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PCR assays for the detection of *Xylella fastidiosa*
Review and comparison of published protocols

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Xylella fastidiosa (Wells *et al.*) is a widespread pathogenic bacterium, causing severe plant diseases with enormous economic impact on agriculture. *X. fastidiosa (Xf)* is regulated in the EU and other countries as quarantine organism. The official surveillance of *Xf* requires valid and appropriate detection tests. Due to the wide host range and the different subspecies and strains, the selection of an appropriate assay is of major concern.

When drafting an international diagnostic protocol for this pathogen (IPPC diagnostic protocol), an extensive literature search (ELS) was performed. One part of the study was to gather information on tests, appropriate for the detection of *Xf* at species level. The detection at species level requires a broad-spectrum detection of different subspecies and strains and the suitability for a wide range of host matrices (plants and insect vectors).

Different literature sources and the EPPO validation database were used to gain data on analytical and diagnostic sensitivity and specificity of currently available PCR assays. Based on the tested strains and non-targets the test performance criteria according to Hughes et al. (2006) were calculated and compared.

Endpoint PCR assays with generic primer for *Xf*

Different endpoint PCR assays with generic primer for *Xf* are available in the literature (1, 2, 3, 4). The primer set Rst31/33 developed by Minsavage et al. (1994) is widely used for surveillance activities. The test results from different sources (1, 8, 10) were compiled (in total 45 different *Xf* strains including subsp. *pauca*, *multiplex*, *sandyi & fastidiosa* and the European strain *Xf* subsp. *pauca* CoDiRO strain and 30 closely related or host related non targets). The compilation of these data revealed that this assay was tested mainly with strains from grapes, citrus and almonds, and that it failed to detect some strains from oaks [*Xf* red oak, US (C. Chang); *Xf* red oak, US (OAK0023 and OAK0024)] as well as from grapes [*Xf V. rotundifolia*, US (C. Chang); *Xf* grapes, US (CR. Almedeida); *Xf* grapes, US (PD0001]. A summary of the test performance criteria for the different endpoint PCR assays are reported in table 1.

Realtime PCR assays with generic primer for Xf

Five different realtime assays for the detection of *Xf* are currently available (5, 6, 7, 8, 9).

The primer set HL5p6 (Francis et al., 2006) was comprehensively tested in different studies (7, 8, 9, 10). The assay failed to detect 5 different *Xf* strains (fig. 1). The primer set *Xf*-fpr (Harper *et al.*, 2010) was tested on 94 different strains, mainly on CVC, PD, OLS and ALS strains and on a broad-spectrum of non targets (fig.2). It showed a high diagnostic sensitivity and selectivity.

Table 1. Compiled data of test performance criteria for endpoint PCR assay.

Reference	Primer	Diagnostic sensitivity	Diagnostic specifity	Relative accuracy	Analytical sensitivity (primary lit. source)	Number of tested *Xf* strains/host combination	Number of tested non-targets
Minsavage *et al*., 1994 (validated by Harper *et al.*, 2010)	Rst 31/33	100/**63.64**	100/100	100/**76.47**	1 x 10^2cfu/ml	93/19	31
Firraro *et al*., 1994	XF 1/6	100	100	100	7.6 x 10^2cfu/ml	5/5	7
Pooler & Hartung 1995 (validated by Huang, 2009)	271-1-int/272-2-int	100/100	100/100	100/100	-	57/13	8
Rodrigues *et al*., 2003	Set A, B, C Gyr	100	100	100	1 x 10^2cfu/ml multiplex: 10 cells	30/10	36

The test performance criteria for the different realtime PCR assays are summarized in table 2.

Table 2

Reference	Primer	Diagnostic sensitivity	Diagnostic specifity	Relative accuracy	Analytical sensitivity (primary lit. source)	Number of tested *Xf* strains/host combination	Number of tested non-targets
Schaad et al., 2002 (validated by Li *et al.*, 2013)	XfF1P1R1	100/100	100/**35.7**	100/**90.1**	1 x 10^3 cfu/ml	93/18	31
Francis et al., 2006 (validated by Harper *et al.*, 2010 and Li *et al.*, 2013)	HLP5p6	100/ **90.5**/ **96.1**	100/ **100**/ 100	100/ **94.1**/ **96.7**	10 copies per reaction	108/21	38
Harper et al., 2010 (validated by Li *et al.*, 2013)	XF-fpr (Rim PCR)	100/100	100/100	100/100	10 copies per reaction	95/20	26
Li et al., 2013	XF16Sfpr	100	100	100	2-3 copies per reaction	77/15	14
Ouyang et al., 2013	Xf.Csp6	100	100	100	3 copies per reaction	27/5	15

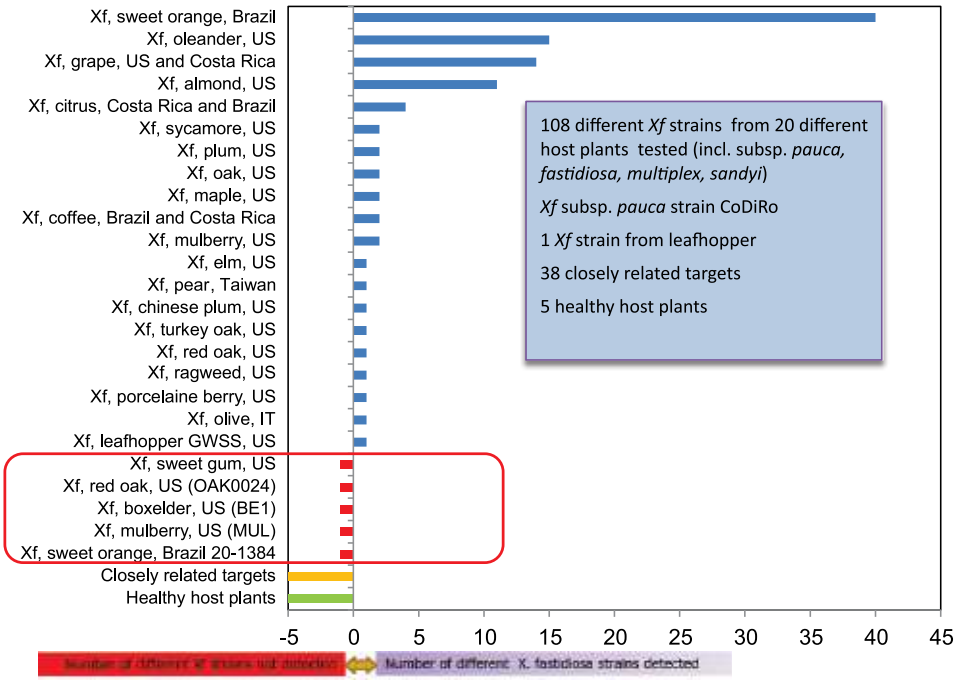

Figure 1. Compiled data from different sources (1, 8, 10) with test results using HL5p6 primer (Francis *et al.*, 2006).

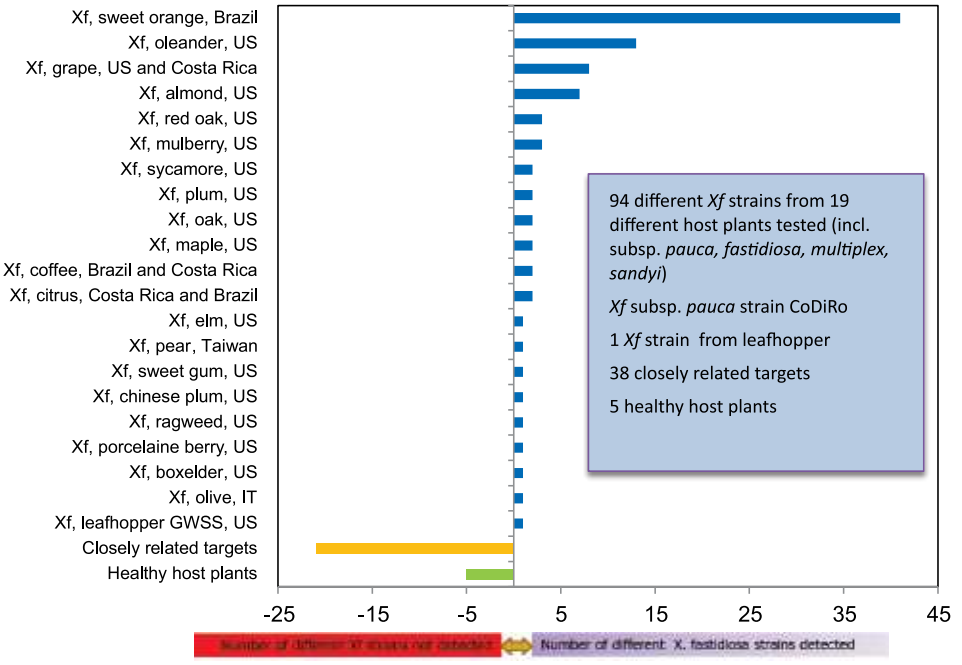

Figure 2. Compiled data from different sources (1, 8, 10) with test results using *Xf*-fpr primer (Harper *et al.*, 2010).

The compiled data from different sources can serve as a decision basis for selecting appropriate PCR assays for the specific requirements of surveillance or research activities. In particular for the detection of a pathogen like *Xf* with different subspecies and strains the limits of an assay can provide important information for further verification and validation studies.

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