

# In vivo Pyro-SIP assessing active gut microbiota of the cotton leafworm, *Spodoptera littoralis*.

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## Abstract

The gut microbiota is of crucial importance for the host with considerable metabolic activity. Although great efforts have been made toward characterizing microbial diversity, measuring components' metabolic activity surprisingly hasn't kept pace. Here we combined pyrosequencing of amplified 16S rRNA genes with in vivo stable isotope probing (Pyro-SIP) to unmask metabolically active bacteria in the gut of cotton leafworm (*Spodoptera littoralis*), a polyphagous insect herbivore that consumes large amounts of plant material in a short time, liberating abundant glucose in the alimentary canal as a most important carbon and energy source for both host and active gut bacteria. With  $(^{13}\text{C})$  glucose as the trophic link, Pyro-SIP revealed that a relatively simple but distinctive gut microbiota co-developed with the host, both metabolic activity and composition shifting throughout larval stages. *Pantoea*, *Citrobacter* and *Clostridium* were particularly active in early-instar, likely the core functional populations linked to nutritional upgrading. *Enterococcus* was the single predominant genus in the community, and it was essentially stable and metabolically active in the larval lifespan. Based on that *Enterococci* formed biofilm-like layers on the gut epithelium and that the isolated strains showed antimicrobial

properties, *Enterococcus* may be able to establish a colonization resistance effect in the gut against potentially harmful microbes from outside. Not only does this establish the first in-depth inventory of the gut microbiota of a model organism from the mostly phytophagous Lepidoptera, but this pilot study shows that Pyro-SIP can rapidly gain insight into the gut microbiota's metabolic activity with high resolution and high precision.

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