International Journal for Parasitology 46 (2016) 83–95

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Transcriptome analysis of stress tolerance in entomopathogenic nematodes of the genus *Steinernema* ☆

Mor Yaari [a,1], Adi Doron-Faigenboim [b,1], Hinanit Koltai [b], Liora Salame [a], Itamar Glazer [a,*]

[a] Department of Entomology and Nematology, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel
[b] Department of Ornamental Horticulture, Agricultural Research Organization, Israel

ARTICLE INFO

Article history:
Received 10 May 2015
Received in revised form 13 August 2015
Accepted 14 August 2015
Available online 17 November 2015

Keywords:
Insect parasitic nematode
Steinernema feltiae
Steinernema riobrave
Desiccation tolerance
Heat tolerance
Stress-related gene

ABSTRACT

Entomopathogenic nematodes of the genus *Steinernema* are effective biological control agents. The infective stage of these parasites can withstand environmental stresses such as desiccation and heat, but the molecular and physiological mechanisms involved in this tolerance are poorly understood. We used 454 pyrosequencing to analyse transcriptome expression in *Steinernema* spp. that differ in their tolerance to stress. We compared these species, following heat and desiccation treatments, with their non-stressed counterparts. More than 98% of the transcripts found matched homologous sequences in the UniRef90 database, mostly nematode genes (85%). Among those, 60.8% aligned to the vertebrate parasites including *Ascaris suum*, *Loa loa*, and *Brugia malayi*, 23.3% aligned to bacteriovores, mostly from the genus *Caenorhabditis*, and 1% aligned to EPNs. Analysing gene expression patterns of the stress response showed a large fraction of down-regulated genes in the desiccation-tolerant nematode *Steinernema riobrave*, whereas a larger fraction of the genes in the susceptible *Steinernema feltiae* Carmiel and Gvulot strains were up-regulated. We further compared metabolic pathways and the expression of specific stress-related genes. In the more tolerant nematode, more genes were down-regulated whereas in the less tolerant strains, more genes were up-regulated. This phenomenon warrants further exploration of the mechanism governing induction of the down-regulation process. The present study revealed many genes and metabolic cycles that are differentially expressed in the stressed nematodes. Some of those are well known in other nematodes or anhydrobiotic organisms, but several are new and should be further investigated for their involvement in desiccation and heat tolerance. Our data establish a foundation for further exploration of stress tolerance in entomopathogenic nematodes and, in the long term, for improving their ability to withstand suboptimal environmental conditions.

© 2015 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The removal of water from cells, the survival of cells in a dry state, and the rehydration of dried cells impose considerable physiological constraints. Most organisms have only a limited ability to survive water loss (Wharton, 2002a,b). Desiccation tolerance, recorded for some organisms, involves activation of anhydrobiosis, a reversible physiologically arrested state of dormancy that results from the loss of 95–98% of the body's water (Crowe et al., 1992). Similarly, heat imposes severe stress on living systems, but evolutionary processes have equipped many organisms with unique mechanisms to withstand high temperatures and repair the resultant damage (Mirimoto, 2008). Despite impressive progress in the study of desiccation and heat tolerance mechanisms, these processes are still not fully understood (Burnell and Tunnacliffe, 2011).

Entomopathogenic nematodes (EPNs) belonging to the genus *Steinernema* are effective biological control agents of soil dwelling insect pests such as grubs and caterpillars, and can serve as alternatives to chemical control (Grewal et al., 2006; Ehlers, 2007). Soil is their natural habitat and similar to other nematodes, EPNs have developed unique mechanisms to facilitate survival in different soil types characterised by various moisture, temperature, texture and chemical composition conditions (Glazer, 2002). These nematodes are capable of anhydrobiosis and can survive in a desiccated state (Glazer, 2002; Gal et al., 2004). Anhydrobiosis is usually reached following a slow rate of water loss (Cooper and Van Gundy, 1971; Crowe and Madin, 1975; Wharton, 1986; Crowe, 2014).

☆ *Note:* Nucleotide sequence data reported in this paper are available in USA Sequence Read Archive (SRA) database under the accession numbers SRR2106521–SRR2106528.

* Corresponding author. Tel.: +972 50 6220290; fax: +972 3 9683903.
E-mail address: glazerit@volcani.agri.gov.il (I. Glazer).

[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.ijpara.2015.08.011
0020-7519/© 2015 Australian Society for Parasitology Inc. Published by Elsevier Ltd. All rights reserved.

Some nematodes form tight coils when exposed to desiccation (Wharton, 1986), which slow the rate of water loss by reducing the area of cuticle exposed to air. The present study focuses on two of the most important stresses affecting nematode survival—desiccation and heat—in *Steinernema* spp. (Glazer, 2002).

We previously studied physiological changes occurring in the EPN *Steinernema feltiae* during desiccation (Solomon et al., 1999, 2000). These included a decrease in glycogen levels (Gal et al., 2001) and increased levels of trehalose (TRE) during gradual dehydration in the adaptation period (24 h at 97% relative humidity (RH); Solomon et al., 1999, 2000). TRE is an osmoprotectant believed to play a major role in protecting cell membrane structure and integrity during dehydration (Crowe et al., 1984; Crowe and Crowe, 1992; Panek, 1995). We also identified elevated production of a known desiccation-related protein from the late embryonic abundant (LEA) group, LEA3, in *S. feltiae* IS-6 strain infective juveniles (IJs) during desiccation stress (Gal et al., 2001). The related genes were first identified in the free-living mycophagous nematode *Aphelenchus avenae* (Browne et al., 2004; see also review by Burnell and Tunnacliffe (2011)), then in the EPNs *Steinernema carpocapsae* (Tyson et al., 2007) and *S. feltiae* (Gal et al., 2003). Silencing the expression of one of the identified homologues (a LEA gene) in *Caenorhabditis elegans* resulted in reduced survival in the face of desiccation as well as heat stress (Gal et al., 2004). Proteomic analysis of *S. feltiae* following desiccation stress (Chen and Glazer, 2004; Chen et al., 2005) showed that out of more than 400 protein spots identified by two-dimensional (2D)-gel electrophoresis, 10 showed changes in abundance upon desiccation, one identified as the chaperonin heat shock protein (HSP) 60.

Similar to desiccation, high temperatures (>32 °C) have an adverse effect on reproduction, growth and survival of nematodes (Zervos et al., 1991; Grewal et al., 1994). Survival of EPNs at a high temperature (37 °C) was substantially increased when they were pre-exposed to mild heat (30 °C), compared with direct exposure to heat stress Grewal et al., 1994; Selvan et al., 1996). These results indicated the need for an adaptation period, perhaps to induce gene regulation and a shift in metabolism for the stress response. HSPs are known to be involved in organisms' survival at elevated temperatures (Schlesinger, 1990), and production of *hsp*70 has also been detected in EPNs (Selvan et al., 1996).

Hao et al. (2010) analysed the transcripts of *S. carpocapsae* following exposure to insect haemolymph and provided a list of stress-related transcripts. Adhikari et al. (2009a,b), reporting on the transcriptional profiling of trait deterioration in the EPN *Heterorhabditis bacteriophora*, also included a list of stress-related genes. However, neither of these studies provided a comparative analysis of gene expression between stressed and non-stressed nematodes.

In the present study, 454 pyrosequencing was used to analyse transcriptome expression in *Steinernema* spp. characterised by high or low tolerance to stress. For this purpose, a high desiccation-tolerant species, *Steinernema riobrave*, was compared with two less tolerant *S. feltiae* strains, as shown in a previous study (Somvanshi et al., 2008). The two *S. feltiae* strains differ in their ability to tolerate heat. The different species/strains were compared, following stress treatments (heat and desiccation), with their non-stressed counterparts.

De-novo assembly of the transcriptome of the *Steinernema* spp. can provide a powerful tool for studying processes in stressed and non-stressed nematodes. However, the assembly may produce erroneous predictions such as chimaeras, fragmented genes, unassembled genes and assembled paralogues. Nevertheless, bioinformatics platforms provide sufficiently accurate and annotated databases to perform state-of-the-art biochemical genomics studies.

2. Materials and methods

2.1. Nematode cultures

The EPN strains were selected on the basis of their differing abilities to tolerate desiccation and heat stresses. *Steinernema riobrave* strain (SR) was isolated from Texas, USA. The *S. feltiae* IS-6 Gvulot strain (SFG) was isolated from the southern region of Israel (Glazer et al., 1991), and the *S. feltiae* Carmiel strain (SFCar) was isolated from the Galilee mountain region in northern Israel. All nematodes were obtained from our collection held at Department of Entomology and Nematology, ARO, Volcani Center, Bet Dagan, Israel and were reared on the last instar of larvae of the greater wax moth, *Galleria mellonella*, according to Kaya and Stock (1997). Freshly emerged IJs of the nematodes were harvested with a modified White's trap, and were stored in 250 ml of distilled water in a culture flask at 5 °C for 2 weeks pending use in desiccation and heat bioassays. Prior to exposure to the stress regimes, the IJs were conditioned at room temperature for 3 h.

2.2. Desiccation and heat tolerance bioassays

The desiccation assay described by Somvanshi et al. (2008)was used. The excess water was drained and approximately one million nematodes were dried using air suction created by a vacuum pump and collected on a Whatman No. 1 filter paper. The filter paper holding the nematodes was air-dried at room temperature for 25–30 min until all excess water had evaporated, and was then immediately transferred to a sealed desiccator. RH levels were controlled with a saturated K_2SO_4 salt solution for 97% RH and KCl for 85% RH, all at 23 °C (Solomon et al., 1999). Anhydrobiosis was induced by exposure of the IJs to 97% RH for 72 h (Solomon et al., 1999) and gradually removing the water from their body. The IJs were then further exposed to 85% RH. Nematode mortality was recorded after 0, 24, 48 and 72 h of exposure to 85% RH. Each treatment consisted of six replicates for each of the three nematode species. The control consisted of non-desiccated nematodes from the same populations kept in distilled water at 23 °C.

The heat-tolerance bioassay consisted of pre-exposure of the IJs from the different strains to 32 °C for 3 h. The nematodes were then transferred to 23 °C for 1 h to recover, and then transferred to 37 °C for 8 h. Nematode viability was recorded after 0, 1, 2, 3, 4, 5 and 6 h at 37 °C. Replicates were performed for each of the three nematode species. The control treatment consisted of nematodes from the same populations kept in distilled water at 23 °C.

2.3. Preparation of total RNA and mRNA

To study gene expression patterns during the adaptation to stress conditions, samples were taken at early stages of exposure (Fig. 1): for desiccation, after exposure to 97% RH for 24 h and for heat, after exposure to 32 °C for 3 h. This was based on our previous experience which indicated that the main events of gene expression take place at early stages of detection of the stress conditions by the nematodes (Solomon et al., 1999; Somvanshi et al., 2008; for review see Gal et al., 2005). Three biological replicates were performed. The control consisted of non-desiccated and non-heated nematodes from the same batch as the desiccated and heated counterparts, kept in distilled water. The desiccated nematodes were removed from the desiccators, and immediately frozen in liquid nitrogen. The heat-treated nematodes were spun down in a 15 ml tube and immediately frozen in liquid nitrogen. All samples were then stored at −80 °C pending RNA extraction.

Total RNA was extracted from the desiccated, heated and control nematodes using TRI reagent (Sigma, Rehovot, Israel)

Download English Version:

https://daneshyari.com/en/article/2435962

Download Persian Version:

https://daneshyari.com/article/2435962

Daneshyari.com