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Comparative transcriptomics to understand the molecular basis of *Bursaphelenchusxylophilus* pathogenicity

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Pinewood nematode (PWN) biology and ecology are strictly associated to pine wilt disease (PWD) and have been extensively investigated. However, the disease molecular mechanism has not yet been established. Aiming to unravel the mechanism of pathogenicity we used a transcriptomics approach to study the gene expression of *Bursaphelenchus xylophilus* and the closely related *Bursaphelenchus mucronatus*. Furthermore, we sequenced the transcriptomes of *B. xylophilus* males, females and dispersal juveniles J_{III}. We then built a transcriptomics platform to carry out educated searches on differential gene expression to highlight the molecular basis of PWN pathogenicity or discover new targets with high interest for nematode control.

The five transcriptomes were sequenced in the 454 platform (Roche). Pyrosequencing generated on average 455,000 reads and 8,500 transcripts per transcriptome; more than 60% of these corresponded to InterPro terms (Table 1). Nucleotide and aminoacid sequences and corresponding annotation were organized in a web-based database. The huge amount of data generated represents an important opportunity to increase the available scientific knowledge on the nematode and to carry out comparative analysis.

Table 1. Summary of sequencing, assembly and annotation data. *B. xylophilus* and *B. mucronatus* mixed stages were collected from fungal cultures, while *B. xylophilus* males, females and JIII were collected from infected pines. Total RNA was isolated from each nematode isolate, and cDNA synthesized according to the SMART technology. Transcript assembly and annotation were performed as described in Bettencourt (2010).

	B. xylophilus (fungi)	*B. mucronatus (fungi)*	*B. xylophilus (pine)*		
			male	female	J_{III}
# Reads	647,641	465,256	407,835	227,307	531,049
# Transcripts	11,006	8,822	6,724	5,760	10,608
# Amino acid sequences	12,038	9,231	6,897	6,013	11,444
# Amino acid sequences assigned to InterPro	7,321	5,547	4,148	4,135	7,120

The platform was queried for three main comparisons: *B. xylophilus* versus *B. mucronatus* grown in fungi; PWN males versus females versus J_{III} grown on pine and PWN grown on fungi versus growth in pine. The transcriptomes were compared for genes exclusively present in each condition according to different annotation strategies such as KEGG and Gene Ontology, and also based on sequences similarity using the CD-HIT program (Huang 2010). In addition, we also studied the gene expression of potential nematode parasitism effetors directly on the database using Myrna as described in Santos (2012). The comparisons identified more than 30 genes potentially involved in PWD parasitism that are being experimentally validated by RT-PCR.

Here, we focus on the comparative gene expression of oxidative response genes, ubiquitination-related genes and a potential secreted purple acid phosphatase (Figure 1). The gene expression experimental validation indicated an overexpression in the oxidative response in males and J_{III} when compared to females grown in pine and also an overexpression in *B. mucronatus* when compared to *B. xylophilus* grown on fungi. Interestly, only *B. xylophilus* males showed an overexpression of 4HPPD (4-hydroxyphenylpyruvate dioxygenase) and an underexpression of HGD (homogentisic acid oxidase), indicating that also homogentisate can be a potentially important nematode oxidative detoxification mechanism in pine (Martin and Batkoff, 1987; Arias-Barrau, 2004). Ubiquitination-related genes were overexpressed in females when compared to the other *B. xylophilus* samples, suggesting an important regulation of internal proteolytic activity, probably to reach normal homeostasis required for reproduction (Comyn, 2013). The marked overexpression of secreted PAP (purple acid phosphatase) in females and J_{III} when compared to *B. xylophilus* grown in fungi and males grown in pine may represent a potential role in the nematode interaction with pine due to the relation of PAPs to a wide range of actions such as reactive oxygen species generation (Schenk, 2013).

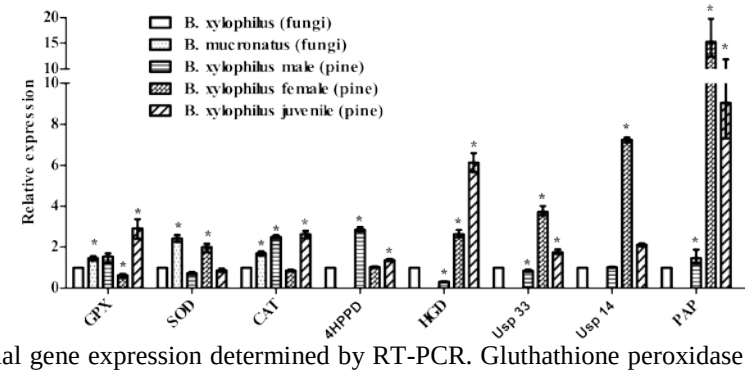

Figure 1. Differential gene expression determined by RT-PCR. Gluthathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) were studied in the five conditions, while 4-hydroxyphenylpyruvate dioxygenase (4HPPD), homogentisic acid oxidase (HGD), Ubiquitin-specific-processing protease 33 (Usp 33), Ubiquitin-specific-processing protease 14 (Usp 14) and purple acid phosphatase (PAP) were studied in the four *B. xylophilus* samples. Data is presented under the form mean±SD (standard deviation). * indicates significative differential expression with $p < 0.05$ as determined with the REST software. The cell division control protein 42 was the endogenous control and *B. xylophilus* grown in fungi the control sample.

Additional differentialy expressed genes corresponded to peptidases and respective inhibitors, carbohydrate-active enzymes, genes involved in oxidative detoxification, phenolic compound degradation or host mimicking. These genes showed higher expression levels for the nematode grown in pine, and also differences between the 3 developmental stages. These results suggest differences between males, females and J_{III} while growing in pine and may elucidate the contribution of the different stages to the PWN pathogenicity. Results and discussion of these genes will be presented at the meeting.

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