

[0224] pGZ25=pos-1=embryonic lethal

[0225] pGZ59=bli-4D=acute lethal

[0226] ACC=acetyl co-enzym A carboxylase=acute lethal

[0227] After 5 days, the phenotype of the *C. elegans* nuc-1 (e1392) worms fed with the bacteria producing dsRNA were compared to the phenotype of worms fed with the empty vector (pGN29) and the other controls. The worms that were fed with the dsRNA were screened for lethality (acute or larval) lethality for the parent (Po) generation, (embryonic) lethality for the first filial (F1) generation, or for growth retardation of Po as follows: (i) Acute lethality of Po: L1's have not developed and are dead, this phenotype never gives progeny and the well looks quite empty; (ii) (Larval) lethality of Po: Po died in a later stage than L1, this phenotype also never gives progeny. Dead larvae or dead adult worms are found in the wells; (iii) Lethality for F1: L1's have developed until adult stage and are still alive. This phenotype has no progeny. This can be due to sterility, embryonic lethality (dead eggs on the bottom of well), embryonic arrest or larval arrest (eventually ends up being lethal); (iv) Arrested in growth and growth retardation/delay: Compared to a well with normal development and normal # of progeny.

[0228] For the target sequences presented in Table 1A, it was concluded that dsRNA mediated silencing of the *C. elegans* target gene in nematodes, such as *C. elegans*, had a fatal effect on the growth and viability of the worm.

[0229] Subsequent to the above dsRNA silencing experiment, a more detailed phenotyping experiment was conducted in *C. elegans* in a high throughput format on 24 well plates. The dsRNA library produced in bacterial strain AB301-105 (DE3), as described above, was fed to *C. elegans* nuc-1 (e1392) worms on 24 well plates as follows: nuc-1 eggs were transferred to a separate plate and allowed to hatch simultaneously at 20 C for synchronization of the L1 generation. Subsequently 100 of the synchronized L1 worms were soaked in a mixture of 500 μ A S-complete fed medium, comprising 5 μ g/mL cholesterol, 4 μ L/mL PEG and 1 mM IPTG, and 500 μ A of bacterial cell culture of OD₆₀₀1 AB301-105 (DE3) of the *C. elegans* dsRNA library carrying each a vector with a *C. elegans* genomic fragment for expression of the dsRNA. The soaked L1 worms were rolled for 2 hours at 25 C.

[0230] After centrifugation and removal of 950 μ L of the supernatant, 5 μ L of the remaining and resuspended pellet (comprising about 10 to 15 worms) was transferred in the middle of each well of a 24 well plate, filled with a layer of agar LB broth. The inoculated plate was incubated at 25° C. for 2 days. At the adult stage, 1 adult worm was singled and incubated at 25° C. for 2 days for inspection of its progeny. The other adult worms are inspected in situ on the original 24 well plate. These experiments were performed in quadruplicate.

[0231] This detailed phenotypic screen was repeated with a second batch of worms, the only difference being that the worms of the second batch were incubated at 20 C for 3 days.

[0232] The phenotype of the worms fed with *C. elegans* dsRNA was compared to the phenotype of *C. elegans* nuc-1 (e1392) worms fed with the empty vector.

[0233] Based on this experiment, it was concluded that silencing the *C. elegans* target genes as represented in Table

1A had a fatal effect on the growth and viability of the worm and that the target gene is essential to the viability of nematodes. Therefore these genes are good target genes to control (kill or prevent from growing) nematodes via dsRNA mediated gene silencing. Accordingly, the present invention encompasses the use of nematode orthologs of the above *C. elegans* target gene to control nematode infestation in a variety of organisms and materials.

Example 2

Identification of *D. melanogaster* Orthologs

[0234] As described above in Example 1, numerous *C. elegans* lethal sequences were identified and can be used for identifying orthologs in other species and genera. For example, the *C. elegans* lethal sequences can be used to identify orthologous *D. melanogaster* sequences. That is, each *C. elegans* sequence can be queried against a public database, such as GenBank, for orthologous sequences in *D. melanogaster*. Potential *D. melanogaster* orthologs were selected that share a high degree of sequence homology (E value preferably less than or equal to 1E-30) and the sequences are blast reciprocal best hits, the latter means that the sequences from different organisms (e.g. *C. elegans* and *D. melanogaster*) are each other's top blast hits. For example, sequence C from *C. elegans* is compared against sequences in *D. melanogaster* using BLAST. If sequence C has the *D. melanogaster* sequence D as best hit and when D is compared to all the sequences of *C. elegans*, also turns out to be sequence C, then D and C are reciprocal best hits. This criterion is often used to define orthology, meaning similar sequences of different species, having similar function. The *D. melanogaster* sequence identifiers are represented in Table 1A.

Example 3

Leptinotarsa decemlineata (Colorado Potato Beetle)

A. Cloning Partial Gene Sequences from *Leptinotarsa decemlineata*

[0235] High quality, intact RNA was isolated from 4 different larval stages of *Leptinotarsa decemlineata* (Colorado potato beetle; source: Jeroen van Schaik, Entocare CV Biologische Gewasbescherming, Postbus 162, 6700 AD Wageningen, the Netherlands) using TRIzol Reagent (Cat. Nr. 15596-026/15596-018, Invitrogen, Rockville, Md., USA) following the manufacturer's instructions. Genomic DNA present in the RNA preparation was removed by DNase treatment following the manufacturer's instructions (Cat. Nr. 1700, Promega). cDNA was generated using a commercially available kit (SuperScriptTM III Reverse Transcriptase, Cat. Nr. 18080044, Invitrogen, Rockville, Md., USA) following the manufacturer's instructions.

[0236] To isolate cDNA sequences comprising a portion of the LD001, LD002, LD003, LD006, LD007, LD010, LD011, LD014, LD015, LD016 and LD018 genes, a series of PCR reactions with degenerate primers were performed using Amplitaq Gold (Cat. Nr. N8080240, Applied Biosystems) following the manufacturer's instructions.