

of the target sequence in both antisense and sense directions since these are flanked by oppositely oriented T7 promoters.

[0381] The optical density at 600 nm of the overnight bacterial culture is measured using an appropriate spectrophotometer and adjusted to a value of 1 by the addition of fresh LB broth. Fifty ml of this culture is transferred to a 50 ml Falcon tube and the culture then centrifuged at 3000 g at 15° C. for 10 minutes. The supernatant is removed and the bacterial pellet resuspended in 50 ml of fresh S complete medium (SNC medium plus 5 µg/ml cholesterol) supplemented with 100 µg/ml carbenicillin and 1 mM IPTG. The bacteria are induced for 2 to 4 hours at room temperature.

Heat Treatment of Bacteria

[0382] Bacteria are killed by heat treatment in order to minimise the risk of contamination of the artificial diet in the test plates. However, heat treatment of bacteria expressing double-stranded RNA is not a prerequisite for inducing toxicity towards the insects due to RNA interference. The induced bacterial culture is centrifuged at 3000 g at room temperature for 10 minutes, the supernatant discarded and the pellet subjected to 80° C. for 20 minutes in a water bath. After heat treatment, the bacterial pellet is resuspended in 1.5 ml MilliQ water and the suspension transferred to a microfuge tube. Several tubes are prepared and used in the bioassays for each refreshment. The tubes are stored at -20° C. until further use.

F. Laboratory Trials to Test *Escherichia coli* Expressing dsRNA Targets Against *Myzus persicae*

Plant-Based Bioassays

[0383] Whole plants are sprayed with suspensions of chemically induced bacteria expressing dsRNA prior to feeding the plants to GPA. The are grown from in a plant growth room chamber. The plants are caged by placing a 500 ml plastic bottle upside down over the plant with the neck of the bottle firmly placed in the soil in a pot and the base cut open and covered with a fine nylon mesh to permit aeration, reduce condensation inside and prevent insect escape. GPA are placed on each treated plant in the cage. Plants are treated with a suspension of *E. coli* AB309-105 harbouring the pGBNJ001 plasmids or pGN29 plasmid. Different quantities of bacteria are applied to the plants: for instance 66, 22, and 7 units, where one unit is defined as 10⁹ bacterial cells in 1 ml of a bacterial suspension at optical density value of 1 at 600 nm wavelength. In each case, a total volume of between 1 and 10 ml s sprayed on the plant with the aid of a vaporizer. One plant is used per treatment in this trial. The number of survivors are counted and the weight of each survivor recorded.

[0384] Spraying plants with a suspension of *E. coli* bacterial strain AB309-105 expressing target dsRNA from pGBNJ003 lead to a dramatic increase in insect mortality when compared to pGN29 control. These experiments show that double-stranded RNA corresponding to an insect gene target sequence produced in either wild-type or RNaseIII-deficient bacterial expression systems is toxic towards the insect in terms of substantial increases in insect mortality and growth/development delay for larval survivors. It is also clear from these experiments that an exemplification is provided for the effective protection of plants/crops from insect dam-

age by the use of a spray of a formulation consisting of bacteria expressing double-stranded RNA corresponding to an insect gene target.

Example 11

Nilaparvata lugens (Brown Plant Hopper)

A. Cloning *Nilaparvata lugens* Partial Sequences

[0385] From high quality total RNA of *Nilaparvata lugens* (source: Dr. J. A. Gatehouse, Dept. Biological Sciences, Durham University, UK) cDNA was generated using a commercially available kit (SuperScript™ III Reverse Transcriptase, Cat N°. 18080044, Invitrogen, Rockville, Md., USA) following the manufacturer's protocol.

[0386] To isolate cDNA sequences comprising a portion of the *Nilaparvata lugens* NL001, NL002, NL003, NL004, NL005, NL006, NL007, NL008, NL009, NL010, NL011, NL012, NL013, NL014, NL015, NL016, NL018, NL019, NL021, NL022, and NL027 genes, a series of PCR reactions with degenerate primers were performed using Amplitaq Gold (Cat N°. N8080240; Applied Biosystems) following the manufacturer's protocol.

[0387] The sequences of the degenerate primers used for amplification of each of the genes are given in Table 2-NL. These primers were used in respective PCR reactions with the following conditions: for NL001: 5 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL002: 3 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL003: 3 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 61° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL004: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 51° C. and 1 minute at 72° C.; for NL005: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL006: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 3 minute 30 seconds at 72° C., followed by 10 minutes at 72° C.; for NL007: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 1 minute 15 seconds at 72° C., followed by 10 minutes at 72° C.; for NL008: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 53° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL009: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL010: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 2 minute 30 seconds at 72° C., followed by 10 minutes at 72° C.; for NL011: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 1 minute at 72° C.; for NL012: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 1 minute at 72° C.; for NL013: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 1 minute 10 seconds at 72° C., followed by 10 minutes at 72° C.; for NL014: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 53° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL015: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 1 minute 40 seconds at 72° C., followed