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Wolbachia infection frequency and phylogenetic affiliation of *Wolbachia* cell division protein gene (ftsZ) in Uzi fly *Exorista sorbillans* (Diptera: Tachinidae) of Karnataka (India)

N. M. Guruprasad, S.K.Jalali, H. P. Puttaraju

ABSTRACT

Wolbachia are a group of obligate intracellular endosymbionts of a wide range of arthropod species. This microorganism is known for its manipulations of host reproduction that include cytoplasmic incompatibility, parthenogenesis, feminization and male killing. *Wolbachia* harbours a temperate bacteriophage named phage WO which may be contribute to the reproductive alterations induced in their hosts. *Wolbachia* and phage WO infections were characterized using polymerase chain reaction (PCR) on the *Wolbachia ftsZ* (*Wolbachia* cell division protein) gene and on minor capsid protein genes (orf 2 and orf 7 markers) of the phage WO. This paper results reports that in *Exorista sorbillans* individuals are infected by *Wolbachia* and its phage WO. Further, construction of molecular phylogeny of *Wolbachia* cell division protein (*ftsZ*) gene revealed double infection of *Wolbachia* strain A and B in Uzi fly *Exorista sorbillans*. Thus by evaluating, *Wolbachia* induced fitness attributes in Uzi fly and its potential role in control of Uzi fly *Exorista sorbillans* a serious menace of silkworm *Bombyx mori* L are discussed.

Keywords: *Wolbachia*, Phage WO, *Exorista sorbillans,* Polymerase Chain Reaction (PCR). Uzi fly.

1. Introduction

Wolbachia infects diverse range of invertebrates [1-3] up to 76% of all known insect species would be infected, making it perhaps the most prevalent symbiotic bacterium on the planet [4]. Eight major *Wolbachia* phylogenetic "super groups" (A–H) are currently recognized. A, B, and E infects diverse arthropods; C and D infects nematodes; G infects spiders; H infects termites; and F infects arthropods and nematodes [5]. Infection with *Wolbachia* is commonly associated with host reproductive phenotypes, such as cytoplasmic incompatibility (sterility) when infected males mate with uninfected females or females infected with a different *Wolbachia* strain), feminization, male-killing parthenogenesis, increased or decreased fitness, and obligate symbiosis [6]. These *Wolbachia* endosymbionts are infected with a bacteriophage named phage WO [7-8].

Several studies have revealed that the phage WO is widespread in *Wolbachia* genomes since 90% of *Wolbachia* strains would be infected [9]. Analysis of the diversity and the evolutionary dynamics of phage WO-*Wolbachia* associations is the first step to understand better the possible implication of phage WO in *Wolbachia* dynamics and effects on hosts, as was already proposed by some authors [10-11]. Phage WO infection is detected using PCR on minor capsid protein genes. Based on such PCR surveys, it has been shown that this phage is harboured by strains that are able to induce all the phenotypic effects known for *Wolbachia* from parasitism of the host reproduction to mutualism [12-14].

The Uzi fly *Exorista sorbillans* (Diptera: Tachinidae) a parasitoid of silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) causes heavy loss to sericulture industry in India. It is known that this parasitoid harbours a *Wolbachia* endosymbiont [15]. Recent investigations revealed that elimination of *Wolbachia* is known to cause cytoplasmic incompatibility (CI) and it is necessary for the normal reproduction of the Uzi fly [16]. In the present study, Uzi fly *Exorista sorbillans* individuals collected from the different geo-climatic conditions of Karnataka (India) were screened for *Wolbachia* and its phage WO infections by using PCR-based on the *Wolbachia* ftsZ gene (*Wolbachia* cell division gene protein) and on minor capsid protein genes of the phage WO. Further, we characterized the molecular phylogenetic affiliation of *Wolbachia* cell division protein.

2. Materials and Methods

2.1 Collection and preservation of Uzi fly

The maggots of Uzi fly, *Exorista sorbillans* were collected from different geographic regions of Karnataka (India) (Table-I). The collected maggots were brought to the laboratory and allowed for metamorphosis to pupa and then to adult. The adults were collected and stored at -80 ^{0}C until further isolation of DNA for further screening of *Wolbachia* and phage WO.

2.2 DNA extraction and PCR assay

The DNA of Uzi fly *Exorista sorbillans* was extracted with phenol-chloroform-isoamyl alcohol (24:24:1) method as described in Sambrook *et al.* [17]. The genomic DNA was resuspended in 50 µl of TE (10 mM Tris-HCl. 1 mM EDTA , pH 8.0).The polymerase chain reaction (PCR) assay was carried out based on specific amplification of the *Wolbachia ftsZ* gene (*Wolbachia* cell division protein) using primers specific of the supergroups A and B (primers: *ftsZ* Adf – 5' CTC AAG CAC TAG AAA AGT CG 3' and *ftsZ* Adr – 5' TTA GCT CCT TCG CTT ACC TG 3' for the group A ; primers *ftsZ* Bf – 5' CCG ATG CTC AAG CGT TAG AG 3' and *ftsZ* Br – 5' CCA CTT AAC TCT TTC GTT TG 3' for the group B)and on the orf2 (orf2 F- 5' GCAGGGCTATATTTTGGCGAGAA 3' ; orf2 R- 5'AACTCCATTAAAACTTCCCTGGC 3') and the orf7 (orf7 F – 5' CCC ACA TGA GCC AAT GAC GTC TG -3' orf7 R – 5' CGT TCG CTC TGC AAG TAA CTC CAT TAA AAC 3') specific phage WO markers [1, 9].

The PCR was carried out with the PTC 200 of MJ Research Thermocycler, in 25 µl reaction mixture containing 2.5µl of 10X PCR buffer, 0.5 µl of dNTP's (10 mM each), 2.5 µl of 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (New England Biolabs, England), 1 µl of both forward and reverse primer (5 pmol), 20 ng of template DNA. The PCR was carried out with a cyclic condition of initial denaturation step at 94 ^{0}C for 5 min followed by 35 cycles with denaturation step at 92 ^{0}C for 1 min, extension at 72 ^{0}C for 1 min, final extension at 72 ^{0}C for 10 min at specific hybridization temperature for ftsZ A 59 ^{0}C, ftsZ B 55 ^{0}C, orf2 57 ^{0}C and orf7 59 ^{0}C. The presence of amplified PCR products was checked by electrophoresis on 1.5% agarose gel running in 1X TBE (89.2 mM Tris HCl, 88.9 mM Boric acid and 2 mM 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.

2.3 Phylogenetic analysis of *Wolbachia* cell division protein (*ftsZ*)

Multiple sequence alignment was carried out by using Clustal W tool of MEGA4 software, aligned with the combined data set of *Wolbachia* cell division protein (*ftsZ*) sequences. The phylogenetic tree was constructed using Kimura 2 distance and N J algorithm. Since, absence of suitable out-group the phylogenetic tree was midpoint rooted. The sequences obtained in this study have been deposited in GenBank under the accession numbers JQ946084, JQ946085.

3. Results and Discussion

3.1 Infection frequency of *Wolbachia* and phage WO in *E sorbillans*

Uzi fly, *E sorbillans* populations collected from twenty different geographic regions of Karnataka (India) (Table-I) were diagnosed for the presence of *Wolbachia* infection and its Phage WO. The primers used for the amplification allowed to amplify the *Wolbachia* cell division protein gene *ftsZ* for *Wolbachia* and open reading frame of minor capsid protein genes (orf) for phage WO. The Uzi fly populations which were positive for *Wolbachia* infection were doubly infected with A and B super group *Wolbachia* (Table I, Figure- 1). The phage WO specific primers *orf2* and *orf7* (minor capsid protein phage WO) detected phage WO by Polymerase Chain Reaction (PCR) (Table-I, Figure- 2). The *Wolbachia* specific primers ftsZ A, and ftsZ B, yielded fragments of 980 bp and 945 bp respectively. The phage WO specific primers *orf2* and *orf7* yielded fragments of 260 bp and 410 bp respectively.

Table 1: Populations of *E. sorbillans* sampled

Sl. No.	Population	Province	*Wolbachia* Infection		Phage WO Infection
			Strain A	Strain B	
01	Chamrajnagar	Karnataka	+	+	+
02	Chitradurga		+	+	+
03	Kolar		+	+	+
04	Mysore		+	+	+
05	Mandya		+	+	+
06	Channarayapatna		+	+	+
07	Hassan		+	+	+
08	Sira		+	+	+
09	Chikkaballapur		+	+	+
10	Ramanagaram		+	+	+
11	Madhugiri		+	+	+
12	Thumkur		+	+	+
13	Bagepalli		+	+	+
14	Doddaballapura		+	+	+
15	Kunigal		+	+	+
16	Vijayapura		+	+	+
17	Kanakapura		+	+	+
18	Madhur		+	+	+
19	Bellary		+	+	+
20	Sidlaghatta		+	+	+

The present results indicated a high prevalence of *Wolbachia* and phage WO infection in *E. sorbillans* populations and revealed that all the *E. sorbillans* samples collected from different geographic regions of Karnataka were found 100% doubly infected with A and B *Wolbachia* super groups. These results paved the way to study the possibility of uninfected individuals which were not detected due to limited sample size. To understand the distribution and prevalence of *Wolbachia* in *E. sorbillans,* additional studies should be extended to examine the populations outside Karnataka and India, particularly those in neighboring sericulture practicing Asian countries so that possible co-evolution can be looked into. Besides *Wolbachia* infection, the present study detected 100% infection of Phage WO in the *E sorbillans.* The observations indicate that the phage WO is widespread in *Wolbachia* infected the *E. sorbillans* populations and suggest that it might be vertically transmitted several recent studies revealed the widespread occurrence of phage WO since it has been detected in 70 to 90% of the strains of *Wolbachia* genomes [18]. However, the PCR survey does not allow to determine whether particles are lytic and free or integrated in the *Wolbachia* genome. The widespread association of *Wolbachia* and the phage WO mean that the phage WO may be beneficial auxiliary to *E. sorbillans, Wolbachia*, as it has been found in various other phages/bacteria couples [19]. Analysis of the diversity and the evolutionary dynamics of phage WO-*Wolbachia* associations is the first step to understand better the possible implication of phage WO in *Wolbachia* dynamics and effects on hosts, as was already proposed by some authors [20].

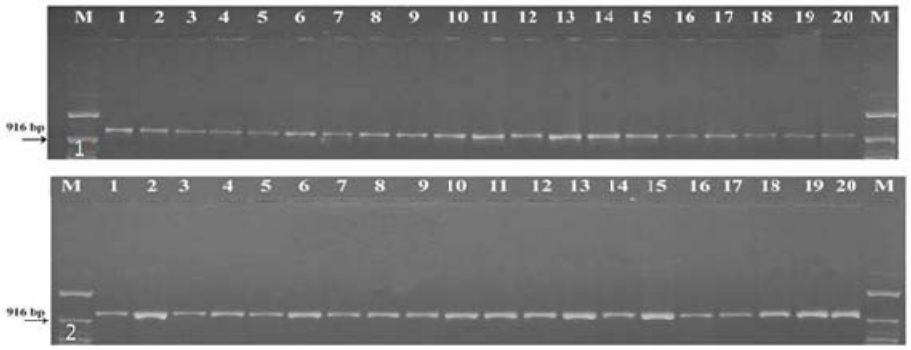

Fig 1: PCR amplification of *Wolbachia* infection in Uzi fly *Exorista sorbillans: A:* Lane M is marker, 1 to 20. Uzi fly populations. 1: ftsZ A gene amplification of *Wolbachia* and 2: ftsZ B gene amplification of *Wolbachia*.

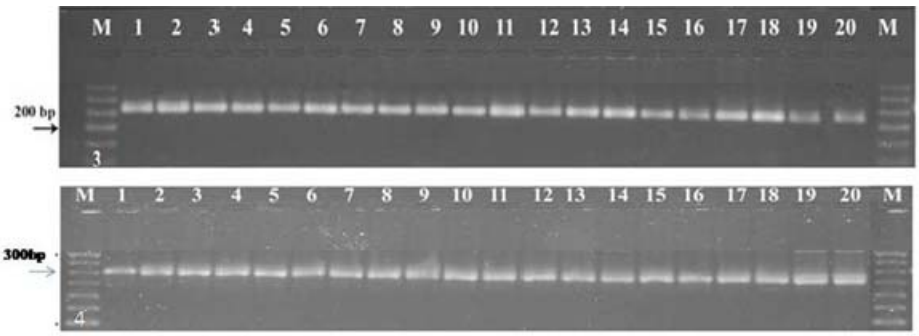

Fig 2: PCR amplification of phage WO infection in Uzi fly *Exorista sorbillans: A:* Lane M is marker, 1 to 20. Uzi fly populations. 3: orf-2 gene amplification of phage WO and 4: orf-7 gene amplification of phage WO.

3.2 Phylogenetic analysis of *Wolbachia* cell division protein (*ftsZ* gene)

The phylogenetic lineage of *Wolbachia* cell division protein in *E. sorbillans* was investigated by evolutionary analysis done with the Neighbor-Joining algorithm. The tree is drawn to scale (0.05 substitutions/site) with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). The sequences obtained with *ftsZA* and *ftsZB* primers have 692 and 700 positions in the final dataset specifically amplify *Wolbachia* cell division protein A and B strain. The direct sequencing of the PCR products gave one sequence without multiple peaks, representative only one strain of *Wolbachia*. These sequences have been submitted to GenBank under the accession numbers JQ946084, JQ946085 and closely related sequences of *Wolbachia* were obtained and used to construct phylogenetic tree (Figure-3). The sequences were correctly assigned to the A and B strain of *Wolbachia* in phylogenetic tree. Phylogenetic analysis of *Wolbachia* using *ftsZ* A gene indicated that *Wolbachia* from the *E. sorbillans* formed a monophyletic lineage with the other arthropod species of *Wolbachia.* The *ftsZ* A gene phylogeny indicated that the *Wolbachia* stran A of *E. sorbillans* is clustered together with the *Wolbachia* of *Asobara tabida* (Figure-3). The strain B showed homology with the butterfly *Eurema hecabe*.

The faster evolving cell-cycle gene *ftsZ* has been used to improve the phylogenetic resolution within the *Wolbachia* clade. Phylogenetic study using the *ftsZ* A and B group primer which amplify 692 and 700 bp of A and B super group *Wolbachia* from *E. sorbillans* revealed that the *E sorbillans* harbour closely related A super group *Wolbachia* of *Asobara tabida* and B super group *Eurema hecabe* composing in a single cluster. A fine-scale phylogenetic analysis have only been able to resolve a number of broad *Wolbachia* strain grouping, designated A and B super groups [21]. It has been estimated that A and B *Wolbachia* diverged around 60-70 MYA based upon synonymous substation rates of *ftsZ* gene.

The *ftsZ* gene sequences has been used to determine the phylogenetic relationships among Cytoplasmic Incompatibility (CI), Parthenogenesis and feminizing *Wolbachia* [22]. In this present study high prevalence of *Wolbachia* infection in field populations clearly indicate that there is a high level of vertical transmission of *Wolbachia* are conserved at the field level upon several generations. This high prevalence of *Wolbachia* infection in wild populations can be having a positive fitness effect on the host *E. sorbillans*. *Wolbachia* is not obligatory in *E. sorbillans*, cured populations survived but there is a rapid decline in the fecundity and hatching due to the *Wolbachia* elimination induced CI [16]. This clearly indicates, presence of *Wolbachia* infection in *E sorbillans* has fitness benefit to host.

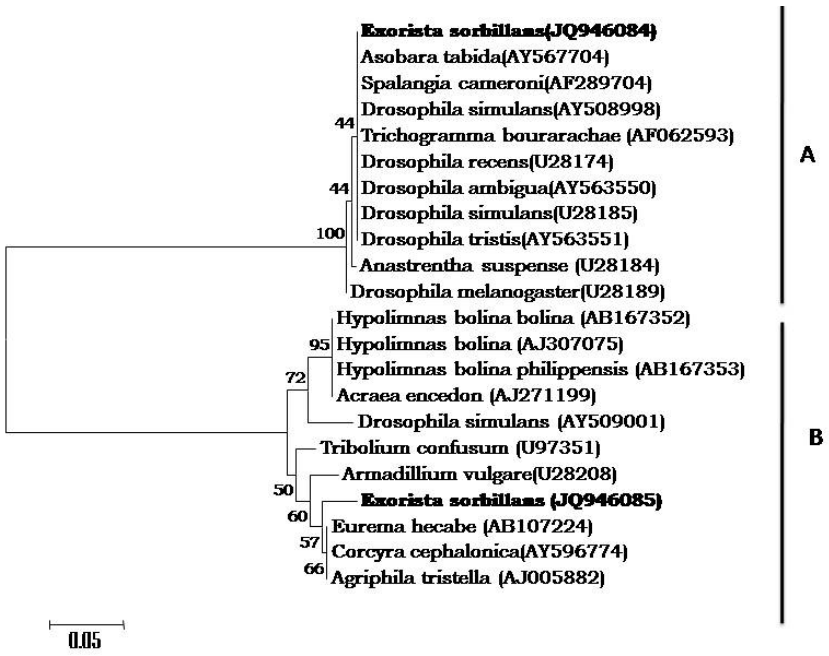

Fig 3: Neighbor joining phylogenetic tree of *Wolbachia* A and B strain based upon sequences of the ftsZ gene. The tree is midpoint rooted. Name of the host species is followed by the name of the *Wolbachia* strain when and the accession numbers in brackets. Sequences obtained in this study are in bold. Bootstrap values are shown at the nodes for NJ analysis.

4. Conclusion

The present study revealed the widespread distribution of *Wolbachia* strain A and B infection in *E. sorbillans* populations. Targeting an influential bacterium for control strategies will be useful if the endosymbiont is ubiquitous and indicate that *Wolbachia* may be useful as a novel potential target for control efforts. As *Wolbachia* infection leads to a reproductive fitness benefit in the *E. sorbillans*, it can be exploited for the suppression of pest, which represents a serious menace to silkworm *Bombyx mori* L.

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