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Wolbachia and phage WO infection in agriculturally important insects

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Abstract
Wolbachia is an endosymbiontic alpha-proteobacteria found in insects. These bacteria are maternally transmitted reproductive manipulators affect the host biology of insects. The reproductive manipulations include cytoplasmic incomparability, feminization, parthenogenesis, male killing. Recent, meta analysis has estimated 65% of insects harbor *Wolbachia*. Significant recent research of using *Wolbachia,* focuses potential in biological control of insect pests and disease vectors. In the present study, *Wolbachia* and phageWO infection in agriculturally important insects was diagnosed and we discussed the its feature application prospects in biological control of agricultural pests and disease vectors.

Keywords: *Wolbachia*, insects, pests, bacteria, disease vectors

1. Introduction
Bacterial symbiosis is generally common in arthropods and other invertebrates, and they play a vital role in host biology and evolutionary success of insects. *Wolbachia* is a maternally inherited alpha-proteobacteria found in arthropods, spiders, isopods and filarial nematodes [1]. These organisms generally fail to survive without their associated symbiont *Wolbachia*. *Wolbachia* may provide some nutrients which is beneficial for host through the metabolic pathways, which are important for fecundity of insect host [2]. The Recent, survey has estimated 65% of arthropod species are infected with *Wolbachia* [3]. *Wolbachia* induces reproductive anomalies in their hosts are cytoplasmic incompatibility, parthenogenesis, Male killing, Feminization and speciation. These reproductive manipulations are boon for infected individuals and allow the *Wolbachia* to spread through insect population [1, 4].

Pest and disease management poses considerable challenge for the agricultural and medical communities. In addition, public distress over pesticide use and additional strict environmental policy creates the need for new technologies. Bacterial symbiosis is common in arthropods that can be devastating pests and efficient disease vectors. One new approach for developing sustainable control of arthropod pest populations or reducing vector competence is by symbiont-based interventions that are environmental friendly and may replace less-efficient chemical control methods that are currently in use [5].

One new approach to control arthropod pest populations or to reduce vector competence is by *Wolbachia* endosymbiont-based control strategies that are environmentally friendly and may replace chemical control methods [6]. Research on *Wolbachia* is an interdisciplinary subject requiring methods and protocols from various fields. It is therefore essential to know the arthropod *Wolbachia* diversity, Arthropod- Host-*Wolbachia* Interactions, *Wolbachia* genomes, *Wolbachia* -based control strategies and regulatory and commercial aspects of *Wolbachia* -based control strategies. The current initiative detection of *Wolbachia* infection will insure in its successful implementation at the forefront of both basic and applied research in the area of *Wolbachia*-arthropod symbiosis for biological control of insect pests and diseases vectors.

2. Materials and Methods
The insect pests and disease vectors were collected in agriculture fields are brought to the laboratory and identified with the help of insect systematic scientists in NBAII. The systematically identified insects were stored at – 20ºC until DNA extraction.

2.1 Genomic DNA Isolation
The genomic DNA of insects was isolated by using DNASe blood tissue extraction kit of Qiagen.

2.2 Detection of *Wolbachia* and Phage WO by using Polymerase Chain Reaction (PCR)
The polymerase chain reaction (PCR) assay was carried out based on specific amplification of the *Wolbachia* WSP (*Wolbachia* Surface Protein) gene primer WSP81F – 5[1]TGGTCCAATAAGTGATGAAGAAAC3[1] and WSP 691R – 5[1]AAAAATTAA ACGCTACTCCA3[1] and phage WO specific primer ORF-7F CCCACATGAGCCAATGACGTCTG and ORF-7R CGTTCGCTCTGCAAGTAACTCCATTAAAAC synthesized in Bioserve Biotechnologies Pvt. Lt., India. The

PCR was carried out with Bio Rad Thermocycler, with 25µl reaction mixture containing 2.5µl of 10X PCR buffer, 0.5µl of dNTP's (10mM each), 2.5µl of 2.5mM $MgCl_2$ and 0.5 U Taq DNA polymerase, 1µl of both forward and reverse primer (5 pmol), 20 ng template DNA; and final volume of milique water to make up 25µl.The PCR was carried out with a cyclic condition of initial denaturation step at 94ºC for 5 min followed by 35 cycles with denaturation step at 92ºC for 1 min, annealing 55 ºC for 1.30 min extension 72ºC for 1.15 min, final extension at 72ºC for 10 min. The PCR amplified products was checked by electrophoresis on 1.5% agarose gel running in 1X TBE (89.2mM Tris HCl, 88.9mM Boric acid and 2mM disodium EDTA) buffer for a length of about 5 cm with a constant voltage of 70V. The gel was stained with 0.5 µg/ml ethidium bromide prior to casting. Gel documentation was done by using Alpha digi doc documentation system.

3. Results and Discussion

A total of 54 arthropod species collected from agricultural importance were PCR tested for *Wolbachia* and Phage WO (Table-1) infection**.** The *Wolbachia* specific primer *WSP* (*Wolbachia* surface protein gene) were detected the fragments of *Wolbachia* genes in pests and disease vectors (Figure-1). The enzymatic amplification of a specific DNA segment is made possible by the highly specific binding of a DNA polymerase that copies the segment. Because each newly made copy can serve as a template for further duplications, the number of copies of the target segment grows exponentially. The mobilities of DNA fragments that were amplified using DNA from different species of insects and pests**.**

The insect pests and disease vectors screened for the presence of *Wolbachia* and Phage WO infection in the 54 taxa screened, 24.07% showed positive for the presence of *Wolbachia* and its Phage WO as shown in the table 1(Figure-2). The agarose gel figure also confirms the presence of *Wolbachia* in the populations of *Exorista sorbillans, Nilaparvata lugens, Trichogramma evanescens, Trichogramma cardubensis, Trichogramma pretiosum, Trichogramma embryophagum, Trichogramma sembliodis, Trichogramma cocaeciae, Bemisia tabaci, Callosobruchus sp, Sitophilus oryzae, Cotesia plutella* and *Callosobruchus chinesis.* Among the *Wolbachia* infected pests and disease vectors causes heavy loss to agriculture are *Exorista sorbillans, Nilaparvata lugens, Bemisia tabaci, Sitophilus oryzae and Callosobruchus chinesis.* The vital role of these pests and disease vectors in destruction of agricultural crops and *Wolbachia* biology of these insects are discussed in the present study.

Table 1: *Wolbachia* infection status in insect pests and disease vector

S. No	Insect name	Order	Family	*Wolbachia* infection	Phage WO infection
1	*Exorista sorbillans*	Diptera	Tachinide	+	+
2	*Nilaparvata lugens (*BPH*)*	Homoptera	Delphacidae	+	+
3	*Trichogramma evanescens*	Hymenoptera	Trichogrammatidae	+	+
4	*Trichogramma cardubensis*	Hymenoptera	Trichogrammatidae	+	+
5	*Trichogramma pretiosum*	Hymenoptera	Trichogrammatidae	+	+
6	*Trichogramma embryophagum*	Hymenoptera	Trichogrammatidae	+	+
7	*Trichogramma sembliodis*	Hymenoptera	Trichogrammatidae	+	+
8	*Trichogramma cocaeciae*	Hymenoptera	Trichogrammatidae	+	+
9	*Trichogramma chilonis*	Hymenoptera	Trichogrammatidae	-	-
10	*Bemisia tabaci*	Hemiptera	Aleyrodidae	+	+
11.	*Apis gossypii*	Hemiptera	Aphididae	-	-
12	*Diaphania pulverulentalis*	Lepidoptera	Pyralidae	-	-
13	*Callosobruchus sp*	Coleoptera	Chrysomelidae	+	+
14	*Hysteraneura setariae*	Hemiptera	Aphididae	-	-
15	*Spilosoma oblique*	Lepidoptera	Arctiidae	-	-
16	*Poekilocerus pictus*	Orthoptera	Pyrgomorphidae	-	-
17	*Triboleum castaneum*	Coleoptera	Tenebrionidae	-	-
18	*Sitophilus oryzae*	Coleoptera	Curculionidae	+	+
19	*Cotesia plutella*	Hymenoptera	Braconidae	+	+
20	*Callosobruchus chinesis*	Coleoptera	Chrysomelidae	+	+
21	*Cimex lectularius*	Hemiptera	Cimicidae	-	-
22	*Thrips franklinella*	Thysanoptera	Thripidae	-	-
23	*Culicoides imicola*	Diptera	Ceratopogonide	-	-
24	*Culex quinquefasciatus*	Diptera	Culicidae	-	-
25	*Tetranyehus felarius*	Isoptera	Termitidae	-	-
26	*Diacrisia obliqa*	Lepidoptera	Arctidae	-	-
27	*Macanellicoccus hirsutus*	Hemiptera	Pseudococcidae	-	-
28	*Oecophylla smargdina*	Hymenoptera	Formicidae	-	-
29	*Aleyrodes disperses*	Hemiptera	Aleyrodidae	-	-
30	*Twany coster*	Lepidoptera	Nymphalidae	-	-
31	*Mylocerus discolour*	Coleoptera	Curuculionidae	-	-
32	*Halys dentatus*	Hemiptera	Pentatomidae	-	-
33	*Camponotus compresus*	Hymenoptera	Formicidae	-	-
34	*Chloealtis conspersa*	Orthoptera	Locustidae	-	-

35	*Pulvinaria maxima*	Hemiptera	Coccidae	-	-
36	*Acanthoscelides obtectus*	Coleoptera	Curculionidae	-	-
37	*Dermestid ater*	Coleoptera	Dermestidae	-	-
38	*Pseudodendrothrips mori*	Thysonoptera	Thripidae	-	-
39	*Hypothenemus hampi*	Coleoptera	Scolytidae	-	-
40	*Xylotrechus quadripes*	Coleoptera	Cerambycidae	-	-
41	*Udonga Montana*	Hemiptera	Pentatomidae	-	-
42	*Xylosandrus compactus*	Coleoptera	Scolytidae	-	-
43	*Planococcus citri*	Hemoptera	Pseudococcidae	-	-
45	*Phyllobis pomaceus*	Coleoptera	Curcullionidae	-	-
46	*Estigmene latinea*	Lepidoptera	Arctidae	-	-
47	*Eurygaster alternates*	Hemiptera	Scutelleridae	-	-
48	*Mylocerus discolour*	Coleoptera	Cuculionidae	-	-
49	*Neoperla edmundri*	Plecoptera	Perlidae	-	-
50	*Coccus viridis*	Homoptera	Coccidae	-	-
51	*Cryptolaemus zastrowi*	Coleoptera	Coccinellidae	-	-
52	*Tetranyehus felarius*	Isoptera	Termitidae	-	-
53	*Plutella xylostella* L(DBM)	Lepidoptera	Yponomeutidae	-	-
54	*Helicoverpa armigera*	Lepidoptera	Noctuidae	-	-

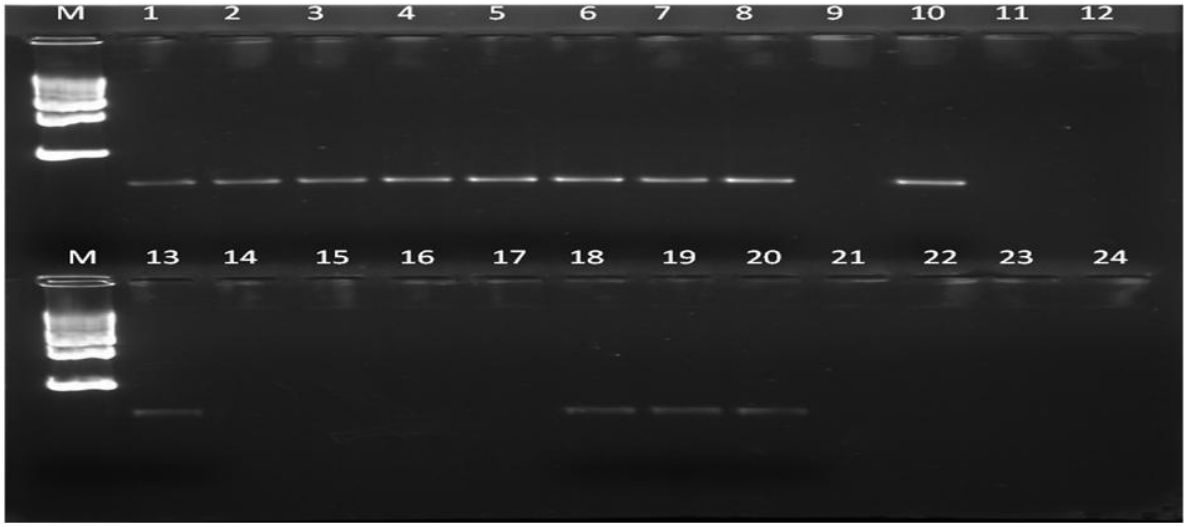

Fig 1: Gel photo showing *Wolhiabac* amplification. M- Molecular weight marker; lane 1 to 24 insect pests and diseases populations (Table-1).

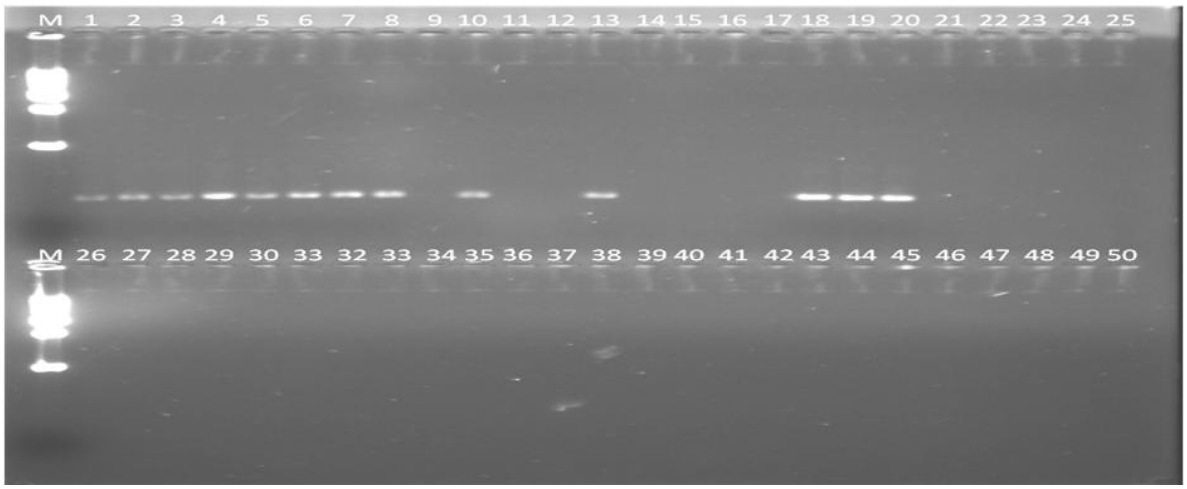

Fig 2: Gel photo showing Phage WO amplification. M- Molecular weight marker; lane 1 to 50 insect pests and diseases populations (Table-1).

In the present study *Wolbachia* was detected in 24% of insect pests and disease vectors sampled. Recent, broad taxon surveys of *Wolbachia* infection frequencies among arthropods found approximately 40% of the tested species to be infected [7]. Among the *Wolbachia* infected insects pests uzifly *Exorista sorbillans,* is a serious pest of Silk worm causing

considerable damage to sericulture industry. *Nilaparvata lugens, Bemisia tabaci* are vectors of Rice ragged virus and Begomavirus causes heavy damage to agricultural crops worldwide. Detection of *Wolbachia* in these pests and disease vectors have paved the way for role *Wolbachia* in biocontrol program. Further, *Wolbachia* is not detected in mosquito *Culex quinquefasciatus* [8]. But, there is a need to extensive survey of mosquitoes for *Wolbachia* infection. The number of species harboring *Wolbachia* is still remarkably justifies further efforts to investigate interactions between these endosymbionts and their arthropod hosts. Insects are reported to have symbiotic associations with a variety of microorganisms that affect many aspects of host biology and physiology. The symbionts usually establish facultative symbiotic associations that range from deleterious to beneficial. Positive effects include the capacity of infected hosts to survive heat stress and to exhibit altered host plant preference. Some guest microbes, however, can negatively affect growth, longevity of the host, and reproduction [9]. Some symbionts such as *Wolbachia*, *Cardinium*, *Rickettsia*, *Spiroplasma* and *Arsenophonus*, manipulate the host through the induction of reproductive alterations such as parthenogenesis, male killing, feminization and cytoplasmic incompatibility [1]. The best-studied symbiont is *Wolbachia* are a diverse group of intracellular bacteria that show impressive adaptations towards living in cells of invertebrates and in manipulating the biology of their hosts. The potential of *Wolbachia*-mediated incompatibilities has been used to control insect pests and associated diseases [4, 5]. Insect pests and predators poses a severe threat to the agriculture, it was felt necessary to explore the possibility of exploiting *Wolbachia* as a means to control them. The present work is an attempt in this direction to provide the information on the prevalence of *Wolbachia* in these insects. Thus, although the number of species harboring *Wolbachia* is still remarkably justifies further efforts to investigate interactions between *Wolbachia* endosymbionts and their arthropod hosts.

Besides *Wolbachia* infection, the present study detected Phage WO in *Wolbachia* infected insects. The phage WO might transmitted vertically and wide spread in these populations. Several recent studies revealed that the widespread occurrence of phage WO in 70 – 90% of the strains of *Wolbachia* genomes [10] and detection of particles has lent support to the idea that WO phage genes are activated and mobile. The widespread association of *Wolbachia* and the phage WO mean that the phage WO may be beneficial auxiliary to *Wolbachia*, as is found in various other phages/bacteria couples [11].

4. Conclusion

During the present study reveals the *Wolbachia* infection and its functional role in some agriculturally important insect pests and disease vectors. This serves as the basic ground data to exploit or biomanipulate *Wolbachia* including their biological control implications to control insect pests and disease vectors. The *Wolbachia* based technologies are environmental friendly and cost effective in biological control programs.

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