Supplementary Information for

Microbial Ecology

Nitrogen fixation and diazotrophic community in plastic-eating mealworms *Tenebrio molitor* L.

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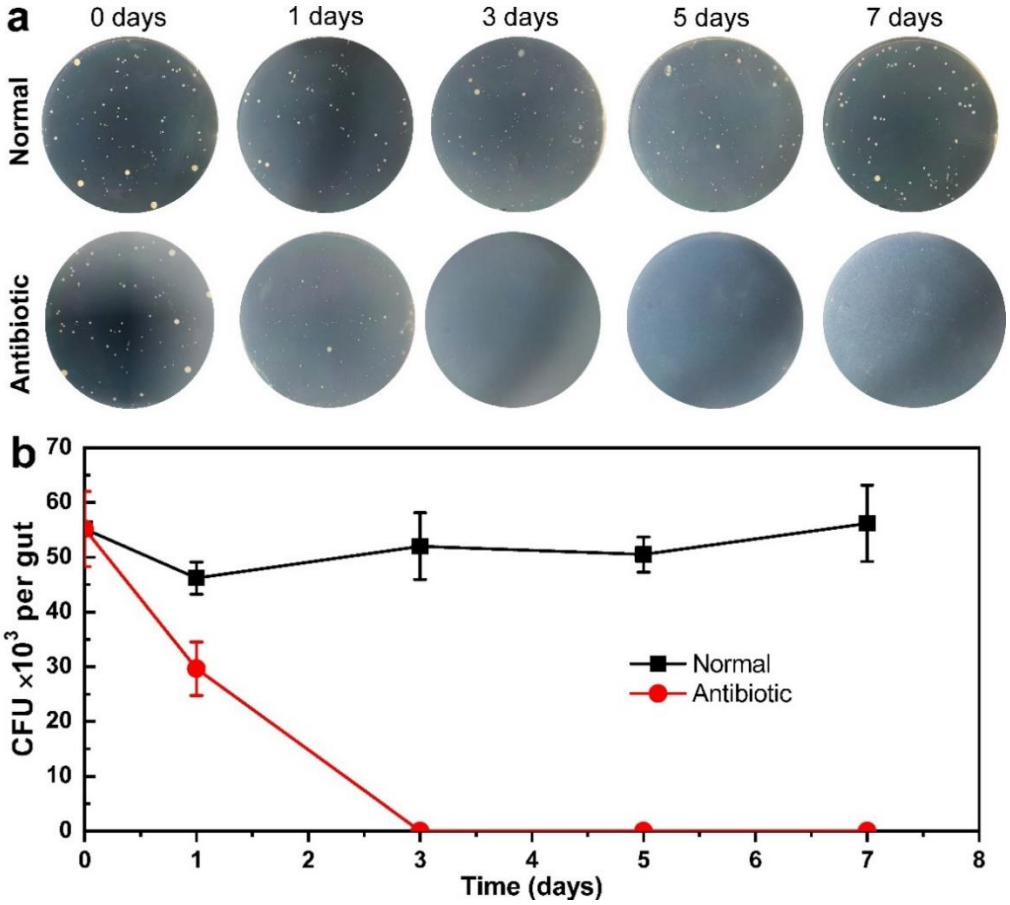

Figure S1. Antibiotic suppression of mealworm' gut microbiota. (a) Photographs of nitrogen-free medium plates inoculated the gut extracts of antibiotic-treated mealworms. (b) Number of total visible bacteria extracted from mealworms fed with gentamicin decreased over a 7 days' incubation period. Gentamicin at 60 mg was mixed with 1 g of bran as antibiotic food, whereas antibiotic-free bran served as the control.

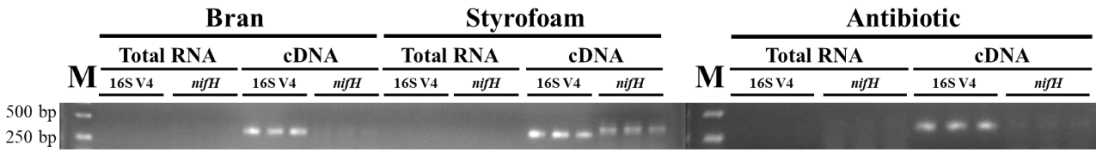

Figure S2. PCR detections of reverse-transcripted *nifH* mRNA in the RNA extracts from the gut microbiota of bran-feeding mealworms, Styrofoam-eating mealworms and antibiotic-treated mealworms (All mealworms sampled from Beijing). The nifH-specific primers PolF-PolR and 16S rRNA v4-spesific primers was used in the PCR reaction. RNA preparations treated with DNase in the absence of RT did not show the presence of PCR products, indicating there no DNA contaminant in the RNA extracts.

Table S1. The average fresh weight, size, acetylene reduction rate (AR) and δ ^{15}N of bran-feeding mealworms, Styrofoam-eating mealworms and antibiotic-treated mealworms from six different sources.

Source	Fresh weight, g	Size, cm	Bran-feeding		Styrofoam-eating		Antibiotic	
			AR activity, nmol·h^{-1}·g^{-1}	δ ^{15}N ‰	AR activity, nmol·h^{-1}·g^{-1}	δ ^{15}N ‰	AR activity, nmol·h^{-1}·g^{-1}	δ ^{15}N ‰
Beijing	0.073 ± 0.016	2.1 ± 0.2	8.7 ± 0.1	1.93 ± 0.02	32.9 ± 9.3	1.74 ± 0.02	12.2 ± 1.3	2.37 ± 0.05
Shanghai	0.086 ± 0.038	2.1 ± 0.3	8.1 ± 2.1	1.97 ± 0.04	17.4 ± 4.3	1.92 ± 0.01	10.1 ± 0.8	2.30 ± 0.10
Chengdu	0.094 ± 0.037	2.2 ± 0.3	10.2 ± 0.4	1.96 ± 0.01	12.3 ± 0.7	1.93 ± 0.02	9.5 ± 0.6	2.35 ± 0.03
Luoyang	0.074 ± 0.027	2.1 ± 0.3	12.2 ± 0.3	2.01 ± 0.02	22.5 ± 5.8	1.82 ± 0.01	11.8 ± 0.9	2.39 ± 0.03
Ningbo	0.100 ± 0.034	2.3 ± 0.3	9.3 ± 5.2	2.05 ± 0.06	17.0 ± 1.0	1.91 ± 0.05	8.7 ± 0.5	2.38 ± 0.12
Guangzhou	0.047 ± 0.013	1.8 ± 0.2	11.7 ± 2.9	1.95 ± 0.02	29.4 ± 9.8	1.79 ± 0.04	19.0 ± 0.4	2.36 ± 0.12

Table S2 *nifH* primer sets and their sequences used in this study.

No.	Primer pairs	References
1	**Forward：** IGK: 5'-ATAGGATCCAARGGNGGNATHGGNAA-3' KAD: 5'-ATAGGATCCTGYGAYCCNAARGCNGA-3' **Reverse：** GEM: 5'-GACCTGCAGADNGCCATCATYTCNCC-3' YAA: 5'-GACCTGCAGATRTTRTTNGCNGCRTA-3'	Ohkuma et al., 1996
2	**Forward：** nifH1: 5’-TGYGAYCCNAARGCNGA-3’ **Reverse：** nifH2: 5’-ADNGCCATCATYTCNCC-3’	Noda et al., 2002
3	**Forward：** PolF: 5'-TGCGAYCCSAARGCBGACTC-3' **Reverse：** PolR: 5'-ATSGCCATCATYTCRCCGGA-3'	Ceja-Navarro et al., 2014
4	**正向引物：** **nif-Fo:** 5’-AAAGGYGGWATCGGYAARTCCACCAC-3’ **反向引物：** **nif-Re:** 5’-TTGTTSGCSGCRTACATSGCCATCAT-3’	Ayayee et al., 2014

Table S3. Sequence similarity of the *nifH* DNA and mRNA from the gut microbiota of plastic-eating mealworms.

Group	Count			Closest Species (accession)	Identity, %	Accession
	DNA	RNA	Isolates			
Cluster I-a	1	1	0	*Klebsiella pneumonia* (ADQ42410.1)	99.2	MW442362 MW442363
	1			*Klebsiella michiganensis* (PLO68789.1)	99.5%	MW442365
		1				MW442366
			1			MW442367
Cluster I-b	1	0	0	*Pseudomonas stutzeri* Gr57 (CAC03734.1)	96.1	MW442364
Cluster I-c	1	0	0	*Leptolyngbya boryana* (D00666.2:925-1280)	86.6	MW442368
Cluster I-d	2	0	0	*Azospirillum brasilense* AWB5 (GQ161232.1)	94.1	MW442369 MW442370