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Phage Diversity within *Wolbachia* Genomes of Drosophila Host

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Abstract

The present work aims the detection of different phage across seven whole genome assemblies of the endosymbiont *Wolbachia* of *Drosophila* host from different eco-geographic regions of Asia (India), USA(Maryland) and Europe (Sweden). Multiple phage (putative) specific to different bacterial species were identified in these *Wolbachia* genomes using computational tool highlighting towards possible phage transmission through horizontal gene transfer. Although, the bacterial species have specific defence mechanism in them to stop invading the phage genome, at the same time they do facilitate their replication, thus, it is left unclear about the mechanism of host-phage interplay. Bacteria-phage interactions influence the gene and genome organization, ecology and evolution of microbial community, however, their contribution to the eukaryotic genome evolution through obligatory bacteria and bacteriophages cannot be ignored. As per our knowledge, it's the first study which reports the presence of multiple phage other than WO phage in *Wolbachia* strains from two different *Drosophila* host species. The results reveal the nature of phage to be more source specific than species specific.

Keywords: *Drosophila, Wolbachia* genome, bacteriophage, horizontal gene transfer

Introduction

Bacteriophages are bacteria infecting viruses that are widespread abundantly in the biosphere and vastly diverse in nature depending upon several features like morphology, genome size and organization, choices of their site and bacterial host (1-3). Bacteriophages use host machinery to survive and replicates by using any one of two-life cycle namely, lytic-virulent (directly infects and kills the bacterial cells by producing viral particles) and lysogenic-temperate (integrates its genome into bacterial genome or sustain as plasmid within bacterial cells) (1, 4, 5). This ability of integrating viral DNA into host genome makes lysogenic bacteriophage an efficient driver of exchanging genetic material horizontally and holds the potential of altering phenotypic traits of the bacterium by remaining stable over generations (2). Thus, the integrated bacteriophages regulate host specific adaptive traits and becomes the part of bacterial genome. Bacteriophage works extensively as therapeutic representor in treatment of several bacterial infections due to alarming hike of multi-drug resistance despite of constant development and production of new antibacterial agents (4, 6).

Wolbachia is an intracellular endosymbiotic bacterium of Rickettsiaceae family which is widely distributed among

Arthropods (7). It is transmitted naturally through eggs to the next progeny and infects host reproductive system which includes parthenogenesis, cytoplasmic incompatibility (CI), distortions in sex ratio, feminization or male killing (8, 9). In spite of being its intracellular nature its genome comprises of large number of mobile genetic elements (8). The WO bacteriophage is reported to be widespread across *Wolbachia* genomes as around 90% of them are highly infected with phages (8, 10). Furthermore, it is depicted from previous studies that WO phage of *Wolbachia* can regulate the horizontal transfer of genes among its different strains whether these genes natively belong to *Wolbachia* only or even to other non-related bacteria (11). The eukaryotic host, temperate WO bacteriophage and the *Wolbachia* together forms the close tripartite relation and this relationship is extremely complex but at the same time it is an emerging model system to understand the importance of mobile elements in adaption of *Wolbachia*, bacteria and also their arthropod host (7, 10). It is reported that *Wolbachia* comprises of many cryptic prophages but usually possess one intact WO phage which is capable of producing active viral particles. Although WO bacteriophage is known to govern the ecology and evolution of *Wolbachia* but still not much information available as this area remains unexplored (10).

Table 1. Details of *Wolbachia* genome (accession number, source) and detected phages

S. No	Wolbachia Strain; Source	Accession No.	Identified Phage
1	wMel_AMD; Ahmedabad India	MNCG00000000	Intact: Salmonella phage BPS15Q2 [NC_031939]
			Intact: Pseudomonas phage PPpW-3 [NC_023006]
			Incomplete: Escherichia phage vB_EcoM-ep3 [NC_025430]
2	wMel_KL; Kerala India	MLZJ00000000	Intact: Salmonella phage BPS15Q2 [NC_031939]
			Intact: Escherichia phage vB_EcoM-ep3 [NC_025430]
3	wRi_AMD; Ahmedabad India	MSYL00000000	Intact: Escherichia phage vB_EcoM_ECO1230-10 [NC_027995]
			Intact: Enterobacter phage Arya [NC_031048]
4	wRi_KL; Kerala India	MKIF00000000	Intact: Escherichia phage vB_EcoM-ep3 [NC_025430]
			Questionable: Vibrio phage vB_VpaM_MAR [NC_019722]
			Incomplete: Escherichia phage vB_EcoM-ep3 [NC_025430]
5	wMel; Maryland, USA	NC_002978	Questionable: Escherichia phage vB_EcoM-ep3 [NC_025430]
			Incomplete: Escherichia phage vB_EcoM_ECO1230-10 [NC_027995]
			Incomplete: Escherichia phage vB_EcoM-ep3 [NC_025430]
			Incomplete: Gordonia phage OneUp [NC_030917]
6	wAna; Maryland, USA	AAGB00000000	Questionable: Clostridium phage phiCT453B [NC_029004]
			Incomplete: Escherichia phage vB_EcoM-ep3 [NC_025430]
			Incomplete: Escherichia phage vB_EcoM-ep3 [NC_025430]
			Incomplete: Paenibacillus phage Tripp [NC_028930]
7	wRi; Uppsala, Sweden	NC_012416.1	Intact: Vibrio phage vB_VpaM_MAR [NC_019722]
			Questionable: Vibrio phage vB_VpaM_MAR [NC_019722]
			Questionable: Vibrio phage vB_VpaM_MAR [NC_019722]
			Incomplete: Phage_Yersin_413C [NC_004745]

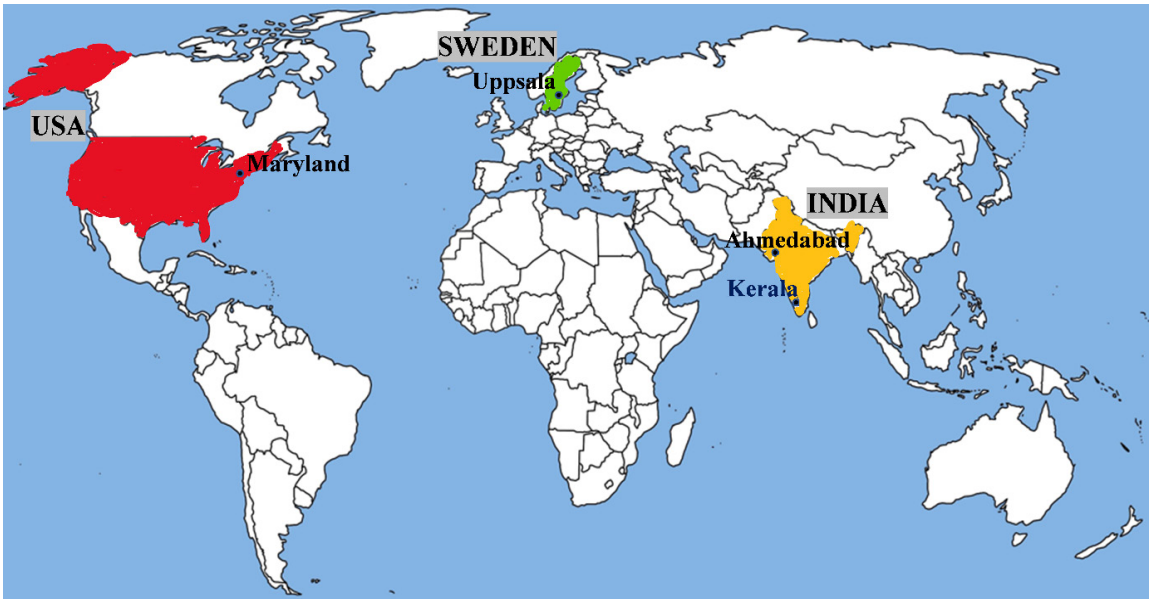

Fig. 1. Map showing the source of studied *Wolbachia* genomes

The purpose of the present study is to detect the presence of different bacteriophages in whole genome assembly of *Wolbachia* strains infecting natural populations of two *Drosophila* host species. As per our knowledge, it is the first attempt to report multiple phage other than WO phage from different strains of *Wolbachia* in *Drosophila* host belonging to different eco-geographical regions.

Materials and Methods

***Wolbachia genomes*:** The study includes whole genome assembly of four *Wolbachia* strains (wMel_AMD, wMel_KL, wRi_AMD and wRi_KL) obtained from two Indian *Drosophila* host species (*D. melanogaster* and *D. ananassae*) collected from two cities; Ahmedabad, West India and Kerala, South India (Fig.1) (7). In addition, the study also includes the whole genome assembly of two *Wolbachia* strain (wMel and wAna) from Maryland, USA and one (wRi) from Uppsala, Sweden (Fig.1). The Indian *Wolbachia* genomes were generated and assembled in our laboratory, whereas the other three genomes were retrieved from 'Genome' bank of NCBI (https://www.ncbi.nlm.nih.gov/), the accession and source details of all seven whole genomes are provided in Table 1.

***Phage Detection*:** Detection of phages were carried out in all 7 *Wolbachia* genomes using web server-based tool known as PHAge Search Tool (PHAST) (12). The PHAST tool library has a huge collection of phage and prophage protein sequences mainly from two sources NCBI and prophage database (12).The tool detects putative phage proteins in the query genome via BLASTP searches and identifies the phages based on their key domains (head, tail, capsids, proteins, transposases, integrases etc.) and also predicts the completeness score of phages as i) intact (score above 90), ii) questionable (score between 60 to 90) and iii) incomplete (score below 60). PHAST is established as one of the best phage finding tool in terms of speed, accuracy with sensitivity of 85.4% and positive predictive value of 94.2% (12, 13).

Results and Discussion

Wolbachia strains from *Drosophila* host were widely researched and found to induce viral resistance, reduce longevity and high cytoplasmic incompatibility in host (14).

Recent use of this obligatory bacteria as one of the biological vector-control methods demands extensive study to understand more of its host- and eco-specific nature. In our laboratory, the presence of *Wolbachia* was observed in only three species i.e *Drosophila ananassae*, *Drosophila simulans* and *Drosophila melanogaster* when 10 different *Drosophila* species from seven locations of India were tested (15). The identification of those *Wolbachia* strains were carried out using multiple marker loci sequences. The whole genomes of *Wolbachia* strains were generated from host gDNA and assembled using reference genome from the NCBI (9). In the present study, four *Wolbachia* genomes generated in our lab were used for detection of phages (Table 1).

Among all 7 whole genome assemblies of *Wolbachia* except one (wRi) from Uppsala, Sweden, the *Escherichia* phage (*Escherichia phage vB_EcoM-ep3*) is found to be common, however, a different strain of this phage (*Escherichia phage vB_EcoM_ECO1230-10*) is observed in wRi from Ahmedabad and both these strains are found in wMel from Maryland, USA. The phylogenetic analysis shows these two *Escherichia* phages to be close relatives (16). The *Escherichia* phage attacks the strains of *E. coli* bacteria and also serves as indicator for the presence of fecal associated coliform bacteria and enteric pathogenic bacteria that resides in host intestinal tracts (17, 18).

The *Vibrio* phage (*Vibrio phage vB_VpaM_MAR*) is detected in wRi from Kerala, India and Uppsala, Sweden. This *Vibrio* phage immensely contributes in controlling the major seafood pathogen namely, *Vibrio parahaemolyticus* which belongs to Vibrionaceae family and is widespread throughout the coastal, marine and estuarine areas (19). It is termed as crucial causative agent of food borne diseases after consumption of seafoods from contaminated sites either raw or partially cooked (19). The possible reasons of finding this phage in *Wolbachia* genome sourced from Kerala and Uppsala may be due to that these two places are biggest hubs for sea-food industries and surrounded by water bodies, which emphasizes the region-specific nature of phages.

As per the PHAST tool detection, wMel from both Ahmedabad and Kerala comprise *Salmonella phage BPS15Q2* within their genome. The *Salmonella* phage works as biocontrol agent to control the harmful effects of *Salmonella* during food production and is regarded as active killer of MDR *Salmonella* strain (20, 21). Some phages are found to be *Wolbachia* strain specific such as: (a) *Pseudomonas phage PPpW-3* detected in only wMel_AMD extensively works against infection caused by *Pseudomonas plecoglossicida* (22), (b) *Enterobacter phage Arya* observed in only wRi_AMD which acts in opposition to *Enterobacter* species by infecting them and shows high extent of synteny with some of identified phages namely, *Escherichia phage vB_EcoM-ep3*, *Escherichia phage vB_EcoM_ECO1230-10* and *Pseudomonas phage PPpW-3* [23], (c) *Gordonia phage OneUp* detected in wMel_Maryland, USA, belongs to the diverse family of *Gordonia* aerobic heterotrophs which are found in terrestrial, aquatic and even to polluted industrial sites. They remain involved with opportunistic infections among immuno-depressed patients and in foaming mechanisms of waste-water treatment plants (24), (d) *Clostridium phage phiCT453B* and *Paenibacillus phage Tripp* are detected in wAna_Maryland, USA. *Clostridium phage phiCT453B* serves potently against strains of *Clostridium tetani* (25) whereas, *Paenibacillus phage Tripp* works in opposition with bacteria responsible for American foulbrood disease among honeybees (26) and (e) *PHAGE_Yersin_413C* identified in wRi_Uppsala, Sweden, are one of the phages specific for *Yersinia pestis* that widely used for plague testing and serves as an important alternative towards antibiotics for drug resistant plagues (27).

The present work contributing towards determination of different phages in different

strains of *Wolbachia*, is the meaningful attempt of understanding the phage-mediated interactions of this endosymbiont, as it can be a major concern for its modulatory effect on the arthropod host physiology (7, 8). Extensive study is required as each phage has particular potential host range to which it can infect and this range could be narrow or broad (28). Presence of different phages in *Wolbachia* strains is evidenced to be more source-specific, which may be the resultant of horizontal gene transfer among the gut microbiome of secondary host (*Drosophila*) (Fig. 2).

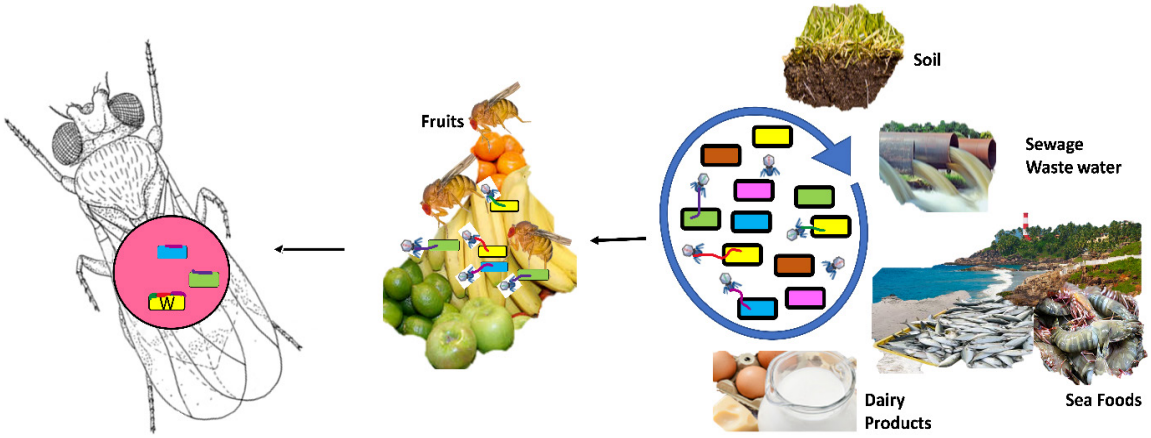

Fig. 2. Possible transmission of phages across primary and secondary (*Drosophila*) host species from various sources

Although, studies have been done to establish the tripartite relationship of Arthropod host-*Wolbachia*-WO phage with respect to the ecology and evolution of *Wolbachia*, very little is known about the nature and possible transmission of phages across bacterial strains or *Wolbachia* to other gut microbiome of the arthropod host (10), which may be of study interest as *Wolbachia* is proposed to be used as a biocontrol agent in one of the vector-control methods (14). Similarly, diversity among phages from various sources (soil, sewage, sea foods, dairy products etc.) may vastly contributes in regulating the composition of bacterial communities but still remains unclear with association of human biome (18, 29). The phage-host interactions and capability of modifying them through modern phage biology have wide applications in clinical science and food & agricultural industry (30).

Conclusion

In one of our previous study, it is reported that diverse ecogeographic region alters the microbial species diversity in *Drosophila* host species which ascertains the role of ecological source in determining the host-microbial association. Similarly, detection of diverse phages in these *Wolbachia* whole genome assemblies of different *Drosophila* host species from different eco-geographical regions has turned out to more source specific rather than strain specific. Hence, extensive studies are required to understand more on host-bacteria-phage interaction, possible phage transmission and their impact on eukaryotic hosts. These kinds of studies may put insight into human disease pathogens, their clinical control through novel disease-specific phage therapy and also to a certain extent to understand genome evolution at organismal level.

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Conflict of interests

The authors declare that they have no conflict of interest.

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