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Molecular detection of *Wolbachia* and phage WO infection in *Spodoptera litura*. (Lepidoptera: Noctuidae)

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ABSTRACT

In nature the kind of association that we observe frequently between the eukaryotes and microorganisms is symbiotic, and range along the continuum between parasitism and mutualism. The genus *Wolbachia* contains well-known intracellular bacteria of arthropods that induce several reproductive phenotypes that benefit the transmission of the bacteria. While this process is facilitated by features of *Wolbachia*, particularly their ability to cause cytoplasmic incompatibility, blocking *Wolbachia* may produce deleterious effects, such as reduced host viability or fecundity, that inhibit successful local introductions and subsequent spatial spread, parthenogenesis, feminization, male killing and many other reproductive manipulations. The present study using PCR technology based on *Wolbachia* specific amplification of the ftsZ A and ftsZ B supergroup and phage WO specific amplification of orf-2 and orf-7 gene fragments not only confirms the presence of B supergroup *Wolbachia* but also reveals the presence of *Wolbachia* infected with phages WO in *Spodoptera litura.* Further, we constructed the molecular phylogeny of *Wolbachia* ftsZ B strain.Thereby this result creates possible implications of cytoplasmic bacteria *Wolbachia* and phage WO infection for the management *Spodoptera litura* a serious menace of agricultural crops.

Keywords: *Wolbachia*, Phage WO, *Spodoptera litura,* Cytoplasmic Incompatibility.

1. Introduction

Wolbachia are alpha–Proteobacteria living as endosymbionts in numerous arthropods and filarial nematodes in which they often alter the reproductive characteristics. In arthropod host, *Wolabchia* can induce cytoplasmic incompatibility (CI) feminization of males, thelytokous parthenogenesis or male killing [1]. In some hosts, it strongly increase fitness attribute of hosts [2]. The usual transmission of *Wolbachia* is vertical, from mother to offspring through the eggs but occasional horizontal transfers between individuals, which may or may not belong to the same species, seem to occur. Estimated frequencies of infected species range from 20% to 76% [3,4,5]. Because of its ability to manipulate host reproduction, *Wolbachia* is attracting increased interest among scientists for application in pest control [6]. Several approaches have been suggested. These include mass-release of laboratory-reared CI males to decrease the number of successful matings in field populations of pest species, and greater reproductive success of *Wolbachia*-infected individuals to allow for the replacement of a pest species with a less destructive strain of the same species [7]. *Wolbachia* also may serve as a vehicle to transfer genes desirable for pest control throughout the pest population [8,9]. The presence of phages in *Wolbachia* was first suspected phage like particals in *Wolbachia* from the mosquito *Culex pipiens*. [10]. Later, characterized the phage WO and showed that it can be either lysogenic and integrated into the *olbachia* chromosome, or lytic and free in the cytoplasm [11]. Because bacteriophages often bring to their bacterial host important functions involved in virulence such as the cholera toxin of Vibrio cholerae or antibiotic resistance as in Pseudomonas aeruginosa [12,13] bacteriophage WO appears to explain the diversity of effects that *Wolbachia* induces in insects. In the present study, an attempt has been made to investigate the presence of A and B supergroup *Wolbachia* and its phage WO using specific primers, in leafworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *S litura* is a serious but sporadic insect pest causes economic losses of crops from 25.8-100% based on crop stage and its infestation level in the field [14]. It has a large host range of more than 120 host plants including crops, vegetables, weeds and ornamental plants [15]. It feeds gregariously on leaves leaving midrib veins only.

An effective time and money saving management practice adopted by the farmers is the utilization of insecticides to control which needs right time, dose and application tools for its proper control. Review of literature has revealed that it has developed resistance against a variety of insecticides belonging to almost all the insecticide groups used against it [16] even against new chemical insecticides like lufenuron [17]. The moth of this species is widespread throught Asia and is present in the Marianas, most of the Carolines and the South Pacific region includes American Samoa. Many vegetables and other crops are damaged by cluster caterpillars. Crops likely to be seriously damaged in this region include the various taros, cabbage and its relatives, and tomatoes.

2. Materials and Methods

2.1 Collection and preservation *Spodoptera litura*

The *Spodoptera litura* moths are collected from Mulberry garden of Department of Sericulture, Bangalore University, Bangalore (India) The collected moths were brought to the Research Lab and stored in -80^0 C until further isolation of DNA for further screening of *Wolbachia* and its Phage WO.

2.2 DNA extraction and PCR assay

The DNA of *Spodoptera litura* was extracted by proteinase – K and SDS lysis method as in Sambrook *et al* [18]. The genomic DNA was resuspended in 50 µl of TE (10 mM Tris-HCl. 1 mM EDTA , P^H 8.0).The polymerase chain reaction (PCR) assay was carried out based on specific amplification of the *Wolbachia* ftsZ Af – 5^1 CTC AAG CAC TAG AAA AGT CG 3^1 ftsZ Ar – 5^1 TTA GCT CCT TCG CTT ACC TG 3^1 and ftsZ B ftsZ Bf – 5^1 CCG ATG CTC AAG CGT TAG AG 3^1 ftsZ Br – 5^1 CCA CTT AAC TCT TTC GTT TG 3^1 super group (*Wolbachia* cell division protein) and bacteriophage WO specific amplification primers orf7 F – 5^1 CCC ACA TGA GCC AAT GAC GTC TG -3^1 WOR - 5^1 CGT TCG CTC TGC AAG TAA CTC CAT TAA AAC 3^1 and orf2 F- 5^1 GCAGGGCTATATTTTGGCGAGAA 3^1 ORF2R- 5^1 AACTCCATTAAAACTTCCCTGGC 3^1 which are synthesized in Sigma Aldrich, Bangalore, India.

The PCR was carried out with PTC 200 of MJ Research Thermocycler, in 25µl reaction mixture containing 2.5µl of 10X PCR buffer, 0.5µl of dNTP's (10mM each), 2.5 µl of 2.5mM $MgCl_2$ and 0.5 U Taq DNA polymerase (New England Biolabs, England), 1µl of both forward and reverse primer (5 pmol), 20 ng of 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 94 ºC for 5 min followed by 35 cycles with denaturation step at 92 ºC for 1 min, extension 72º C for 1 min, final extension at 72 ºC for 10 min at specific hybridization temperature. The amplified PCR 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.

2.3 Molecular Phylogenetic analysis of *Wolbachia*

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. The phylogenetic tree was midpoint rooted. The sequences obtained in this study have been deposited in GenBank under the accession numbers JF713684.

3. Results and Discussion

3.1 Detection of *Wolbachia* and Phage WO in *Spodoptera litura*

Amplification of ftsZ A and ftsZ B a cell division gene sequences of *Wolbachia* and bacteriophage WO from the *Spodoptera litura* was positive for both *Wolbachia* and bacteriophage WO (Figure-1). The *Spodoptera litura* is infected with only the B supergroup *Wolbachia,*(Figure-1A) A supergroup *Wolbachia* is absent in the *Spodoptera litura.* *Wolbachia* are commonly found in natural populations of various insects. PCR surveys have found demonstrated that diverse groups of insects sampled in tropical and temperate environments consistently exhibited infection rates around 16- 76% of species examined [3] . To date, a number of arthropod species have been examined for *Wolbachia* infection in their local populations, including fruit fly *Drosophilla simulans* [19,20] , rice planthopper *Laodelphax striatellus* [21], tsetse flies *Glossina sp* [22], raspberry beetle *Byturus tomentosus* [23], fire ants *Solenopsis sp*. [24] woodlouse *Armadillidium vulgare* [25] and other isopod crustaceans [26]. The most common effect that *Wolbachia* can have on arthropod host reproduction is CI. CI is widespread in insects and has been reported in different insect orders, including Coleoptera, Diptera, Homoptera, Hymenoptera, Orthoptera, and Lepidoptera [27]. The detection of *Wolbachia* in *S.litura* a serious menace of agricultural crops have lent to for further use in biological control program.

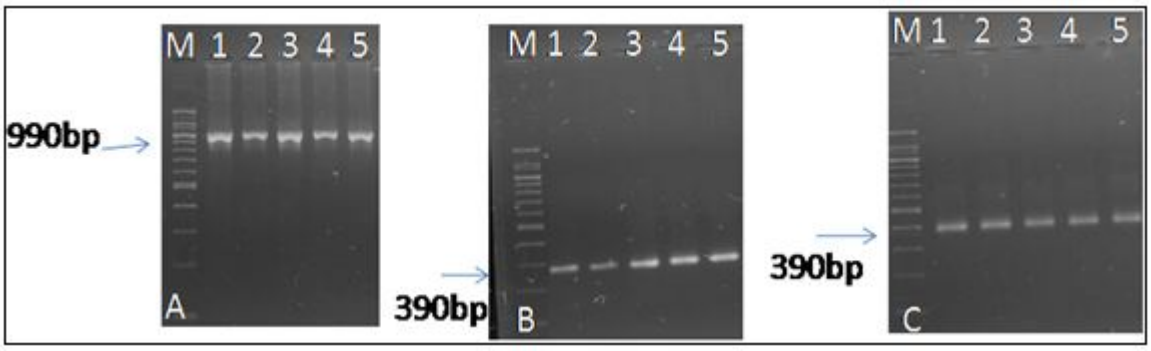

Fig 1: PCR amplification of *Wolbachia* and phage WO in *Spodoptera litura.* Lane M is marker 1 to 5 is PCR amplification of specific gene. A. *Wolbachia* fts Z B strain amplification. B. Phage WO amplification by using orf-2 primer C. Phage WO amplification by using orf-7 primer.

Phages are widespread viruses infecting bacteria that use the host cell molecular systems for replicating their own nucleic acid and for synthesizing their proteins. At the end of the phage infection cycle, the accumulation of phage particles in bacterial cytoplasm induces cell lysis and bacterial death. However, some bacteriophages can establish a not immediately lethal association with bacteria when they enter lysogenic cycles [28]. The detection of Phage WO in *Wolbachia* of *S.litura* (Figure-1B&C) have lent to further implications that it may also have secret some beneficial proteins to *Wolbachia*. Sveral studies revealed there is a relationship between the presence of active phage WO and cytoplasmic incompatability induced by *Wolbachia* [29].

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

The phylogenetic lineage of *Wolbachia* cell division protein in *S. litura* was investigated by evolutionary analysis done with the Neighbour-Joining algorithm. The tree is drawn to scale (0.01 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 *fts Z*B primer have 700 positions in the final dataset specifically amplify *Wolbachia* cell division protein B strain. The direct sequencing of the PCR products gave one sequence without multiple peaks, representative only one strain of *Wolbachia*. These sequence have been submitted to Gen-Bank under the accession number JF713684 and closely related sequences of *Wolbachia* were obtained and used to construct phylogenetic tree (Figure-2). The sequences were correctly assigned to the B strain of *Wolbachia* in phylogenetic tree. Phylogenetic analysis of *Wolbachia* using fts ZB gene indicated that *Wolbachia* from the *S. litura* formed a monophyletic lineage with the other arthropod species of *Wolbachia.* The *fts Z*B gene phylogeny indicated that the *Wolbachia* strain B of *S. litura* is clustered together with the *Wolbachia* of *Agriphilla tristella, Corcyra cephalonica and Eurema hecabe* (Figure-2).

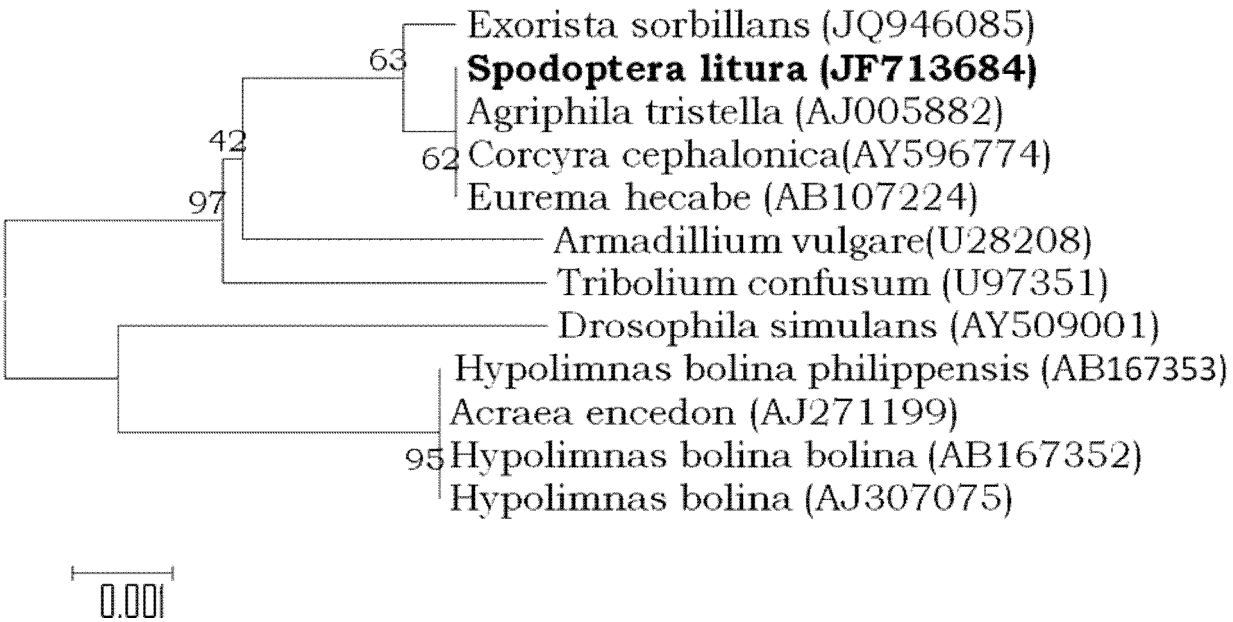

Fig 2: Neighbor joining phylogenetic tree of *Wolbachia* B strain based upon sequences of the ftsZ B strain gene in *Spodoptera litura*.

Molecular phylogenies analysis signify the prime basis of information on the evolution of *Wolbachia* endosymbiont. Researchers have been engaged in studying host-Wolbachia associations, the origin of *Wolbachia* behavior, and divergence dates [30,31]. The estimation of phylogenetic relationship have provided useful information about the evolution and biology of these bacteria [32]. The phylogenies *S. litura* B strain have been found to be homology with the Lepidopteron insects *Agriphilla tristella, Corcyra cephalonica and Eurema hecabe.* Topologies inferred from sequences of above said sequences were consistent. Therefore, the phylogenetic relationships of *Wolbachia* bacteria can now be inferred between lineages of ftsZ as simple sequence-based classification of wsp gene [33].

4. Conclusion

Wolbachia populations, raises novel issues on the dynamics of *Wolbachia* infection and warrants the use of the phage WO markers to analyze the genetic structure of *Wolbachia* infecting field populations. This may open routes for the control of insect pests and disease vectors since *Wolbachia* are considered as a promising driving force for manipulating gene pools of their host populations. The ability of *Wolbachia*, particularly to cause cytoplasmic incompatibility in their hosts produce deleterious fitness effects, such as reduced host viability or fecundity. The presence of cytoplasmic incompatibility inducing *Walachia* and phage WO creates lot of scope to exploit the both *Wolbachia* and phage WO for the control of *Spodoptera litura* a serious menace of agricultural crops.

5. Acknowledgements

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6. Reference:

1. Werren JH, Baldo L, Clark ME. Wolbachia: Master Manipulators of Invertebrate Biology. Nat Rev Micro 2008; 6:741–751.
2. Guruprasad NM, Mouton L, Puttaraju HP. Effect of antibiotic, temperature curing of Wolbachia and seasonal variation on the reproductive fitness of the Uzifly Exorista sorbillans (Diptera:

Tachinidae). Symbiosis 2011; 54:151-158.
3. Jeyaprakash A, Hoy MA. Long PCR improves Wolbachia DNA amplification: wsp sequences found in 76% of sixty-three arthropod species. Inse Mol Bio 2000; 9:393–405.
4. Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. How many species are infected with Wolbachia? A statistical analysis of current data. FEMS Micro Lett 2008; 281: 215-220.
5. Sumithra, Guruprasad NM, Puttaraju HP. A Comparative analysis of Long PCR and Standard PCR technique in detecting the Wolbachia Endosymbiont. Curr Trend in Biotech Pharm 2012; 6(4):472–478.
6. Ioannidis P, Bourtzis K. Insect symbionts and applications: The paradigm of cytoplasmic incompatibility-inducing Wolbachia. Ento Res 2007; 37:125–138.
7. Saridaki I, Bourtzis K. Wolbachia: more than just a bug in insect's genitals. Curr Opin in Micro 2010; 13:67-72.
8. Kambris Z, Blagborough AM, Pinto SB, Blagrove MS, Godfray HC, Sinden RE *et al* . Wolbachia stimulates immune gene expression and inhibits plasmodium development in Anopheles gambiae. PLoS Patho 2010; 6:e1001143.
9. Ormaetxe II, Walker T, O'Neill SL. Wolbachia and the biological control of mosquito-borne disease. EMBO reports, 2011, doi:10.1038/embor.2011.84.
10. Wrighit JD, Sjoatrand FS, Portreo JK, Barr AR. The ultrastructure of the Rickettsia-like microorganism Wolbachia pipientis and associated virus-like bodies in the mosquito Culex pipientis. J Ultrastruct Res 1978; 63:79-85.
11. Musui S, Kamoda S, Sasaki T, Ishikawa H. Distribution and evolution of bacteriophage WO in Wolbachia, the endosymbiont causing sexual alterations in arthropods. J Mol Evol 2000; 51:491-497.
12. Faruque SM, Alim ARA, Albert MJ, Islam KM, Mekalanos JJ. Induction of the lysogenic phage encoding cholera toxin in naturally occurring strains of toxigenic Vibrio cholerae O1 and O139. Infect Immunol 1998; 66:3752-3757.
13. Blahova J, Kralikova K, Bartionikova N. High-frequency transduction of antibiotic resistance in pseudomonas aeruginosa by a wild-type bacteriophage with restricted specificity for recipient strains. Eur J Clin Microbiol Infect Dis 1999; 18:152-154.
14. Dhir BC, Mohapatra HK, Senapati B. Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F.). Indian J Plant Protect 1992; 20(7-10):215-217.
15. Ramana VV, Reddy GPV, Krishnamurthy MM. Synthetic pyrethroids and other bait formulation in the control of *Spodoptera litura* (Fab.) attacking rabi groundnut. Pesticides 1988; 1(13-16):522- 524.
16. Kranthi KR, Jadhav DR, Kranthi S, Wanjari RR, Al i SS, Russell DA. Insecticide resistance in five major insect pests of cotton in India. Crop Protect 2002; 21(6):449-460.
17. Sudhakaran R. Efficacy of lufenuron (Match 5% EC) against *Spodoptera litura* (F.) under in vitro condition. Insect Environ 2002; 8(1):47-48.
18. Sambrook J, Fritishi EF, Miniatis GD. Molecular cloning: A laboratory manual. 2nd Ed, Cold Spring Harbor Laboratory Press, New York, 1989.
19. Turelli M, Hoffmann AA. Rapid spread of an inherited incompatibility factor in California Drosophila. Nature. 1991; 353: 440–442.
20. James AC, Ballard JW. Expression of cytoplasmic incompatibility in Drosophila simulans and its impact on infection frequencies and distribution of *Wolbachia pipientis*. Evolution 2000; 54(5):1661-1672.
21. Hoshizaki S, Shimada T. PCR-based detection of Wolbachia, cytoplasmic incompatibility microorganisms, infected in natural populations of *Laodelphax striatellus* (Homoptera : Delphacidae) in central Japan: has the distribution of Wolbachia spread recently? Insect Mol Biol 1995; 4:237-243.
22. Cheng Q, Ruel TD, Zhou W, Moloo SK, Majiwa P, O'Neill SL *et al*. Tissue distribution and prevalence of Wolbachia infection in tsetse flies, Glossina spp. Med Vet Ento 2000; 14:44-50.
23. Malloch G, Fenton B, Butcher RDJ. Molecular evidence for multiple infections of a new subgroup of Wolbachia in the European raspberry beetle Byturus tomentosus. Mol Ecol 2000; 9:77-90.
24. Shoemaker DD, Ross KG, Keller L, Vargo EL, Werren JH. Wolbachia infections in native and introduced populations of fire ants (Solenopsis spp.). Ins Mol Biol 2000; 9:661-673.
25. Rigaurd T. Inherited microorganisms and sex determination of arthropod hosts. In Influential passengers (Eds O'Neill SL, Hoffmenn AA, Werren JH), Oxford University Press, Oxford, 1997, 81-101.
26. Bouchon D, Rigaud T, Juchault P. Evidence for widespread Wolbachia infection in isopod crustaceans: molecular identification and host feminization. Proc Roy Soci of Lond B 1998; 265:1081-1090.
27. O'Neill SL, Hoffman AA, Werren JH. Influential Passenger: Inherited Microorganisms and Arthropod Reproduction. Oxford Univ. Press, New York, 1997, 1–214.
28. Serbus LR, Lindley CC, Landmann F, Sullivan W. The genetics and cell biology of Wolbachia–host interactions. Ann Rev Genet 2008; 42:28.1-28.25.
29. Lwoff A. Lysogeny Bacteriol Rev 1953; 17:269-337.
30. Werren JH, Zhang W, Guo LR. Evolution and phylogeny of Wolbachia: reproductive parasites of arthropods. Pro of the Roy Soc Lon B 1995; 261:55–63.
31. Guruprasad NM, Mouton L, Sumithra, Puttaraju HP. Survey of Wolbachia and its phage WO in the Uzifly Exorista sorbillans (Diptera: Tachinidae). Cur Micro 2011; 63:267–272.
32. Baldo L, Werren JH. Revisiting Wolbachia supergroup typing based on WSP: spurious lineages and discordance with MLST. Curr Microbiol 2007; 55:81–87.
33. Zhou W, Rousset F, O'Neill S. Phylogenetic and PCR based classification of Wolbachia strains using wsp gene sequences. Proc R Soc Lond B 1998; 265:509–515.