

Differences in Gut Microbiota between Silkworms (*Bombyx mori*) Reared on Fresh Mulberry (*Morus alba* var. *multicaulis*) Leaves or an Artificial Diet

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Supplementary Information

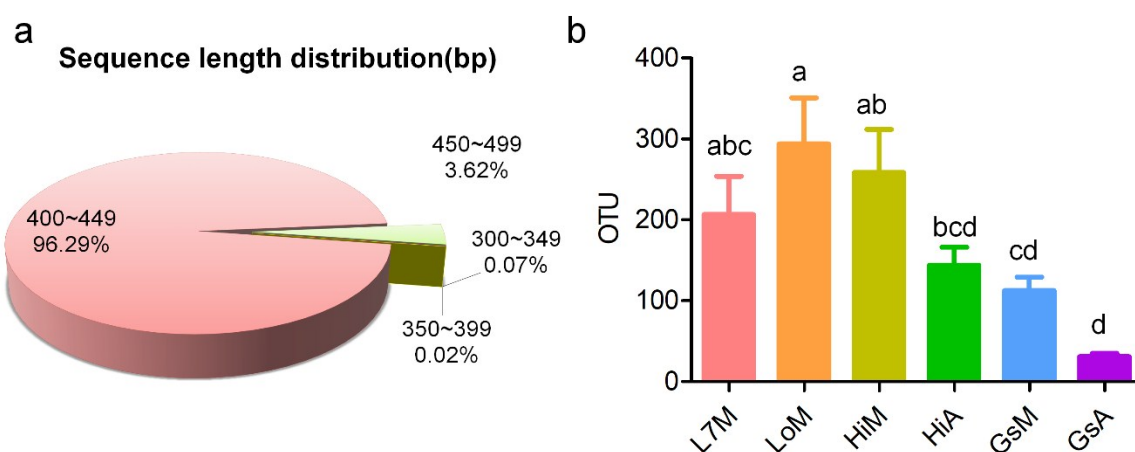


Figure S1 Statistics of the 16SrRNA gene obtained from metagenomic sequencing. (a) Sequence length distribution. (b) Reads were clustered into the operational taxonomic units (OTU) at 97% identity. Several different metrics were used to calculate OTU richness (Ace and Chao) and diversity index (Shannon and Simpson). Sample coverage for 16SrRNA libraries of the different samples was estimated. Four silkworm strains were used in this study. They were the Guangshi (Gs) and Lu7 (L7) strains, a Jingsong strain with high ingestive habit (Hi) for artificial diets, and a Jingsong strain with a low ingestive habit (Lo) for artificial diets. In this study, we used four silkworm strains that were reared on fresh mulberry (*Morus alba* var. *multicaulis*) leaves, named HiM, GsM, LoM, and L7M, and two strains that were reared on an artificial diet, HiA and GsA. Samples marked with the same letter were not significant difference from each other, $P < 0.05$ ($n = 3$ repeats).



Figure S2 Comparison of the impact of differences between fresh mulberry leaves and the artificial diet on silkworm gut microbiome. Two silkworm strains that were reared on fresh mulberry (*Morus alba* var. *multicaulis*) leaves, named HiM and GsM, and two strains that were reared on an artificial diet, HiA and GsA. They were reared on fresh mulberry (*Morus alba* var. *multicaulis*) leaves, named HiM and GsM, and were reared on an artificial diet, named HiA and GsA. Predicted functional analysis of the silkworm gut microbiomes based on the PICRUSt software tool. The barplot show pathways that were significantly enriched ($P < 0.05$) as determined by one-way ANOVA. Fold change represents value of HiM / HiA or GsM / GsA.

Table S1 The differences in relative abundance of eight major bacterial phyla between HiA and GsA.

Taxon	Average value			Fold change	
	HiA	GsA	P-value	HiA/GsA	GsA/HiA
<i>Cyanobacteria</i>	0.271857	0.003925	0.00906	69.26	0.01
<i>Firmicutes</i>	0.704003	0.308146	0.00588	2.28	0.44
<i>Proteobacteria</i>	0.021370	0.687796	0.00002	0.03	32.18
<i>Bacteroidetes</i>	0.001508	0.000045	0.00229	33.53	0.03
<i>Actinobacteria</i>	0.000544	0.000052	0.01642	10.42	0.10
<i>Acidobacteria</i>	0.000163	0.000005	0.01044	35.79	0.03
<i>Chloroflexi</i>	0.000132	0.000005	0.01008	25.73	0.04
<i>Gemmatimonadetes</i>	0.000050	0	0.02255	∞	0

Note: Average value of 5 repeated measurements. pvalue, t-test significance. Fold change of the main discriminant between HiA and GsA or GsA and HiA.

Table S2 The differences in relative abundance of eight major bacterial phyla between HiA and GsA.

Taxon	Average value			Fold change	
	HiA	GsA	P-value	HiA/GsA	GsA/HiA
<i>Streptophyta(o)</i>	0.27161	0.00393	0.00906	69.20	0.01
<i>Enterococcaceae</i>	0.70112	0.30583	0.00593	2.29	0.44
<i>Pseudomonadaceae</i>	0.00509	0.00024	0.00149	20.94	0.05
<i>Enterobacteriaceae</i>	0.00897	0.68721	0.00001	0.01	76.60
<i>Flavobacteriaceae</i>	0.00055	0.00001	0.00093	50.98	0.02
<i>Caulobacteraceae</i>	0.00051	0.00005	0.01564	9.32	0.11
<i>Xanthomonadaceae</i>	0.00042	0.00001	0.03299	45.66	0.02
<i>Aerococcaceae</i>	0.00016	0.00000	0.02942	∞	0.00
<i>Brucellaceae</i>	0.00013	0.00001	0.03514	20.76	0.05
<i>Corynebacteriaceae</i>	0.00016	0.00001	0.00386	23.79	0.04
<i>Sphingomonadaceae</i>	0.00017	0.00001	0.00096	24.81	0.04
<i>Sphingobacteriaceae</i>	0.00021	0.00001	0.00043	16.17	0.06
<i>Aurantimonadaceae</i>	0.00007	0.00000	0.03415	∞	0.00
<i>Streptococcaceae</i>	0.00011	0.00001	0.01508	16.01	0.06
<i>Bacteroidaceae</i>	0.00005	0.00000	0.02060	∞	0.00
<i>Hyphomicrobiaceae</i>	0.00003	0.00000	0.02735	∞	0.00
<i>Propionibacteriaceae</i>	0.00002	0.00000	0.04661	∞	0.00
<i>Sinobacteraceae</i>	0.00003	0.00000	0.01247	∞	0.00

Note: Average value of 5 repeated measurements. pvalue, t-test significance. Fold change of the main discriminant between HiA and GsA or GsA and HiA.