Exploring the relation between virulence and oxidative stress response of *Bursaphelenchus xylophilus* and *Bursaphelenchus mucronatus*

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Tolerance to host-mediated oxidative stress (OS) conditions is an essential characteristic of plant-parasitic organisms. Susceptible *Pinus thunbergii* reacts to *Bursaphelenchus xylophilus* invasion with a strong oxidative burst (Hirao et al., 2012), which may indicate that virulent *B. xylophilus* must possess an efficient antioxidant system to cope with these conditions. Previous studies have suggested that PRX (2-cysteine peroxiredoxin), GST (glutathione S-transferase) and GAPDH, all localized in the surface coat, are potential scavengers of *B. xyophilus* to plant reactive oxygen species (ROS) (Shinya et al., 2010; Li et al., 2011). More recently, 12 antioxidant proteins were identified in the *B. xylophilus* secretome after plant extract stimuli, emphasizing their importance in the control of global oxidative stress of *B. xylophilus* (Shinya et al., 2013). In this work, our main concern was to study of OS tolerance of *B. xylophilus* isolates and *B. mucronatus* and the relation with their pathogenicity (virulence level) to susceptible pine species. Previous results (Vicente et al., submitted) have already suggested a relation virulence-OS tolerance among *B. xylophilus* isolates virulent Ka4 and avirulent C14-5. So, firstly, three *B. xylophilus* isolates, Ka4 and T4 (virulent) and C14-5 (avirulent), and one *B. mucronatus* (avirulent) were tested for OS tolerance using hydrogen peroxide as oxidative agent, in concentrations ranging from 0-40 mM H$_2$O$_2$. After 24h-exposure to this oxidant agent, nematode survival was checked. A clear difference between virulent and avirulent isolates was recorded in OS conditions, even in the lowest H$_2$O$_2$ concentration. The virulent isolates (Ka4 and T4) presented lower mortality percentage in all concentrations than avirulent ones (C14-5 and *B. mucronatus*). Statistical differences between Ka4 and T4 were also found until 30mM H$_2$O$_2$ treatment, being Ka4 the most resistant isolate. Concerning avirulent isolates, mortality percentage was higher than 90% in all concentrations, with no statistical differences found between C14-5 and *B. mucronatus*. Next, we assessed transcription levels of 5 main antioxidant and detoxifying enzymes during the OS conditions (15mM H$_2$O$_2$, 24h-exposure), and compared with normal conditions (no stress applied) by qRT-PCR. The following enzyme genes were analysed:
CTL (catalases, Bxy-ctl-1 and Bxy-ctl-2), SOD (superoxide dismutase, Bxy-sod-1, Bxy-sod-2 and Bxy-sod-3), GXP (glutathione peroxidase, Bxy-gxp-1, Bxy-gxp-2 and Bxy-gxp-3), GST (glutathione S-transferase, Bxy-gst-1 and Bxy-gst-3) and PRDX (peroxiredoxin, Bxy-prdx-2). In the case of B. mucronatus, this analysis was not possible to conduct since no information is available about its genome. From the selected enzymes, only Bxy-ctl-1 and -ctl-2 were significantly upregulated ($P<0.05$) in virulent isolates Ka4 and T4. In the case of C14-5, only Bxy-ctl-2 was significantly downregulated ($P<0.05$) in comparison with normal conditions. For SODs and GPXs, there were no statistical differences between isolates, although we could assess that Bxy-sod-1 and -2 were nearly 1-fold upregulated for T4; Bxy-sod-3, Bxy-gxp-2 and Bxy-gxp-3 for Ka4 and T4 were expressed at the same level than normal conditions; and that Bxy-sod-2 and -3, and Bxy-gxp-2 and -3 were downregulated for avirulent C14-5. Concerning the detoxifying enzymes GST and PRDX: Bxy-gst-1 of isolates Ka4 and T4 were, respectively, downregulated and unchanged under OS conditions, and that expression of Bxy-prdx-2 for both virulent isolates was suppressed in stress conditions. In contrast, Bxy-gst-1 of avirulent C14-5 was upregulated in OS conditions and Bxy-prdx-2 remained unaltered. GST-3 was not detected in all isolates. Following, we will analyse gene expression of these enzymes in in vivo conditions for all B. xylophilus isolates to ascertain the global oxidative status of the nematode as result of natural oxidative stress conditions. We were able check 100% sequence similarity of coding sequences of CTLs, SODs and GXP for Ka4, T4 and C14-5, suggesting that if different enzymatic activities are presented may be due to posttranslational modifications.

Based in these results, we hypothese a possible positive correlation between the level of OS tolerance and the level of virulence of B. xylophilus, which can be further investigated as a virulence marker.

REFERENCES


