Wolbachia limits pathogen infections through induction of host innate immune responses

PLOS One

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Feb 21, 2020

1 Works for me

dx.doi.org/10.17504/protocols.io.xcafise

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EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0226736

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Zhang D, Wang Y, He K, Yang Q, Gong M, Ji M, Chen L (2020) *Wolbachia* limits pathogen infections through induction of host innate immune responses. PLoS ONE 15(2): e0226736. doi: 10.1371/journal.pone.0226736

Mosquitoes

1 The Cx. pipiens pallens larvae were collected from NJ (Nanjing, Jiangsu Province□32°3'30.11"N, 118°47'47.28"E), and TK (Tangkou, Shandong Province, 34°52'34.97"N, 117°22'53.69"E) in 2017 from July to August. All collection was done on public land. After morphology identification, the larvae were then maintained in the insectary. Mosquitoes were kept at 28°C, 75% relative humidity and a photoperiod of 14h light: 10h darkness. Adult mosquitoes were fed 10% (w/v) glucose solution prior to blood meals[27].

Tetracycline treatment to eliminate Wolbachia from Culex populations

2 Tetracycline treatment to eliminate Wolbachia from Culex populations was carried out according to published methods [28]. Tetracycline (Amresco) at a concentration of 0.05 mg/ml was used for the treatment through both larval and pupal stages. Eggs were placed on tetracycline water solution to hatch. Surviving larvae were transferred to fresh tetracycline solution every 24 hours. A normal infusion was prepared in parallel and fed to larvae in tetracycline solution. After continuous tetracycline treatment for 6 generations, Wolbachia-negative Culex populations were established.

Establishment of new host-Wolbachia symbiosis

3 Establishment of new host-Wolbachia symbiosis
To separate virgin females and males, pupae from each population were put into 15 ml tubes with water for individual emergence. Then, male and female adults were raised in 30.5×30.5×30.5 cm cages. Females 1 day post-eclosion and males 2 days post-eclosion were used in mating experiments. Each set of crossings included combination groups of virgin tetracycline treated males from TK/NJ and females from NJ/TK populations (NJ♀×TKtet♂ and TK♀×NJ tet♂), with combinations of males and females from the same populations as controls (NJ♀×NJ♂ and TK♀×TK♂). Females and males placed in the same cages were given 2 days to mate. Females were blood fed after mating, then the egg rafts were given 48 hours after oviposition to hatch. Females of (NJ♀×TKtet♂)F1, (TK♀×NJ tet♂)F1, NJ and TK were collected 2 days post-eclosion for RNA extraction and sequencing.

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RNA sequencing and analysis

4 RNA sequencing and analysis

Total RNA of 15 female mosquitoes of each group was extracted using TRIzol reagent (Thermo Fisher Scientific,USA) following the manufacturer's protocol. cDNA library construction and sequencing were performed according to standard procedures by Beijing Genomics Institute (BGI-Shenzhen, China) using BGISEQ-500 platform. At least 60 Mb clean reads of sequencing were obtained for each sample. Since no genomic sequence in any database was available for Cx. pipiens pallens, Trinity [29] was used to perform de novo assembly with clean reads, then Tgicl [30] was used on cluster transcripts to remove abundance and retain Unigenes. After assembly, Unigenes functional annotation was performed with 7 functional databases (NR, NT, GO, KOG, KEGG, SwissProt and InterPro), then all the clean reads of each sample were mapped to the Unigenes with Bowtie2 [31] software and the gene expression levels were calculated with RSEM [32]. Based on the gene expression levels, the DEGs (differential expression genes) between samples or groups were identified with PossionDis [33] (Fold Change >= 2.00 and FDR<= 0.001). The DEGs were classified based on the GO annotation results and official classification. Pathway analysis was performed to provide further information on the DEGs' biological functions. The DEGs were also subjected to KEGG pathway classification and functional enrichment.

Validation of immunity-related DEGs by real-time quantitative PCR

5 Validation of immunity-related DEGs by real-time quantitative PCR

Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific,USA). The cDNA was synthesized with PrimeScript RT reagent kit (Takara, Otsu, Shiga, Japan) according to the manufacturer's protocol. PCR was performed on the LightCycler 96 Real-Time PCR System (Roche, Switzerland) using SYBR Green Master Mix kit (Roche, Switzerland). Primers specific for real-time quantitative PCR are listed in Table 1. For each reaction, 10 µl of SYBR Green Master Mix was used, 1.0 µl of each primer solution at 10 µM and 8 µl of diluted cDNA were added. PCR cycling protocol was as follows: initial 50 °C for 2 min, denaturation for 10 min at 95 °C, followed by 40 cycles at 95 °C for 15 s, 60 °C for 1 min. The housekeeping gene Rps6 was used as an internal control and the data were analyzed with LightCycler 96 Software v1.1 (Roche, Switzerland). Quantitation of relative mRNA expression was calculated using $2^{-\Delta\Delta Ct}$ method [34]. Each experiment was performed twice, shown here is a representative result.

Microbial challenge and survival experiments

6 Microbial challenge and survival experiments

Microbial challenge and survival experiments were performed in the same way as described in[35]. In brief, an acupuncture needle (0.20×25mm) was dipped into a concentrated overnight bacterial culture of Gram-negative (Escherichia coli) or Gram-positive (Micrococcus luteus) bacteria or sterile LB culture (negative control) and pricked mosquitoes (2 days old female) in the rear part of the abdomen. For each group, three parallel experiments of 15-20 mosquitoes per container were performed [36].

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