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Fungal community composition in the gut of rove beetles (Coleoptera: Staphylinidae) from the Canadian boreal forest reveals possible endosymbiotic interactions for dietary needs

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ABSTRACT

The goal of this study was to investigate the fungal community composition in the gut of Staphylinidae from boreal forest in order to better understand the diversity and the complexity of fungus-insect relationships. DNA gut content analyses of nine abundant rove beetle species (Coleoptera, Staphylinidae) living in the boreal balsam fir forest ecosystem (Montmorency Forest, Quebec, Canada) were performed to identify the fungal taxa present either as endosymbiotic taxa or as a source of nutrition. A total of 42 fungal operational taxonomic units (OTUs) were recorded from the analysis of 441 fungal ITS rDNA sequences recovered from gut extracts. The OTU richness per species ranged between four in *Tachinus quebecensis* and 16 in *Atheta ventricosa*. The fungal mycobiota in posterior gut extracts was dominated by Saccharomycetales (12 OTUs), followed by Sordariomycetes (nine OTUs). No significant difference was observed between the OTU richness recorded within each of the three subfamilies of rove beetles investigated. The core mycobiome of the posterior gut extracts was dominated by three OTUs related to yeasts, with ITS sequences having pairwise similarities equal to or greater than 99% with *Candida mesenterica*, *Debaryomyces* spp. and *Ophiostoma pluriannulatum*. These results provide some evidence of the consumer-resource relationships of these beetles. Predominance of yeast and fungal spores in the posterior gut of rove beetles suggests that they may play an important role in their dietary requirements and as endosymbionts.

1. Introduction

Forest ecosystems consist of diverse groups of species ranging from hyper-diverse micro-organisms and invertebrates to less diverse plants and vertebrates. These different groups of species interact in complex and often poorly understood ways, and represent the structural and functional building blocks of forest ecosystems (Wisz et al., 2013). Insects are the dominant group of forest invertebrates and those inhabiting the forest litter play significant roles in the decomposition processes (Seastedt, 1984). One of the most abundant and speciose litter-inhabiting insect groups is the rove beetles (Coleoptera: Staphylinidae). Rove beetles are useful indicators of forest disturbance and recovery because they are sensitive to environmental perturbations, are diverse in species and trophic roles, and are easily sampled (Boháč, 1990, 1999; Pohl et al., 2007, 2008; Klimaszewski et al., 2013; Work et al., 2013). Rove beetles occupy numerous microhabitats in forest ecosystems where they co-occur with fungi.

Associations with fungi have been important in the evolution of Staphylinidae (Lawrence, 1989). Fungi are key players in ecosystems (Christensen, 1989) and particularly in forest ecosystems where they facilitate the movement of carbohydrates, nutrients, and water between plants and soil (Högberg and Högberg, 2002; Read and Perez-Moreno, 2003; Read et al., 2004; Clemmensen et al., 2013). They represent a significant amount of the microbial biomass in the boreal forest (Högberg and Högberg, 2002) and their interactions with insects come in various forms from parasitism to mutualism. Fungi are an important food source for insects since they accumulate high amounts of nitrogen, phosphorus and organic compounds such as chitin. Although many Aleocharinae

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(e.g., *Aleochara*, some *Atheta*) and Staphylininae (e.g. *Philonthus*, *Staphylinus*, *Ontholestes*) are known to be predators of other small arthropods, mycophagy is also part of the feeding habits of Staphylinidae. Many species of Tachyporinae and some Aleocharinae (e.g., Homalotini) eat the flesh or spores of agaricoid fungi such as *Russula* spp. (Ashe, 1981), or polypores such as *Ganoderma* spp. (Newton, 1984; Thayer, 1987; Klimaszewski et al., 2013), and obligate mycophagy is observed in the subtribe Gyrophaenina (Aleocharinae) and in Oxyporinae (Hanley and Goodrich, 1995). Epps and Arnold (2010) observed that 98% of the adult beetles in the sporocarps of 68 fungal species in mature hardwood forests in Virginia belong to Staphylinidae.

Despite the growing awareness that the gut microbiota play key roles in the metabolism and health of their hosts, relatively little is known about the gut fungal associates of rove beetles in boreal forest. Identification of the fungal taxa that inhabit the gut of rove beetles is very valuable since it provides information on the diet of their host, on their potential as endosymbionts, and on the fungal communities that live in rove beetle microhabitats.

To obtain a better view of the fungal community interacting with rove beetles in boreal forest, we analyzed the gut 'ecosystem' of nine species belonging to three subfamilies of Staphylinidae using both microscopic observations and cloning and sequencing of the fungal internal transcribed spacers (ITS) of the nuclear ribosomal DNA (rDNA) present in the gut extracts.

2. Materials and methods

2.1. Ethics statement

No specific permits were required for the described field studies. This work did not involve endangered or protected species.

2.2. Sampling sites and rove beetle species

Rove beetles were collected as part of a larger field experiment examining the impact of biomass harvesting on forest ecosystem functioning (Work et al., 2013) within the Montmorency Forest (ranges of latitude and longitude: 47°13′–47°22′ N, and 71°05′–71°11′ W) approximately 70 km north of Quebec City, Quebec, Canada. This site is part of a 70-year-old boreal balsam fir-white birch dominated forest in the Laurentian Mountains. The site and experimental layout were described in detail by Work et al. (2013). All beetles were collected using pitfall traps deployed between June and August 2013 (eleven sampling weeks). Beetles were collected in pitfall traps from both harvested and unharvested stands and preserved in 75% ethanol with some vinegar, and later cleaned with 75% ethanol and dissected. Nine rove beetle species representing three subfamilies were targeted to investigate the fungal diversity present in gut extracts: Aleocharinae (*Atheta strigosula*, *Atheta ventricosa*, *Liogluta aloconotoides* (synonym of *Liogluta terminalis*), *Lypoglossa franclemonti*), Staphylininae (*Atrecus macrocephalus*, *Gabrius brevipennis*, *Quedius labradorensis labradorensis*), and Tachyporinae (*Ischnosoma fimbriatum*, and *Tachinus quebecensis*).

2.3. Gut extraction for microscopic analysis

Six dried and mounted specimens of each species were selected from samples collected in 2013. Individual specimens were softened in distilled water and ammonia solution for about 15 min and their guts were dissected in distilled water under a stereoscopic microscope. The colon and rectum of the hindgut were transferred directly to absolute alcohol, then placed on a glass slide with Canada balsam, and pressed by dissecting needles to liberate gut contents and then covered with a cover slip. Slides were studied under a compound microscope (Reichert, Vienna, Austria) and photographs were taken using an Olympus DP73 digital camera. The work by Hanlin (1990, 1998) was consulted for fungal spore illustrations.

2.4. DNA extraction, PCR amplification, cloning and sequencing

The rove beetle specimens were originally trapped using pitfalls with 75% ethanol, and subsequently specimens were card mounted and dried, and later used for DNA extractions. Total genomic DNA (gDNA) was isolated using the QIAamp DNA Micro Kit from Qiagen (Qiagen, Toronto, ON) according to the manufacturer's specifications. A total of 33 specimens per species (maximum of three specimens per one trapping week), representing the catch of an 11-week trapping season (June–August) was used for molecular analyses of the gut extracts, gDNA was eluted from the columns in 100 μl of PCR grade nuclease-free water. Negative controls were included among the extracted samples. gDNA concentration was determined spectrophotometrically by reading absorbance at 260 and 280 nm with the Synergy Mx Microplate Reader (BioTek Instruments, Winooski, VT).

For each sample, 30 ng of gDNA was amplified using 2X HotStarTaq Plus Master Mix (Qiagen, Toronto, ON), which contains one unit of HotStarTaq Plus DNA Polymerase, PCR Buffer with 1.5 mM $MgCl_2$, 200 μM of each dNTP and 0.3 μM of each primer in a 30 μl final reaction. The fungal internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA (rDNA) were amplified using the primer sets ITS5 (5′- GGAAGTAAAAGTCGTAACAAGG-3′)/ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). ITS5 was replaced with ITS9mun (5′-TGTACACACCGCCCGTCG-3′) (Egger, 1995) to re-amplify samples that yielded a limited number of colonies at the PCR cloning step. Negative controls that contained sterile water instead of gDNA were included among the samples to amplify. PCR amplification was carried out using an initial denaturation step at 95 °C for 15 min, followed by 35 cycles: 15 s at 95 °C, 30 s at 52 °C, 30 s at 72 °C, and a final extension for 10 min at 72 °C. Cycling was performed on a PTC-200 Peltier Thermal Cycler (MJ Research, Watertown, MA). Amplified fragments were inserted directly in the Zero Blunt® TOPO® PCR Cloning Kit (Invitrogen, Carlsbad, CA) and transformed into *E. coli* strain DH10B. Plasmids were isolated using the Qiacube with the Qiagen miniprep columns and sequenced with an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA).

2.5. Bioinformatics and diversity analyses

Sequences were edited, trimmed, cleaned and assembled in Vector NTI Advance 11 (Invitrogen). The similarity threshold for ITS sequences belonging to the same operational taxonomic unit (OTU) was set to 97% (uncorrected pairwise distance) to serve as a proxy for 'species'. OTU clustering and chimera filtering were performed using USEARCH v8.0.1623_86linux64 (Edgar, 2013). Taxa assignment was done in QIIME v1.9.0–20140227 (Caporaso et al., 2010) with the RDP classifier using a minimum confidence of 0.8. Hierarchical classification of the fungal OTUs was performed against the UNITE (version 7) reference data set. Scripts from the Brazilian Microbiome Project were used to convert the USEARCH map file into an OTU table (Pylro et al., 2014). QIIME scripts were used for rarefying the data sets and for performing alpha- and beta-diversity analyses. Consensus sequences of each OTU were aligned with MAFFT v7.017 (Katoh et al., 2002) as implemented in Geneious v8.1.6 (Biomatters) and the phylogenetic tree was calculated using FastTree v2.1.5 (Price et al., 2010) using the GTR model. Each OTU was also compared with reference sequences contained in the