evaluated in the light of improving food safety of agricultural products. The use of chemotype determination in population studies, toxin prediction as well as for breeding purpose is described.

Contents

1. Introduction

Among the most studied and harmful toxins produced by *Fusarium* spp. are the sesquiterpene epoxides trichothecenes, secondary metabolites that inhibit eukaryotic protein synthesis and cause severe toxicosis in humans and other animals upon ingestion of contaminated grain or their derivatives, affecting intestinal, immune endocrine and neurologic functions (Maresca, 2013). Trichothecenes are also highly phytotoxic and play a role in virulence on the host plants (Arunachalam and Doohan, 2013; Desmond et al., 2008; Ilgen et al., 2009; Proctor et al., 2009; Scherm et al., 2011).

Fusaria may produce different types of toxins depending on differences in the core trichothecene cluster (*TRI* cluster), which includes two regulatory genes (*TRI6* and *TRI10*) and most of the biosynthetic enzymes required for the production of trichothecenes (Alexander et al., 2009, 2011; Kimura et al., 2003; Lee et al., 2001). Depending on the species and chemotype the number of functional core genes in the cluster varies. In *F. graminearum*, for example, the trichothecene gene cluster consists of 10–12 contiguous genes as well as two other genes,

* Corresponding author. Tel.: +352 470261436.
E-mail address: pasquali@lippmann.lu (M. Pasquali).

http://dx.doi.org/10.1016/j.ijfoodmicro.2014.08.011
0168-1605/

TRI1 and *TRI101*, which are at separate loci outside the main cluster. *Fusarium* trichothecenes can be grouped in two classes based on the presence (B-trichothecenes) versus absence (A-trichothecenes) of a keto group at the C-8 position (Ueno et al., 1973). The difference is due to catalytic divergence of the cytochrome P-450 enzymes encoded by *TRI1*. While in *F. graminearum* Tri1p oxygenates both C-7 and C-8 (which results in a hydroxyl at C-7 and a carbonyl at C-8), in *F. sporotrichioides*, only C-8 is hydroxylated by Tri1p (Rep and Kistler, 2010). Among type B-trichothecenes, those having a significant impact on safety issues are: deoxynivalenol (DON), nivalenol (NIV), and their acetylated derivatives 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), and 4-acetylnivalenol (4-ANIV, *syn*. fusarenone-X).

Based on the type of trichothecene produced, different chemotypes have been described so far for *Fusarium* species: chemotype I, producing DON and/or its acetylated derivatives, and chemotype II, producing NIV and/or 4-ANIV (Sydenham et al., 1991). The DON chemotype can be further split into chemotype IA (producing 3-ADON) and IB (producing 15-ADON; Miller et al., 1991). The intact gene cluster in *F. graminearum* results in strain producing NIV where TRI13 cytochrome P450 monoxygenase and TRI7, the associated acetyltransferase, catalyze the C-4 hydroxylation and acetylation. In DON/ADON producers, *TRI13* and *TRI7* show insertions and deletions which determine the lack of functional enzymes able to hydroxylate in C-4 and transacetylate (Lee et al., 2002). The acetylation position determining the 3-ADON or 15-ADON seems to be caused by differential activity of *TRI8* which encodes for a C-3 esterase (Alexander et al., 2011).

Structural differences among toxin chemotypes may have relevant consequences on strain fitness, since the specific pattern of oxygenation and acetylation can modify the bioactivity and hence the (phyto)toxicity of these compounds (Alexander et al., 2009, 2011; Brown et al., 2002, 2004; Ward et al., 2002; Lee et al., 2002).

The discovery of a vast array of secondary metabolites produced by *Fusarium* species has fostered surveys of mycotoxin diversity in many different epidemiological and agricultural conditions. Surveys are routinely conducted in different geographic areas (Barros et al., 2012; Clear et al., 2000a, 2000b; Del Ponte et al., 2012; Desjardins et al., 2000; Edwards, 2009; Giraud et al., 2010; Goertz et al., 2010; Kim et al., 1993; Ok et al., 2011, 2014; Park et al., 2005; Seo et al., 1996; Tanaka et al., 1986; Vanheule et al., 2014; Wagacha et al., 2010; Yoshizawa and Jin, 1995) to identify major toxigenic risks in affected grains. Indeed, trichothecenes are continuously found in cereals and derived food products around the world (Adejumo et al., 2007; Bosch and Mirocha, 1992; González et al., 2008; Nielsen et al., 2014; Poapolathep et al., 2008; Roscoe et al., 2008; Scudamore and Patel, 2009). DON and NIV now represent the two major concerns for safety of wheat and barley products, being the two most abundant toxins detected, as recently reported in a large survey on Canadian grains (Tittlemier et al., 2013).

The purpose of this review is to summarise genetic methods used for chemotype determination of type B-trichothecene producing *Fusarium* spp. Papers published during the last 15 years and reporting on the chemotype identified for sets of isolates were selected, and information on the investigated area as well as on the species and crop have been retained to generate a virtual description of known chemotype distribution worldwide. Moreover, potential applications and limits of genetic chemotyping of *Fusarium* are discussed.

We focus on the *Fusarium graminearum* species complex (FGSC, O'Donnell et al., 2000) which presently includes at least 16 species (Aoki et al., 2012), *F. culmorum* and *F. cerealis* (Cooke) Sacc., since these species are considered among the most relevant pathogens on wheat and other cereals (Moss and Thrane, 2004; Osborne and Stein, 2007). Production of a type B tricothecene (nivalenol) has been reported also from *F. poae* (Peck) Wollenw. (Jestoi et al., 2008; Thrane et al., 2004; Vogelgsang et al., 2008b) and *F. equiseti* (Corda) Sacc. (Kosiak et al., 2005). However, since they rarely produce significant amounts of other trichothecene B toxins (Kristensen et al., 2005), genetic chemotype determination does not offer additional valuable information and it is therefore not treated in detail here.

1.1. Part I

1.1.1. Why determine the chemotype of an isolate?

Determining the chemotype of an isolate is carried out for two main reasons:

1) to obtain epidemiological information on the population colonising a crop in a given area, using chemotype as a proxy in the field;
2) to inform on the toxigenic risk that the presence of a certain chemotype may determine on the food or feed that is produced, with the long term perspective of developing preventive models to decrease the toxigenic risk.

Ward et al. (2002) demonstrated that polymorphism within *TRI* genes is trans-specific and appears to have been maintained by balancing selection acting on chemotype differences. Different trichothecene-type isolates do not just have different trichothecene profiles but can in some instances be regarded as different genetic populations (Mishra et al., 2009), even if they co-occur within the same area and some gene flow may take place between them as shown using VNTR and RFLP markers (Gale et al., 2007; Karugia et al., 2009b; Ward et al., 2008). Gene flow between different populations, yet within a species, seems comparatively limited even if they co-exist, although the factors that inhibit gene flow between populations in the same area are unknown so far (Karugia et al., 2009b). This original observation leads to the idea that monitoring chemotype diversity can be informative for characterising a field population. Indeed, the evolutionary dynamics of the core trichothecene cluster were demonstrated to be essentially uncoupled from the rest of the genome (Ward et al., 2008; Proctor et al., 2009). However, as long as recombination frequency is low, chemotype could be considered as a marker for a genomic background specific to populations or individuals that are distinguished by a variety of phenotypic traits beyond chemotype. Because trichothecene production is associated with the spread of the disease after initial infection in wheat (Mesterházy, 2002), and trichothecene production is a factor affecting not only FHB but also seed diseases (Wang et al., 2006), finding a population with higher toxin production may suggest a stronger impact of the disease. For example, *F. graminearum* populations with 3-ADON chemotype seem to have a higher average toxigenic capacity in wheat and barley (as well as growth rate) in North America compared to NIV and 15-ADON populations (Foroud et al., 2012; Gilbert et al., 2010; von der Ohe et al., 2010; Ward et al., 2008). Conversely, 3-ADON populations do not differ for pathogenicity and sexual reproduction in different *Fusarium* species from different locations (Alvarez et al., 2010; Gilbert et al., 2010; Purahong et al., 2014; Schmale et al., 2011; Spolti et al., 2014b; von der Ohe et al., 2010). On another set of isolates, differences in the aggressiveness among chemotypes were reported by Malihipour et al. (2012), who suggested a gradient of aggressiveness from NIV to 15-ADON to 3-ADON chemotypes. Analysing populations carrying the NIV chemotype compared to local DON populations, lower virulence for the NIV populations were observed in *F. asiaticum* from China (Puri et al., 2012; Shen et al., 2012; Zhang et al., 2012), in *F. graminearum* (Foroud et al., 2012; Miedaner et al., 2008), as well as in *F. culmorum* in rye (Miedaner and Reinbrecht, 2001). On the contrary, NIV population did not differ in pathogenicity to its DON population counterpart when two different pathogenicity scorings were used (Purahong et al., 2014). Discrepancies between results on aggressiveness among chemotypes can be attributed to the use of chemotype as a proxy of a population. Depending on the gene flow and variability of a certain population in a sampled area, pathogenicity characters may or may not be associated with chemotype data. Aggressiveness is a factor being influenced not only by the characters of the pathogen, but also by its interaction with the host and the environment. Indeed, when