



DETECTION OF GENES OF WOLBACHIA IN FIVE SPECIES OF INSECTS FROM VIDARBHA REGION OF MAHARASHTRA (INDIA)

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Abstract

Wolbachia are maternally inherited endosymbiotic bacteria widely distributed among arthropods, especially insects. These bacteria are known to influence host reproduction through mechanisms such as cytoplasmic incompatibility, parthenogenesis, feminization, and male killing. Due to these characteristics, Wolbachia has gained attention in evolutionary biology and biological control studies. The present study aimed to detect the presence of different Wolbachia genes in selected insect species collected from the Vidarbha region of Maharashtra, India. Molecular detection was carried out using PCR amplification of four commonly used Wolbachia gene markers: *wsp*, *wspec*, *WO-orf7*, and *ftsZB*. The amplified gene fragments were sequenced and the resulting sequences were submitted to the GenBank database of the National Center for Biotechnology Information (NCBI). The results confirm the presence of Wolbachia in five insect hosts and provide baseline molecular data for future studies related to strain typing and multilocus sequence typing (MLST).

Keywords: Wolbachia, *wsp* gene, *wspec* gene, *WO-orf7* gene, *ftsZB* gene, insect symbionts

Introduction

Wolbachia is a genus of intracellular bacteria that infects a wide variety of arthropods including insects, spiders, mites, and crustaceans, as well as some nematodes. These bacteria are transmitted maternally through host eggs and are typically located within the reproductive tissues of infected hosts. Because of their unique mode of inheritance, Wolbachia can spread efficiently through insect populations.

Several studies have demonstrated that Wolbachia can manipulate host reproductive biology to enhance its transmission. The most well-known reproductive effects include cytoplasmic incompatibility, parthenogenesis induction, feminization of genetic males, and male killing. These mechanisms increase the proportion of infected females within host populations and thereby facilitate bacterial persistence.

Surveys conducted in different geographical regions indicate that a large percentage of insect species may harbor Wolbachia infections. In some host species the relationship is parasitic,



whereas in others it may evolve toward a mutualistic association. Due to its widespread distribution and biological influence on hosts, Wolbachia has become an important model system for studying host–microbe interactions.

Molecular detection of Wolbachia is commonly performed using polymerase chain reaction (PCR) targeting specific bacterial genes. Genes such as *wsp*, *wspec*, *WO-orf7*, and *ftsZB* are frequently used as molecular markers for identifying Wolbachia infection and for studying genetic diversity among strains.

The present study focuses on detecting Wolbachia genes in selected insect species collected from the Vidarbha region of Maharashtra, India. The study also reports the sequencing of amplified gene fragments and submission of the sequences to the GenBank database, thereby contributing valuable molecular information for future comparative and evolutionary studies.

Materials and Methods

Insect Collection

Insect specimens were collected from different locations within the Vidarbha region of Maharashtra using sweep nets and hand collection methods. The collected specimens were preserved and identified using standard taxonomic keys and morphological characteristics.

DNA Extraction

Genomic DNA was extracted from individual insect specimens to determine the Wolbachia infection status. The insects were dissected under sterile conditions and abdominal tissues were isolated for DNA extraction. A commercial insect DNA extraction kit was used following the manufacturer's protocol. Prior to extraction, flies were starved for 24 hours to minimize contamination from gut contents. The extracted DNA samples were stored at appropriate temperatures until further molecular analysis.

PCR Amplification

Polymerase chain reaction (PCR) was performed to detect the presence of Wolbachia genes including *wsp*, *wspec*, *WO-orf7*, and *ftsZB*. Each reaction was carried out in a 50 µL reaction mixture containing genomic DNA template, PCR master mix, gene-specific primers, MgCl₂, Taq DNA polymerase, and nuclease-free water. The amplification reactions were performed in a thermocycler under standard PCR conditions consisting of an initial denaturation step followed by repeated cycles of denaturation, primer annealing, and extension, with a final extension step to complete DNA synthesis. Negative controls containing DNA-free water were included in each run.



Gene	Primer	Sequence	Product size in bp
<i>wspec</i>	Wspec F wspec R	CATACCTATTCGAAGGGATAG AGCTTCGAGTGAAACCAATTC	438~bp
<i>wsp</i>	Wsp F wsp R	TGG TCC AAT AAG TGA TGA AGA AAA AATTAAACGCTACTCCA	600~bp
<i>WO-orf7</i>	WO-orf7F WO-orf7 R	CCC ACA TGAGCC AAT GAC GTC TG CGT TCG CTC TGC AAG TAA CTC CATTAA AAC	400~bp
<i>ftsZB</i>	FtsZB F FtsZB R	CCG ATG CTC AAG CGT TAG AG CCA CTT AAC TCT TTC GTT TG	900~bp

Gel Electrophoresis

The amplified PCR products were separated by agarose gel electrophoresis using 1% agarose gel prepared in 1× TAE buffer. Electrophoresis was performed at constant voltage and the separated DNA fragments were visualized using a gel documentation system with a molecular weight DNA ladder as reference.

DNA Sequencing

The PCR amplified products were purified and subjected to DNA sequencing using an automated DNA sequencer. The obtained sequences were analyzed and submitted to the GenBank database maintained by the National Center for Biotechnology Information (NCBI).

Results and Discussion

Screening of the collected insect samples using Wolbachia-specific primers revealed the presence of infection in five insect species. PCR amplification of the *wsp* gene (~600 bp) was observed in the butterfly *Catopsilia pyranthe pyranthe* and the mosquito *Culex quinquefasciatus*. Amplification of the *wspec* gene (~438 bp) was detected in the honeybee *Apis cerana indica*. The *WO-orf7* gene (~400 bp) was amplified in the rice weevil *Sitophilus oryzae*, whereas the *ftsZB* gene (~900 bp) was detected in the housefly *Musca domestica*.

These genes are widely used as molecular markers for detecting Wolbachia infections and studying strain diversity. The amplified gene fragments were sequenced and sequence comparison using BLAST analysis showed 98–100% similarity with previously reported Wolbachia sequences in the GenBank database, confirming the identity of the detected bacteria.

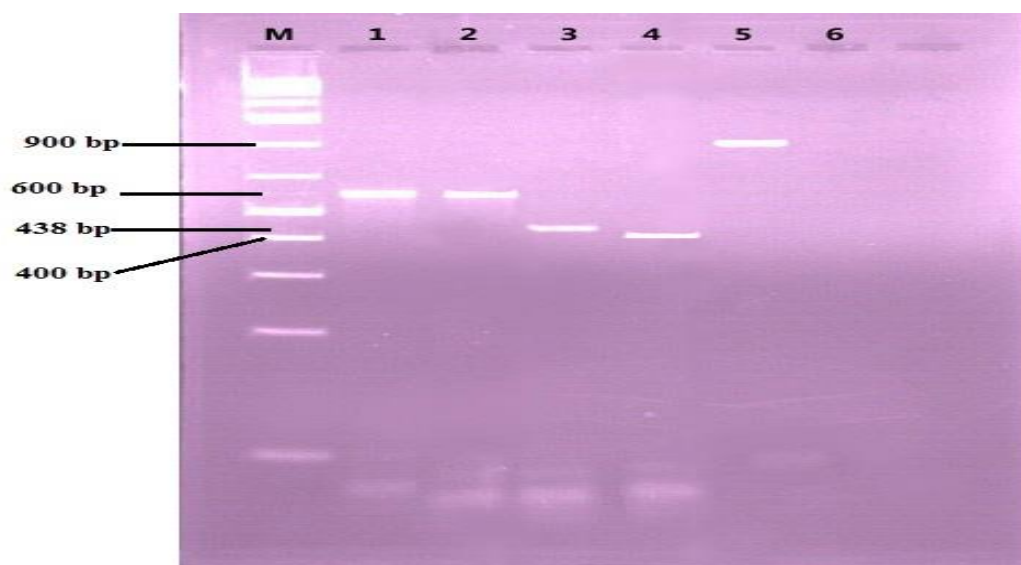


Figure 1: Gel image of *wsp*, *wspec*, *WO-orf7* and *ftsZB* gene amplification in five species of insects .

Lane M :Molecular weight Marker, **Lane 1:** *Catopsiliapyranthepyranth* (~600bp), **Lane 2:** *Culex quinquefasciatus*(~600bp) ;**Lane 3** : *Apis cerena indica* (~438bp); **Lane 4:** *Sitophilus oryzae* (~400bp); **Lane 5:** *Musca domestica*(~900bp) **Lane 6** :Negative Control

Table 1: Taxonomic details of *Wolbachia*'s hosts and accession numbers of analyzed sequences

Host species	Phylum	Class	Order	Gene	GenBank accession number
<i>Catopsiliapyranthepyranth</i>	Arthropoda	Insecta	Lepidoptera	<i>wsp</i>	LC276756.1
<i>Culex quinquefasciatus</i>	Arthropoda	Insecta	Diptera	<i>wsp</i>	LC276757.1
<i>Sitophilus oryzae</i>	Arthropoda	Insecta	Hymenoptera	<i>WO-orf7</i>	LC276758.1
<i>Musca domestica</i>	Arthropoda	Insecta	Hymenoptera	<i>ftsZB</i>	LC276759.1
<i>Apis cerena indica</i>	Arthropoda	Insecta	Hymenoptera	<i>wspec</i>	KT832793.1



Conclusion

The present investigation confirms the occurrence of *Wolbachia* infection in five insect species collected from the Vidarbha region of Maharashtra. Detection and sequencing of multiple *Wolbachia* genes including *wsp*, *wspec*, *WO-orf7*, and *ftsZB* provide valuable molecular data for future research on strain diversity and host-symbiont interactions.

The obtained gene sequences were successfully deposited in the GenBank database, contributing important molecular information for comparative and phylogenetic studies. The findings of this study may also support future research related to biological control strategies and vector management programs involving insect hosts.

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