seconds at 50° C. and 1 minute at 72° C., followed by 10 minutes at 72° C. The resulting PCR fragment was analyzed on agarose gel, purified (QIAquick Gel Extraction kit, Cat. Nr. 28706, Qiagen), blunt-end cloned into Srf I-linearized pGNA49A vector (reference to WO00188121A1), and sequenced. The sequence of the resulting PCR product corresponds to SEQ ID NO: 488 as given in Table 8-PC. The recombinant vector harbouring this sequence was named pGCDJ001.

F. Expression and Production of a Double-Stranded RNA Target in Two Strains of *Escherichia coli* AB309-105

[0303] The procedures described below are followed in order to express suitable levels of insect-active double-stranded RNA of insect target in bacteria. In this experiment, an RNaseIII-deficient strain, AB309-105 is used.

Transformation of AB309-105

[0304] Three hundred ng of the plasmid were added to and gently mixed in a 50 μl aliquot of ice-chilled chemically competent *E. coli* strain AB309-105. The cells were incubated on ice for 20 minutes before subjecting them to a heat shock treatment of 37° C. for 5 minutes, after which the cells were placed back on ice for a further 5 minutes. Four hundred and fifty μl of room temperature SOC medium was added to the cells and the suspension incubated on a shaker (250 rpm) at 37° C. for 1 hour. One hundred μl of the bacterial cell suspension was transferred to a 500 ml conical flask containing 150 ml of liquid Luria-Bertani (LB) broth supplemented with 100 μg/ml carbenicillin antibiotic. The culture was incubated on an Innova 4430 shaker (250 rpm) at 37° C. overnight (16 to 18 hours).

Chemical Induction of Double-Stranded RNA Expression in AB309-105

[0305] Expression of double-stranded RNA from the recombinant vector, pGBNJ003, in the bacterial strain AB309-105 was made possible since all the genetic components for controlled expression are present. In the presence of the chemical inducer isopropylthiogalactoside, or IPTG, the T7 polymerase will drive the transcription of the target sequence in both antisense and sense directions since these are flanked by oppositely oriented T7 promoters.

[0306] The optical density at 600 nm of the overnight bacterial culture was measured using an appropriate spectrophotometer and adjusted to a value of 1 by the addition of fresh LB broth. Fifty ml of this culture was transferred to a 50 ml Falcon tube and the culture then centrifuged at 3000 g at 15° C. for 10 minutes. The supernatant was 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 were induced for 2 to 4 hours at room temperature.

Heat Treatment of Bacteria

[0307] Bacteria were killed by heat treatment in order to minimize 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 was 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 was resuspended in a total volume of 50 ml of 0.05% Triton X-100 solution. The tube was stored at 4° C. until further use

G. Laboratory Trials to Test *Escherichia coli* Expressing dsRNA Targets Against *Phaedon cochleariae*

Leaf Disc Bioassays

[0308] The leaf-disc bioassay method was employed to test double-stranded RNA from target PC010 produced in *Escherichia coli* (from plasmid pGCDJ001) against larvae of the mustard leaf beetle. Leaf discs were prepared from oilseed rape foliage, as described in Example 4. Twenty μl of a bacterial suspension, with an optical density measurement of 1 at 600 nm wavelength, was pipetted onto each disc. The leaf disc was placed in a well of a 24-multiwell plate containing 1 ml gellified agar. On each leaf disc were added two neonate larvae. For each treatment, 3 replicates of 16 neonate larvae per replicate were prepared. The plates were kept in the insect rearing chamber at 25±2° C. and 60±5% relative humidity, with a 16:8 hours light:dark photoperiod. At day 3 (i.e. 3 days post start of bioassay), larvae were transferred to a new plate containing fresh treated (same dosage) leaf discs. The leaf material was refreshed every other day from day 5 onwards. The bioassay was scored on mortality and average weight. Negative controls were leaf discs treated with bacteria harbouring plasmid pGN29 (empty vector) and leaf only.

A clear increase in mortality of *P. cochleariae* larvae with time was shown after the insects were fed on oilseed rape leaves treated with a suspension of RNaseIII-deficient *E. coli* strain AB309-105 containing plasmid pGCDJ001, whereas very little or no insect mortality was observed in the case of bacteria with plasmid pGN29 or leaf only control (FIG. **3**-PC).

Plant-Based Bioassays

[0309] Whole plants are sprayed with suspensions of chemically induced bacteria expressing dsRNA prior to feeding the plants to MLB. 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. MLB 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 10^9 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.

[0310] Spraying plants with a suspension of *E. coli* bacterial strain AB309-105 expressing target dsRNA from pGBNJ003 leed 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