insects

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Supplementary Material

Diagnosis of secondary symbionts in the pea aphids

We used PCR to detect the presence of eight known symbionts in the aphids after over 30 generations on alfalfa and broad beans, respectively, to see if rearing on different host plants changes the secondary symbiotic bacteria in our pea aphid colony. Five adult pea aphids were used for DNA extraction with a TIANamp Stool DNA Kit (Tiangen Biotech, China). PCR detection of eight secondary symbionts (*Hamiltonella defensa*, *Regiella insecticola*, *Serratia symbiotica*, *PAXS*, *Rickettsia sp.*, *Spiroplasma sp.*, *Rickettsiella sp.*, *and Wolbachia sp.*) was performed using specific primers targeting the symbiotic bacterial genes (Supplemental table 1). The total PCR reaction volume was 50 μl including 0.25 μl TaKaRa Taq (R001A, Takara Bio Inc., China), 5 μl 10X PCR Buffer (with Mg^{2+}), 4 μl dNTP mixture (2.5 mM), 1.75 μl DNA template, 1μl forward and reverse primers each, and 37 μl ddH_2O. The PCR reactions were performed on a MiniAmp™ Plus Thermal Cycler (A37835, Thermal Fisher Scientific) with the following condition: 94℃ for 3 min, 35 cycles consisting of 94℃ for 30 sec, 52℃ for 45 sec and 72℃ for 1 min. Four microlitres of PCR reaction was electrophoresed on 1% agarose gel and visualized under a UV detector. Only *Serratia symbiotica* was found in these two groups fed alfalfa and broad beans (Supplemental figure 1), indicating that the host plants had no effect on the composition of secondary symbionts in the pea aphid colony used in this study.

Table S1. Primer sequences used for detection of secondary symbionts in the pea aphid.

Symbionts	Primer sequences (5′→ 3′)	References
Hamiltonella defensa	Forward: 10F AGTTTGATCATGGCTCAGATTG Reverse: T419R AAATGGTATTCGCATTTATCG	[27]
Regiella insecticola	Forward: 10F Reverse: U443R GGTAACGTCAATCGATAAGCA	[27]
Serratia symbiotica	Forward: 10F Reverse: R443R CTTCTGCGAGTAACGTCAATG	[27]
PAXS	Forward: 10F Reverse: GCAACACTCTTTGCATTGCT	[27]
Rickettsia	Forward: 16SA1 AGAGTTTGATCMTGGCTCAG Reverse: TTTGAAAGCAATTCCGAGGT	[28]
Spiroplasma	Forward: 16SA1 Reverse: ATCATCAACCCTGCCTTTGG	[28]
Rickettsiella	Forward: GGGCCTTGCGCTCTAGGT Reverse: TGGGTACCGTCACAGTAATCG	[29]
Wolbachia	Forward: GGGTCCAATAAGTGATGAAGAAAC Reverse: TTAAAACGCTACTCCAGCTTCTGC	[30]

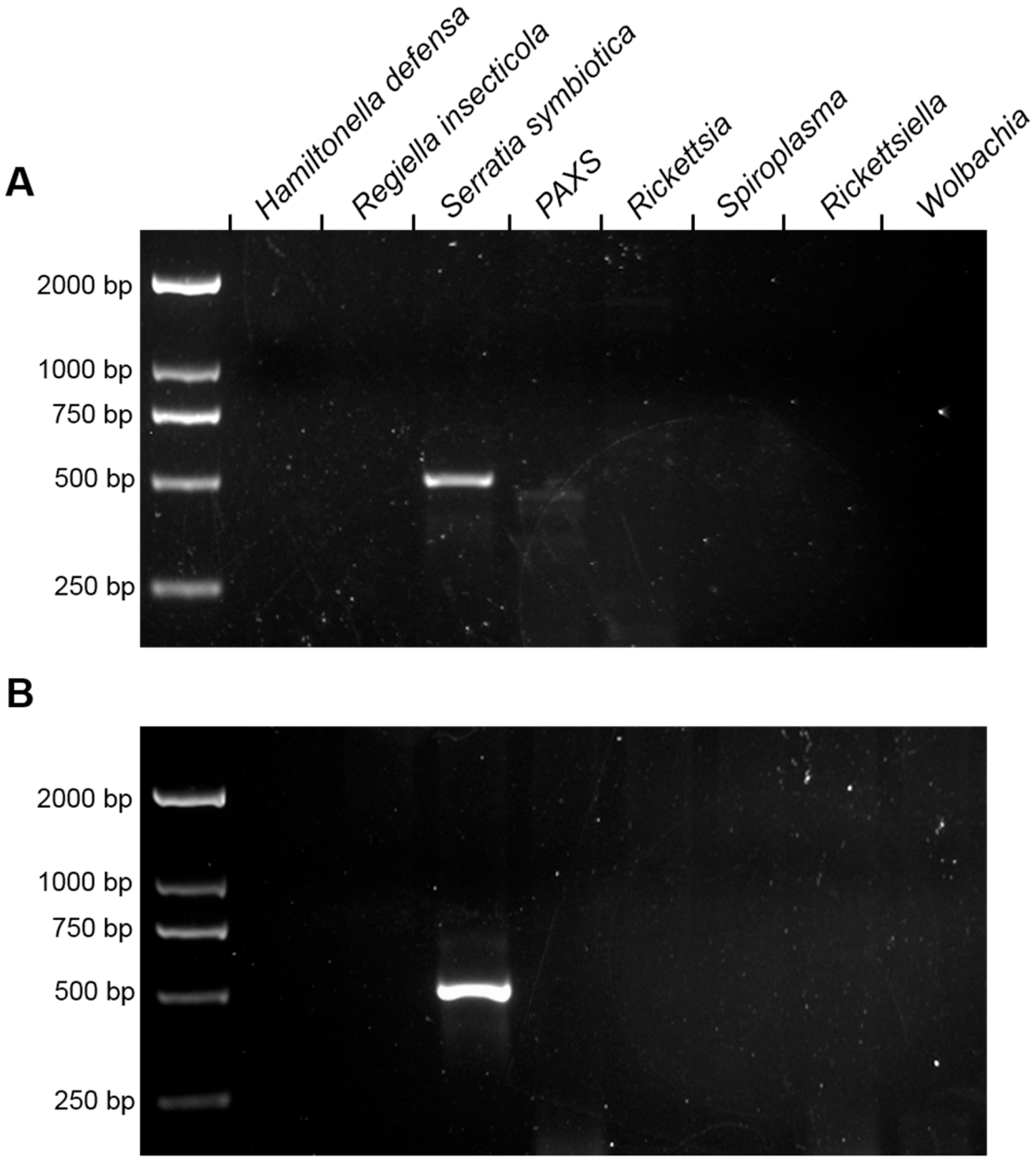

Figure S1. Diagnosis of eight known secondary symbionts in the pea aphids feeding on alfalfa (A) and on broad beans (B). DNA from five aphids on alfalfa or broad beans was prepared and used as the templates for diagnosis PCR. The reactions were separated on 1% agarose gels and photographed under a UVP image system.

References

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