

# Microbial Community Changes in Silkworms Suspected of Septicemia and Identification of *Serratia* sp.

## Supplementary Material

Table S1. Raw data statistics

Sample name	Total read bases (bp)	Total reads	GC (%)	AT (%)	Q20 (%)	Q30 (%)
Norm 1	66,619,728	221,328	52.90	47.10	91.15	81.47
Norm 2	65,049,110	216,110	53.56	46.44	90.76	81.08
Norm 3	45,110,870	149,870	53.05	46.95	90.65	80.97
Sept 1	57,657,754	191,554	55.15	44.85	90.15	80.33
Sept 2	67,107,348	222,948	55.02	44.80	91.05	81.34
Sept 3	58,414,468	222,948	55.21	44.79	91.34	81.77

\*Total read bases: Total number of bases sequenced

\*Total reads: Total number of reads. For Illumina paired-end sequencing, this value refers to the sum of read 1 and read 2

\*GC(%): GC content

\*AT(%): AT content

\*Q20(%): Ratio of bases that have a phred quality score of over 20

\*Q30(%): Ratio of bases that have a phred quality score of over 30

Table S2. Preprocessing summary of trimmed and filtered read counts for each step.

<b>Sample Name</b>	<b>Adapter and primer trimming</b>	<b>Quality filter</b>	<b>Denoised for</b>	<b>Denoised rev</b>	<b>Merged pair</b>	<b>Non-chimeric</b>
Norm 1	107,109	91,969	91,570	91,741	90,786	74,562
Norm 2	104,258	87,783	87,717	87,739	87,510	85,796
Norm 3	71,501	60,519	60,461	60,480	60,335	60,208
Sept 1	92,832	75,956	75,867	75,948	75,848	75,680
Sept 2	108,185	91,166	91,146	91,128	91,064	90,406
Sept 3	94,097	80,268	80,181	80,265	80,125	79,870

Tools for analysis of adapter and primer trimming, Cutadapt; quality filtering, denoising, merging, and chimera removal, DADA2.

Table S3. Sample statistics estimated by ASV analysis corresponding to the six analyzed samples.

Sample Name	ASVs	Chao1	Shannon	Gini-Simpson	Good's Coverage
Norm 1	126.0	126.71	2.16	0.64	0.99
Norm 2	129.0	129.0	2.99	0.73	0.99
Norm 3	81.0	81.0	2.75	0.70	1.0
Sept 1	7.0	7.0	0.66	0.26	1.0
Sept 2	8.0	8.0	0.71	0.2	1.0
Sept 3	6.0	6.0	0.72	0.27	1.0

\* ASVs: amplicon sequence variant (ASV) is a term used to refer to single DNA sequences recovered from a high-throughput marker gene analysis.

\* Chao1: returns the Chao1 richness estimate for an ASV definition.

\* [Shannon](#): the Shannon index takes into account the number and evenness of species.

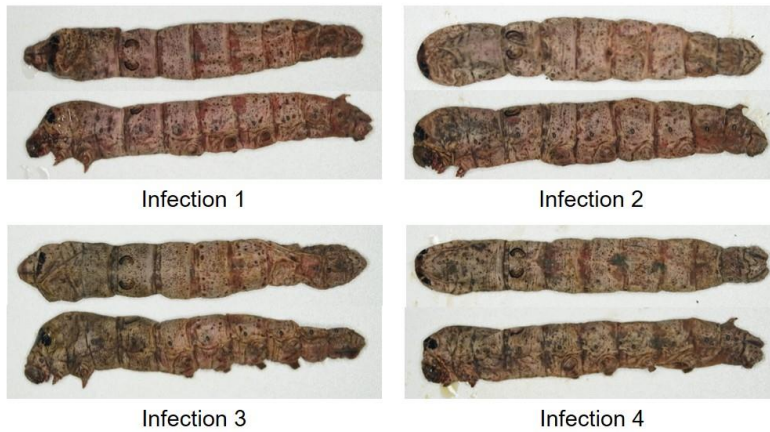
\* [Gini-Simpson](#): the Gini-Simpson index represents the probability that two randomly selected individuals in the habitat will belong to the same species.

\* Good's Coverage: coverage is calculated as  $C=1-(s/n)$ , where  $s$  is the number of unique ASVs and  $n$  is the number of individuals in the sample. This index provides a relative measure of how effectively the sample represents the larger environment.

This_study	GCTGGCGGCGAGGCTTAACACATGCAAGTCGAGCGGTAGCACAGGGGAGCTTGCTCCCCGG	60
LC645982.1	GCTGGCGGCGAGGCTTAACACATGCAAGTCGAGCGGTAGCACAGGGGAGCTTGCTCCCCGG	60
NR114043.1	GCTGGCGGCGAGGCTTAACACATGCAAGTCGAGCGGTAGCACAGGGGAGCTTGCTCCCCGG	60
*****		
This_study	GTGACGACGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCTGATGGAGGGGATAACT	120
LC645982.1	GTGACGACGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCTGATGGAGGGGATAACT	120
NR114043.1	GTGACGACGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCTGATGGAGGGGATAACT	120
*****		
This_study	ACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAGAGGGGGACCTTCGGGC	180
LC645982.1	ACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAGAGGGGGACCTTCGGGC	180
NR114043.1	ACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAGAGGGGGACCTTCGGGC	180
*****		
This_study	CTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGTGGGGTAATGGCTCACCTA	240
LC645982.1	CTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGTGGGGTAATGGCTCACCTA	240
NR114043.1	CTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGTGGGGTAATGGCTCACCTA	240
*****		
This_study	GGGACGATCCCTAGCTGGTCTGAGAGGATGACGAGCCACACTGGAACCTGAGACACGGTC	300
LC645982.1	GGGACGATCCCTAGCTGGTCTGAGAGGATGACGAGCCACACTGGAACCTGAGACACGGTC	300
NR114043.1	GGGACGATCCCTAGCTGGTCTGAGAGGATGACGAGCCACACTGGAACCTGAGACACGGTC	300
*****		
This_study	CAGACTCCTACGGGAGGACAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG	360
LC645982.1	CAGACTCCTACGGGAGGACAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG	360
NR114043.1	CAGACTCCTACGGGAGGACAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG	360
*****		
This_study	CCATGCGCGGTGTGTGAAGAAGGCCCTTCGGGTTGTAAGCACCTTTCAGCGAGGAGGAAG	420
LC645982.1	CCATGCGCGGTGTGTGAAGAAGGCCCTTCGGGTTGTAAGCACCTTTCAGCGAGGAGGAAG	420
NR114043.1	CCATGCGCGGTGTGTGAAGAAGGCCCTTCGGGTTGTAAGCACCTTTCAGCGAGGAGGAAG	420
*****		
This_study	TGGTGAGCTTAATACGCTCATCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCC	480
LC645982.1	TGGTGAGCTTAATACGCTCATCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCC	480
NR114043.1	TGGTGAGCTTAATACGCTCATCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCC	480
*****		
This_study	GTGCCACGACCGCGGTAATACGGAGGGTGCACAGCSTTAATCGGAATTACTGGGCGTAAA	540
LC645982.1	GTGCCACGACCGCGGTAATACGGAGGGTGCACAGCSTTAATCGGAATTACTGGGCGTAAA	540
NR114043.1	GTGCCACGACCGCGGTAATACGGAGGGTGCACAGCSTTAATCGGAATTACTGGGCGTAAA	540
*****		
This_study	GCGCACGACGGCGGTTTGTAAAGTCAGATGTGAAATCCCGGGGCTCAACCTGGGAACCTGC	600
LC645982.1	GCGCACGACGGCGGTTTGTAAAGTCAGATGTGAAATCCCGGGGCTCAACCTGGGAACCTGC	600
NR114043.1	GCGCACGACGGCGGTTTGTAAAGTCAGATGTGAAATCCCGGGGCTCAACCTGGGAACCTGC	600
*****		
This_study	ATTTGAAACTGGCAAGCTAGAGTCTCGTAGAGGGGGGTAGAAATCCAGGTGTAGCGGTGA	660
LC645982.1	ATTTGAAACTGGCAAGCTAGAGTCTCGTAGAGGGGGGTAGAAATCCAGGTGTAGCGGTGA	660
NR114043.1	ATTTGAAACTGGCAAGCTAGAGTCTCGTAGAGGGGGGTAGAAATCCAGGTGTAGCGGTGA	660
*****		
This_study	AATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGCGGCCCCCTGGACGAAGACTGAC	720
LC645982.1	AATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGCGGCCCCCTGGACGAAGACTGAC	720
NR114043.1	AATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGCGGCCCCCTGGACGAAGACTGAC	720
*****		
This_study	GCTCAGGTGCGAAAGCGTGGGGAGCAACAGGATTAGATACCCCTGGTAGTCCACGCTGTA	780
LC645982.1	GCTCAGGTGCGAAAGCGTGGGGAGCAACAGGATTAGATACCCCTGGTAGTCCACGCTGTA	780
NR114043.1	GCTCAGGTGCGAAAGCGTGGGGAGCAACAGGATTAGATACCCCTGGTAGTCCACGCTGTA	780
*****		
This_study	AACGATGTGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCGGGAGCTAACGCGTTAAA	840
LC645982.1	AACGATGTGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCGGGAGCTAACGCGTTAAA	840
NR114043.1	AACGATGTGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCGGGAGCTAACGCGTTAAA	840
*****		
This_study	TCGACCGCTGGGGAGTACGCGCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCGC	900
LC645982.1	TCGACCGCTGGGGAGTACGCGCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCGC	900
NR114043.1	TCGACCGCTGGGGAGTACGCGCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCCGC	900
*****		
This_study	ACAAGCGGTGGAGCATGTGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGA	960
LC645982.1	ACAAGCGGTGGAGCATGTGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGA	960
NR114043.1	ACAAGCGGTGGAGCATGTGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGA	960
*****		
This_study	CATCCAGAGAACTTTCCAGAGATGGATTGTCCTTCGGGAACTCTGAGACAGGTGCTGC	1020
LC645982.1	CATCCAGAGAACTTTCCAGAGATGGATTGTCCTTCGGGAACTCTGAGACAGGTGCTGC	1020
NR114043.1	CATCCAGAGAACTTTCCAGAGATGGATTGTCCTTCGGGAACTCTGAGACAGGTGCTGC	1020
*****		
This_study	ATGGCTGTCGTAGCTCGTGTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC	1080
LC645982.1	ATGGCTGTCGTAGCTCGTGTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC	1080
NR114043.1	ATGGCTGTCGTAGCTCGTGTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC	1080
*****		
This_study	TTATCCTTTGTGCCAGCGGTTTCGGCCGGGAACCTCAAAGGAGACTGCCAGTGATAAATG	1140
LC645982.1	TTATCCTTTGTGCCAGCGGTTTCGGCCGGGAACCTCAAAGGAGACTGCCAGTGATAAATG	1140
NR114043.1	TTATCCTTTGTGCCAGCGGTTTCGGCCGGGAACCTCAAAGGAGACTGCCAGTGATAAATG	1140
*****		
This_study	GAGGAAGG 1148	
LC645982.1	GAGGAAGG 1148	
NR114043.1	GAGGAAGG 1148	
*****		

Figure S1. Sequence alignment analysis. Isolated bacteria (this study), *Serratia nematodiphila* (LC645982.1), *Serratia marcescens* (NR\_114043.1) were compared using Clustal Omega for the 16s rRNA region.

A



B

<i>Serratia</i> sp.	-----CCAGCAGCCGCGGTA	15
Infection 1	CATCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTA	60
Infection 2	CATCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTA	60
Infection 3	CATCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTA	60
Infection 4	CATCAATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTA	60
	*****	
<i>Serratia</i> sp.	ATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTG	75
Infection 1	ATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTG	120
Infection 2	ATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTG	120
Infection 3	ATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTG	120
Infection 4	ATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTG	120
	*****	
<i>Serratia</i> sp.	TTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATTTGAAACTGGCAAGCT	135
Infection 1	TTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATTTGAAACTGGCAAGCT	180
Infection 2	TTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATTTGAAACTGGCAAGCT	180
Infection 3	TTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATTTGAAACTGGCAAGCT	180
Infection 4	TTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAAGTGCATTTGAAACTGGCAAGCT	180
	*****	
<i>Serratia</i> sp.	AGAGTCTCGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGA	195
Infection 1	AGAGTCTCGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGA	240
Infection 2	AGAGTCTCGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGA	240
Infection 3	AGAGTCTCGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGA	240
Infection 4	TGAGTCTCGTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGA	240
	*****	
<i>Serratia</i> sp.	GGAATACCGGTGGCGAAGGCGGCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGT	255
Infection 1	GGAATACCGGTGGCGAAGGCGGCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGT	240
Infection 2	GGAATACCGGTGGCGAAGGCGGCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGT	240
Infection 3	GGAATACCGGTGGCGAAGGCGGCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGT	240
Infection 4	GGAATACCGGTGGCGAAGGCGGCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGT	240
	*****	
<i>Serratia</i> sp.	GGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGTCGATTTGGAG	215
Infection 1	GGGGAGCAAACA-----	312
Infection 2	GGGGAGCAAACA-----	312
Infection 3	GGGGAGCAAACA-----	312
Infection 4	GGGGAGCAAACA-----	312
	*****	

Figure S2. Microbial identification from silkworms re-infected with the isolated bacteria. (A) Among the silkworms re-infected with the isolated bacteria, four silkworms showing signs of disease were randomly selected. (B) Bacteria from the selected silkworms were extracted using PBS and plated on LB medium, and the most abundant morphological colonies were selected and the 16S rRNA gene sequence (the size of about 312 bp) was amplified using primers 518F and 907R and sequenced for comparison with the sequence of the infecting bacteria.