A multiplex quantitative real time PCR to detect *Xanthomonas axonopodis* pv. *allii* from onion seeds

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Bacterial blight of onion is a seed-borne emerging disease threatening world onion production, and causing damage to other *Allium* crops. Its causal agent, *Xanthomonas axonopodis* pv. *allii* (Xaa) is listed in quarantine European and Mediterranean Protection Plant Organization (EPPO) A1 list since 2009. The development of a reliable tool is necessary to manage Xaa spreading via international seed trade. We developed a triplex quantitative real-time PCR capable of detecting Xaa using the Taqman® technology. This quantitative PCR targets two markers specific from Xaa (1) and an internal control chosen in NADH dehydrogenase from Alliaceae. The multiplex real-time PCR was assayed on a large collection of Xaa strains isolated worldwide and pathogenic to onion or to other *Allium* species. Xaa strains were detected by the amplification of one or both of the two specific markers. In case of poor or no amplification of these Xaa markers, the internal control signal validates both the extraction process and the reaction itself. Specificity was assayed on 80 Xaa strains, and 120 non target strains belonging to other *X. axonopodis* pathovars including strains from the same *X. axonopodis* subgroup 9.2 *sensu* Rademaker, other species, other genera, and saprophytic strains isolated from onion seed and plants. We obtained standard curves with high correlation coefficients (r^2> 0,99) and amplification efficiencies of more than 90% on bacterial suspension (10^7 to 10^3 CFU/ml), and efficiencies of more than 80% from seed macerate, allowing the detection of 5.10^3 to 5.10^7 CFU/g for seed artificially inoculated with Xaa strains. We successfully detected both bacterial DNA and internal control DNA from plant by performing two successive steps: homogenization of a seed macerate with a stomacher®, followed by DNA extraction using DNeasy Plant minikit (Qiagen). We are currently validating our assay on naturally contaminated seed lots. This tool would be useful for the international sanitary surveillance of seed exchanges

References:

1. Robene-Soustrade *et al.,* 2010. *Appl. Environ. Microbiol*. (76) 9, in press