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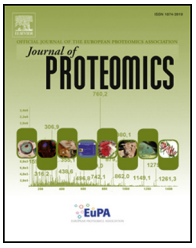

Quantitative proteome analysis of *Caenorhabditis elegans* upon exposure to nematicidal *Bacillus thuringiensis*

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ABSTRACT

Caenorhabditis elegans can be infected by a plethora of pathogens, most of them are also pathogenic for humans. Consequently, the nematode has emerged as a powerful surrogate host to model microbial human infectious diseases in a non-vertebrate, for the study of innate immunity and host–pathogen interactions. Signaling cascades are well investigated that face bacterial or fungal pathogens. We analyzed the downstream processes of these cascades, i.e. the differential expression of effector and regulatory molecules due to a microbial challenge with a pathogenic strain of the bacterium *Bacillus thuringiensis* (Bt) in comparison to a non-pathogenic Bt strain. The protein abundance profile of the nematode was studied by quantitative proteomics using iTRAQ labeling and 2D-LC–MS analysis. We developed (i) a novel method for the preparation of defined *C. elegans* samples; (ii) a pooling strategy for fractions in 2D-LC separation schemes; and (iii) an isobaric labeling scheme reducing the number of necessary LC–MS experiments.

More than 3,600 proteins were quantified, 288 of which showed altered abundances, implicating protein classes such as lectins, lysozymes, and transthyretin-like proteins to be involved in the nematode innate immune defense. A number of gene products previously only identified by transcriptomic profiling could be verified at the protein level. Moreover, several other protein classes such as proteases, proteins related to autophagy and apoptosis, structural proteins, and proteins involved in chromatin organization were detected.

The results provide an overview of the physiological response towards a pathogen at protein level in the important model organism *C. elegans*, giving insights into highly complex host–pathogen interactions.

Biological significance

This study identified system-wide effects of Bt intoxication on *C. elegans* at protein level, expanding the catalogue of immune effectors potentially acting towards the pathogen, and provide verification for numerous gene products implicated in previous transcriptomic studies. The data present evidence in support of both a general defense response as well as

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a specific reaction against the *Bt* toxin within the nematode. The described findings will also contribute to a deeper understanding of host–microbe interaction in other organisms, including humans, and may provide key information that touches far reaching aspects of coevolutionary processes.

1. Introduction

Understanding the molecular mechanisms involved in the interaction of higher organisms with their microbiota is an important key for understanding host–parasite coevolution [1] and for the identification of processes related to both the maintenance of homeostasis and the development of diseases. The nematode *Caenorhabditis elegans* is an important model organism that has been extensively used for both the study of host–pathogen interactions and the elucidation of host innate immunity [2,3]. Besides general aspects making it a suitable model organism, e.g. its short generation time, a well curated genomic repository [4] and a wide repertoire of methods for manipulation and assessment of genetic information, a major benefit provided by this organism is the ability to generate sterile and synchronous cultures by hypochlorite treatment [5]. Such precise control over the presence of microbes enabled the development of a number of robust and simple infection assays which allow the unbiased investigation of interactions between *C. elegans* and a range of microbial pathogens [6–8].

In its natural environment, *C. elegans* interacts with a diverse range of microorganisms, including bacteria, fungi, and protists, that can serve as food sources or act as pathogens. Among these microorganisms, the gram-positive soil bacterium *Bacillus thuringiensis* (*Bt*) coexists with *C. elegans* in its natural habitat [9]. *Bt* produces several classes of proteinogenic crystal (Cry) toxins that are highly toxic to specific susceptible insect larvae, while showing negligible biological or environmental toxicities. In this study we utilized two *Bt* isolates, one of which is nematicidal and the second one is non-nematicidal. The pathogenic strain (B-18247) shows nematicidal activity in both its spore crystal mixtures and purified endotoxin crystals [10]. The second strain (DSM350) has been shown to be non-pathogenic by using survival assays [11–15]. Furthermore, exposed nematodes did not show signs of intestinal damage [11,12] and no reduction in nematode population growth, nematode body size, or feeding behavior was observed, in contrast to the effects caused by the nematicidal B-18247 [12–15]. These observations make this strain a suitable non-nematicidal control for evaluation of the induced proteome response to the nematicidal *Bt* strain.

Several aspects of the interactions of *Bt* and Cry toxins with different host organisms, e.g. insects and nematodes including *C. elegans*, have been studied [13,16]. However, many aspects of these interactions are still under investigation, not least due to growing concerns about the emergence of *Bt*-toxin resistant strains [17].

Several classes of effector proteins, such as lysozymes [18], neuropeptide-like proteins [19,20], caenacins [21], thaumatins [22], lipases [23], saposin-like proteins (caenopores) [24–26], medritin-like Shk toxins [3], proteins containing CUB-like domains [27,28], C-type lectins and collagens [29,30] have been identified as being involved in the interaction of *C. elegans* with microorganisms. Bioinformatic annotation of the *C. elegans* genome sequence assigns more than 750 genes to one of these protein families [31] which shows the diversity of potential effectors.

The identification of these particular protein families aside, it can be expected that the host organism will undergo global changes while interacting with microorganisms. The majority of system wide studies have analyzed gene expression at the transcript level [29]. However, the study of the host proteome can deliver complementary data to provide deeper insights into the underlying systemic mechanisms at the molecular and hence the functional level [32]. Proteomic analyses of *C. elegans* host–microbe interactions are limited in number, however, they represent an important bridge towards the understanding of far more complex host–microbiome interactions [33].

Differential two-dimensional gel-electrophoresis based proteomics (DIGE) was applied to study the interaction of *C. elegans* with the gram-positive bacterium *Staphylococcus aureus* [34] and the gram-negative bacterium *Aeromonas hydrophila* [35]. In these two studies, 108 and 65 proteins, respectively, were identified as differentially abundant following infection. In both cases, the effect of the pathogenic bacteria and the non-pathogenic *Escherichia coli* (OP50) on the host was studied independently. In another study, the interaction of *C. elegans* with the pathogenic *E. coli* strain LF82 in comparison to the *E. coli* food source strain OP50 was investigated using $^{14}N/^{15}N$-metabolic labeling followed by GeLC–MS [36]. In the two biological situations, 117 proteins were identified at differential abundances.

Multidimensional chromatography coupled online or offline to ESI- or MALDI-MS in combination with isobaric labeling, e.g. iTRAQ [37], provides a number of advantages for quantitative proteomics. A major benefit is the multiplexing capacity of isobaric labeling, which allows comparison of several biological situations in a single experiment. However, depending on the number of biological replicates and samples to be analyzed the overall measurement time is still a serious issue. Another critical parameter is the chromatographic separation scheme applied; collecting many fractions in the first dimension separation results in significantly increased instrument times for the second dimension separation and MS analysis.

In the present study, we investigated the protein abundance profile of *C. elegans* exposed to a pathogenic in comparison to a non-pathogenic *Bt* strain. In both treatments, *E. coli* OP50 was present as a food source to prevent starvation in order to keep experimental conditions a close as possible to the real biological situation. For the same reason, we were interested in the reaction of the host to the presence of the whole bacterium rather than studying the influence of