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Research Article

Detection and characterization of *Wolbachia* in *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), a parasitoid of the diamond back moth *Plutella xylostella* (Linn.)

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ABSTRACT: *Cotesia plutellae* (Kurdjumov) is an indigenous, larval endoparasitoid that attacks mid instar larvae of the diamond back moth (DBM). Although the parasitoid is distributed widely, not all local populations appear to be equally effective in controlling the DBM. Bacterial endosymbionts may play regulatory role in determining their efficiency. Field collected *C. plutellae* populations from cauliflower fields were assessed for the prevalence of the bacterial endosymbionts. Bacterial endosymbionts in the genus *Wolbachia* were detected in the populations obtained from Hoskote (Karnataka) and Thirupathi (Andhra Pradesh). PCR amplification using specific primers for *Wolbachia* revealed 528 and 518 bp for the populations, respectively. Sequencing of the *Wolbachia* surface protein, *wsp*, revealed *Wolbachia* infection to be related to *Wolbachia* endosymbiont of *Cotesia glomerata* outer surface protein, *wsp* gene (Genbank Accession No. AB094202) with maximum identity of 99% with BLAST search of NCBI. The sequence was submitted to the GenBank with the Accession No. JF421566. The detection of *Wolbachia* in the parasitoid signifying its role in biological manipulations of the parasitoid for enhanced efficiency is discussed.

KEY WORDS: *Wolbachia*, characterization, *Cotesia plutellae*, cabbage, *Plutella xylostella*

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INTRODUCTION

Cotesia plutellae (Kurdjumov) (Hymenoptera: Braconidae), a solitary larval endoparasitoid, is one of the most important biological control agents of the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), regarded as the most significant pest of *Brassica* crops. The key factors for species/strain selection in biocontrol release programs include acceptance of the host plant for foraging, acceptance of the host insect, tolerance to local environmental conditions, ability to parasitize hosts of varying ages, and searching efficiency (Brand *et al*., 1984; Doutt, 1958; Trumble and Alvarado-Rodriguez, 1998). Parasitoids have developed a natural arsenal and a number of physiological mechanisms to enable them to successfully colonise the host and regulate host development to their own benefit. One of these is through their association with symbionts. Obligate symbionts are required for the successful parasitism and suppression of the host immune system, as well as for inducing physiological alterations in the parasitized host (Consoli and Elliot, 2006).

Symbiotic bacteria have been reported to be associated with a number of parasitoid species and are known to inflict various types of metabolic, physiological and reproductive alterations, with the sex regulators bacteria being the most frequent ones. The bacteria infect the reproductive tissues (ovaries and testes) of arthropods and are transmitted through the egg cytoplasm and alter reproduction in their hosts. The infection affects feminization, parthenogenesis, male killing and cytoplasmic incompatability (Weeks *et al.,* 2002).

Among the sex regulators, *Wolbachia* infect a large number of parasitic hymenopterans and has been reported around the globe in different insect species including the major insect orders such as Diptera, Coleoptera, Lepidoptera, Hymenoptera and Orthoptera (Werren and Windsor, 2000). About 2/3 of all insect species are infected with *Wolbachia*. *Wolbachia* enhance their own spread through the host population by phenotypes and in doing so drive the associated host mitochondrial haplotypes to high frequencies, causing dramatic changes in host mtDNA patterns (Delgado and Cook, 2009). These have been investigated for their potential use to host control

because of their ability to modulate sex ratio or act as vectors for introducing foreign genes in to their hosts, which could open new opportunities for insect manipulations (Shoemaker *et al*., 2002). In the present study, the occurrence of the *Wolbachia* symbiont in *C. plutellae* was investigated to assess their potential usefulness in insect management programmes.

MATERIALS AND METHODS

Insect culture

Two populations from Hoskote (Karnataka) and Thirupathi (Andhra Pradesh) were used in this study. Individual cocoons of *C. plutellae* obtained from *P. xylostella* larvae were collected from field-grown cauliflower plants. The colony of *P. xylostella* was maintained on potted mustard seedlings, *Brassica juncea* L. Czern for oviposition, in ventilated oviposition cages for the development of larval stages. Host larvae at early L3 stage were exposed to *C. plutellae* on mustard seedlings in ventilated cages and maintained on the plant until cocoon formation. Cocoons were collected and held in plastic cages until adult emergence. Adult wasps were fed on honey.

Insect DNA isolation

Two adults of *C. plutellae* were freeze-killed at –80°C and transferred to an Eppendoff tube and homogenization was done by crushing the adult in 20µl of 5% Chelex 100 MB (BIO-RAD). This was followed by incubation for 3 h at 56°C and then at 100ºC for 10 min. Eight microliters of 2.5 mg/ml Proteinase K solution were added to the tubes. Solutions were incubated at 55°C for 1 h, heated twice to 90°C for 15 min, and centrifuged for 2 min at 14,000 rpm. The supernatant was refreshed by a 1 min 14,000 rpm centrifugation

Wolbachia specific PCR

A molecular diagnostic approach was adapted for the detection of *Wolbachia* infection, since *Wolbachia* cannot be cultured. The assay was based on PCR mediated amplification and sequence determination of *wsp* gene. The presence of *Wolbachia* was verified by a PCR method based on the *Wolbachia* surface protein (*wsp*) gene. Diagnostic PCR using the *Wolbachia* specific primer set (forward: 52-CAT ACC TAT TCG AAG GGA TAG-32; reverse: 52-AGA TTC GAG TGAAAC CAA TTC-32) was performed to determine the *Wolbachia* infection status of adults of the wasps. The PCR reaction was performed in a 500 ìl PCR tube with a 25 µl reaction mixtures each containing 1 mM dNTPs mix (3 µl), 5 ng/ µl specific primer (5 µl), 2.0 U Taq Polymerase (MBI, Fermentas) and 2 µl of template DNA solution (30 ng) in Taq reaction buffer. The reaction was set in the Thermal Cycler (Biorad Laboratories). The temperature profile for *Wolbachia* specific PCR was a pre-denaturing step of 2 min at 94°C, followed by 38 cycles of 60 s at 94°C, 60 s at 60°C, and 60 s at 72°C, with a final extension step of 10 min at 72°C. The amplified PCR – products were resolved by horizontal gel electrophoresis in 1.8 percent Agarose gel with a low range ladder (Fermentas Mass Ruler 100bp), visualized under UV trans-illuminator and size of the amplified *Wolbachia* specific bands was estimated by comparison with a co-migrating molecular weight standard. The *wsp* gene fragments from *Wolbachia* bacteria in *C. plutellae* were sequenced. Again, these sequences were aligned and blasted against sequences present in GenBank.

Sequence analysis

The PCR product was purified with MinElute PCR purification kit (Qiagen). The PCR product sequenced using an ABI prism 310 DNA sequencer using Big Dye Terminator reaction. The sequence was edited by BioEdit software and aligned using BLAST2 and verified. The BLAST2 (www.ncbi.nlm.nih.gov) and BioEdit tools were used to find the similarity between the two populations for the *wsp* gene. The pair-wise alignment was done by DNASTAR Lasergene 8 software.The sequences were uploaded to SeqBuilder and the pair-wise alignement was done by MegAlign of DNASTAR.

RESULTS AND DISCUSSION

The presence of *Wolbachia* infection was detected in the adult parasitoids obtained from both the populations of Hoskote (Karnataka) and (Andhra Pradesh) using the *Wolbachia* specific primers for the *wsp* gene. The PCR amplification of the *Wolbachia* strains revealed 528 and 518 bp size for the population from Hoskote and respectively (Fig. 1). Sequencing of the *wsp* gene of Hoskote population revealed *Wolbachia* infection to be related to *Wolbachia* endosymbiont of *Cotesia glomerata* outer surface protein, *wsp* gene partial cds (Genbank Accession No. AB094202) with maximum identity of 99% with BLAST search of NCBI. The DNA sequence of *C. plutellae* from Hoskote (Karnataka) was submitted to GenBank (Acc. no JF421566). The pair-wise alignment of the *C. plutellae* (Karnataka and Andhra Pradesh) populations were done by DNA Star software. The pairwise alignment from MegAlign report revealed that the bacteria from both the populations were identical.

The detection of *Wolbachia* in the parasitoid populations of *C. plutellae* may prove to be useful for biological manipulations of the parasitoid. These bacteria

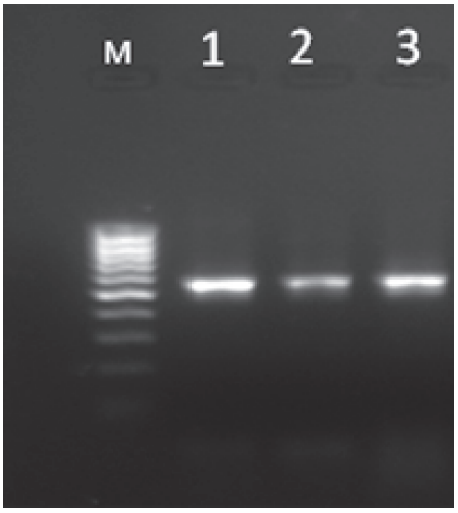

Fig. 1. PCR amplification of *wsp* gene of *Wolbachia* from *Cotesia plutellae* (Lane M: 100bp ladder, Lane 1: *C. plutellae* (Hoskote, Karnataka) Lanes 2: *C. plutellae* (Thirupathi, Andhra Pradesh) Lane 3: *Wolbachia* + from *Trichogramma pretiosum*)

can drive particular mtDNA haplotypes through populations and alter reproductive biology (Delgado and Cook, 2009). Therefore, prescreening of agents for *Wolbachia* has been advocated in biocontrol programs, since, *Wolbachia* can manipulate host reproduction through parthenogenesis, feminization and cytoplasmic incompatability (Ngi-Song and Mochiah, 2001), In *Cotesia sesamiae,* a braconid wasp that attacks stem borers in Africa, *Wolbachia* is known to cause cytoplasmic incompatability (Mochiah *et al.,* 2002), while, in the species of *Trichogramma* wasps, the egg parasitoid of several lepidopteran pests, parthenogenesis is often induced. However, infected individuals have reduced fecundity and dispersal in laboratory studies (Hosokawa *et al.,* 2006) Nevertheless, because of the greater production of female progeny by parthenogenesis (Huigens *et al.,* 2000), infected females are predicted to be more efficacious in pest control. The feasibility of such utilization depends heavily upon how the transmission of *Wolbachia* and the genes being driven in to a population occurs.

Sequence submitted to GenBank

ATACAACGGTGAAATTTTACCTTTTTATACAA
AAGTTGATGGTATTATAAATGCAATAGGTAAA
GAAAAGGATAGTCCCTTAACAAGATCTTTTAT
AGCTGGTGGTGGTGCATTTGGTTATAAAATGG
ATGACATTAGAGTTGATGTTGAAGGGCTTTAC
TCACAATTGGCTAAAGATACAGCTGTAGTAAA
TACTTCTGAAACAAATGTTGCAGACAGTTTA
ACAGCGTTTTCAGGATTGGTTAACGTTTATTA
CGATATAGCGATTGAAGATATGCCTATCACTC
CATACGTTGGTGTTGGTGTTGGTGCAGCATAT
ATCAGCAATCCTTCAAAAGCTGATGCAGTTA
AAGATCAAAAAGGATTTGGTTTTGCTTATCA
AGCAAAAGCTGGTGTTAGCTATGATGTAACT
CCAGAAATCAAACTCTTTGCTGGAGCTCGT
TACTTCGGTTCTTATGGTGCTAGTTTTGATA
AGGCAGCTAAGGATGATACTGGTATCAAA
AATGTTAACTTCAAG

Translated protein

YNGEILPFYTKVDGIINAIGKEKDSPLTRSFIAG
GGAFGYKMDDIRVDVEGLYSQLAKDTAVVNT
SETNVADSLTAFSGLVNVYYDIAIEDMPITPYV
GVGVGAAISNPSKADAVKDQKGFGFAYQAKA
GVSYDVTPEIKLFAGARYFGSYGASFDKAAK
DDTGIKNVNFK

The infections may influence the host directly, eg., by affecting fecundity, activity or longevity or may have no discernable effect. *Wolbachia* infection types in parasitoids may give clues as to which parasitoid strain can be used effectively. If parasitoid strains with *Wolbachia* infection are released in an area where a *Wolbachia* –free population occurs, sterile mating will diminish the reproductive potential of the introduced parasitoid (Mandrioli, 2009). Thus, mating between males from populations that harbor *Wolbachia* and females free from *Wolbachia* infection could theoretically contribute to failure in biocontrol programmes. Incorporation of *Wolbachia* in biocontrol research strategies may be prohibited by technical challenges. Infections can be manipulated by elimination, transfection or genetic modifications.

The detection of *Wolbachia* in *C. plutellae,* a dynamic and effective parasitoid of *P. xylostella* in India would emphasize upon the need to intensify further research on investigating the role of *Wolbachia* in insect biology

and population dynamics. The application of *Wolbachia* as a form of sterile-insect technique for population replacement or to reduce the insect survival and their ability to transmit the infection (Kevin *et al.,* 2006), as vehicle for expressing foreign genes and rendering arthropods incapable of transmission of diseases, genetic manipulations of virus-vectoring capabilities of target insects (Kokoza *et al.* 2000; Pidiyar *et al.* 2003) would provide clues to novel pest management strategies.

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