Genomics and Genetics of *Wolbachia*: A review

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Abstract:
*Wolbachia*are one of the most ubiquitous obligate intracellular maternally inherited bacteria that have been found in several arthropod groups.*Wolbachia*behave as areproductive parasite by manipulating host reproduction to enhance their vertical transmission. Their reproductive modification and cytoplasmic incompatibility (CI) has received attention for use in applied strategies targeting economically important insect pests and disease vectors. The genetic mechanism of incompatibility in the cytoplasm has been discovered to be due to the asynchrony of the male and female pronuclei at the initial stage of mitosis. Genetic investigations using molecular tools have yielded two *Wolbachia*genes (ftsZ anddnaA).It is opined that both comparative and post genomics analysis will facilitate the development of genetic transformation system.There has been a significant increase in the study of this parasite of recent. In this paper, the phylogeny, genetics, cytoplasmic inheritance and genomics of *Wolbachia*are reviewed. The article aimed at reviewing the contemporary knowledge of the parasite; identify the research gaps and future challenges in the study of these bacteria.

Key words: Genomics, Genetics, Cytoplasmic incompatibility and wolbachia

Introduction:
Wolbachia are α-proteobacteriaendosymbionts that are found in many arthropods and nematodes (Werren*et al*., 2008). These intracellular parasites are transmitted vertically through host eggs and alter host biology in many ways. A recent meta-analysis revealed that more than 65% of insect species harbor*Wolbachia*, making it one of the most ubiquitous endosymbionts on earth (Hilgenboecker*et al*., 2008). About one fifth of the arthropods are infected with*Wolbachia*.Among these arthropod species are the insects, mites, isopods, terrestrial, spiders as well as some filarial nematodes (Werren, 1997and O'Neill *et al*., 1992). The genus, *Wolbachia* contains many species among which is *W. pipientis,* first described by Hertig and Wolbach, (1924).

Attention has been drawn to this intracellular parasite because of its potential application in pest control. *Wolbachia* are maternally inherited, and they behave more like a reproductive parasite by inducing: feminization of genetic males, parthenogenesis, male-killing, and cytoplasmic incompatibility (CI) (Werren*et al*., 2008 and Werren, 1997). These modifications typically give a reproductive advantage to infected individuals and allow for the spread of *Wolbachia*through a population (Xi *et al.*, 2005a; Dobson, 2003; Dobson *et al.*, 2002a; Dobson *et al.*, 2002b;Turelli and Hoffman, 1991). Wolbachia can also affect nutritional and metabolic pathways of host development; (Casiraghi, *et al*. 2005) lifespan; (Verne *et al*., 2007; Baldo and Werren, 2007; Baldo, *et al.* 2006 and Funk *et al*., 2000) and provide protection of hosts from pathogens and parasites (Hilgenboecker*et al*., 2008; Baldo*et al.,* 2006a; Baldo*et al*., 2006b; Kose and Karr, 1995), as well as affect host mating behaviour and facilitate host speciation (Bressac and Rousset, 1992).

2.0 Phylogeny of Wolbachia
The application of molecular phylogenetic methods to identify these intracellular microorganisms

(Nikoh*et al*., 2008 and Jigginset *al.,* 2002) has been a major breakthrough in *Wolbachia* research. Studies using 16S rDNA, 23S rDNA, and protein-coding genes have shown that CI bacteria and isopod feminizing bacteria form a monophyletic bacterial group—the *Wolbachia* (Nikoh*et al*., 2008). Research on *Wolbachia* has increased drastically in recent years. This fascinating bacterial group appears to have evolved as specialists in manipulating reproduction and development in their eukaryotic hosts.

The challenge of *in vitro* culture of these parasites has been overcome by the development of polymerase chain reaction (PCR). The use of 16S rDNA sequences for microbial phylogeny, have greatly facilitated studies of these bacteria (Arakaki*et al*., 2001; Roux and Raoult; 1995 and Weisburg*et al*., 1991). Phylogenies based on 16S rDNA sequences show that *Wolbachia* are monophyletic relative to the *Neorickettsia and Rickettsia* (Baldo, 2006). The genus *Wolbachia* contains two major subdivisions that show around 2% 16S rDNA sequence divergence. The closest bacteria to the *Wolbachia* are a group of rickettsiae that include *Ehrlichiaequii, E. canis*, *Ehrlichiachaffeensis, Ehrlichiaruminantium, E. sennetsu and E. risticii*, Cowdriaruminata, *Anaplasmamarginale*, *A platys* and *A. phagocytophilum*.

Bacteria in the genus Rickettsia are still more distantly related. This genus includes several arthropod vector disease agents, including the causative agents of Rocky Mountain spotted fever, murine typhus, and scrub typhus, as well as a cytoplasmically inherited male killing bacterium found in ladybird beetles (Werren*et al*., 1994). Wolbachia comprises an un-rooted phylogenetic tree of eight super groups. The ninth group is however controversial because its status is yet to be ascertained (Bourtzis, 2008). *Wolbachia* participates exclusivelyin either reproductive parasitism or mutualism.

3.0 The genomics of Wolbachia

*Wolbachia*have a relatively small genomes ranging from 1.08–1.7mb. This is however in line within the range of other Rickettsia (0.8–2.1 mb) and are in accordance with a reductive trend following host adaptation. However, *Wolbachia*genomes lack the typical minimal genome content and high stability that is observed in other obligate endosymbionts (Salzberg*et al*., 2005). There is a slight deviation of the *Wolbachia* genome from other Rickettsia. Theirgenomes contain a high number of repetitive elements with each repeats constituting more than 14% of the *w*mel genome. A high number of these repeats are represented by ankyrin domains, but unusual in bacteria (Wu *et al*., 2004).

Extensive duplications of short open reading frames results in additional redundancy of the genome. This constitutes the unique nature of *Wolbachia* genome (Wu *et al*., 2004). There are two well-known *Wolbachia* genomes discovered on arthropod and nematode which include: the cytoplasmic incompatibility inducing strain from *Drosophila melanogaster* and the wBm strain from the *Brugiamalayii*(Foster *et al*., 2005). The table below shows the assembled genome of *Wolbachia*. This project has provided insight into the evolutionary study and mechanisms of host manipulation by *Wolbachia*.

Table 1: The Wolbachia genome project

Strain	Host organism	Super group	Phenotype	Genome size (mb)	Reference

wMel	*Drosophila melanogaster*	A	Cytoplasmic Incompatibility	1.27	Xi *et al.,* 2005
wBm	*Brugiamalayi*	D	Mutualist	1.08	Foster, 2005
wMelPop	*D.melanogaster*	A	Cytoplasmic incompatibility	1.3	O'Neill*et al*.,1992
wPip	*Culexpipiens*	B	Cytoplasmic incompatibility	1.48	Klasson*et al*., 2008
*w*Ri	*Drosophila simulans*	A	Cytoplasmic incompatibility	1.44	Klasson*et al*., 2009
*w*Bol1	*Hypolimnasbolina*	B	Male killing	~1.6	Duplouy*et al*., 2013
*w*Vul	*Armadillidiumvulgare*	B	Feminization	~1.7	Bourtzis et al., 2008
wDim	*Dirofilariaimmitis*	C	Mutualist	~1.0	Ling *et al*., 2001
*w*Ov	*Onchocerca volvulus*	C	Mutualist	~1.1	McNulty *et al.,* 2010
wAna	*Drosophila ananassae*	A	Cytoplasmic incompatibility	Not known	Salzberg*et al*., 2005
wSim	*D. simulans*	A	Cytoplasmic incompatibility	Not known	Salzberg*et al*., 2005
wAu	*D. simulans*	A	Not cytoplasmic incompatibility	Not known	Shereee*t al*., 2009

4.0 Genetics of *Wolbachia*

Genetic investigations using molecular tools have yielded two *Wolbachia* genes, ftsZ (Holden *et al*., 1993) and dnaA in *Drosophila* (Bourtzis*et al*., 1994). Genomic libraries now exist for more systematic studies. It is yet to be ascertained whether *Wolbachia* carry plasmids. However, some researchers have suggested an infectious agent of *Wolbachia* may exist (Williams *et al*., 1993). These could be useful in future molecular genetic studies. Genetic recombination between strains i.e. in double-infected host cytoplasm is not yet known (Werren*et al*., 1995). Researchers recently found that the presence of wMelPop in mosquitoes up-regulated the host immune gene expression which results in the inhibition of pathogen replication. The up-regulated six immune genes observed in wMelPop infected *Anophelesgambiae*are LRIM1, TEP1, CEC1, DEF1, CTL4 and CLIPB3 as reported by (Clancy and Hoffmann, 1996). When *Wolbachia* strain wMelPop was trans-infected into *A. gambiae*, there was up-regulation of immune genes (LRIM1 and TEP1) which inhibits the parasite development by interfering in the opsonization pathway (Conner and Saul 1986; Coyne, 1992).

4.1 Genetics of cytoplasmic incompatibility in Wolbachia

The genes involved in the mechanisms of cytoplasmic incompatibility (CI) are of great importance for future research on Wolbachia. There are two main components; modification and rescue in the fertilized egg. Therefore, four categories has been gathered so far: modCrescC (wildtype), mod–rescC (modification defective, but rescue capable), modCresc– (modification capable but rescue deficient), and mod–resc– (modification deficient and rescue deficient). Both modCrescC and what appear to be

mod–resc– isolates have been found in natural populations (Werren, 1997; Stouthamer and Kazmer, 1994). The other two categories have not been isolated, although population genetic theory predicts that

mod–rescC can be selectively favoured (Turelli, 1994). Thus, screening natural populations could provide a number of mutant categories forstudying mechanisms of CI action.

The incompatible cross is due to the asynchrony of the male and female pronuclei at the initial stage of mitosis; the delay of male nuclear envelope breakdown and histone H3 phosphorylation (a histone modification that is required for the initiation of mitosis) indicates that the activity of Cdk1, a key kinase that drives the cell into mitosis, is delayed in the male pronucleus(Tram and Sullivan, 2002). As a result, chromatids from the female pronuclei are properly condensed and lie at the first metaphase plate, but male pronuclear chromosomes are only in a semi-condensed state. During anaphase, the female chromosomesseparate normally, whereas the male pronuclei are either stretched to the centrosome poles or excluded entirely (Lassy and Karr, 1996; Tram *et al*., 2006). Cytogeneticist have unraveled a method of detecting incompatible cross by carefully observing for chromatin bridges between the nuclei at anaphase. A result of the incompatible cross is often haploid development, which has been observed in some dipterans and hymenopterans. In diploid organisms, this normally results in embryonic lethality, but in haplodiploids, haploidy can result in normal male development (Reed and Werren, 1995)

5.0 Conclusion:

An exponential increase has been recorded in *Wolbachia* research most especially in the areas of molecular biology and genomics. Recent trends have evolved such as *Wolbachia* genome sequencing, *Wolbachia* tissue culturing and strain typing. Despite the recent surge in *Wolbachia* research in the last decade, there is dearth of information on the biochemical mechanisms of cytoplasmic incompatibility. Questions arising from their distribution and how they migrate from one species to the other are left unanswered. Can it be effectively used in disease control? Does*Wolbachia* play any important role in the evolution of their hosts? Do they alter the rates of speciation and contribute to the acquisition of novel genes? There is a great challenge in the genetic transformation of *Wolbachia* as it has been reported by researchers (Cheng andAksoy, 1999)working in the field of insect symbionts. It is therefore suggested that both comparative and post genomics analysis will facilitate the development of genetic transformation system and answer some salient questions regarding *Wolbachia*induced reproductive alterations.

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