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### **WOLBACHIA-HOST INTERACTION STUDY: A METHOD TO INVESTIGATE THE ROLE OF WOLBACHIA AND ITS PROTEINS**

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**Abstract:** *Wolbachia* is maternally inherited gram negative, obligate intracellular endosymbiotic, alpha proteo bacteria that are found in a wide range of arthropods and filarial nematodes. The ability to transfer *Wolbachia* into new hosts can create novel *Wolbachia*-host associations. *Wolbachia*-host association can range from parasitic to symbiotic, depending upon the interaction with the host. *Wolbachia*-host interactions can be examined to know the refractoriness (resistance) or recalcitrance (uncooperativeness) of host to *Wolbachia* infection. The role / effects of *Wolbachia* and its product on the host and vice versa can also be known. Here, in the present review we summarize the current understanding of *Wolbachia* and its proteins interaction with the host with focus on cell lines and its application with special emphasis on Anti-pathogenic effects/Immune responses of the host to *Wolbachia* infection. *Wolbachia* and its proteins have been reported to act on the immune responses of its host and inhibit pathogenic microbes.

**Keywords:** *Wolbachia*, *Wolbachia* –host associations, Refractoriness, Recalcitrance, Cell lines, Immune responses



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## INTRODUCTION

*WOLBACHIA* are obligate maternally inherited intracellular alpha – proteo - bacteria that infect a wide spectrum of insects. Despite considerable progress in understanding *WOLBACHIA*, *WOLBACHIA*-host interactions remain largely unknown [1]. Infection allows the creation and examination of diverse novel *WOLBACHIA*–host associations and also to disentangle (unravel) the role of the bacteria on the host. Examination of *WOLBACHIA*-host association involves the understanding of refractoriness (resistance) or recalcitrance (uncooperativeness) of host to *WOLBACHIA* infection. In this review, we describe the current understanding of the *WOLBACHIA* and its proteins interaction with the host and vice versa with focus on cell lines. In the following paragraphs, *WOLBACHIA*-host interactions with special emphasis on Anti–pathogenic effects/Immune responses of the host has been outlined.

### ***WOLBACHIA*-host interactions with special emphasis on Anti–pathogenic effects/Immune responses in the cell lines**

#### ***WOLBACHIA*-host interactions**

*WOLBACHIA* are known to act as nutritional mutualists or reproduction facilitators [2, 3]. They affect host evolution, causing sexual selection [4], sex determination [5] or speciation [6, 7, 8]. *WOLBACHIA* have attracted considerable interest over the last two decades due to their potential application as biocontrol agents [9, 10]. *WOLBACHIA* can be used to directly suppress arthropod populations [11, 12], or as a vector for the expression of transgenes [13, 14, 15], or as a tool to drive desirable genotypes into arthropod populations [16, 17].

*WOLBACHIA* has been linked to viral resistance in *Drosophila melanogaster*. Flies infected with the bacteria are more resistant to RNA viruses such as *Drosophila C virus*, Flock house virus and Cricket paralysis virus [18]. In the filarial nematode species *Brugia malayi*, *WOLBACHIA* has become an obligate endosymbiont and provides the host with chemicals necessary to its survival and is being exploited in Anti-*WOLBACHIA* consortium.

Generally, any gram negative bacteria invades into *Drosophila*, it activates the Immune Deficiency (IMD) pathway inducing the synthesis of potent antimicrobial peptides (AMPs) such as attacin, cecropin, drosocin and dipterucin [19].

The study by S E Osborne was extended by research in *Drosophila* using different *WOLBACHIA* strains that vary in a variety of characteristics including genome sequence, infection density, pathogenicity and the strength of reproductive parasitism they impose upon their hosts. This has led us to conclude that not all but some strains of

*WOLBACHIA* induce up-regulation in the host and it also depends on host background [20].

A study by Z S Wong on experimentally infected mosquitoes has demonstrated the antipathogen protection in it is associated with *WOLBACHIA* induced up-regulation of a range of host immune genes [21].

The presence of the *WOLBACHIA* in the mosquito completely blocks the ability of the dengue virus to grow [22]. The researchers believe that the bacteria might boost the immune system of the insects to help it to fight off the virus or that the bacteria competes for key molecules such as fatty acids, cellular nutrients, proteins detrimental to the viruses [23].

Antipathogenic effects are frequently observed when *WOLBACHIA* are transfected into hosts that are either naturally uninfected or infected with a different strain. In such cases, infection induces the upregulation of host immune genes, in particular genes involved in the Toll and IMD pathway, leading to the generation of AMPs. Such immune upregulation of Toll/IMD pathway genes is assumed to underlie antipathogenic effects in novel hosts, especially antiviral effects in mosquitoes [24, 25, 26, 27, 28, 29].

These observations suggest that *WOLBACHIA* infection primes (increases) the immune system of the host, especially when infected insects are challenged with a pathogen.

#### ***WOLBACHIA* -host interactions with focus on insect and mammalian cell lines**

Being an endosymbiont, it was not possible to culture *WOLBACHIA* in in-vivo conditions. However, recently *WOLBACHIA* have been cultured in cell lines of insects and mammals. Through these cultures several interesting findings have been observed. When *WOLBACHIA* were cultured in *Drosophila* cell lines, the gram negative bacteria activate the Immune Deficiency pathway inducing the synthesis of potent antimicrobial peptides (AMPs) such as attacin, cecropin, drosocin and dipteracin [19]. In other such studies it has been shown *WOLBACHIA* provide antiviral protection from RNA viruses [18, 30]. *WOLBACHIA* was also established in cell cultures of silkworm and differential expression of host genes involved in immune response of silkworm cells associated with *WOLBACHIA* were identified [31, 32]. In Asian tiger mosquito, *Aedes albopictus*, two different *WOLBACHIA* strains, have remained in the continuous cell line as a carrier culture [33] and are being extensively used in assaying insect immunology. Similarly, *WOLBACHIA* that infect the small brown planthopper, *Laodelphax striatellus*, were cultured and maintained not only in insect cell line but also in mammalian cell lines. In mammals, mouse cell line L929 has been tested for *WOLBACHIA* cultivation [33]. These studies have indicated that

*WOLBACHIA* play a major role in inducing pathogenesis (mechanism of development of a disease) and activating the innate immune responses.

Apart from culturing live *WOLBACHIA*, investigations were made on the *WOLBACHIA* derived proteins such as *WOLBACHIA* Surface Protein (WSP) and heat shock protein 60 with the cell lines. An antiapoptotic effect was observed by *WOLBACHIA* Surface Protein (WSP) on human Polymorphonuclear cells (PMNs) [34]. Whereas *WOLBACHIA* heat shock protein 60 induces pro-inflammatory cytokines and apoptosis in monocytes invitro [35]. WSP in *Anopheles gambiae* cell lines stimulates increased transcription of immune genes by up-regulating *TEP1* and *APL1* which are known for Plasmodium killing in this species. WSP was the first protein that showed Anti-apoptotic activity in human cell line [34]. *WOLBACHIA* heat shock protein 60 (rWmhsp60) induces gene expression of pro-inflammatory cytokines in human monocytic cell line. In addition, it inhibits the phagocytic activity and does not alter the nitric oxide production [35]. Apart from these proteins, several ankyrin proteins and WO phage encoded proteins show specific responses in vertebrate immune response [36].

The human cell lines have so far been not able to support *WOLBACHIA*, however when *WOLBACHIA* related proteins are introduced in mammalian cell lines there was an immunological response. Humans are not naturally infected by *WOLBACHIA*, but few proteins have shown to have an anti-apoptotic activity (WSP and HSP) [37, 38, 39, 40, 41].

## SUMMARY

In this review, the information on the associations and interactions of *WOLBACHIA* and its proteins with the hosts has been analyzed. *WOLBACHIA* and its proteins have been reported to act on the immune responses of its host and inhibit pathogenic microbes. Usually in infected hosts, *WOLBACHIA* has triggered an immune response which is aimed at eliminating the pathogen infection. *WOLBACHIA*'s impact on the host might probably open up exciting avenues (ways or paths) by giving novel insights in new therapeutic approaches.

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