Partial CO1 sequence of the cotton bollworm, *Helicoverpa armigera*

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ABSTRACT

This paper reports the comparison of the partial CO1 sequence of Indian, field collected Helicoverpa armigera *and a laboratory reared, insecticide susceptible, homogeneous strain of* H. armigera. *Using primers C1-J-2090 and C1-N-2659 the near mid region of the CO1 sequence was obtained. In the resulting amino acid sequence substitutions at 16 positions were noticed in the insecticide susceptible strain compared to Indian* H. armigera.

Introduction

Animal mitochondrial genome is a small circular molecule ranging in size from 15-18 Kb (Wilson *et al.*, 1985). Insect mitochondrial DNA consists of 37 genes (Mitchel, 1993). With rare exceptions, insect mitochondrial genome is inherited exclusively maternally (Khambampathi and Smith, 1995). The CO1 region is the largest of the three mitochondria encoded cytochrome oxidase (CO) encoded subunits and is one of the largest protein coding genes in the metazoan mitochondrial genome. The CO1 region of the Indian *H. armigera* has not been reported in literature.

We proposed the existence of host races in the Indian *H. armigera* on the basis of its feeding preference, cornutal spine numbers in male genitalia and RAPD studies (Kranthi, unpublished). An attempt was being made to differentiate the two strains on the basis of their CO1 sequence and is not being discussed herein. Partial CO 1 sequence was obtained as a part of these studies and is being reported.

The cotton bollworm, *H. armigera,* is a major pest of cotton in addition to being a polyphagous pest. It is found damaging several crops throughout the year. The main cause of concern to scientists and administrators is control failures of the insect on cotton that is attributed largely to the phenomenon of insecticide resistance. Different populations of *H. armigera* available in the country have exhibited resistance against all the major groups of insecticides (Kranthi *et al.,* 2002)

In stark contrast we have a universal insecticide susceptible, homogeneous strain of *H. armigera*. This paper reports the comparison of partial gene sequence of the CO1 region, of the two strains.

Experimental Procedure

Rearing of insects

Field collected *H. armigera* larvae and the insecticide susceptible strain of *H. armigera* were reared in the laboratory on a semi synthetic diet (Armes *et al.*, 1992) at 27 ^{0}C+ 1 ^{0}C at 75% RH.

Conserved primers, PCR amplification and direct sequencing

DNA was purified from eight individual female live moths of each strain using a phenol/chloroform procedure described by Livak (1984). PCR was carried out in a 25 μl reaction containing 10x assay buffer with 15 mM MgCl2, dNTPs 10 mM each (0.5 μl), sample DNA (0.5 μl), Taq 1Unit per reaction, forward and reverse primer each at 5 pmol per reaction finally made up to 25 μl with sterile water.

Following an initial denaturation at 94 °C for 2 min and 80 °C for 2 min, Taq was added to the PCR reaction, forty cycles were performed in a DNA thermal cycler (MJ Research), each consisting of melting at 94 °C for 45 s, annealing at 50 °C for 45 sec and extension at 72 °C for 1.30 min. PCR was terminated in the last cycle at 72 °C for 10 min. Amplification products were electrophoresed on 1% agarose. No contamination was detected in the negative controls. PCR product was purified on Geneclean Turbo from Q Biogene and used for DNA sequencing in both directions using the forward and reverse primer, each, separately on CEQ 2000, Beckman Coulter, following the protocol described by the company.

Oligonucleotide primers

DNA sequences for primer sets used for amplification of the CO1 region are given in Table1. Two sets of primers each comprising of a forward and reverse primer was used. Primers were designed based on published sequences of other organisms (Simon *et al.*, 1994) and were synthesized by Thermo Hybaid, Germany.

Analysis of the data was done using Clustal X software (for determining the homology of sequences obtained).

Result and Discussion

The two primer sets were designed to amplify 569 bp and 645 bp of the CO1 region. Sequences obtained with the forward and reverse primers were highly reproducible in each of the eight samples tested, especially with C1-J-2090 and C1-N-2659. This sequence corresponds to the near middle region of the CO 1 sequence (Lunt *et al.*, 1996). Homology between the forward and reverse sequence reads was found to be good and hence the sequence reads obtained were considered reliable. However sequence reads obtained with C1-N-2659 and C1- J- 2369 were unsatisfactory. Therefore a full-length sequence of 1214 bp was not obtained. Instead, a 556 bp region with high fidelity of which 450 bp matched well with the sequence of the insecticide susceptible strain was obtained as indicated in Figure 1. An additional 79 bp were added to the sequence obtained with the first primer pair with the sequence obtained with the sec-

ond primer set. The partial CO1 sequence of *H. armigera* bears accession number AY 264943 of the NCBI data bank.

The sequenced CO1 region of *H. armigera* was represented by 30.0% A, 40.1% T, 15.3% G and 14.6% C of the 556 bp sequence. This composition was different in the insecticide susceptible strain (31.1% A, 37.7% T, 16.4% G and 14.8% C of the 562 bp sequence). Difference of six base pairs between the two strains was due to insertion of nucleotides in the insecticide susceptible laboratory strain.

CO1 is the terminal catalyst in mitochondrial respiratory chain and has been well studied at the biochemical level. Reaction centers of this subunit have been mapped (Gennis, 1992) and it provides a background that enables interpretation of sequence differences in terms of gene function. Base pair substitutions occurred in at least fifteen positions in the insecticide resistant strain with respect to the insecticide susceptible strain. This led to a difference in software generated amino acid sequence (Figure 2). In the 160 amino acid sequence obtained, substitutions at 16 positions were noticed in the insecticide susceptible strain compared to Indian *H. armigera*. It was interesting to note that amino acid iso-leucine and tryptophan in the susceptible strain was substituted with methionine and glutamate/ glutamine in the Indian insecticide resistant strain in at least seven and four positions respectively of the amino acid sequence. It remains to be seen whether these substitutions are in any way responsible for insecticide resistance seen in the Indian strain.

The primer sequences of C1-J-2090 and C1-J-2410 were identical in both strains. However C1-J-2369 demonstrated the presence of a base substitution at the sixth base pair position where C of *Helicoverpa* was replaced with T in the insecticide susceptible strain.

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References

- Armes, N.J., Bond, G.S and Cooter, R.J (1992). The laboratory culture and development of *Helicoverpa armigera*, Natural Resources Institute, Bulletin 57 (Chatham, NRI, UK).
- Gennis, R.B. (1992). Site directed mutagenesis studies on subunit of the aa3-type cytochrome C oxidase of *Rhodobacter sphaeroides*: a brief review of progress to date. *Biochemical Biophysical Acta*, **1101**: 184-187.
- Khambampathi, S. and Smith, P.T. (1995). PCR primers for the amplification of four insect mitochondrial gene fragments. *Insect Molecular Biology*, **4**: 233-236.
- Kranthi, K.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, S.S. and Russell, D.A. (2002). Insecticide resistance in five major pests of cotton in India. *Crop Protection*, **21**: 449-460.
- Livak, K.J. (1984) Organization and mapping of a sequence on the Drosophila melanogaster X and Y-chromosomes that are transcribed during spermatogenesis. *Genetics*, **107**, 611-634
- Lunt, D.H., Zhang, D.X., Zsymura, J.M. and Hewitt, G.M (1996). The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology*, **5**: 153-165.
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. and Flook, P. (1994). Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological society of America*, **87**: 652-701.
- Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllensten, U.B., Helm-Bychowski, K.M., Higuchi, R.G., Palumbi, S.R., Prager, E.M., Sage, R.D and Stoneking, M. (1985). Mitochondrial DNA and two perspectives on evolutionary genetics. *Biology Journal of Linnaeus Society,* **26**: 375-400.

Table 1. *Sequences of primers used in the study.*

S. no	Primer code	Sequence
	C1-J-2090	5'-AGT TTT AGC AGG AGC AAT TAC TAT-3'
2	C1-N-2659	5'-GCT AAT CCA GTA AAT AAA GG-3'
3	C1-J-2369	5'- TAC AGT TGG AAT AGA CGT TGA TAC-3'
4	TL-2N-3014	5'-TCC AAT GCA CTA ATC TGC CAT ATT-3'

(Source: Simon *et al*., 1994).

H. armigera	*GGAGCAATTA*	*CTATA*CTTTT	AACAGATCGA	AACCTTAATA	CAT CTTTTT
German	*GGAGCAATTA*	*CTATA*CTTTT	AACAGATCGA	GACCGTAATA	CATGCTTTTT
	TTGACCCTGC	TGG AGGAG	GTGATCCTAT	TTTATATCAC	C ATTTATTTT
	TTGACCCTGC	CTGGGAGGAG	GTGATCCTAT	TTTATA CAA	CTATTTATTTT
	GATTTTTTG0	GCCATCCAGA	AGTGTATATT	TTAATTTTAC	CGGGATTTGG
	GATTTTTTGT	GCCATCCAGA	AGTGTATATT	TTAATTTTAC	CAGGATTTGG
	TATAATTTCT	CACATTATTT	CCCAAGAAAG	AGGAACAAAA	GAAACATTTG
	TATAATTTCT	CACATTATTT	CCCAAGAAAG	AGGAAAAAAA	GAAACATTTG
	GTTGTTTAGG	GATAATTTAT	GCTATATTAG	CTATTGGATT	ATTAGGATTT
	GTTGTTTAGG	GATAATTTAT	GCTATATTAG	CTATTGGATT	ATTAGGATTT
	ATTGTATGAG	CTCACCATAT	ATT*TACAGCT*	*GGTATAGATA*	*TTG*ATACTCG
	ATTGTATGAG	CTCACCATAT	ATA*TACAGTT*	*GGTATAGATA*	*TTG*ATACTCG
	AGCTTATTTC	ACATCAGCTA	CTATAATTAT	TGCAGTA*CCA*	*ACAGGAATTA*
	AGCTTATTTC	ACATCAGCTA	CTATAATTAT	CGCAGTA*CCA*	*ACAGGAATTA*
	AAATTTTTAG	*TTGAT*TAGCT	ACTTTTCATG	GAACTCAACT	TAATTACTCC
	AAATTTTTAG	*TTGAT*AAGCT	ACTTTTCATG	GAACTCAAAT	CAATTACTCC
	CCATCTATTT	TATGAAGTTT	AGGATTTGTA	TTTTTATTTA	CTGTTGGAGG
	CCATCTATTT	TATGAAGTTT	AGGATTCGTA	TTTTTATTTA	CTGTTGGAGG
	GTTAACAGGA	GTAATTTTAT	CTAA TTCTT	CTATTGATAT	TACATTACAT
	ATCAACAGGA	GTAATTCTAT	CTAAATTCTC	CTATTGATAT	TACATGACAT

Figure 1. *Comparison of CO1 sequences of* H. armigera *and the insecticide susceptible strain. Underlined nucleotides indicate differences between two strains. Nucleotide sequences in italics refer to primer sequences.*

H. armigera	GAITMLLTDR	NLNTSFFDPA	GGGDPILYHH	LFWFFGHPEV	YILILPGFGM
German strain	GAIT**ILX**TDR	**DR**NTSFFDPA	GGGDPILY**PN**	LF**Z**FFGHPEV	YILILPGFG**I**
	ISHIISQESG	TKETFGCLGM	IYAMLAIGLL	GFIVWAHHMF	TAGMDIDTRA
	ISHIISQERG	KKETFGCLG**I**	IYA**I**LAIGLL	GFIV**Z**AHH**I**F	T**V**G**I** DIDTRA
	YFTSATMIIA	VPTGIKIFSW	LATFHGTQLN	YSPSILWSLG	FVFLFTVGGL
	YFTSAT**I**IIA	VPTGIKIFS**Z**	LATFHGTQLN	YSPSIL**Z**SLG	FVFLFTVGGL
	TGVILSNSSI	DITLHD			
	TGVILSNSSI	DITLH**A**			

Figure 2. *Comparison of amino acid sequences of* H. armigera *and the insecticide susceptible strain generated through software (Clustal X). The letters in bold refer to the amino acid change in the insecticide susceptible strain with reference to* H. armigera.