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Detection of putative secreted proteins in the plant-parasitic nematode *Heterodera schachtii*

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Abstract The beet cyst nematode *Heterodera schachtii* is an important pathogen worldwide, but its molecular characterization has been limited to studying individual genes of interest. We undertook a high-throughput genomic approach and drastically increased the number of available sequences for this parasite. A total of 2,662 expressed sequence tags were grouped into 1,212 clusters representing a nonredundant catalog of *H. schachtii* genes. Implementing a bioinformatic workflow, we identified 50 sequences coding for candidate secreted proteins. All of these contain a putative signal peptide required for entry into the secretory pathway and lack any transmembrane domain. Included are previously postulated cell-wall-degrading enzymes and other parasitism-related genes. Moreover, we provide the first report of an arabinogalactan endo-1,4- β -galactosidase enzyme (EC 3.2.1.89) in animals. As sequence data increase at a rapid rate, developing high-throughput genomic screening is a necessity. The *in silico* approach described here is an effective way to identify putative secreted proteins and prioritize candidates for further studies.

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Abbreviations aa: amino acid · BLAST: basic local alignment search tool · CBD: cellulose-binding domain · CBP: cellulose-binding protein · cDNA: complementary DNA · EST: expressed sequence tag · HMM: hidden Markov model · J2: second stage juvenile · NN: neural network · nt: nucleotides · ORF: open reading frame · SL: spliced leader · SP: signal peptide · SPT: secreted proteins identified from tags · SPW: standard pore water · TM: transmembrane

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Introduction

Plant-parasitic nematodes are major parasites of most crops and cause considerable economic losses in agriculture (Müller 1999). Traditional management strategies, like crop rotation and host resistance, are often inefficient when compared to nematocidal agrochemicals, which in turn are neither environmentally safe nor nematode-specific. Hence, there is a strong demand for the development of novel control strategies. Characterizing parasites molecularly and unraveling the mechanisms these animals use to infect plants can aid in the development of new control strategies. Molecular studies have long been hampered by the species' obligate parasitic life cycle. However, research is now beginning to benefit from new methods for gene identification. Among the most powerful and cost-effective approaches for rapid gene discovery is the single-pass sequencing of randomly sampled cDNA clones or expressed sequence tags (ESTs). Different high-throughput EST projects have now brought the total number of publicly available sequences from parasitic nematodes to more than 300,000 (McCarter et al. 2003; Wylie et al. 2004). Organizing and analyzing these data by using standard similarity-based searches can greatly contribute both to basic understanding of nematode biology and applied

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