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Genomics of entomopathogenic nematodes and it's impact in pest control

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Abstract

Entomopathogenic nematodes (EPNs) have been utilized in biological control but improvement is required to know their full potential for broader application in agriculture. Some improvements are gained through selective breeding and also the isolation of further species and populations. Having genomic sequences for a minimum of six EPNs opens the possibility of genetic improvement, either by facilitating the choice of candidate genes for hypothesis-driven studies of gene-trait relationships or by genomics-assisted breeding for desirable traits. However, the genomic data are getting to be of limited use without a more mechanistic understanding of the genes underlying traits vital for biological control. In addition, molecular tools are used to completely translate the genomic resources into further functional studies and better biological control.

Keywords: biological control, entomopathogenic nematodes, genomics

Introduction

In World annual crop loss due to herbivore by pests is 32.1%. Farmers and researchers have applied many methods to reduce this crop loss, one of which is the application of specialized insect-parasitic nematodes called entomopathogenic nematodes (EPNs). EPNs differ from other insect-parasitic nematodes in two significant ways: EPNs associate with symbiotic bacteria to facilitate pathogenesis and they rapidly kill their hosts, usually within seventy-two hours after infection (Dilman *et al.*, 2012; Kaya and Gaugler 1993) [30]. Entomopathogenic species within the genera *Heterorhabditis* and *Steinernema* are the most extensively studied and most frequently employed in biological control (Zhang *et al.*, 2008) [45]. EPNs are extremely pathogenic and are used as biological control agents of many insect pests. They have been commercialized on several continents and are used in large-scale agriculture and individual home gardens.

Despite their promise as biological control agents, lack of consistent efficacy within the sector has prevented these nematodes from being more widely used. Researchers have worked on improving efficacy against arthropod pests under field conditions for decades, employing two main strategies: 1) artificial selection and 2) genetic improvement via mutagenesis or other molecular methods. Artificial selection is improved by the continued collection of the newest EPN species and/or populations that are adapted to certain environmental conditions and pests. Often, locally adapted EPNs givesuperior control in comparison to non-native species or populations (Gaugler 1988; Hiltpold 2015) [23, 27]. Several new EPN isolates have been identified, which may cause increased genetic variation and also the development of new nematode strains (Adams *et al.*, 2007) [2]. Isolation and/or breeding of EPNs for improved insect pest suppression relieson the identification and manipulation of certain traits (Burnell *et al.*, 2002; Glazer *et al.*,2015) [9, 25]. These traits include but are not limited to increased tolerance to temperature, desiccation, and ultraviolet light, as well as increased or modified host-seeking ability, virulence, and resistance to nematicides. Improving these traits in EPNs has been done primarily by classical genetic techniques such as breeding and selection. However, traits improved this way are not always stable and individual trait gains can sometimes be lost once the selective pressure is removed.

Moreover, selection of some traits can lead to the inadvertent reduction of others or of overall fitness (Gaugler *et al.*, 1989; Gaugler *et al.*, 1990) [22, 21]. Inbreeding depression or other means of fitness loss throughout EPN-mass production or as a result of continuous laboratory culture are also concerns (Bilgrami *et al.*, 2006; Chaston *et al.*, 2011) [7, 13]. The second major strategy to enhance EPN field efficacy is to use modern genetic and molecular tools. These tools have not yet beenused to improve EPN field efficacy in biological control. Progress has been made toward tool development and technology transfer from the Caenorhabditiselegans research community, but the application of modern techniques to improve EPN efficacy is still in its infancy. EPNs are model nematode parasites for studies of ecology (Campos-Herrera *et al.*, 2012; Hadson *et al.*, 2012) [11], behaviour (Cambell *et al.*, 1993; Lewis *et al.*, 2006) [34] neurobiology (Hallem *et al.*, 2011) [26], and host-parasite interactions (Catillo *et al.*, 2015; Pena *et al.*, 2015) [37]. Manipulation of the bacterial partner may be a strategy that will yield improvements in field efficacy-related traits, however here we focus on the nematodes and also the recently sequenced genomes. The availability of multiple EPN genomes should facilitate new and powerful studies of EPN biology and will be used to decipher the function of individual genes in parasitism (Bai *et al.*, 2013; Dilman *et al.*, 2015) [3]. Here we discuss the implications that the recently available EPN genomes will have on their efficacy as biological control agents.

Traits important

Traits necessary for biological control are often sorted into three main categories: infectivity, persistence, and storage stability. Infectivity refers to the characteristics involved with finding, infecting and killing a target host. Persistence refers to traits that increase survival after application in the field such as temperature, desiccation, and ultraviolet tolerance. Storage stability of EPNs involves traits that increase the shelf life necessary for the distribution of EPNs. Improving these traits and many others have the potential for ultimately increasing field efficacy.

For EPNs to be effective in biological control they must be able to find and kill insect hosts. Thus attempt to increase and modify host-seeking behavior have been popular. Host-seeking is a highly heritable trait, which can be enhanced through selective breeding efforts for species such as *Steinernema feltiae* and *Steinernema carpocapsae* (Gaugler *et al*., 1990; Gaugler *et al*., 1991) [21,20]. The research done to enhance host-seeking traits has relied solely on selective breeding and the genes that are implicated in these processes are unknown. Host seeking improvements were correlated with an increase in overall fitness. Unfortunately, the trade-off is a reduction in desiccation tolerance, affecting storage stability. The use of new genomic data may reveal how the genes controlling these traits are genetically linked and provide new strategies for enhancing these traits without compromising others.

Persistence is as important as infectivity for EPN efficacy. Desiccation tolerance is very important for EPN persistence and production. EPN species that forage close to the soil surface tend to have better desiccation tolerance. Desiccation can induce EPN quiescence, which leads to longer shelf life and may also contribute to their longevity in the soil (Koppenhofer *et al*., 1997) [33]. Storage stability is essential for EPN longevity in the soil and commercial production and distribution. Artificial selection and hybridization can enhance desiccation tolerance, but the removal of selection pressure ultimately results in the loss of the desired traits (Nimcingrat *et al*, 2013; Salamae *et al.*, 2010). Deep knowledge of EPN genomes (Bai *et al.*, 2013; Dilman *et al*., 2015) [3], especially the genetic regulatory networks controlling the traits, coupled with improvements in genetic engineering for EPNs may provide new means to stabilize enhanced traits.

Genome of *Heterorhabditis bacteriophora*

The genome sequence of *H. bacteriophora* revealed numerous genes putatively implicated in parasitism and survival that could be manipulated for field applications (Bail *et al.*, 2013) [3]. It has also raised many questions since the 77 Mb genome contains more than 10,000 genes encoding proteins of unknown function. The obligate association of *H. bacteriophora* with the bacterium *Photorhabdus luminescens* has shaped the architecture and content of the nematode genome as it relies on the bacteria for nutrient acquisition and metabolism. The bacteria also serve as a means by which the insect's immune response is overcome (Kaya and Gaugler 1993; Dilman *et al*., 2015) [30]. *P. luminescens* produces an arsenal of enzymes including proteases to beat host immunity, degrade host tissues and make them available for the developing nematodes. Further, the bacteria prevent opportunistic fungi and other bacteria from making use of the nutrient-rich insect cadaver. Certain species of EPNs such as *H. bacteriophora* rely on bacteria to overcome host immunity and kill the host, whereas other EPNs such as *Steinernema carpocapsae* are lethal even without their bacteria and therefore may be reliant on the bacteria for nutrient acquisition or sequestration of host resources from opportunistic soil microbes (Elftherianos *et al*., 2010; Han *et al*., 2010). However, it's the nematode that has to find hosts, gain entry into the hemolymph, and persist in the soil until a host is found, leaving many rooms for genetic improvements to enhance field efficacy. Genes that function in the symbiotic association between EPNs and the insect-pathogenic bacteria they carry might be used to enhance the biological control potential of EPNs.

H. bacteriophora seems to have a reduced or modified immune response compared to *C.elegans* (Bale *et al.*, 2013). It has far fewer C-type lectin domain-containing, which function in the immune response of *C. elegans* to bacterial infection (Bale *et al.*, 2013 and Schulenburg *et al*, 2008) [39]. This might be related to the association between *H. bacteriophora* and *P. luminescens* (Ciche *et al*., 2008) [14]. However, it is not yet known to what extent environmental bacterial infection might impair the efficacy of EPNs as biocontrol agents and more research needs to be done on this subject. If it is demonstrated that bacterial infection diminishes EPNs field efficacy against insect pests, this might be another area worth investigating further.

EPN secreted proteases are known to influence the penetration of the nematode into the host hemolymph (Abuhatab *et al*, 1995) [1], tissue degradation of insect hosts (Balasubramanian *et al*, 2009) [5], as well as immune suppression, and could be used to increase the field efficacy of EPNs in biocontrol. *H. bacteriophora* has fewer than 30 predicted protease and protease inhibitors in its secretome (Bai *et al.,* 2013) [3]. This may reflect the nematode's reliance on *P. luminescens* for immune suppression and tissue degradation of the insect host. Host-killing by EPNs might be improved just by adding further copies of genes already present, similar to the transgenic inclusion of multiple endogenous cuticle-degrading proteases in entomopathogenic fungi (St Legel *et al*., 1996).

The genomic sequence of H. bacteriophora provides a long list of candidate genes that would be used to improve infectivity and/or survival and this list will be refined as our understanding of the underlying biology increases. The successful application of tools from (e.g *C. elegans*. transformations and RNAi) (Ward *et al.,* 2015) [42] in H. bacteriophora coupled with this archive of genetic information is expected to lead to significant advances in the application of molecular genetics to enhance the field efficacy of EPNs in biological control.

Steinernema carpocapsae

The genomes of *Steinernema carpocapsae* and four congeners (*S. feltiae, S. glaseri, S. monticolum, and S. scapterisci*) have recently been sequenced and annotated. Analyses of these genomes revealed numerous genes that could be involved in parasitism by EPNs and are candidates for use in programs to improve traits for biological control (Dilman *et al*., 2015). Similar to what has been found in the H. bacteriophora genome, more than 10,000 predicted seems to have no orthologues in other animals or even other nematodes. Studying the function of these orphan proteins might reveal genes important for infection or survival and persistence and so be helpful to future transgenic endeavors in EPNs. Comparing sequenced EPN genomes confirms that they are similar in size but differ considerably in nucleotide prevalence (G+C content), which may affect the application of recombinant DNA techniques for genetic enhancement. Gene expression and regulation are affected by codon usage preferences, which has implications for technology transfer from *C. elegans* to the EPNs (Ward *et al.,* 2015) [42], with techniques developed in *C. elegan* potentially being more easily applied to *H. bacteriophora* due to their closer ancestry and similar nucleotide prevalence (Ciche *et al*., 2008) [14]. This needs to be further explored experimentally as there are not many reports of molecular techniques developed in *C. elegans* being applied to EPNs. In contrast to the *H. bacteriophora* genome, *steinernematids* have a large variety of predicted proteases and protease inhibitors with signal peptides. A multi-genome comparison revealed Steinernema-specific expansions of serine and metallo proteases (Dilman *et al*., 2015). Proteases and protease inhibitors are an important group of proteins for investigation in the future selection and recombinant studies since they are known to be important in invasion and host-killing for Steinernema (Abuhatab *et al.,* 1995; Balasubramanian *et al.,* 2009) [1, 5]. Proteases in steinernematids have been shown to play an important role in suppressing insect host immunity as well as tissue degradation (Balasubramanian *et al.,* 2009 & 2010; Toubarro *et al.,* 2010) [5, 41]. Functional studies show that protease inhibitors play a key role in nematode evasion of host immunity (Milstine *et al.,* 2000; Zang and Maizels 2001) [44], and genome analysis revealed that many families of protease inhibitors are expanded in steinernematids.

One provocative possibility is that the host range and specificity of EPNs could also be influenced by their repertoire of secreted products and which use genetic transformation, the host range and/or specificity may be altered by the addition or removal of certain secreted products from the secretome. Not enough is throughout regarding the evolution of insect immunity, however as more genomes are being studied it seems that insect immunity could differ dramatically between orders and that niche partitioning among EPNs could be based on individual species' abilities to overcome or avoid the immune response of certain hosts (Elsik 2010; Gerardo *et al*., 2010) [18, 24]. Fatty acid- and retinol-binding (FAR) proteins are another interesting gene family that was expanded in the genomes of *Steinernematids* (Dilman *et al*., 2015). FAR proteins are thought to play a key role in parasitism by functioning in the sequestration of host retinoids similarly by contributing to immune evasion or suppression, although their actual functional role is not well understood (Garofraco *et al*., 2002; Kennedy *et al.,* 2013) [31]. FARs seem to be involved in nematode parasitism of animals, insects, and plants (Iberkleid *et al.,* 2013) [29], which makes understanding their mechanistic function important for both biocontrol as well as disease treatment and prevention. The availability of genomic sequence from multiples species of Steinernema provides many candidate genes and gene families that would be used to improve infectivity and/or survival. It also highlights the importance of more mechanistic studies of EPN biology and the need for molecular tools to be more commonly applied in EPN research (Ward *et al.,* 2015) [42].

Methods for trait improvement

As mentioned above, artificial selection and genetic engineering are the two main options for trait improvement in EPNs. Artificial selection does not require an understanding of genetic mechanisms underlying selected traits. Having been done for thousands of years with crops and livestock, individuals with certain traits are selected and crossed. This generates new cultivars/breeds with improved or desired traits. We can now employ genomic tools to understand what genetic changes introduced by domestication and artificial selection are actually behind the selected traits. This knowledge, combined with genetic engineering has resulted in the more efficient production of enhanced traits or novel combinations in many systems (Kole *et al*., 2015) [32].

There are many tools and traits which will aid in the effort of enhancing EPNs for better field efficacy: (1) advanced genomic tools to identify the genes underlying desired traits; (2) genetic tools that can be applied to modify genes; (3) short generation time of EPNs and their ability to be cultured *in vitro* and *in vivo*; and (4) a large collection of EPN species and strains with rich genetic diversity to select from.In addition to genomics-assisted breeding, genome sequences of EPNs can even be used for direct genetic modification, which includes mutagenesis, transgenesis, and targeted gene modification. Mutagenesis might generate novel genetic variations, yielding more effective genes than those that exist. One common practice is to do random mutagenesis followed by selection (Xu *et al.,* 2012) [43]. This will accelerate the selection process, thereby allowing for the analysis of larger ranges of genetic variation than are found in nature. Successful gains in desired traits may then be useful themselves or in combination with other techniques such as transgenesis. Transgenes could be derived from the same species (intraspecific), different species (interspecific), or even non-nematode organisms. Because releasing transgenic organisms into the environment remains controversial, transgenic nematodes may not be the first option for EPN trait improvement. One alternative is the utilization of CRISPR/Cas9, a targeted gene editing procedure allowing for direct changing of specific alleles. CRISPR-mediated gene targeting can generate defined modifications in specific genes that mimic natural alleles (Bortesi and Fischer, 2015) [8]. Genetically modified EPNs generated by this process are more likely to be publically accepted since the final strains only contain modified alleles rather than genes from other organisms. Of course, the prerequisite of successful CRISPR-mediated gene targeting is the identification of genes that control the traits under selection.

Along with the advanced bioengineering tools and also the availability of genomic information, it is essential to remember the importance of genetic diversity in EPN improvement. One reason is that large genetic diversity is an invaluable natural resource to select for useful traits and the underlying genes. Another reason is that one may need to employ integrated biocontrol using a collection of EPN strains and/or species to better control multiple insect pests in one application, instead of attempting to develop a magic bullet.

Conclusion

The availability of the genomic sequence data and putative proteomes provides anoutsized number of genes that would be useful in increasing the infectivity of EPNs. We highlighted proteases, protease inhibitors, FAR proteins, and GPCRs as potential targets for improvement, though there are certainly many more genes and gene families waiting to be discovered in these species that could be exploited. Many genes could be used to increase infective juvenile persistence and survival in the soil. Several known stress-tolerance genes like heat shock proteins, trehalose-related molecules and pathways, as well as their orthologs and paralogs that have been expanded in EPN genomes, remain to be functionally tested. EPN research is burgeoning with possibility, but much remains intractable without the application of more molecular tools. The field advanced significantly with the sequencing of these genomes, but whether this will lead to actual improvements in the field efficacy of EPN bio-control remains to be seen.

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