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ABSTRACT BOOK 2019

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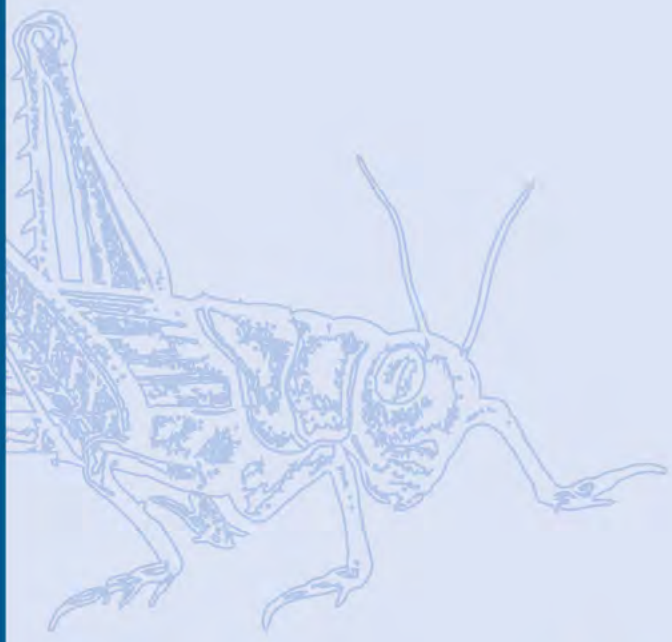

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Systematics & Molecular Biology

METHODOLOGICAL OPTIMIZATION AND STANDARDIZATION OF THE METABARCODING OF INSECTS GUT MICROBIOME

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Metabarcoding analysis of microbiota could help understand how Orthopteran species cope with challenges associated with environmental changes. Since microbial symbionts have a mutually beneficial relationship with its host and play important roles in the immune and physiological systems, they likely impact its ecology and evolution (i.e. plant range, life history, behaviour). In addition, the analysis of the complex pathogenic communities associated with locusts could be useful to discover unexplored pathogens and develop future research on biological control innovation. Yet, current knowledge of Orthopteran-associated microbial communities is limited.

This is partly because recognizing cryptic, diverse, and numerous microorganisms hosted by insects is a difficult task. Despite the design of standard genes for their identification and the latest advances in high throughput sequencing, difficulties persist when we look at the microbiota of insects, including Orthopterans. (1) DNA purification is an essential step in all cultivation-independent approaches to characterize microbial diversity. Indeed, the microbial composition is mainly biased by the efficiency of cell lysis. (2) Another critical step for unbiased representation analysis and high taxonomic resolution is the choice of amplicon and primers. In particular, we showed that Enterobacteriacea, common in insects, were poorly resolved with some of currently used amplicons. (3) Moreover, in the case of phytophagous insects, it is necessary to avoid the amplification of plant remains contained in the digestive tract.

In this study, we use (1) three mock community standards that contained equal and logarithmic numbers of eight species (ZymoBIOMICS), and equal numbers of twenty other species (ATCC), and (2) six samplesrepresenting the six main orders of insects (Orthoptera, Diptera, Hemiptera, Coleoptera, Hymenoptera and Lepidoptera). On these dedicated samples, we first statistically evaluate the most commonly used DNA purification kit (Qiagen DNeasy Blood and Tissue), two microorganisms-specific DNA purification kits (ZymoBIOMICS-96 bashing beads and DNeasy UltraClean 96 Microbial Kit) and two homemade procedures (bashing beads and enzymatic cocktails added to Qiagen DNeasy Blood and Tissue). These methods are compared on the basis of DNA yield, DNA shearing, reproducibility, and most importantly representation of microbial diversityin 16S rRNA gene sequences. . Secondly, we are currently evaluating the taxonomic representativity and resolution of different 16S gene primers to avoid plant chloroplast genes amplifications.

Second, we evaluate, using in silico analyses, (1) the PCR efficiency (representativity), (2) the taxonomic resolution and (3) the risk to amplify plant chloroplasts of already published primers on various variable regions of the 16S gene (V3, V4, V6, V9) and of the rpoB gene. We then test and validate in vitro the best primer candidates on the dedicated samples.

Key Words: Gut microbiota, Next Generation Sequencing, metabarcoding.