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ABSTRACT

Identification of *Eutypa lata* by PCR-RFLP

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ABSTRACT

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Eutypa lata is a vascular canker pathogen of woody plants commonly diagnosed by isolating the pathogen from infected tissue. Related fungi from the same family, the *Diatrypaceae,* also have been found in association with grapevine in Californian vineyards. An in situ polymerase chain reaction (PCR) method has been developed for detection of *E. lata* in infected wood tissue. However, our results indicate that this method also would amplify other Diatrypaceous fungi, which could potentially lead to an incorrect diagnosis. Therefore, we developed a PCR-restriction fragment length polymorphism (PCR-RFLP) assay. The internal transcribed spacer (ITS)1/5.8S/ITS2 ribosomal DNA region was amplified by PCR using universal primers, and RFLP patterns were determined after digestion with *Alu*I. The restriction profiles obtained served to distinguish *E. lata* from wood trunk pathogens of grapevine (*Phomopsis viticola*, *Botryodiplodia* sp., *Phaeoacremonium aleophilum*, and *Phaeomoniella chlamydospora*), Diatrypaceous fungi (*Diatrype* sp., *Diatrypella* sp., *Eutypella vitis*, and *Eutypa leptoplaca*), and *Cryptovalsa* sp. found on dead wood of grapevine, and other *Eutypa* spp. (*E. petrakii* var. *hederae*, *E. astroidea*, *E. crustata,* and *E. lejoplaca*), with the exception of *E. armeniacae,* which we regard as a synonym for *E. lata,* and *E. laevata*, which has not been found on grapevine.

Additional keywords: vegetative compatibility groups

Eutypa lata (Pers.) Tul. & C. Tul (= *E. armeniacae* Hansf. & M. V. Carter) is a major pathogen of cultivated crops such as apricot and grapevine and has been found all over the world (3). It is responsible for significant economic damage to the wine industry in California (22,25).

E. lata (*Ascomycetes, Diatrypaceae*) can be identified based on morphological features of the meiosporic stage on the dead wood of infected hosts (3,15,24). For disease diagnosis, positive identification of *E. lata* often is based on the mitosporic stage isolated from infected wood on potato dextrose agar (PDA). On this medium, *E. lata* grows as a white cottony mycelium, which can produce conidia from conidiogenous cells aggregated into enclosed or open conidiomata (15).

Other fungi placed in members of *Diatrypaceae* are difficult to separate from each others based solely on morphological features of their mitosporic stage. Also, the biology and taxonomy of this group of fungi is not well known. Glawe and Rogers (15) found other fungal species in this family on dead or declining hosts, suggesting they may be somewhat pathogenic. *Cryptosphaeria populina* (Pers.) Sacc. (16) and *Eutypella parasitica* R. W. Davidson & Lorentz (8) are the only species other than *E. lata* reported to be pathogenic on aspen and maple, respectively. Trouillas et al. (26) reported the presence of several Diatrypaceous fungi in Californian vineyards on dead wood (*Eutypa lata*, *Diatrypella* sp., *Diatrype* sp., *Eutypella* sp., and *Cryptovalsa* sp.) including a second *Eutypa* sp., *Eutypa leptoplaca* Rappaz (27). These findings suggest that such fungi could be isolated from wood cankers instead of or in concert with *E. lata.* Therefore, incorrect identification of the target pathogen could occur.

Other techniques currently available to identify *E. lata* include serology (12,23), fatty acid analysis (11), and a polymerase chain reaction (PCR)-based method (19). However, neither fatty acid analysis nor serological methods are used extensively for disease diagnosis. Lecomte et al. (19) developed an in situ detection method by PCR using primers designed to specifically amplify 5.8S ribosomal DNA of *E. lata*. However, the three primer pairs (Lata 1/Lata 2-1, Lata 1/Lata 2-2, and Lata 3/Lata 2-1) were not tested on other Diatrypaceous fungi. Given the potential for other fungi in this family to co-occur with *E. lata* in grape wood tissue (26,27), the possible lack of specificity of these primers may render this in situ detection procedure inadequate for accurate detection of *E. lata*.

After testing the specificity primer pairs Lata 1/Lata 2-1, Lata 1/Lata 2-2, and Lata 3/Lata 2-1 designed by Lecomte et al. (19), our objective was to develop an alternative molecular method to quickly and reliably identify *E. lata* in culture and distinguish it from morphologically similar and taxonomically related fungi. For this purpose, we used a PCR-restriction fragment length polymorphism (PCR-RFLP) method, which has been used effectively for identification of other fungal pathogens (10,17,21).

MATERIAL AND METHODS

Isolates of *E. lata*. Over 30 *E. lata* isolates were collected over the years at the University of California, Davis (UC Davis) from several plant hosts, including tree hosts and cultivated crops, and from several geographic locations worldwide. A subsample of 11 *E. lata* isolates was presented for this study (Table 1). Isolates

Table 1. Designation, geographic origin, plant host, and source of 11 *Eutypa lata* isolates used in this study[a]

E. lata isolate	Geographic origin	Plant host	Isolate source
E30	United States—California	*Vitis vinifera*	Canker
E31	United States—California	*V. vinifera*	Canker
E38	United States—California	*V. vinifera*	Canker
E454	United States—California	*Prunus armeniaca*	Canker
E455	United States—California	*P. armeniaca*	Canker
ESW	Switzerland	*V. vinifera*	Ascospore