Bacteriophage: a useful tool for studying gut bacteria function of housefly larvae, musca domestica

Xinyu Zhang\textsuperscript{1, 2*}, Shumin Wang\textsuperscript{2*}, Ting Li\textsuperscript{2}, Qian Zhang\textsuperscript{1, 2}, Ruiling Zhang\textsuperscript{1, 2*}, Zhong Zhang\textsuperscript{1, 2*}

\textsuperscript{1} Collaborative Innovation Center for the Origin and Control of Emerging Infectious Diseases, Shandong First Medical University (Shandong Academy of Medical Sciences), No. 619, Changcheng Road, 271016 Taian, Shandong, China

\textsuperscript{2} School of Basic Medical Science, Shandong First Medical University (Shandong Academy of Medical Sciences), Taian 271016, Shandong, China

* Correspondence:

Zhong Zhang, E-mail: nasonia@163.com

Ruiling Zhang, E-mail: rlzhang@tsmc.edu.cn

#These authors have contributed equally to this work.
Supplementary Tables:

Table S1  Table showing the name, location, year of isolation and morphology of the phage used in this study.

| Name | Location and year of isolation | Morphology        |
|------|--------------------------------|-------------------|
| Y12Pw| Taian China (36° 12' 26" E, 117° 6' 4" N) 2019 | Caudovirales (order) |
|      |                                 | Myoviridae (family) |
Table S2 Information about 16S rRNA gene analysis in this study. Data are expressed as the mean ± standard deviation of three replicate samples in each sampling.

| Samples | Clean Reads | Normalized reads | OTU number | Shannon    | Simpson    | Chao1       | Coverage |
|---------|-------------|------------------|------------|------------|------------|-------------|---------|
| Ctea1   | 53042       | 48059            | 360.33±84.68 | 1.39±0.04  | 0.4008±0.0274 | 735.33±166.63 | 0.9965±0.0006 |
| Ctea2   | 59119       | 48059            | 458.00±66.02 | 1.14±0.86  | 0.3494±0.0194 | 819.33±215.22 | 0.9959±0.001 |
| Ctea3   | 63412       | 48059            | 529.67±6.66  | 1.40±0.21  | 0.441±0.1091 | 907.33±98.74  | 0.9957±0.0003 |
| Ctea4   | 62649       | 48059            | 645.00±499.77 | 1.21±0.83  | 0.613±0.1984 | 1019.00±274.31 | 0.9962±0.0014 |
| Ppca1   | 53042       | 48059            | 278.33±22.59 | 1.32±0.18  | 0.8739±0.8053 | 643.00±93.74  | 0.997±0.0002  |
| Ppca2   | 64404       | 48059            | 453.00±183.28 | 1.24±0.08  | 0.4136±0.0097 | 833.33±263.21 | 0.9964±0.0018 |
| Ppca3   | 63580       | 48059            | 412.00±20.66 | 1.43±0.15  | 0.3976±0.0755 | 865.67±91.54  | 0.9961±0.0006 |
|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pca3 | 3   | 54303 | 48059 |     |     |     |     |     |     |
| Pca4   | 1   | 53875 | 48059 |     |     |     |     |     |     |
| Pca4   | 2   | 55875 | 48059 | 392.00±16.52 | 1.80±0.07 | 0.2824±0.0188 | 744.00±54.34 | 0.9963±0.0003 |     |
| Pca4   | 3   | 61849 | 48059 |     |     |     |     |     |     |
**Table S3** Topological properties of bacterial co-occurrence networks associated with no-phage and single-phage treatments.

| Group | Network index | Total nodes | Total links | Average clustering coefficient | Average path distance | Maximal betweenness |
|-------|---------------|-------------|-------------|-------------------------------|-----------------------|---------------------|
| Ctea  | 0.820         | 183         | 405         | 0.186                         | 5.074                 | 3996.842            |
| Ppca  | 0.740         | 166         | 778         | 0.249                         | 3.397                 | 2073.992            |
Table S4 Information on all the driver taxa associated with bacterial communities exposed to phage and not exposed to phage based on network analysis.

| Genera                | Jaccard Index | NESH score | Delta-betweenness | Degree in Cteca | Degree in PPCa | Degree in both |
|-----------------------|---------------|------------|-------------------|-----------------|----------------|----------------|
| Bordetella            | 0.25          | 1.845      | 0.041             | 5               | 15             | 4              |
| Morganella            | 0.368         | 1.708      | 0.143             | 7               | 19             | 7              |
| Stenotrophomonas      | 0.091         | 2.189      | 0.007             | 1               | 11             | 1              |
| Kurthia               | 0.143         | 2.159      | 0.028             | 2               | 13             | 2              |
| Desulfovibrio         | 0.111         | 2.074      | 0.006             | 1               | 9              | 1              |
| Staphylococcus        | 0             | 2.279      | 0.001             | 1               | 10             | 0              |
| Vagococcus            | 0.286         | 1.69       | 0.0017            | 5               | 13             | 4              |
| Enterobacter          | 0.308         | 1.49       | 0.013             | 6               | 11             | 4              |
| Proteus               | 0.167         | 2.315      | 0.072             | 2               | 19             | 2              |
| Lactococcus           | 0.083         | 2.12       | 0.012             | 2               | 11             | 1              |
| Empedobacter          | 0.438         | 1.458      | 0.068             | 7               | 16             | 6              |
| Lactobacillus         | 0             | 2.079      | 0.003             | 1               | 6              | 0              |
| Pseudomonas            | 0.429        | 1.331      | 0.016             | 8               | 13             | 6              |
| Serratia              | 0.5           | 1.148      | 0.012             | 4               | 8              | 4              |
| Novosphingobium       | 0.333         | 1.407      | 0.001             | 1               | 3              | 1              |
| Paenarthrobacter       | 0             | 2.233      | 0.006             | 1               | 8              | 0              |
| Enterobacteriaceae_unclassified | 0.652      | 0.992      | 0.069             | 16              | 24             | 16             |
Supplementary Figures:

Figure S1  Biological characteristics of phage Y12Pw. (A) One-step growth curve of the phage Y12Pw. The Y-axis shows the log of plaque forming units per milliliter (PFU/mL). Experiments were repeated three times with duplicate samples. (B) The resistance of *Pseudomonas aeruginosa* and other cultivable bacterial isolates in the housefly larval intestine to phage used in the experiments. The infectivity of Y12Pw phage against host bacteria non-host bacterial isolates (other cultivable bacteria) from the housefly larval intestine.
Figure S2 Annotated genome maps for the phage Y12Pw. In the circular genome map, the outermost black circle represents the full length of the genome, the innermost multicolored circle represents annotated functional proteins, the second outermost blue circle represents the GC skew, and the third outermost purple circle represents the GC skew content.
Figure S3 VIRFAM analysis is used to the recognition of head-neck-tail modules. A clustering of the query phage with respect to those of same Type in the Aclame database. The head-neck-tail modules protein of Y12Pw (in text box with red border and white background) is similar with K139 (K139 belongs to the Mycoviridae family).
Figure S4 Changes in body weights and body lengths of housefly larvae treated with sterile water and sterile water containing less than $10^8$ PFU/mL bacteriophage. (A) Body weight and body length (B) changed over time in housefly larvae with different treatments. Ctsa and Ppsa represent housefly larvae samples treated with sterile water and sterile water containing less than 108 PFU/mL bacteriophage, respectively. Data are shown as the mean ± SEM. Each treatment included 12 biological replicates. Repeated measures ANOVA was followed by Sidak correction for multiple comparisons.
Figure S5 Housefly larvae intestinal bacteria diversity and richness index information and shared and unique genera of the intestinal bacteria in housefly larvae samples. (A) Dynamics of the ACE, Chao1, Shannon and Simpson indices of intestinal bacteria in the two groups of housefly larvae. (B) UpSet plot showing differences in the levels of bacterial genera in the intestinal bacteria of housefly larvae between the two groups within 1-4 days. (C) Venn diagram comparing unique and overlapping bacterial genera in the groups. The numbers represent the numbers of unique genera in each sample and common genera shared by two or more samples.
Figure S6 Antagonism experiment of *Pseudomonas aeruginosa* and *Lactococcus lactis* in the housefly larval intestine in a microaerobic environment. (A) Left panel: *P. aeruginosa* was seeded on the right side of the plate, while sterile water was spread on the opposite side as a control. The filters paper was dipped into *Lactococcus lactis*. Right panel: *Lactococcus lactis* was seeded on the right side of the plate, while sterile water was spread on the opposite side as a control. The filter paper was dipped into *P. aeruginosa*. (B) Competitive inhibition between *Pseudomonas aeruginosa* and *Lactococcus lactis* in the housefly larval intestine. Data are shown as the mean ± SEM. The t-test was used for the statistical analysis.