

Development of a sensitive real-time PCR detection protocol for *Xanthomonas fragariae* in strawberry plant tissue

J. Vandroemme, S. Baeyen, P. De Vos and M. Maes.

Institute for Agricultural and Fisheries Research, Unit Plant - Crop Protection, Burg. van Gansberghelaan 96, 9820 Merelbeke, Belgium. joachim.vandroemme@ilvo.vlaanderen.be

Xanthomonas fragariae (Xf) is the causal agent of angular leafspot on strawberry and is considered a quarantine organism in strawberry propagation material within the EU. Reliable screening of planting material for latent infections is an important challenge in the control of this disease. A real-time PCR assay based on Taqman chemistry was developed for the detection of Xf. Primers and probe sequences were based on a DNA fragment amplified in the Xf-specific multiplex PCR of Pooler *et al.*, 1996. Specificity of the designed assay was tested with an extended range of Xf collection strains and isolates, with other *Xanthomonas* spp. and with unidentified bacterial isolates from strawberry plants. The DNeasy Plant Mini Kit (Qiagen) was adapted to allow preparation of PCR-quality DNA from 100 mg of fresh strawberry tissue. For this, extra purification measures had to be taken; polyvinylpyrrolidone (PVP) was added to the kit lysis buffer and the eluted DNA was filtrated over polyvinylpolypyrrolidone (PVPP). The PCR master mix was supplemented with T4 protein 32 (GP32), which further improved the sensitivity of detection. The final protocol enabled Xf detection in undiluted DNA extracts from strawberry tissue, while with the default sample preparation and real-time PCR mix, the sample had to be diluted 100 times to obtain reliable detection. Xf was detected down to 300 colony forming units in a 100 mg of strawberry leaf material. The assay offers a new tool for sanitary control of plant material with low or latent *X. fragariae* infections.