

293, proline 129.33, serine 124.28, threonine 127.16, tryptophane 42.75, tyrosine 38.63, L-valine 190.85. The amino acids were dissolved in 30 ml Milli-Q H₂O except for tyrosine which was first dissolved in a few drops of 1 M HCl before adding to the amino acid mix. The vitamin mix component of the diet was prepared as a 5× concentrate stock as follows: in mg/L, amino benzoic acid 100, ascorbic acid 1000, biotin 1, calcium pantothenate 50, choline chloride 500, folic acid 10, myoinositol 420, nicotinic acid 100, pyridoxine hydrochloride 25, riboflavin 5, thiamine hydrochloride 25. The riboflavin was dissolved in 1 ml H₂O at 50° C. and then added to the vitamin mix stock. The vitamin mix was aliquoted in 20 ml per aliquot and stored at -20° C. One aliquot of vitamin mix was added to the amino acid solution. Sucrose and MgSO₄·7H₂O was added with the following amounts to the mix: 20 g and 242 mg, respectively. Trace metal stock solution was prepared as follows: in mg/100 ml, CuSO₄·5H₂O 4.7, FeCl₃·6H₂O 44.5, MnCl₂·4H₂O 6.5, NaCl 25.4, ZnCl₂ 8.3. Ten ml of the trace metal solution and 250 mg KH₂PO₄ was added to the diet and Milli-Q water was added to a final liquid diet volume of 100 ml. The pH of the diet was adjusted to 7 with 1 M KOH solution. The liquid diet was filter-sterilised through an 0.22 µm filter disc (Millipore).

[0373] Green peach aphids (*Myzus persicae*; source: Dr. Rachel Down, Insect & Pathogen Interactions, Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK) were reared on 4- to 6-week-old oilseed rape (*Brassica napus* variety SW Oban; source: Nick Balaam, Sw Seed Ltd., 49 North Road, Abington, Cambridge, CB1 6AS, UK) in aluminium-framed cages containing 70 µm mesh in a controlled environment chamber with the following conditions: 23±2° C. and 60±5% relative humidity, with a 16:8 hours light:dark photoperiod.

[0374] One day prior to the start of the bioassay, adults were collected from the rearing cages and placed on fresh detached oilseed rape leaves in a Petri dish and left overnight in the insect chamber. The following day, first-instar nymphs were picked and transferred to feeding chambers. A feeding chamber comprised of 10 first instar nymphs placed in a small Petri dish (with diameter 3 cm) covered with a single layer of thinly stretched parafilm M onto which 50 µl of diet was added. The chamber was sealed with a second layer of parafilm and incubated under the same conditions as the adult cultures. Diet with dsRNA was refreshed every other day and the insects' survival assessed on day 8 i.e. 8th day post bioassay start. Per treatment, 5 bioassay feeding chambers (replicates) were set up simultaneously. Test and control (gfp) dsRNA solutions were incorporated into the diet to a final concentration of 2 µg/11. The feeding chambers were kept at 23±2° C. and 60±5% relative humidity, with a 16:8 hours light:dark photoperiod. A Mann-Whitney test was determined by GraphPad Prism version 4 to establish whether the medians do differ significantly between target 27 (MP027) and GFP dsRNA.

[0375] In the bioassay, feeding liquid artificial diet supplemented with intact naked dsRNA from target 27 (SEQ ID NO: 1061) to nymphs of *Myzus persicae* using a feeding chamber, resulted in a significant increase in mortality, as shown in FIG. 1. Average percentage survivors for target 27, GFP dsRNA and diet only treatment were 2, 34 and 82, respectively. Comparison of target 027 with GFP dsRNA groups using the Mann-Whitney test resulted in a one-tailed P-value of 0.004 which indicates that the median of target 027 is significantly different (P<0.05) from the expected larger

median of GFP dsRNA. The green peach aphids on the liquid diet with incorporated target 27 dsRNA were noticeably smaller than those that were fed on diet only or with GFP dsRNA control (data not presented).

D. Cloning of a GPA Gene Fragment in a Vector Suitable for Bacterial Production of Insect-Active Double-Stranded RNA

[0376] What follows is an example of cloning a DNA fragment corresponding to a GPA gene target in a vector for the expression of double-stranded RNA in a bacterial host, although any vector comprising a T7 promoter or any other promoter for efficient transcription in bacteria, may be used (reference to WO0001846).

[0377] The sequences of the specific primers used for the amplification of target genes are provided in Table 8-MP. The template used is the pCR8/GW/topo vector containing any of target sequences. The primers are used in a PCR reaction with the following conditions: 5 minutes at 98° C., followed by 30 cycles of 10 seconds at 98° C., 30 seconds at 55° C. and 2 minutes at 72° C., followed by 10 minutes at 72° C. The resulting PCR fragment is 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 the respective sequence as given in Table 8-MP. The recombinant vector harbouring this sequence is named pGBNJ00XX.

E. Expression and Production of a Double-Stranded RNA Target in Two Strains of *Escherichia coli*: (1) AB309-105, and, (2) BL21(DE3)

[0378] The procedures described below are followed in order to express suitable levels of insect-active double-stranded RNA of insect target in bacteria. An RNaseIII-deficient strain, AB309-105, is used in comparison to wild-type RNaseIII-containing bacteria, BL21(DE3).

Transformation of AB309-105 and BL21(DE3)

[0379] Three hundred ng of the plasmid are added to and gently mixed in a 50 µA aliquot of ice-chilled chemically competent *E. coli* strain AB309-105 or BL21(DE3). The cells are incubated on ice for 20 minutes before subjecting them to a heat shock treatment of 37° C. for 5 minutes, after which the cells are placed back on ice for a further 5 minutes. Four hundred and fifty µl of room temperature SOC medium is 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 is 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 is 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 and BL21(DE3)

[0380] Expression of double-stranded RNA from the recombinant vector, pGBNJ003, in the bacterial strain AB309-105 or BL21(DE3) is 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