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Detection of Wolbachia in Human Lice

Matthew Anderson

Georgia Southern University, ma03313@georgiasouthern.edu

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ABSTRACT

Background: Bioinformatic analysis of the *Riesia* genome revealed that the vitamin B1 pathways are not complete in this microorganism. These findings suggest that a secondary louse endosymbiont may be present and responsible for the missing functions. *Wolbachia*, an obligate intracellular alpha-Proteobacteria, is a candidate for this role.

Methods: DNA samples of human lice from the USA, Madagascar, and Russia were tested by PCR using Wsp81F and Wsp691R primers that can detect the single copy of conserved Wsp protein gene of *Wolbachia*. PCR products were detected by 1% agarose gel electrophoresis.

Results: Sites were compared for Wsp protein gene presence. Out of thirty samples tested, none tested PCR positive for Wsp protein gene.

Conclusions: Confirming the widespread presence of *Wolbachia* in lice would provide an immediate impetus to new approaches in the research effort to control of lice and improve the treatment of pediculosis in humans. Since it was not present in our samples, it may be that another bacterium may play the role posited for *Wolbachia*.

BACKGROUND

Between 6 to 12 million human head louse infestations occur each year in young children in the United States indicating continued needs for effective pediculicides. Lice are metabolically dependent on their primary endosymbiont, *Riesia pediculicola*, so targeting *Riesia* appears to be an attractive approach for designing new countermeasures against lice.

Bioinformatic analysis shows that vitamin B1 pathways are not complete in *Riesia*, suggesting that a secondary complementary louse endosymbiont may be present which is responsible for the missing functions (1). *Wolbachia*, an obligate intracellular alpha-Proteobacteria, is one possible candidate for this role. It has been suggested that 20% to 75% of arthropod species contain a *Wolbachia*. Kyei-Poku *et al.* detected the presence of A and B *Wolbachia* genetic groups in a limited number of human lice (2), but whole genome sequencing of a laboratory strain of the human body louse detected only the presence of *Riesia* but no *Wolbachia*. The Wsp protein gene is a quickly evolving gene found in *Wolbachia*; it is often used for detection and phylogenetic analysis of *Wolbachia* (5). The purpose of this study is to PCR test for *Wolbachia* in *Pediculus humanus capitis* and *Pediculus humanus corporis* using the Wsp protein gene in different louse samples and to determine if *Wolbachia* prevalence rates differ in geographically distinct louse populations.

METHODS

Human head lice were collected in Georgia, USA and Madagascar, and body lice were collected in Russia. All lice were identified using standard taxonomic keys and DNA was extracted from individual samples. Polymerase chain reaction with Wsp81F and Wsp691R primers targeting a single copy of a conserved Wsp protein gene were used to test DNA extracted from louse samples (4). PCR products were separated by 1% agarose gel electrophoresis. The gels were stained with ethidium bromide and observed under UV-light. A positive control from a *Wolbachia* positive spittle bug DNA sample and a negative control was included in each set of reactions.

Table 1. Wsp 81F and Wsp 691R primers used for detection of *Wolbachia*

Primer	Sequence 5' to 3'
Wsp 81F	TGG TCC AAT AAG TGA TGA AGA AAC
Wsp 691R	AAA AAT TAA ACG CTA CTC CA

PHYLOGENY AND VISUALIZATION OF *WOLBACHIA*

Figure 1 shows the genetic relationship of *Wolbachia* detected in different species of nematodes and insects, including lice. At present 5 genetic groups of *Wolbachia* are identified based on diversity of the Wsp gene sequence (Perotti *et al.*). Obligatory intracellular lifestyle of *Wolbachia* is depicted on electron microphotograph in Figure 2; this picture shows three *Wolbachia* residing in the cytoplasm of an insect cell (O'Neill).

Figure 1. Phylogeny of *Wolbachia* strains from lice and other insects (Perotti *et al.*).

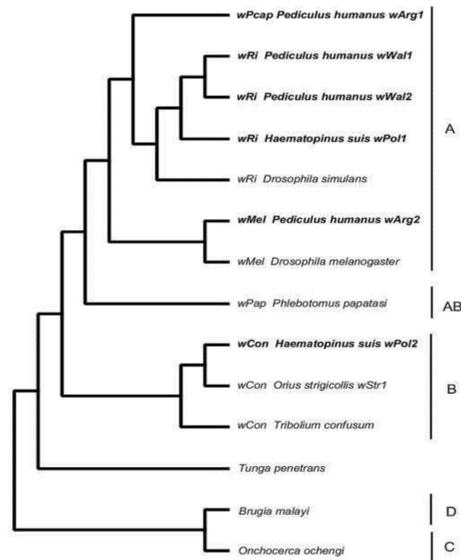
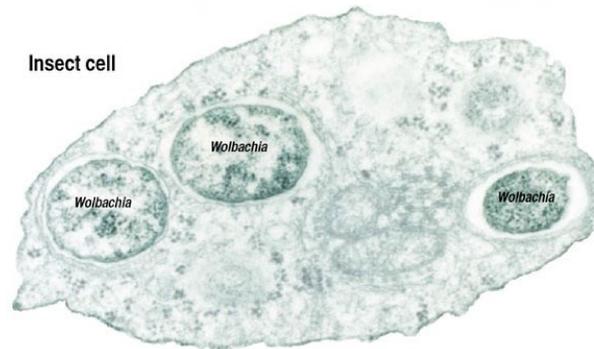


Figure 2. *Wolbachia* endosymbiont in an insect cell (Public Library of Science/Scott O'Neill).



RESULTS

Table 2. Results of PCR Wsp Testing

Location	Number of Head Lice	Number of body lice	Positive Results for Wsp
USA	10	0	0/10
Madagascar	10	0	0/10
Russia	0	10	0/10
Total	20	10	0/30

10 head lice from USA, 10 head lice from Madagascar, and 10 body lice from Russia were tested for *Wolbachia* using the methods described. All PCR results were negative for *Wolbachia* with positive controls present at 612 bp.

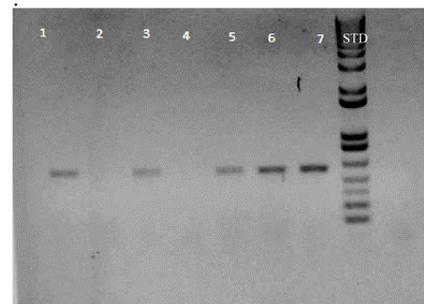


Figure 3. Positive results for 5 of 7 spittle bugs. Numbers 1-7 indicate spittle bug sample. STD indicates base marker lane.

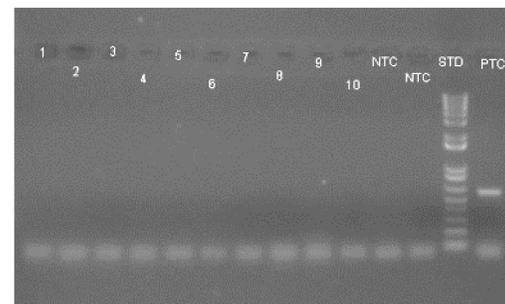


Figure 4. *Wsp* PCR from USA head lice. Numbers 1-10 indicate louse samples. NTC indicates negative controls. STD indicates base marker lane. PTC indicates positive control from spittle bug.

CONCLUSIONS & FUTURE WORK

- I. No *Wolbachia Wsp* DNA was detected in 30 louse samples of different geographic origins tested by routine PCR. These observations are in agreement with data of the published whole genome sequence of laboratory reared human body louse strain Orlando, but differ from other observations accumulated upon testing lice collected from humans.
- II. Since Wsp gene PCR is based on routine single-round PCR amplification, nested PCR or fluorescence based PCR detecting this or other gene targets (*ftsZ* and 16 sRNA gene) may provide better sensitivity and efficiency of *Wolbachia* detection in human lice (4).
- III. Several more genome sequences of human head and body lice became recently available from Next Generation Sequencing projects; therefore annotating these genomes and searching for *Wolbachia* sequences will be another approach to confirm or refute previous findings about an association of this microorganism with human lice.
- IV. Once completed, this study will contribute to further understanding of the biology of human lice and their endosymbionts, and their interactions. In particular, development of drugs targeting endosymbionts might be useful to treat and control lice in the human population (2).

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