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WOLBACHIA ASSOCIATION AND ITS PHYLOGENETIC AFFILIATION OF *BRUGIA MALAYI* PARASITES FROM INDIA

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ABSTRACT

Wolbachia have established a mutualistic association with filarial nematodes and has a phenomenal implication in its normal development, reproduction and survival. Elimination of *Wolbachia* by tetracycline class of antibiotic compounds have been suggested and successfully implemented for the treatment of lymphatic filarial parasites. Thereby, is necessary to assess the prevalence of the *Wolbachia* in *B. malayi* before such new strategies are employed, across the world. In the present communication, the presence of *Wolbachia* and phylogenetic affiliation in *B. malayi* collected from Sevagram, Maharashtra, India, has been addressed.

Keywords: *Wolbachia*, Lymphatic filarial parasites.

INTRODUCTION

Filarial nematodes like *Brugia malayi*, *Wuchereria bancrofti* and *Onchocerca volvulus* cause several important human diseases across tropics and subtropics. They belong to the order Spirurida and family Onchocercidae, have been reported to harbour the *Wolbachia* endosymbionts [1]. These bacteria have been implicated not only in establishing a mutualistic association with filarial nematodes but also in the pathogenesis of filarial disease [2]. *Wolbachia* acts on the host immune system and accelerates the rate of inflammation. During pathogenesis the β cell proliferation of the host is directed specifically towards *Wolbachia* surface antigens which strengthen the possible role of *Wolbachia* in filarial pathogenesis [3]. After the death of the nematodes, the host respond to *Wolbachia* by releasing stimulatory and modulatory factors from neutrophils and monocytes [4].

The typical immunological response of the host in producing mimics of lipopolysaccharide and activation of Toll like receptor-4 (TLR4) are known to be induced by *Wolbachia* [6]. The regulation of Th1 and Th2 cytokines, which are the potential targets for filarial pathogenesis, is known to be governed by TLR4 [7]. The strategies of combating filarial nematodes through *Wolbachia* by tetracycline class of antibiotic compounds directly hinder their proliferation and manage their pathogenesis [8]. In view of the importance of *Wolbachia* for the survival, developmental stages, reproduction and pathogenesis, it is necessary to assess the prevalence of *Wolbachia* in *B. malayi* from different geographical locations in India. However, earlier research by Hoti, et al., [9] and Gayen, et al., [10] identifies the occurrence of *Wolbachia* only in *W. bancrofti* collected from various geographical locations in India. The present study is an attempt to screen for the presence of *Wolbachia* in *B. malayi* (Bm-I) collected from the Jamnalal Bajaj Tropical Disease Research Centre at Mahatma Gandhi Institute of Medical Sciences, Sevagram, Maharashtra, India and to fill this impending gap.

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MATERIALS AND METHODS

Genomic DNA was extracted from microfilariae (approximately 1500 in number) by Column based Animal tissue kit (Chromous Biotech™ Pvt, Ltd, Bangalore, India) with manufacturer's protocol. DNA was quantified through

Bio-photometer (Eppendorf AG, Hamburg, Germany). A polymerase chain reaction (PCR) assay was done through thermocycler (Eppendorf AG, Hamburg, Germany), with the reaction mixture containing 10 µl 10X buffer (5 Prime Eppendorf), 3 µl 25 mM MgCl2, 1.25 µl dNTPs (10 mM each), 1 µl 10 pmoles of both forward and reverse primers, 1.5 unit of Taq DNA polymerase(5 Prime Eppendorf) and template DNA. The PCR conditions followed for each step included 3 min at 95^0C for the initial denaturation step followed by 35 cycles of 45 s at 94^0C (denaturation), 1 min at 51^0C (annealing), 1min at 72^0C (primer extension) and 7 min at 72^0C for the final extension. For amplication filarial-specific 28 S rRNA, *Wolbachia* 16 S rRNA and *Wolbachia* surface protein (wsp) gene primers [9,10,11]. PCR products were resolved in 1.2% agarose gel and stained with green view dye (Chromous Biotech^{TM} Pvt, Ltd, Bangalore, India). The amplicons were observed and recorded in a gel documentation unit (Alpha Imager (R) EP, Canada).The size of the PCR product was determined using a 1-kb ladder (GeNei^{TM}, Bangalore, India).

The PCR product of *wsp* gene were purified using Chromous PCR Clean-up kit (Chromous Biotech^{TM}, Bangalore, India) and directly sequenced with respective primers using an automated sequencer (3130 Genetic Analyzer, ABI, Foster city, California, USA). The sequences obtained have been deposited in GenBank under the accession number JX506736. Phylogenetic analysis of the wsp gene sequence was done at BLAST-x program at NCBI. Multiple sequence alignment was done by using CLUSTAL W program. The phylogenetic trees were constructed using Kimura-2-distances and the Neighbor-Joining algorithm was computed using MEGA 4 program [12].

Fig. 1. PCR amplification of *B. malayi* (Bm-I) using filarial 28S rRNA-specific primers (Lane 1), *Wolbachia*- specific 16S rRNA primers (Lane 2), *Wolbachia* surface protein-specific primers (Lane 3) and Lane M is the migration pattern of the 1 kd DNA ladder.

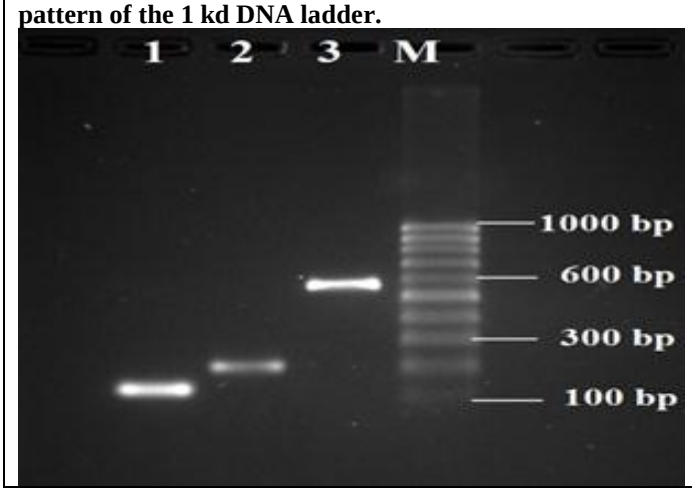
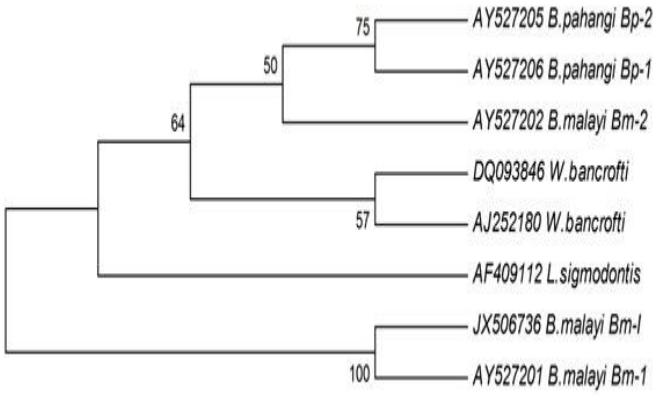


Fig. 2. Phylogenetic tree of *Wolbachia* based on the wsp sequences, constructed from Kimura-2-distance and the Neighbour-Joining algorithm. The numbers near the node indicate percentage of 1000 bootstrap replicates. Names correspond to host species. The GenBank accession numbers are also mentioned.



RESULTS AND DISCUSSION

PCR amplification was carried out with *Wolbachia*- specific 16S rRNA and wsp gene primers to confirm the presence of *Wolbachia* infection in *B. malayi*. 16S rRNA gene amplified at 207 bp and wsp gene amplified around 590 bp fragments, whereas filarial-specific 28S rRNA primers was used confirming the quality of template DNA and authenticity of the experimental protocol yielded distinct band at 150 bp as shown in the figure-1.

The evolutionary history of *Wolbachia* lineages in the *B. malayi* of Indian populations was investigated by phylogenetic analysis performed with Neighbour-Joining algorithm using Kimura-2-distance. Direct sequencing of the PCR products gave only one sequence without double peaks, indicating the presence of only one strain in the Bm-I. These sequences have been submitted to the Genbank database and phylogenetic tree based *wsp* gene was constructed. *B. malayi* grouped in D supergroup as shown in the figure 2. The wsp gene sequences of *Wolbachia* from Bm-I strain showed highly homology with the previously reported *B. malayi* strain Bm-1, proving they belonged to the same strain.

The study investigated the presence and phylogenetic affiliation of *Wolbachia* in *B. malayi* (Bm-I) from India. The presence of *Wolbachia* in *W. Bancrofti* has been well documented in India by Hoti et al [8] and Gayen et al [9]. *B. malayi* is considered to be high endemic to several states of India since decades. But there is a lack of information on Indian strain Bm-I. Thus, the current preliminary study has shown that *Wolbachia* is present in *B. malayi* (Bm-I) and the phylogenetic analysis shows the sequence similarity with members of Bm-1 strain of D

super group and correlated well with the earlier works of Bazzocchi et al [11] and Casiraghi et al [1]. *Wolbachia* has displayed a mutualistic relationship with nematodes, and therefore elimination of *Wolbachia* by tetracycline class of antibiotic compounds decreases the host fitness and ultimately leads to its mortality [10]. The prevalence of *Wolbachia* in *B. malayi* from various geographical areas across India, and the extent of mutualism, its application in filarial management programmes is an interesting proposition for future studies.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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