

SHORT COMMUNICATION

Presence of *Wolbachia* Endosymbionts in Microfilariae of *Wuchereria bancrofti* (Spirurida: Onchocercidae) from Different Geographical Regions in India

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In view of the recent discovery of rickettsial endosymbionts, Wolbachia in lymphatic filarial parasites, Wuchereria bancrofti and Brugia malayi and subsequently of their vital role in the survival and development of the latter, antibiotics such as tetracycline are being suggested for the treatment of lymphatic filariasis, by way of eliminating the endosymbiont. But, it is essential to assess their presence in parasites from areas endemic for lymphatic filariasis before such a new control tool is employed. In the present communication, we report the detection of Wolbachia endosymbionts in microfilariae of W. bancrofti parasites collected from geographically distant locations of India, such as Pondicherry (Union Territory), Calicut (Kerala), Jagadapur (Madhya Pradesh), Thirukoilur (TamilNadu), Chinnanergunam (TamilNadu), Rajahmundry (Andhra Pradesh), and Varanasi (Uttar Pradesh), using Wolbachia specific 16S rDNA polymerase chain reaction.

Key words: *Wolbachia* - *Wuchereria bancrofti* - polymerase chain reaction - India

Wolbachia endosymbionts are maternally inherited intracellular α -proteobacteria, which infect broad range of invertebrate hosts like insects, mites, isopods, and filarial nematodes (Kellen et al. 1981, Noda 1984, Richardson et al. 1987, Bazzocchi et al. 2000). *Wolbachia* symbionts have been reported to play important role in the development and pathogenesis of the filarial nematode such as *Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus*. Filariasis is mainly characterized by the occurrence of inflammatory pathogenesis and *Wolbachia* bacteria present in filarial nematodes appear to contribute to this inflammation (Bandi et al. 1998, 1999, Taylor & Hoerauf 1999, Taylor et al. 2000). The lipopolysaccharide (LPS) of *Wolbachia* appears to play an important role in elicitation of Toll like receptor-4 (tlr-4) signalling which in turn governs the proliferation and regulation of Th1 and Th2 cytokines associated with differential disease manifestations observed in onchocerciasis (André et al. 2002). In view of the importance of *Wolbachia* endosymbionts for the survival, development and pathogenesis of lymphatic filarial parasites in the human host, a new chemotherapeutic strategy targeting the endosymbionts with tetracycline class of compounds is being envisaged for the treatment of filariasis (Taylor & Hoerauf 1999, Taylor et al. 2000). But, before

such an approach is implemented on large scale in wider geographical areas, it is necessary to assess the prevalence of the endosymbiont in *W. bancrofti* from wide geographic locations. At present *Wolbachia* has been detected only in *W. bancrofti* microfilariae (mf) from two individuals from Kimuta Island, Papua New Guinea, and Sri Lanka (Taylor et al. 1999, Bazzocchi et al. 2000) and there is no data on their universal prevalence. Hence, a preliminary study was undertaken to screen for the presence of these endosymbionts in mf of *W. bancrofti* from different geographic regions of India. Five-milliliter blood samples (heparinized) were collected from two or three microfilariaemic individuals residing in each of the five geographically distant locations of India and also from two rural areas near Pondicherry (Figs 1, 2). Microfilariae were separated from blood samples using Millipore membrane – 5.0 μ M (Millipore, US) following a standard protocol (Sasa 1976) and then purified using Percoll gradient (Chandrasekhar et al. 1984).

DNA was extracted from mf following the method described by Hoti et al. (2003) which is briefly as follows: the mf pellet was washed in phosphate buffered saline (PBS) and digested in 500 μ l of lysis buffer [0.1 M Tris-pH 8.5, 0.05 M EDTA, 0.2 M NaCl, 1% sodium dodecyl sulphate (SDS) and proteinase K 2 mg/ml]. The lysate was subjected to phenol: chloroform: isoamylalcohol (25:24:1) extraction followed by ethanol precipitation and pellet was stored in 20 μ l of 6 mM Tris, 6 mM NaCl, 0.2 mM EDTA; pH 7.0.

Wolbachia specific 16S rDNA primers, forward primer 27f (5'-GAGATTTGATCCTGGCTCAG-3' and reverse primer 1495r (5'-CTACGGCTACCTTGTTACGA-3') were used to amplify *Wolbachia* ribosomal genes from the genomic DNA of mf (Taylor et al. 1999). The genomic positions of forward primer and reverse primer in 16S

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Fig. 1: map showing different geographic locations of India from where microfilariae of *Wuchereria bancrofti* were collected.

ribosomal gene lie at nucleotide position 27 to 46 and 1495 to 1515, respectively. The polymerase chain reaction (PCR) mixture consisted of dNTP - 20 mM of each nucleotide (Finnzymes, Finland); $MgCl_2$ - 2.5 mM (Finnzymes); Forward and Reverse Primer (Synthesized from Metabion, Germany) - 10 pmol each, 10X Dynazyme buffer, Dynazyme II Taq polymerase - 3 units (Finnzymes, Finland), template mf DNA 10 ng and distilled water (Sigma, US). *B. malayi* mf DNA known to harbor *Wolbachia* endosymbiont ribosomal gene was used as positive control and distilled water instead of template DNA was used as negative control for PCR. Amplification was run on a Master Cycler Gradient (Eppendorf). The PCR programme consisted of an initial denaturation at 96°C for 4 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 2 min with a final extension step of 72°C for 7 min. The amplified products were detected by running a 1.5% agarose gel with Lambda DNA/Hind III and Phi X 174 DNA/Hae III Mix molecular weight marker (NEB, US). The gels were stained in ethidium bromide, observed under UV transilluminator and then photographed (Fig. 2a, b).

Results of the study (Fig. 2a, b) showed that the PCR amplification using the eubacterial primers yielded an amplicon of 1400 bp which corresponds to 16S rDNA gene

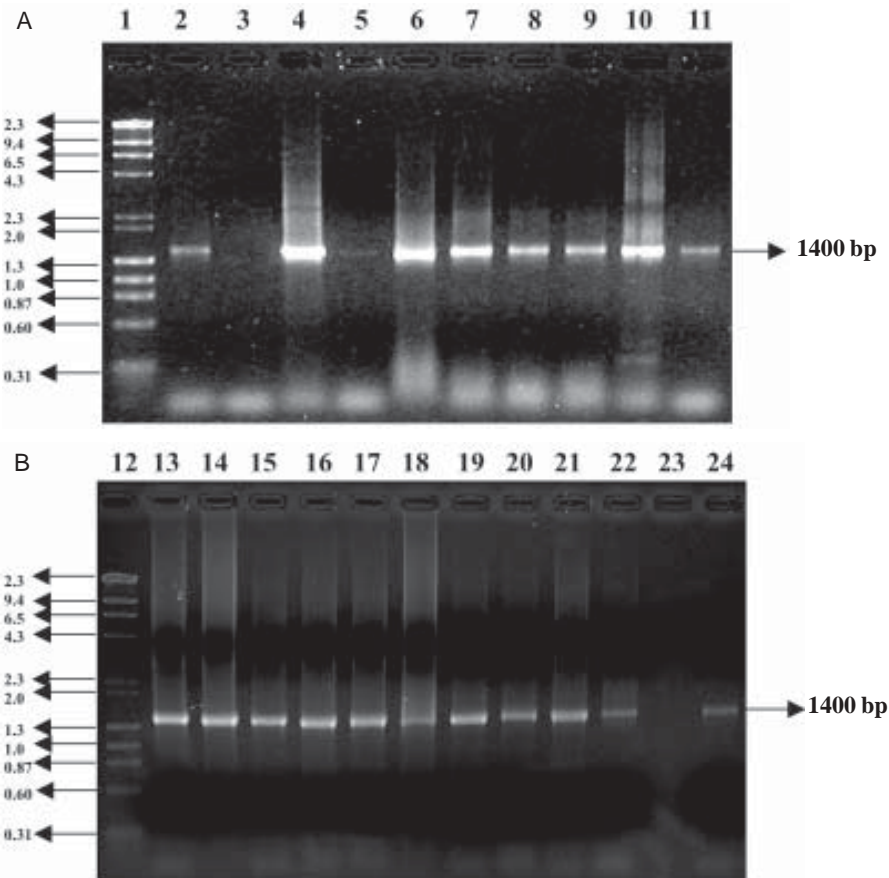


Fig. 2: amplification of 16s rDNA of *Wolbachia* from *Wuchereria bancrofti* microfilariae collected from different geographic regions of India. A: lanes - 1: mol. wt marker; 2: positive control; 3: negative control; 4-6: Pondicherry isolates; 7-9: Calicut isolates; 10-11: Varanasi isolates; B: lanes - 12: mol. wt marker; 13-15: Jagadapur isolates; 16-17: Thirukoilur isolates; 18-20: Chinnanergunam isolates; 21-22: Rajahmundry isolates; 23: negative control; 24: positive control

of *Wolbachia*, from *W. bancrofti* mf isolates collected from different geographic locations of India viz., Pondicherry (Union Territory), Calicut (Kerala State), Jagadapur (Chattisgarh State), Thirukoilur (TamilNadu), Chinanergunam (TamilNadu), Rajahmundry (Andhra Pradesh), and Varanasi (Uttar Pradesh) spanning about 2000 km. This is similar to what has been reported for *Wolbachia* endosymbionts of filarial nematodes by other workers (Taylor et al. 1999). Thus, the preliminary study showed that *Wolbachia* endosymbionts were present in populations of *W. bancrofti* originating from wider geographic areas of India.

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