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Molecular interactions of the phytotoxins destruxin B and sirodesmin PL with crucifers and cereals: Metabolism and elicitation of plant defenses

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ABSTRACT

Destruxin B and sirodesmin PL are phytotoxins produced by the phytopathogenic fungi *Alternaria brassicae* (Berk.) Sacc. and *Leptosphaeria maculans* (asexual stage *Phoma lingam*), respectively. The molecular interaction of destruxin B and sirodesmin PL with cruciferous and cereal species was investigated using HPLC–ESI-MS^n. It was determined that crucifers transformed destruxin B to hydroxydestruxin B, but sirodesmin PL was not transformed. Overall, the results suggest that the five cruciferous species *Arabidopsis thaliana, Thellungiella salsuginea, Erucastrum gallicum, Brassica rapa* and *Brassica napus* are likely to produce a destruxin B detoxifying enzyme (destruxin B hydroxylase), similar to other cruciferous species reported previously. In addition, HPLC analyses and quantification of the phytoalexins elicited in each cruciferous species by these phytotoxins indicates that sirodesmin PL elicits a larger number of phytoalexins than destruxin B. Interestingly, transformation of destruxin B appears to occur also in the cereals *Avena sativa* and *Triticum aestivum*; however, the various destruxin metabolites detected in these cereals suggest that these reactions are non-specific enzymatic transformations, contrary to those observed in crucifers, where only a main transformation pathway is detectable. None of the toxins appear to elicit production of metabolites in either *A. sativa* or *T. aestivum*.

1. Introduction

Plant microbial pathogens produce phytotoxic secondary metabolites that facilitate their colonization of plant tissues, i.e. phytotoxins. While host-selective (phyto)toxins (HSTs) affect mainly host-plants, non-host selective (phyto)toxins (NSTs) damage a wider range of plants that include both hosts and non-hosts. In other contexts, phytotoxins, more properly named plant toxins, are defined as metabolites produced by plants that are toxic to animals (Strobel, 1982).

Destruxin B is a HST produced by the fungal pathogen *Alternaria brassicae* (Berk.) Sacc. (Pedras et al., 2002), while sirodesmin PL is a NST produced by the fungal pathogen *Leptosphaeria maculans* (Desm.) Ces. et de Not. [asexual stage *Phoma lingam* (Tode ex Fr.) Desm.] (Pedras and Yu, 2009). Diseases caused by these pathogens (Alternaria blackspot, *A. brassicae*, and Phoma blackleg, *L. maculans*) have enormous negative economic impact on yields of rapeseed (*Brassica napus* L. and *Brassica rapa* L.) and canola (*B. napus* L. and *B. rapa* L.). Alternaria blackspot is one of the most damaging and widespread fungal diseases of canola (*B. napus* and *B. rapa*) and brown mustard (*B. juncea*). Currently, sources of blackspot resistance within the family Brassicaceae (syn. Cruciferae) include white mustard (*Sinapis alba* L.) (Mishra et al., 2010), used as condiment and vegetable, and wild species such as false flax (*Camelina sativa* L.) and Shepherd's purse (*Capsella bursa-pastoris* L.) (Conn et al., 1988; Conn and Tewari, 1986). By contrast, several cultivars of canola resistant/tolerant to blackleg are commercially available (Canola Council Canada, http://www.canolacouncil.org/pcvt.aspx).

Plants respond to fungal attack by producing a wide range of metabolites such as phytoalexins, phytoanticipins and macromolecules (proteins and polysaccharides) that determine the outcome of the interaction. For example, plant enzymes catalyzing the detoxification of HSTs may impart resistance to the toxin-producing pathogen (Karlovsky, 1999; Strange, 2007; Moebius and Hertweck, 2009). Previously, it was established that white mustard (blackspot resistant) detoxified ^{14}C-labeled destruxin B (**1**) to hydroxydestruxin B (**2**) and glucosyl hydroxydestruxin B (**3**), substantially faster than any of the susceptible *Brassica* species (Fig. 1, Pedras et al., 2001). As well, destruxin B (**1**) was metabolized via identical intermediates in three unrelated cruciferous species, *C. sativa*, *C. bursa-pastoris*, and *Eruca sativa* L. (Pedras et al., 2003). That is, the destruxin B (**1**) detoxification pathway present in *S. alba* is present in several unrelated cruciferous species, suggesting a conservation of this pathway across crucifers. By contrast, the molecular interaction of sirodesmin PL (**4**) (Fig. 2) with cruciferous species or any other plant species has not been described, although its biological effects on a wide range

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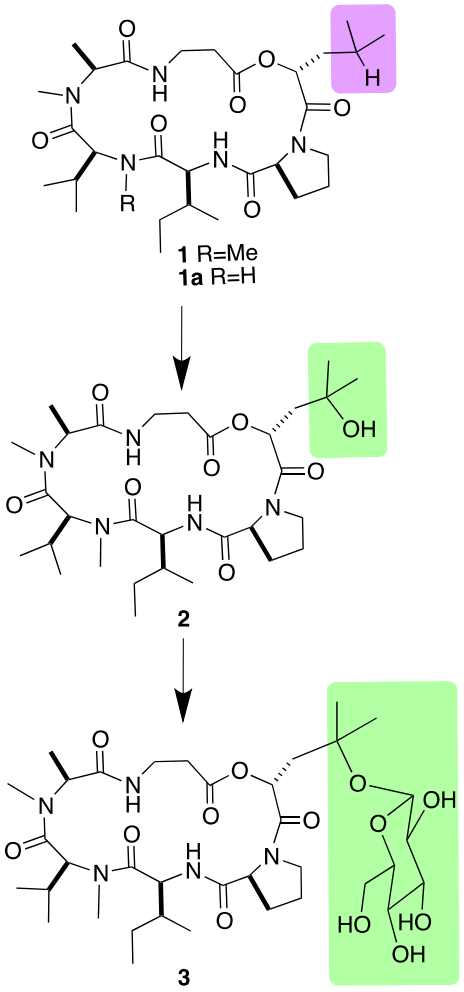

Fig. 1. Metabolism of the host-selective toxin destruxin B (**1**) in crucifers (Pedras et al., 2001).

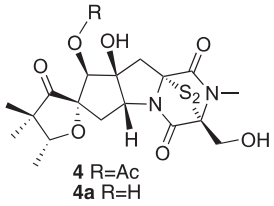

Fig. 2. Structure of the phytotoxins sirodesmin PL (**4**) and deacetylsirodesmin PL (**4a**).

of organisms have been reported (Fox and Howlett, 2008; Pedras and Yu, 2009). As well, indirect evidence using a sirodesmin-deficient mutant of *L. maculans* in which the non-ribosomal peptide synthetase has been disrupted, suggested that it contributes to pathogen virulence (Elliott et al., 2007). Besides causing necrotic lesions on plant leaves, destruxin B (**1**) was shown to elicit the phytoalexins sinalexin and sinalbin A in *S. alba* (resistant to blackspot) (Pedras et al., 2001), whereas sirodesmin PL (**4**) was reported to elicit the phytoalexins brassilexin, cyclobrassinin, rutalexin, and spirobrassinin in *B. juncea* (resistant to blackleg) (Pedras and Yu, 2008).

To better understand the interactions between the fungal pathogens *A. brassicae* and *L. maculans* with host and non-host plants, the phytotoxins destruxin B (**1**) and sirodesmin PL (**4**) were used as surrogates of these pathogens. The molecular interactions of phytotoxins **1** and **4** with crucifers (roots of turnip, *B. rapa* L., and rutabaga, *B. napus* L.; leaves of thale cress, *Arabidopsis thaliana* L., dog mustard, *Erucastrum gallicum* L., and salt cress, *Thellungiella salsuginea* L.) and cereals (leaves of oat, *Avena sativa* L. and wheat, *Triticum aestivum* L.) were investigated for the first time. Specifically, the fate of each phytotoxin in plant tissues and elicitation of plant defense responses (production of phytoalexins and phytoanticipins) were examined. Analyses of the model species *A. thaliana* and *T. salsuginea* was particularly important, given the availability of their genome sequences and mutants (Pedras and Zheng, 2010; Amtmann, 2009).

Now we report for the first time that destruxin B (**1**) is metabolized via identical intermediates in all cruciferous species investigated (*B. rapa*, *B. napus*, *A. thaliana*, *E. gallicum* and *T. salsuginea*), although at different rates. By contrast, in cereals, destruxin B (**1**) was transformed to new metabolites, whose chemical structures were determined by HPLC–DAD–ESI-MS^n, whereas sirodesmin PL (**4**) did not appear to be transformed.

2. Results

2.1. Production and isolation of destruxins and sirodesmins from fungal cultures

Destruxin B (**1**) and desmethyldestruxin B (**1a**) were isolated from cultures of *A. brassicae* following a procedure modified as reported in the Experimental (Pedras and Smith, 1997). In brief, fungal cultures of *A. brassicae* grown for 20 days in the dark, were homogenized, extracted and separated by chromatography to yield destruxin B (**1**) and desmethyldestruxin B (**1a**). The authenticity and purity of each compound was confirmed by HPLC, ^{1}H NMR, HREI-MS and comparison with authentic synthetic samples (Ward et al., 2001).

Sirodesmin PL (**4**) and deacetylsirodesmin PL (**4a**) were isolated from cultures of *L. maculans* grown in minimal medium, as previously reported and described in the Experimental (Pedras et al., 1990; Pedras and Yu, 2008). The authenticity and purity of each compound was confirmed by HPLC, ^{1}H NMR, HREI-MS and comparison with authentic samples (Pedras et al., 1990).

2.2. Metabolism of the phytotoxins destruxin B (*1*) and sirodesmin PL (*4*)

Previous work established that destruxin B (**1**) was successively converted to hydroxydestruxin B (**2**) and hydroxydestruxin B β-D-glucopyranoside (**3**) in *S. alba*, *B. napus*, *B. juncea*, *B. rapa*, *C. bursa-pastoris* and *C. sativa* (Pedras et al., 2001, 2003) using ^{14}C-radiolabeled compounds. The chemical structures of both metabolites **2** and **3** were confirmed by chemical synthesis (Pedras et al., 2001). In this work, the response of additional cruciferous species to destruxin B (**1**) was analyzed to establish a general metabolic map in crucifers and compare it with that of distinct species such as cereals. In addition, similar experiments were carried out using sirodesmin PL (**4**), to compare responses of the same species to two intrinsically different phytotoxins, i.e. unique chemical structures and diverse biological activities.

In work described in this report, no radiolabeled material was used because our current analytical LC–MS instruments can detect minimal differences between extracts of plants treated with toxins and control extracts (non-treated). Application of LC–MS in such studies is technically simpler because handling "cold" compounds is less demanding than handling radioactive materials. However, it was essential to have in hand authentic samples to determine the ESI–MS^n fragmentation patterns of destruxin B (**1**), related