

ing using an aluminum block template shaped in the form of a 96-well microtiter-plate. To each such formed dome was added 40 ul of a 1:10 (v/v) dilution of test solution in diet. The dome-molded Parafilm™ is then heat sealed with a sheet of Mylar. The resulting Parafilm dome sheet (96-wells) is placed onto a 96-well flat-bottomed microtiter plate containing one *Lygus* nymph each. The assay is typically scored after 4 days for mortality and stunting (0-3).

[0255] Insect inhibition results from all tests is shown in Table 7.

TABLE 7

Bioactivity of Strain Xs85816												
BCW	WTB	BWV	CEW	TBW	WCR	Rs	Fg	Sc	MI	Bc	Sa	
-	+++	++	-	-	-	+	-	++	+	+	+++	

Legend:

- BCW = Black Cut Worm;
- WTB = Western Tarnished Plant Bug;
- BWV = Boll Weevil;
- CEW = Corn Ear Worm;
- TBW = Tobacco Bud Worm;
- WCR = Western Corn Root Worm;
- Rs = *Rhizoctonia solani*;
- Fg = *Fusarium graminearum*;
- Sc = *Saccharomyces cerevisiae*;
- MI = *Micrococcus luteus*;
- Bc = *Bacillus cereus*;
- Sa = *Staphylococcus aureus*.
- = no activity;
- + = low activity;
- ++ = medium activity;
- +++ = strong activity

Example 7

[0256] This example illustrates the alignment of insect inhibitory amino acid sequences identified from publicly available databases to sequences encoded by genomic sequences disclosed herein. Also, thermal amplification primers are described based on conserved regions identified in the alignments which can be used to isolate DNA sequences encoding insect inhibitory proteins from both *Xenorhabdus* and *Photorhabdus* species. Surprisingly, primers designed to isolate insect inhibitory proteins based on regions of substantial homology between proteins from diverse species fail to produce amplification products from strains which are believed to be phylogenetically more closely related and which have been shown to produce insect inhibitory proteins active against the same target pest insect species. In this example, *Xenorhabdus* and *Photorhabdus* strains other than strain Xs85816 and W14 were selected for thermal amplification and southern blot analysis based on their having demonstrated activity against southern corn root-worm.

[0257] Translation of *Xenorhabdus* genomic data indicated several sequences encoding proteins which exhibited homologies to previously identified *Photorhabdus* insect inhibitory polypeptides available in public databases such as GenBank (see, for example Table 2). Thermal amplification primers were designed to amplify DNA sequences from within insect inhibitory coding sequences. Based on *Xenorhabdus* sequences which were aligned with *Photorhabdus* sequences, the top BLAST hits were used along with regions of greatest amino acid sequence conservation for primer design. *Xenorhabdus* polypeptide XIP8 most closely aligned with polypeptide TccB from *Photorhabdus* strain W14. Based on this alignment, regions of greatest amino acid sequence homology were used to design a XIP-8 primer set consisting of the primers 5'-GAGATCGATCCGGATACAG-3' and 5'-AATATTCAAACGGCGCTC-3'. As indicated in Table 8, this primer set amplified DNA xip8 sequences from *Xenorhabdus* strain Xs85816 but not from other *Xenorhabdus* strains or from at least one other *Photorhabdus* strain. Interestingly, a primer set designed from regions of identity between XIP9 and XIP10 protein coding sequences comprising the primers 5'-CCGGAACCKCARTTRGGYRAAGG-3' and

5'-GCCTGAGTYTGTCYTGTGCTG-3' was able to amplify xip9 and xip 10 coding sequences from *Xenorhabdus* strain Xs85816 as well as sequences from several other *Xenorhabdus* strains. However, the XIP9/10 primer set failed to amplify sequences from every *Xenorhabdus* or *Photorhabdus* strain analyzed, even though degeneracies (R=A+G, Y=C+T, W=A+T) were engineered into the primer set to compensate for possible wobble. A further primer set similar in nature to the set designed for XIP9 and XIP10 was also designed from DNA sequences encoding regions of amino acid sequence identity between XIP 1 and XIP2 consisting of the sequences 5'-CGTGATGCGGAAAACCTGGTATCA-3' and 5'-TGRC-TRACRCGWGGATTRGAAAG-3'. Primer set XIP1/2 failed to amplify any sequences from *Xenorhabdus* or *Photorhabdus* strains other than *Xenorhabdus* strain Xs85816.

[0258] The product derived from thermal amplification of Xs85816 genomic DNA using the primers designed to amplify XIP8 coding sequences was labeled and used to probe total genomic DNA from Xs85816 as well as other *Xenorhabdus* and *Photorhabdus* strains. A thermal amplification product derived from *Xenorhabdus* strain Xs85816 ompR, a highly conserved gene at the DNA sequence level among gram negative Enterobacteriaceae, was used as a control. The ompR sequence was able to hybridize to sequences in all strains analyzed, however, the xip8 sequence failed to hybridize to any sequences in the strains tested other than Xs85816. In addition, ompR thermal amplification primers based on the ompR gene in Xs85816 also amplified a sequence of equivalent size from each strain tested. These results, taken together, suggest a large diversity in the proteins exhibiting insect inhibitory activity from both *Xenorhabdus* and *Photorhabdus* species.

TABLE 8

Characterization of <i>Xenorhabdus</i> and <i>Photorhabdus</i> Strains							
Bacterial Strain	Symbiotic nematode	WCR Activity	XIP-8 PCR product	XIP-9/10 PCR product	XIP-1/2 PCR product	OmpR PCR product	Southern (XIP-8)
85816	<i>Steinernema</i> sp	no	yes	yes	yes	yes	yes
85825	<i>S. intermedium</i> ¹	yes	no	yes	no	yes	no
85826	<i>S. intermedium</i> ¹	yes	no	yes	no	yes	no