SUPPLEMENTARY INFORMATION

Burkholderia insecticola triggers midgut closure in the bean bug *Riptortus pedestris* to prevent secondary bacterial infections of midgut crypts

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Supplementary Materials and Methods

Microscopic observations of dissected symbiotic organs

The insects administered with the GFP-labeled or RFP labelled strains were dissected in phosphate-buffered saline (PBS: 137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4 [pH7.4]) using fine forceps under a dissection microscope (S8APO, Leica; MZ FZ III, Leica). Pictures of the dissected tissues were taken by a digital camera (EC3, Leica) and the width of the CR and M4B were measured using ImageJ software (Schneider *et al.* 2012). To investigate the infection process, freshly dissected midguts were stained with 4',6-diamidino-2-phenylindole (DAPI) and observed under an epifluorescence microscope (DMI4000B, Leica). For analysis of phalladoidin-stained tissues, the dissected midguts were fixed with 4% paraformaldehyde for 10 min at room temperature, washed twice in PBS, incubated in PBS containing 0.1% Triron X-100 for 5 min, stained with 0.5 μM of SYTOX Green and 5 units/ml of Alexa Fluor555 phalloidin (Thermofisher) in PBS for 20 min, washed twice in PBS, and mounted on silane-coated glass slides and observed with a confocal laser scanning microscope (TCS SP8, Leica).

Oral inoculation of colonization-deficient mutants and non-symbiotic bacteria

Immediately after 1st instar nymphs molted to the 2nd instar, DWA was removed from the rearing containers so that the nymphs were kept without drinking water overnight and

became thirsty. Then DWA containing 10^4 cells/μl of each GFP-labelled strain was supplied to the rearing containers for 24 h. After 24 h, the symbiont-containing DWA was replaced by symbiont-free DWA, and the nymphs were further reared in the absence of bacteria during the instar. After the 2nd instar nymphs molted to 3rd instar, DWA containing 10^4 cells/μl of the RFP-labelled KT39 was supplied to the rearing containers for 24 h. These nymphs were further reared for two days with symbiont-free DWA, and then dissected to check their infection status of the GFP and RFP strains.

Bottleneck estimation

To estimate how many symbiont cells can infect the symbiotic region before the midgut closure, co-inoculation of the GFP-labelled strain RPE225 (Rf^r, Km^r) and the non-labelled wild type strain RPE75 (Rf^r) was performed. The non-labelled symbiont and GFP-labelled symbiont were mixed in different ratios (GFP-labelled symbiont:non-labelled symbiont = 1:10, $1:10^2$, $1:10^3$, $1:1.65x10^3$, $1:5.0x10^3$, $1:10^4$, $1:1.5x10^4$, $1: 4.6x10^4$ or $1:2.2x10^5$). Symbiont cells were diluted in DWA to a cell density of 10^5 CFU/μl, and 2nd instar nymphs were fed with 1 μl of the solution as above. Six to sixteen insects were inoculated per dilution. Three days after inoculation, the symbiotic organ (M4+M4B) was dissected and its content was plated on a YG plate containing kanamycin 30 μg/ml to check whether GFP-labelled symbionts entered the symbiotic organ. Insects were counted as positive for infection with the GFP-labelled symbiont if at least one colony of the GFP-labelled symbiont was detected.

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012; 9: 671-675.