

**Functional characterization of ShK domain-containing protein in the plant-parasitic nematode *Bursaphelenchus xylophilus***

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Plant pathogens cause severe economic and ecological problems in a wide range of crops and forestry plant species worldwide. The migratory plant parasitic nematode *Bursaphelenchus xylophilus* (pinewood nematode, PWN) is a European quarantine organism which causes economic and ecological damage to the forestry industry leading to the death of the tree. The interactions of plant-parasitic nematodes with their hosts are mediated by parasitism-related proteins: secreted proteins produced by the pathogen that modify the host to their benefit and to protect itself against host defenses. Several proteins have been identified in *B. xylophilus* as important during the interaction with the host, including toxins, venom-like proteins, and other peptides which their function is unknown. From the previous PWN transcriptomic dataset, we have identified 96 transcripts encoding ShK domain-like proteins. The ShK domain-like protein is a 35/37-residue peptide toxin capable of blocking the potassium channels, originally found in sea anemones. The ShK superfamily is characterized by a small and conserved domain (ShKT) distributed across plant and animal kingdoms, involving small toxic peptides and larger multifunctional proteins with several functions.

This study aimed to characterize and understand the functional role of ShK-domain like proteins in *B. xylophilus* biology. To achieve these objectives, we used different approaches such as *in silico* analysis, *in situ* hybridization to validate the spatial expression of the transcripts in the nematode tissues, and an oxidative stress assay to understand the nematode response to the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). From the results of a sequence similarity analysis, we have selected nine candidate genes with a predicted ShK domain-like protein that are only found in *B. xylophilus*, have a predicted signal peptide and, most of them, are highly expressed during infection of the host. The results showed that those genes are expressed in different nematode tissues, such as in the pharyngeal gland cells (a parasitism-related tissue), in the intestine and nerve cords, suggesting a diversity of functions in the nematode. Furthermore, eight genes have an upregulation the expression levels in response to H<sub>2</sub>O<sub>2</sub>, suggesting that they might be involved in the ROS scavenging and possibly have a protective role against host defenses enabling a successful parasitism. Understanding the role of ShK-domain like proteins may increase our knowledge of how nematodes modulate hosts during infection and develop new target molecules for nematode control.

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