## **1** Supporting Information

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3 Fig. S1. Axenic A. aegypti and A. atropalpus first instars rapidly ingested bacteria. 4 Conventional first instars were hatched in open containers containing distilled water and 5 sterilized diet (Non-sterile). Axenic first instars were hatched in fully sterile conditions 6 and fed sterilized diet (Axenic). Other axenic larvae were fed sterilized diet plus the 7 indicated bacterial isolate. For each treatment, DNA was isolated from a pooled sample 8 of 10 larvae that was collected 6 h post-inoculation after repeated washing and surface 9 sterilization per Coon et al. [21]. DNA was also isolated from cultures of each bacterial 10 isolate. DNA samples were then used as templates with universal or genus-specific 11 primers (see Methods and Table S2). The agarose gel shows ethidium bromide stained 12 PCR products. Lane 1, molecular mass markers labeled in base pairs (bp); Lane 2, 13 universal primers plus DNA from conventional larvae; Lane 3, universal primers plus 14 DNA from axenic larvae; Lane 3-4, Chryseobacterium-specific primers plus template 15 from Chryseobacterium (Control) axenic first instars inoculated with or 16 Chryseobacterium (Larva). The same treatments are then shown for Sphingobacterium 17 (Lanes 5-6), Microbacterium (Lanes 7-8), Leucobacter (Lanes 9-10), Paenibacillus (Lanes 11-12), Aquitalea (Lanes 13-14), and Comamonas (Lanes 15-16). 18 This 19 experiment was repeated four times with independently collected samples and each 20 time yielded identical outcomes for both A. aegypti and A. atropalpus.



Figure S1.

Mosquito species isolate was collected from	Colony color and morphology	Culture medium	Genus	
A. aegypti	White/pink + sec	Nutrient agar (BHI)	Paenibacillus	
A. aegypti	Yellow	Nutrient agar (BHI)	Chryseobacterium	
A. aegypti	White	Nutrient agar (BHI)	Sphingobacterium	
A. aegypti	White (small)	Blood agar (TSA base)	Microbacterium	
A. aegypti	White (small)	Nutrient agar (BHI)	Leucobacter	
A. atropalpus	Green	R2A	Aquitalea	
A. atropalpus	White (small) + sec	R2A	Comamonas	

## **Table S1**. Bacterial isolates used in the study.

Name	Sequence	Target gene	Target	Tm (°C)	Reference			
General primers (product > 300 bp)								
27F	GAGAGTTTGATCCTGGCTCAG	16S rRNA	Most bacteria	50	1			
1492R	GGTTACCTTGTTACGACTT	16S rRNA	Most bacteria	50	1			
1492F	AAGTCGTAACAAGGTAACC	16S rRNA	Most bacteria	55	1			
129R	GGTTBCCCCATTCRG	23S rRNA	Most bacteria	55	2			
Quantitative PCR primers (product < 300 bp)								
*HDA1	ACTCCTACGGGAGGCAGCAGT	16S rRNA	Most bacteria	55	3			
*HDA2	GTATTACCGCGGCTGCTGGCA	16S rRNA	Most bacteria	55	3			
Aqui-qF	GTTGAGCACTCTAATGGGAC	16S-23S ITS	<i>Aquitalea</i> sp.	55				
Aqui-qR	TCGGTTTTATGAGATTGGCT	16S-23S ITS	<i>Aquitalea</i> sp.	55				
Chryseo-qF	GAAACTGCCATTGATACTGC	16S-23S ITS	Chryseobacterium sp.	55				
Chryseo-qR	CCCAAAAACGAGTTAGCATC	16S-23S ITS	Chryseobacterium sp.	55				
Coma-qF	GAGTCGAAAATCAGCGTTGC	16S-23S ITS	Comamonas sp.	55				
Coma-qR	GCACAAGAACCCAAGCAACT	16S-23S ITS	Comamonas sp.	55				
Micro-qF	AACTAGTTGTGGGGTCCA	16S-23S ITS	Microbacterium sp.	55				
Micro-qR	TGTATGTCAAGCCTTGGTAA	16S-23S ITS	Microbacterium sp.	55				
Paeni-qF	TAGGTGTTGGGGGATTCGAT	16S-23S ITS	Paenibacillus sp.	55				
Paeni-qR	CACCTGTCTCCTCTGTCC	16S-23S ITS	Paenibacillus sp.	55				

Table S2. Primers designed and used during the study.

\*Universal primers were used for qPCR analysis of Sphingobacterium- and Leucobacter-associated mosquitoes

1. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, editors. Nucleic acid techniques in bacterial systematics. New York: John Wiley and Sons; 1991. p. 115-175.

2. Gurtler V, Stanisich VA. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology. 1996;142:3-16.

3. Walter J, Tannock GW, Tilsala-Timisjarvi A, Rodtong S, Loach DM, Munro K, Alatossava T. Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Appl Environ Microbiol. 2000;66:297-303.

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