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Use of conventional DNA- and protein-based techniques for the detection and characterization of *Xylella fastidiosa* in Italy

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The recent finding of *Xylella fastidiosa* (*Xf*) in southern Italy and its fast expansion in that region prompted the necessity to apply techniques that guarantee a fast, simple and efficient detection tool to be applied at large spectrum in different host-plant species, environments and insects, *i.e*. *ca.* 30 different plant species for *Xf* (EFSA, 2015) and 3 insect species, for which only *Spumarius philaenus* (L.) (Hemiptera: Aphrophoridae) was experimentally ascertained to be a vector of *Xf* in Italy (Elbeaino *et al*., 2014; Saponari *et al*., 2014). The Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assays (ELISA) were among the first techniques, afterward combined with the Immuno-fluorescence microscopy and isolation, to shed light on the bacteriological nature and the etiology of the severe disease of olive in Italy, named as "Olive quick decline syndrome" (OQDS; Cariddi *et al*., 2014).

Numerous serological techniques and genotyping approaches have been used for the detection, diagnosis and characterization of *Xf* worldwide (Holt, 1994). However, ELISA assay remains the first choice detection tool for this pathogen, being mostly adapted to screen high number of samples in a short lapse of time without a laborious work and high costs. In fact, based on an inter-laboratory validation study that was completed in November 2013 (Loconsole *et al.*, 2014), its application to detect *Xf* in infected olive plants was found suitable, and therefore it was included in the EFSA and EPPO protocols as a conventional method to screen thousands of suspected olive samples collected from areas adjacent to already claimed infected zones in Salento region (Italy). At the same time, results of screening olive samples using this serological test were in harmony with those obtained by PCR assays. Two commercial antisera, provided by Agritest s.r.l (*Xylella fastidiosa* Cat. No. K-27B, Italy) and Loewe Biochemica GmbH (*Xylella fastidiosa* Cat. No. 07119S, Germany) are essentially used to investigate the presence of this bacterium in infected samples through a DAS-ELISA test. The validation of both antisera to detect *Xf* in other susceptible host plants (oleander, almond, cherry, ornamentals, etc.) is in due course of evaluation through an inter-laboratory test in the frame of many projects at the European level, *i.e*. Euphresco, POnTe, *Xf*-actors, etc.

For the PCR assay, the bacterial genomic DNA is extracted using a CTAB buffer (Hexadecyl trimethyl-ammonium bromide) and\or a commercial kit designed for pathogens with DNA genomes.

A portion of the RNA polymerase sigma-70 factor gene is amplified using a conventional set of primers (RST31\33) generating an amplicon of 733 bp in size (Minesavage *et al*., 1994), previously adopted in quarantine programs (EPPO, 2004). Two additional couples of primer pairs targeting a hypothetical protein HL (Francis *et al*., 2006) and the 16S rDNA genes (Firrao and Bazzi, 1994) are also recommended since they are more suitable for accurate detection of a wider number of genetically diverse strains of *Xf* (Harper *et al*., 2010). However, a multiplex PCR for detection of all *Xf* strains in both plant tissue and insects, using primers against *Xf*-gyrase b gene and 16S rDNA genes are also reported in the literature (Rodrigues *et al*., 2003).

An additional conventional DNA-based technique, i.e. the Multilocus sequence typing system (MLST; Maiden *et al*., 1998), was also used and helped to acquire more genetic data on the type of *Xf* strain affecting the Salentinian olive groves (Elbeaino *et al*., 2014) that was found to belong

to the subspecies pauca with a sequence type 53 (ST53). The application of this technique is relatively simple, since it is based on a preliminary amplification operation of seven housekeeping genes of *Xf* (*leuA, petC, lacF, cysG, holC, nuoL and gltT*), conventionally used as key factors for strains characterization, followed by gene sequence concatenation (4161 nucleotides), profile and phylogenetic analyses. However, the application of this technique is conditioned by the success of isolating *Xf* in a culture medium, besides to being applied on genomic DNA extracted from living cells of a single bacterial colony. It is noteworthy mentioning that in this era of Next-Generation Sequencing (NGS), many conventional DNA- and protein-based techniques were left archaically behind since they cannot overtake the huge and complete information generated by this new technology to unveil or characterize the identity of many plant pathogens.

References

Cariddi C., M. Saponari, D. Boscia, A. De Stradis, G. Loconsole, F. Nigro, F. Porcelli, O. Potere, G.P. Martelli, 2014. Isolation of a *Xylella fastidiosa* strain infecting olive and oleander in Puglia, Italy. Journal of Plant Pathology 96(3), 1–5.

EFSA, 2015. Update of a database of host plants of *Xylella fastidiosa*: 20 November 2015. doi:10.2903/j.efsa.2016.4378.

Elbeaino T., Yaseen T., Valentini F., Ben Moussa I.E., Mazzoni V., D'Onghia A.M., 2014. Identification of three potential insect vectors of *Xylella fastidiosa* in Southern Italy. Phytopathologia Mediterranea 53(1), 126–130.

EPPO, 2004. Diagnostic protocols for regulated pests. *Xylella fastidiosa*. Bulletin OEPP/EPPO Bulletin 34: 187-192.

Firrao G., Bazzi C., 1994. Specific identification of *Xylella fastidiosa* using the polymerase chain reaction. Phytopathologia Mediterranea 33, 90–92.

Francis M., Lin H., Cabrera-La Rosa J., Doddapaneni H., Civerolo E.L., 2006. Genome-based PCR Primers for Specific and Sensitive Detection and Quantification of *Xylella fastidiosa*. European Journal of Plant Pathology 115, 203–213.

Harper S.J., Ward L.I., Clover G.R.G., 2010. Development of LAMP and Real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. Phytopathology 100: 1282-1288.

Holt J.G., 1994. Bergey's Manual of Determinative Bacteriology. 9th ed. Williams and Wilkins, Baltimore, MD.

Loconsole G., Potere O., Boscia D., Altamura G., Palmisano F., Pollastro P., Silletti M.R., Trisciuzzi N., Djelouah K., Elbeaino T., Frasheri D., Lorusso D., Valentini F., Savino V., Saponari M., 2014. Detection of *Xylella fastidiosa* in olive trees by molecular and serological methods. Journal of Plant Pathology, 96 (1), 7-14.

Maiden M.C.J., Bygraves J.A., Feil E., Morelli G., Russell J.E., Urwin R., Zhang Q., Zhou J., Zurth K., Caugant D.A., Feavers I.M., Achtman M., Spratt B.G., 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proceedings of the National Academy of Science 95, 3140–3145.

Minesavage G.V., Thompson C.M., Hopkins D.L., Leite M.V.B.C., Stall R.E., 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84, 456–461.

Rodrigues J.L.M., Silva-Stenico M.E., Gomes J.E., Lopes J.R.S., Tsai S.M., 2003. Detection and diversity assessment of *Xylella fastidiosa* in field-collected plant and insect samples by using 16S rRNA and gyrB sequences. Applied and Environmental Microbiology 69: 4249-4255.

Saponari M., Loconsole G., Cornara D., Yokomi R.K., De Stradis A., Boscia D., Bosco D., Martelli G.P., Krugner R., Porcelli F., 2014. Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Puglia, Italy. Journal of Economic Entomology 107(4), 1316–1319.