International Journal of Food Microbiology 221 (2016) 61–68

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Fungal biotransformation of chlorogenic and caffeic acids by *Fusarium graminearum*: New insights in the contribution of phenolic acids to resistance to deoxynivalenol accumulation in cereals

Léa Gauthier [a,b], Marie-Noelle Bonnin-Verdal [a], Gisèle Marchegay [a], Laetitia Pinson-Gadais [a], Christine Ducos [a], Florence Richard-Forget [a], Vessela Atanasova-Penichon [a,*]

[a] INRA, UR1264 MycSA, 71 Avenue Edouard Bourlaux, CS20032, 33882 Villenave d'Ornon, France
[b] Euralis, Domaine Sandreau, 6 chemin de Panedeautes, Mondonville CS 60224, 31705 Blagnac Cedex, France

ARTICLE INFO

Article history:
Received 9 September 2015
Received in revised form 18 December 2015
Accepted 10 January 2016
Available online 12 January 2016

Chemical compounds studied in this article:
Chlorogenic acid (PubChem CID: 1794427)
Caffeic acid (PubChem CID: 689043)
Protocatechuic acid (PubChem CID: 72)
Deoxynivalenol (PubChem CID: 40024)
15-Acetyldeoxynivalenol (PubChem CID: 10382483)
3-Acetyldeoxynivalenol (PubChem CID: 5458510)
Nivalenol (PubChem CID: 5284433)
Fusarenon X (PubChem CID: 24832112)

Keywords:
Fusarium
Type B trichothecenes
Chlorogenic acid
Caffeic acid
Biotransformation

ABSTRACT

Fusarium Head Blight and *Gibberella* Ear Rot, mainly caused by the fungi *Fusarium graminearum* and *Fusarium culmorum*, are two of the most devastating diseases of small-grain cereals and maize. In addition to yield loss, these diseases frequently result in contamination of kernels with toxic type B trichothecenes. The potential involvement of chlorogenic acid in cereal resistance to *Fusarium* Head Blight and *Gibberella* Ear Rot and to trichothecene accumulation was the focus of this study. The effects of chlorogenic acid and one of its hydrolyzed products, caffeic acid, on fungal growth and type B trichothecenes biosynthesis were studied using concentrations close to physiological amounts quantified in kernels and a set of *F. graminearum* and *F. culmorum* strains. Both chlorogenic and caffeic acids negatively impact fungal growth and mycotoxin production, with caffeic acid being significantly more toxic. Inhibitory efficiencies of both phenolic acids were strain-dependent. To further investigate the antifungal and anti "mycotoxin" effect of chlorogenic and caffeic acids, the metabolic fate of these two phenolic acids was characterized in supplemented *F. graminearum* broths. For the first time, our results demonstrated the ability of *F. graminearum* to degrade chlorogenic acid into caffeic, hydroxychlorogenic and protocatechuic acids and caffeic acid into protocatechuic and hydroxycaffeic acids. Some of these metabolic products can contribute to the inhibitory efficiency of chlorogenic acid that, therefore, can be compared as a "pro-drug". As a whole, our data corroborate the contribution of chlorogenic acid to the chemical defense that cereals employ to counteract *F. graminearum* and its production of mycotoxins.

1. Introduction

Fusarium Head Blight (FHB) and *Gibberella* Ear Rot (GER) are two of the most devastating diseases of small-grain cereals and maize, respectively. In addition to direct losses as a result of alteration of grain filling, FHB and GER pose potential health risks to domestic animals and humans due to the production of type B trichothecene mycotoxins (TCTB) by the associated pathogens. In Europe, FHB and GER are principally caused by *Fusarium graminearum*, although many different *Fusarium* species can be involved (Bottalico and Perrone, 2002). *F. graminearum*, and *Fusarium culmorum* are major producers of TCTB, including deoxynivalenol (DON) and its acetylated forms, 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), and nivalenol (NIV) and its acetylated form 4-acetylnivalenol or fusarenon X (FX). DON is of major concern due to its frequent occurrence in cereal crops and its recognized toxicity. Maximum contamination levels acceptable for cereals and maize-based food were set by the European Commission in June 2005 (EC No. 856/2005) and revised in July 2007 (EC No. 1126/2007). These limits were fixed to 1250 μg/kg for DON in unprocessed

Abbreviations: CHLO, chlorogenic acid or 5-*O*-caffeoylquinic acid; CA, caffeic acid; PA, protocatechuic acid; HCHLO, hydroxychlorogenic acid; HCA, hydroxycaffeic acid; DON, deoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; 3-ADON, 3-acetyldeoxynivalenol; NIV, nivalenol; FX, fusarenon X; TCTB, type B trichothecenes; FHB, *Fusarium* Head Blight; GER, *Gibberella* Ear Rot; PDA, Potato Dextrose Agar; MS medium, Mycotoxin Synthetic medium.

* Corresponding author.
E-mail addresses: lea.gauthier@bordeaux.inra.fr (L. Gauthier), mnverdal@bordeaux.inra.fr (M.-N. Bonnin-Verdal), marchega@bordeaux.inra.fr (G. Marchegay), lpinson@bordeaux.inra.fr (L. Pinson-Gadais), christine.ducos@bordeaux.inra.fr (C. Ducos), fforget@bordeaux.inra.fr (F. Richard-Forget), vessela.atanasova-penichon@bordeaux.inra.fr (V. Atanasova-Penichon).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.01.005
0168-1605/

common wheat and 1750 μg/kg in unprocessed durum wheat and maize. Grains or derived products exceeding the established limits for DON cannot be commercialized for human consumption.

TCTB are heat-stable molecules and are not fully degraded by the processes currently used in cereal-based food manufacture (Hazel and Patel, 2004). Thus, the best way to prevent the contamination is to limit TCTB biosynthesis at the field level during plant cultivation. Three major factors influencing fungal development and mycotoxin production in kernels are environmental conditions, agricultural practices and susceptibility of the plant genotypes (Edwards, 2004). During the past decade, increasing efforts in the cereal seed industry have been undertaken to identify genetic sources for cereal resistance to FHB and GER. However, despite significant progress (Martin et al., 2011), the success of selection for FHB and GER resistance is still challenging.

Combined with genetic approaches, biochemical ones aiming at deciphering the chemical mechanisms plants use to fight against *F. graminearum* and reduce toxin production hold great potential for assisting breeding programs for FHB or GER resistant plant genotypes. In addition to detoxification processes involving enzymatic chemical modifications of DON, e.g., glycosylation (Pasquet et al., 2014), some metabolites can interfere with fungal growth and DON production (Boutigny et al., 2008). Recent metabolomic strategies were implemented to identify these inhibitory metabolites (Gauthier et al., 2015). Based on a comparison of metabolomic profiles of wheat and barley cultivars with different levels of FHB sensitivity and challenged or not with *F. graminearum*, these approaches have highlighted the potential inhibitory contribution of more than 300 compounds. These compounds can be roughly divided in three major chemical groups: alkaloids, isoprenoids and shikimates. Half of these compounds were putatively assigned as phenylpropanoids, with phenolic acids the most frequently identified (Gauthier et al., 2015). These data support the results of several previously published studies showing that resistance to FER and/or GER may be linked with high levels of phenolic acids in wheat (Siranidou et al., 2002) and maize (Atanasova-Penichon et al., 2012; Bily et al., 2003; Cao et al., 2011).

Phenolic acids have been frequently described for their contribution to defense to plant fungal pathogens, either through direct interference with the fungus, or through the reinforcement of plant structural components acting as a mechanical barrier (Lattanzio et al., 2006; Siranidou et al., 2002). Phenolic acids also are suspected of specifically reducing mycotoxin accumulation in planta because of their in vitro demonstrated ability to inhibit the production of various mycotoxins including TCTB (Boutigny et al., 2009) and fumonisins (Atanasova-Penichon et al., 2014; Dambolena et al., 2008; Samapundo et al., 2007). Among phenolic acids, derivatives of cinnamic acid, e.g. caffeic, ferulic and *p*-coumaric acids, are the best recognized as contributors to FHB resistance (Gauthier et al., 2015). Cinnamic acid derivatives also have high antioxidant properties, which are an important primary factor of the ability of phenolic acids to modulate mycotoxin production (Ponts et al., 2011).

Chlorogenic acid or 5-*O*-caffeoylquinic acid (CHLO), formed by the esterification of caffeic acid (CA) with quinic acid, belongs to the cinnamic acid derivative group, and may play a key role in plant defense against *F. graminearum* and DON accumulation. CHLO is the main free phenolic acid that *F. graminearum* is likely to encounter at the beginning of maize ear colonization, i.e. when the production of DON is initiated in planta (Atanasova-Penichon et al., 2012). More resistant maize varieties have higher levels of CHLO, and CHLO has been reported as a potential resistance factor in several pathosystems, including maize—*Fusarium verticillioides* (Atanasova-Penichon et al., 2014), peach—*Monilinia laxa* (Villarino et al., 2011), chrysanthemum-thrips (Leiss et al., 2009), and tomato—*Alternaria alternata* (Wojciechowska et al., 2014).

However there are still large gaps in our knowledge concerning the ability of CHLO to restrain the growth of DON-producing fungi and interfere with DON production. Little is known of the capacity of *F. graminearum* strains to metabolize and detoxify CHLO. The only available knowledge is restricted to the occurrence of genes coding feruloyl esterase enzymes in the *F. graminearum* genome, and their expression profile determined in ferulic acid supplemented media (Balcerzak et al., 2012). Feruloyl esterases are subclasses of carboxylic acid esterases and include CHLO hydrolases. These enzymes cleave the ester linkages between cinnamic acid derivatives and plant cell wall and catalyze the hydrolysis of CHLO into CA and quinic acid.

The present work was aimed at providing new insights on the role CHLO could play in resistance of cereals to TCTB-producing fungi and TCTB accumulation. As a first step towards understanding the role of CHLO in plant defense, its antifungal activity and inhibitory effect on TCTB production were evaluated. In a second step, the hypothesis that CHLO can act as a "prodrug", i.e. a chemical compound that can be enzymatically transformed to yield more toxic metabolites was investigated. This paper reports for the first time the capability of *F. graminearum* to biotransform CHLO into metabolites, some more toxic to the fungus and more efficient in inhibiting mycotoxin production than CHLO.

2. Material and methods

2.1. Fusarium strains

Thirteen different *F. graminearum* and *F. culmorum* strains were used in this study: six *F. graminearum* (four with the DON/15-ADON chemotype — Fg 605, Fg 156, 245AP4 and CBS 185.32, and two with the NIV/FX chemotype — Fg 91 and Fg 183) and seven *F. culmorum* (four with the DON/3-ADON chemotype - MCf21, Fc 124, Fc 233 and Fc 305, and three with the NIV/FX chemotype — Fc 130, Fc 319 and Fc 337). Characteristics of the 13 strains are summarized in Table 1. All strains have been purified by single-spore isolation. The fungal species was confirmed using the species-specific primers Fc01 and Fg16N markers (Nicholson et al., 1998). Toxigenic potential of each strain was assessed according to the procedure described by Bakan et al. (2001). INRA-MycSA strains are deposited in the International Center for Microbial Resources — Filamentous Fungi (CIRM-CF, http://www6.inra.fr/cirm_eng/Filamentous-Fungi/Strains-catalogue). For kinetic studies, the CBS 185.32 strain was used. Stock cultures were maintained at 4 °C on Potato Dextrose Agar (PDA) (Difco, France) slants under mineral oil. When inoculum was required, the *Fusarium* strains were grown on PDA slants at 25 °C in the dark for 7 days and spore suspensions prepared by adding 6 mL of sterile distilled water to the PDA slant with gentle shaking.

2.2. Media and culture conditions

Liquid-culture experiments were performed in a Mycotoxin Synthetic medium (MS medium) (Boutigny et al., 2010). Sterile petri dishes (55 mm in diameter) containing 8 mL of MS medium supplemented or not with 0.5 mM CHLO, CA or protocatechuic acid (PA) (purchased from Sigma-Aldrich — France), were inoculated with 2×10^4 spores/mL. Fungal liquid cultures (static) were incubated in dark at 25 °C for 11 days. Additional incubation times were included for kinetic studies (4, 8, 11, and 21 days). Following incubation, cultures were centrifuged at 3000 *g* for 10 min. Supernatants were stored at −20 °C until analysis. Fungal biomass was measured by weighing the mycelial pellet after 48 h of freeze-drying (Flexi-Dry®, Oerlikon Leybold, Germany). Lyophilized mycelia were stored at −20 °C until analysis. Cultures were made in triplicate. Appropriate controls using phenolic acid-free control media and non-inoculated control media were performed. It was verified that the initial pH (pH = 6.5) of the culture medium was not affected by the phenolic acid treatment and the final pH conditions did not vary between treatments (pH = 2).

Growth inhibition tests were performed on PDA medium in petri dishes as described by Ponts et al. (2011). PDA media were supplemented with CA or CHLO at 0, 2, 4, 6, 8 and 10 mM.