Bacterial Community of the Spined Soldier Bug Gut

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SUMMARY

OBJECTIVE:
Characterize the bacterial community in the gut of the spined soldier bug.

METHODS:
Culture-dependent and culture-independent characterizations were used.

RESULTS:
A total of 3,000 clones were examined for the culture-independent analysis.

CONCLUSIONS:
The bacterial community was found to be limited in terms of species diversity. A high level of turnover between sampling years was found, which is not unusual for predators. Potential symbionts were identified for further investigation.

BACKGROUND

The spined soldier bug (Pentatomidae: *Podisus maculiventris*) has been demonstrated to be a highly effective biocontrol agent. However, its deployment in field cropping systems is limited by the high cost associated with mass rearing and, subsequently, the price of eggs/adults available for purchase by producers. The development of the zoophytogenous diet has helped advance mass rearing of this insect. Thus, is it possible that the inclusion of beneficial probiotic bacteria might further improve the artificial diet for *P. maculiventris*? As a first step to answering this question, we sought to characterize the species that constitute the bacterial community present in the gut of *P. maculiventris.*

Above photos: images of *P. maculiventris* nymphs and adults. Feeding and at rest. The photos were obtained from the University of Georgia *Insect Images* database.

OBJECTIVE

Characterize the bacterial community in the gut of the spined soldier bug and assess the potential for manipulating the community to develop a better medium for mass rearing.

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RESULTS

On the basis of distinct colony morphology, we chose 116 isolates for species identification. In addition, we analyzed over 2,500 16S clones. The results are shown in Figures 1-3. Of particular interest was the observation that the community changed very rapidly between the two years sampled (Fig. 2). This may be due to changes in prey sources between years. We also found that commonly used web-based software for performing species assignment (e.g, RDP and EZ-Taxon) are prone to significant error. In Figure 2, *Pseudomonas ficuserectae* was found to be the most common species detected in the culture-independent survey of 2010. However, a phylogenetic analysis of all known type strains of the genus *pseudomonas*, indicated that all of these clones were actually *P. saponiphila* (Fig. 4). Thus, caution should be applied when making inferences on the basis of results from these programs. Our phylogenetic analyses also revealed a potential clade of secondary symbiont closely related to *Ewingella* and *Rahnella* genera (Fig. 5), although confirmation of symbiont status is needed. Finally, our analyses revealed an interesting vista into feeding behavior. Specifically, we detected several species (e.g., Enterococcus spp., Enterobacter spp., etc.) closely associated with dairy cattle and associated food products (e.g., yogurt, cheese, etc.). In addition, two clones (F106 and L2_155; Fig. 5) were found to be closely related to uncultured bacterial clones recovered from the feces of humans and red pandas, respectively. If *P. maculiventris* probes cattle feces, it would be a reasonable explanation that could account for the presence of these microbes in its GI tract.

METHODS

Field Sampling

Twenty-five adult *P. maculiventris* were collected from a dairy farm near Columbia, Missouri. The complete GI tracts were separately extracted in phosphate buffered saline and stored at 4°C for 24 hours prior to processing. Gut samples were mashed with the end of a pipette tip and pooled into a single sample which was subsequently divided into two larger "master" samples: one for culturing strains and another for conducting a molecular analysis of bacterial community diversity. An additional 25 individuals were analyzed from a laboratory population reared at the USDA-ARS facility at Columbia, Missouri.

Strain Isolation and Culturing Analysis

Serial dilutions of guts and fluid were streaked onto six different media and allowed to grow until colonies appeared (24-48 h). A variety of conditions were used in both aerobic and anaerobic atmospheres under temperatures ranging from 15° C to 37° C. Strains were selected for species identification on the basis of their unique morphologies. The 16S ribosomal RNA (rRNA) gene sequence of all known species in the genus to which the strain was assigned were downloaded from GenBank and aligned using CLUSTAL-X to the sequences for the isolates in question. A phylogenetic analysis was subsequently performed with the computed software MEGA4 to determine the phylogenetic placement of each colony.

Culture-Independent Analysis

Gut samples were initially processed by "bead-beating" for 10 minutes, followed by processing with a gram positive DNA purification kit to obtain both gram positive and gram negative bacterial DNA. Following DNA extraction, polymerase chain reaction amplification of the 16S gene was performed for 35 cycles, each consisting of a 30 sec. denaturation step at 94°C, a 30 sec. an annealing step at 50°C, and a 1 min. extension step at 72°C. The amplification product was purified and cloned into a plasmid vector. Clones were screened using standard restriction digest assays with three enzymes *(RSA*1, *Hinf*1, and *Sma*1) in order to identify those with distinct banding patterns. Two representatives of each banding type were chosen for sequencing. The resulting sequences were edited visually using Sequencher version 4.1.2. Initial species assignments were conducted using the RDP and EZ-Taxon webserver software. The former was also used to conduct rarefaction analyses. Species assignment was verified through independent phylogenetic analyses that included the type strains of all known species for the genus assigned to each strain.

RESULTS

Figure 1. Results of the rarefaction analysis of the cloned sequence data. The flattening lines for sequences that show divergences less than 15%, 10%, and 7% suggest that the total diversity at these levels has been captured. The line for divergences less than 3% is approaching flatness, suggesting that a few hundred more clones might be sequenced to capture the total diversity at this level

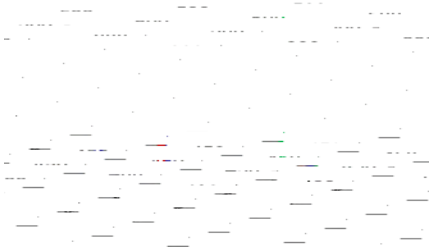

Figure 2. Frequency of bacterial genera present in the field and lab samples. Note that the population detected in field samples collected in 2010 differs greatly from the population detected in field samples collected in 2009 as well as from the lab samples.

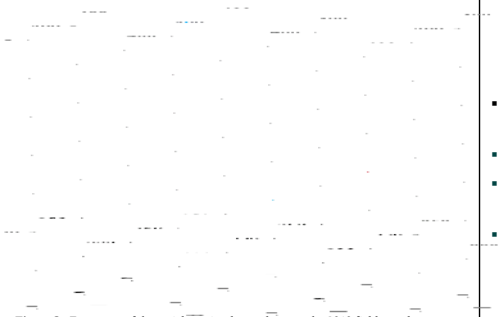

Figure 3. Frequency of bacterial species detected among the 2010 field sample clones compared with the species detected through direct culturing. Note that the populations are dissimilar for the most part, but there is some overlap.

RESULTS

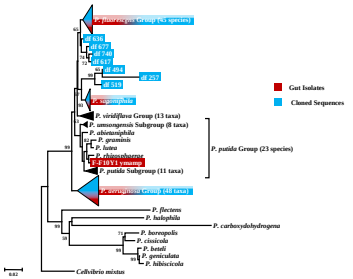

Figure 4. Phylogenetic placement of cultured bacterial isolates and cloned sequences assigned to the genus *Pseudomonas* on the basis of 16S rRNA gene similarity. Species names in black are type strains. Note that the predominant species of Pseudomonas is *P. saponiphila*. The phylogeny was reconstructed from 16S rRNA gene sequences by the using the neighbor-joining method.

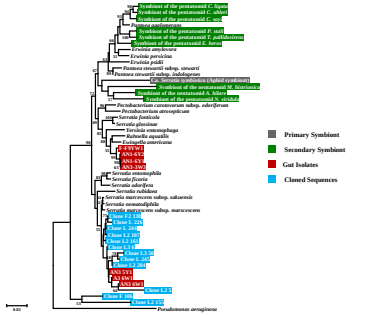

Figure 5 . Phylogenetic placement of cultured bacterial isolates and cloned sequences assigned to initially to the genus *Serratia* on the basis of 16S rRNA gene similarity. Species names in black are type strains. The phylogeny was reconstructed from 16S rRNA gene sequences by the using the neighbor-joining method. A potentially secondary symbiont clade was found to cluster to with *Ewingella* and *Rahnella*.

CONCLUSIONS

- Overall levels of bacterial diversity are low within the GI tract of *P. maculiventris.*
- Low levels of diversity are consistent with a predatory feeding mode.
- The total gut bacterial community shows rapid turnover between years in terms of species composition.
- Several previously unknown species, including a potential symbiont, were detected. Confirmation of symbiont status for the latter is needed.

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