

(Zukowsky et al., 1983) which encodes a catechol dioxygenase that can convert chromogenic catechols; an α -amylase gene (Ikatu et al., 1990); a tyrosinase gene (Katz et al., 1983) which encodes an enzyme capable of oxidizing tyrosine to DOPA and dopaquinone which in turn condenses to melanin; an α -galactosidase, which catalyzes a chromogenic α -galactose substrate.

[0179] A transformation vector can be readily prepared using methods available in the art. The transformation vector comprises one or more nucleotide sequences that is/are capable of being transcribed to an RNA molecule and that is/are substantially homologous and/or complementary to one or more nucleotide sequences encoded by the genome of the insect, such that upon uptake of the RNA there is down-regulation of expression of at least one of the respective nucleotide sequences of the genome of the pest.

[0180] VIII. Methods for Genetic Engineering

[0181] The present invention contemplates introduction of a nucleotide sequence into an organism to achieve pest inhibitory levels of expression of one or more dsRNA molecules. The inventive polynucleotides and polypeptides may be introduced into a host cell, such as bacterial or yeast cell, by standard procedures known in the art for introducing recombinant sequences into a target host cell. Such procedures include, but are not limited to, transfection, infection, transformation, natural uptake, calcium phosphate, electroporation, microinjection biolistics and microorganism-mediated transformation protocols. The methods chosen vary with the host organism.

[0182] A transgenic organism of the present invention is one that comprises at least one cell in its genome in which an exogenous nucleic acid has been stably integrated. Thus, a transgenic organism may contain only genetically modified cells in certain parts of its structure.

[0183] Accordingly, the present invention also encompasses a transgenic cell or organism comprising any of the nucleotide sequences or recombinant DNA constructs described herein. The invention further encompasses prokaryotic cells (such as, but not limited to, gram-positive and gram-negative bacterial cells) and eukaryotic cells (such as, but not limited to, yeast cells or plant cells).

[0184] For example, the present invention contemplates introducing a target gene into a bacterium, such as *Lactobacillus*. The nucleic acid constructs can be integrated into a bacterial genome with an integrating vector. Integrating vectors typically contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (EP 0 127,328). Integrating vectors may also be comprised of bacteriophage or transposon sequences. Suicide vectors are also known in the art.

[0185] Construction of suitable vectors containing one or more of the above-listed components employs standard recombinant DNA techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required. Examples of available bacterial expression vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript™ (Stratagene, La Jolla, Calif.), in which, for example, a *D. v. virgifera* protein or fragment thereof, may be ligated into the vector in frame with sequences for the

amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke and Schuster, 1989); and the like.

[0186] The invention also contemplates introducing a target gene into a yeast cell. A yeast recombinant construct can typically include one or more of the following: a promoter sequence, fusion partner sequence, leader sequence, transcription termination sequence, a selectable marker. These elements can be combined into an expression cassette, which may be maintained in a replicon, such as an extrachromosomal element (e.g., plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 (Botstein et al., 1979), pCl/1 (Brake et al., 1984), and YRp17 (Stinchcomb et al., 1982). In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and typically about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20.

[0187] Useful yeast promoter sequences can be derived from genes encoding enzymes in the metabolic pathway. Examples of such genes include alcohol dehydrogenase (ADH) (EP 0 284044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EP 0 3215447). The yeast PHOS gene, encoding acid phosphatase, also provides useful promoter sequences (Myanohara et al., 1983). In addition, synthetic promoters that do not occur in nature also function as yeast promoters. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (U.S. Pat. Nos. 4,876,197 and 4,880,734). Examples of transcription terminator sequences and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes, are known to those of skill in the art.

[0188] Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors typically contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome (Orr-Weaver et al., 1983). An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver et al., supra. One or more expression constructs may integrate, possibly affecting levels of recombinant protein produced (Rine et al., 1983).

[0189] IX. Quantifying Inhibition of Target Gene Expression

[0190] Inhibition of target gene expression may be quantified by measuring either the endogenous target RNA or the protein produced by translation of the target RNA and the consequences of inhibition can be confirmed by examination of the outward properties of the cell or organism. Techniques for quantifying RNA and proteins are well known to one of ordinary skill in the art. Multiple selectable markers are available that confer resistance to ampicillin, bleomycin, chloramphenicol, gentamycin, hygromycin, kanamycin, lincomycin,