

Effects of three esca-associated fungi on *Vitis vinifera* L.: III. Enzymes produced by the pathogens and their role in fungus-to-plant or in fungus-to-fungus interactions

Giovanni Bruno, Lorenzo Sparapano*

Dipartimento di Biologia e Patologia vegetale, University of Bari, Via G. Amendola 165/A, 70126 Bari, Italy

Accepted 30 April 2007

Abstract

When the esca-associated fungi *Phaeoconiella chlamydospora* (*Pch*), *Togninia minima* (*Tmi*) and *Fomitiporia mediterranea* (*Fme*) were grown in liquid stationary cultures, it was seen that they were able to live in media containing resveratrol (RES) or tannic acid (TA) as the sole carbon source and that the fungi were able to convert both compounds. Particular attention is paid here to detecting RES and TA conversion. *Pch*, *Tmi* and *Fme* were partially inhibited by RES or TA. *Pch*, *Tmi* and *Fme* produced extracellular tannase, laccase and peroxidase enzymes in liquid or agarized cultures, whether glucose was present or not. When colonies of *Pch*, *Tmi* and *Fme* were confronted, they showed spatially and temporally heterogeneous patterns of laccase and peroxidase activity. The results indicate the non-synergistic, competitive association of *Pch* and *Tmi* and the inhibition of *Fme* growth. Muconic acid, a well-known intermediate in a large number of lignin and phenol oxidative processes, can partly or completely inhibit the lignolytic agent *Fme*, but is tolerated by *Pch* and *Tmi*. An explanation for wood pigmentation patterns by *Pch*, *Tmi* and *Fme* is given.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: *Vitis vinifera*; *Phaeoconiella chlamydospora*; *Togninia minima*; *Fomitiporia mediterranea*; Esca syndrome; Plant–pathogen interactions; Phenolics; Phytoalexins; Pentaketides; Tannase; Laccase; Peroxidase

1. Introduction

Recent studies have ascertained that the ascomycete *Togninia minima* (Tul. and C. Tul.) Berl. (*Tmi*) (anamorph: *Phaeoacremonium aleophilum* W. Gams, P.W. Crous, M.J. Wingfield and L. Mugnai), the anamorphic ascomycete *Phaeoconiella chlamydospora* P.W. Crous and W. Gams (*Pch*) and the basidiomycete *Fomitiporia mediterranea* M. Fisch. (*Fme*) are associated with esca syndrome on grapevines [1–4]. The consistent isolation of these fungi from the discolored or decayed wood of esca-diseased vines indicates a close relationship between the individual stages of wood deterioration and particular species of fungi [2,5,6]. Pathogenicity of the three fungi was confirmed by inoculating them singly and in all possible combinations on the spurs, branches or trunks of standing esca-free cv.

Italia and cv. Matilde vines [3,6,7]. Esca syndrome can be either chronic or acute; chronic esca causes symptoms inside the trunk and branches, on the shoots and smaller branches, on the leaves, and on the berries, while acute esca causes “vine apoplexy”, consisting of rapid basipetal wilt of the entire vine, including clusters [2].

The results of recent studies have also provided new information on the production of toxic metabolites by *Pch* and *Tmi* and host response compounds in naturally infected vines, indicating that the pathogens, their by-products and defense substances are translocated from the infected woody tissue of the trunk to the aerial part of the affected vines [8,9].

Plant pathogen attacks are perceived through pathogen-issued compounds or plant-derived molecules which elicit defense reactions comprising both constitutive and inducible barriers. Active defense mechanisms mainly involve the accumulation of phytoalexins [10], rapid and localized cell death [11], synthesis of pathogenesis-related proteins [12],

*Corresponding author. Tel.: +39 080 5443085; fax: +39 080 5442906.
E-mail address: sparlor@agr.uniba.it (L. Sparapano).

and reactive oxygen species (ROS) produced in the plant oxidative burst [13,14]. After infection, grapevines synthesize several compounds such as phytoalexins (e.g., the viniferins), phenolics and glycolic acid [8,15–17]. Phytoalexins from the *Vitaceae* seem to constitute a rather restricted group of molecules belonging to the stilbene family, the skeleton of which is based on the *trans*-3,5,4'-trihydroxystilbene structure [12]. Because of their *in vitro* antimicrobial activity, specific classes of flavonoid and isoflavonoid compounds have long been thought to play a role in plant–pathogen interactions as part of the host-plant defense arsenal [18].

The capacity of the pathogens to produce enzymes degrading antifungal compounds released by the host or plant cell constituents may also play a significant role in the outcome of the interaction between grapevine and esca-associated fungi.

Degradation of hydrolysable tannins, particularly gallo-tannins, is best understood in fungal systems [19]. Tannase is active on galloyl residues of galloyl esters, as well as hexahydroxydiphenol and other residues of ellagitannins. The enzyme tannase, which plays a prominent role in the degradation of gallotannins, has been described and purified from various fungal strains [19,20]. Tannins inhibit the growth of a number of microorganisms, resist microbial attack and are recalcitrant to biodegradation. Despite the antimicrobial properties of tannins, many fungi, yeasts and bacteria are quite tolerant to tannins, and can grow and develop on them [21–23]. The oxidative degradation of hydrolyzable tannin has been studied in detail in *Aspergillus* spp. and the pathway of gallic acid degradation has been determined [24]. The decomposition of tannic acid (TA) and gallic acid by *Penicillium chrysogenum* Thom. was maximum in shake culture, and both these acids had completely decomposed after a few days, whereas sugars present as an additional carbon source retarded their degradation [25].

Laccases occur widely in fungi, and their presence has been reported in an increasing number of fungal species [26,27]. Fungal laccases have been reviewed by Thourston [27] and by Mayer and Staples [26]. Basically, any substrate with characteristics similar to a *p*-diphenol will be oxidized by laccases. The reduction of oxygen to water is typically accompanied by the oxidation of a phenolic substrate, and laccase activity displayed by *Botrytis cinerea* Pers. is assumed to detoxify resveratrol (RES) and to facilitate colonization of grapevines [28].

Peroxidases are enzymes which use hydrogen peroxide to catalyze the oxidation of a variety of inorganic and organic compounds such as phenol and aromatic amines, hydroquinones and hydroquinoid amines, especially benzidine derivatives [29]. Lignin and manganese peroxidases are part of the extracellular oxidative system developed by rot fungi to degrade lignin [30].

Browning of fungal mycelium resulting from enzymatic oxidation of phenolic substrates by phenoloxidase and peroxidase is commonly observed *in vitro* [31–33]. The

initial products of oxidation are usually *o*-quinones, which are highly unstable and so undergo polymerization to yield dark melanin-like pigments [27].

All organisms in their natural environment (soil or plant) come into contact or proximity with a diversity of others, so that frequent interactions must be a salient feature of their pattern of life. The interactive behaviors of wood-decay fungi (e.g., *Fme*) on simple, nutrient-rich media like malt-agar, often correlate well with their patterns of occurrence in the field. Both inter- and intra-specific interactions between fungi, and between fungi and other organisms, are of vital significance in the complex etiology of decay processes. The pairing of microorganisms on artificial substrates can lead either to deadlock, in which neither mycelium can enter the other's domain, or to replacement of one by the other. Interactive events can begin before contact in situations allowing free chemical diffusion through the substrate and implying involvement of antimicrobial substances, generation of an unfavorable pH, and so on, or can begin more selectively, implying specific recognition signals which in the case of mutual inhibition, are reciprocally exchanged [31].

The fungus-to-fungus interaction is interesting from the point of view of fungal ecology and pathogenicity. However, in the case of esca syndrome this type of interaction may help to clarify the role of each fungus in the infection process of this disease complex. In our *in vitro* bioassays, confronted fungi did not directly attack each other, but interfered with each other using metabolites as fungicides. Phenol-oxidizing enzymes then play a role in detoxification of the substances, and the fungus which has a greater capacity to detoxify becomes predominant [31]. However, fungal competition for organic resources can be further sub-divided into primary resource capture and combat; the former consists of gaining initial access to and influence over an available uncolonized resource, and the second consists of capturing territory from fungi which are already colonizing a resource or defending territory from potential invaders.

The present paper deals with the production of extracellular tannase, laccase and peroxidase by the three esca-associated fungi in axenic cultures or during intra- and interspecific fungal interactions, with a view to explaining any patterns produced during specific interaction outcomes between fungi and host plants, or between fungi themselves. The fungi may have an enzymatic pool able to lyse cell walls and woody tissue or to oxidize phenolics with extracellular enzymes like laccases.

2. Materials and methods

2.1. Fungal strains

Pch strain PVFi56, *Tmi* strain PVFi69 and *Fme* strain DBPV-1 were isolated from grapevines showing esca symptoms in Italy and grown on slants or plates of malt-agar (MA) in darkness at 25 °C [3,4,7].

Download English Version:

<https://daneshyari.com/en/article/2836762>

Download Persian Version:

<https://daneshyari.com/article/2836762>

[Daneshyari.com](https://daneshyari.com)