Uncovering Active Bacterial Symbionts in Pollen-Feeding Beetles

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Abstract

Microbial symbionts enable many phytophagous insects to specialize on plant-based diets through a range of metabolic services. Pollen comprises one plant tissue consumed by such herbivores. While rich in lipids and protein, its nutrient content is often imbalanced and difficult-to-access due to a digestibly recalcitrant cell wall. Pollen quality can be further degraded by harmful allelochemicals. To identify microbes that may aid in palynivory, we performed cDNA-based 16S rRNA metabarcoding on three related pollen beetles (Nitidulidae: Meligethinae) exhibiting different dietary breadths: Brassicogethes aeneus, B. matronalis, and Meligethes atratus. Nine bacterial symbionts (i.e. 97% OTUs) exhibited high metabolic activity during active feeding. Subsequent PCR surveys revealed varying prevalence of those from three Rickettsiales genera - Lariskella, Rickettsia and Wolbachia - within beetle populations. Our findings lay the groundwork for future studies on the influence of phylogeny and diet on palynivorous insect microbiomes, and roles of symbionts in the use of challenging diets.

Main Text

Many phytophagous insects harbor symbiotic microorganisms that play fundamental roles in their host-plant interactions [1]. Symbiotic bacteria can synthesize essential diet-limited metabolites, break down defensive or recalcitrant plant compounds, or recycle/upgrade metabolites that are metabolically inaccessible to insects [1, 2]. Through such activities, they can have a large impact on the adaptation of insects with varying degrees of dietary specialization.

The beetle subfamily Meligethinae includes ~700 species that develop in budding flowers [3, 4] of Dicots and Monocots (palms), with both larvae and adults consuming pollen as their primary food. Evolution within this subfamily has been characterized by repeated host shifts towards plants of different tribes or genera within the same botanical family, and more rarely towards members of unrelated families [5, 6, 7, 8]. Overall, Meligethinae are remarkably variable in their degrees of host-plant specialization within several genera [3]. These factors position this subfamily as a suitable group for studies on how phylogeny and diet shape symbiont communities [9, 10]. Given the precedent for microbial digestion of pollen [11], this group holds further promise for exploring symbionts’ impacts on diet use and diversification.

To initiate such investigation, we studied microbiomes of three Meligethinae species varying in host-plant specialization. Brassicogethes aeneus is a renowned pest of oilseed rape crops [12] and feed on various Brassicaceae (e.g. Brassica, Sinapis, Cakile, and Biscutella). Its congener, B. matronalis, is monophagous, feeding exclusively on Hesperis matronalis (Brassicaceae). Meligethes atratus is a more distantly related oligophagous beetle, associated with genera from the Rosaceae (i.e. Rosa, Crataegus and Prunus) [5, 7]. Highlighting active bacterial communities in these beetles, we performed 16S rRNA metabarcoding on cDNA libraries from a total of 66 dissected individuals collected while feeding on their respective host plants (Supplementary Methods). cDNA was synthesized from two pooled RNA extractions per species - one from dissected head tissue and one from abdomens - aiding in preliminary
ascertainment of whether bacteria are confined to the midgut and hindgut, or more broadly distributed throughout tissues beyond. After quality control and filtering of our six Illumina V4 sequencing libraries, we obtained 69,382 reads (B. aeneus = 21,337; B. matronalis = 19,549; M. atratus = 28,496; **Supplementary data**). Sequences were clustered into Operational Taxonomic Units (OTUs) at 97% sequence similarity, enabling us to compute the relative ‘activity’ [13] of distinct symbionts.

Among the 47 detected 97% OTUs, nine (i.e. 5, 6, 17, 18, 34, 40, 42, 46, 99) had a relative abundance higher than 3% in at least one of the six tissue pools gathered from the three species: eight belonged to Proteobacteria (92.6% of all reads) and one to Tenericutes (7.2% of reads), the latter represented by *Spiroplasma* (Mollicutes: Entomoplasmatales) in *B. aeneus* (**Supplementary data**). Among these OTUs, eight corresponded to distinct bacterial genera, whereas *Pseudomonas* was represented by OTU40 and by the collectively rare (i.e. <0.01%) OTU67 and OTU136 (**Supplementary data**). The eight bacterial genera appeared differently distributed across the three Meligethinae and localized beyond midgut and hindgut tissues (**Fig. 1**).

Among the Proteobacteria, three abundant/active OTUs (i.e. 5, 18 and 42; **Supplementary data**), comprising 54.2% of all reads, were classified to *Wolbachia*, *Rickettsia*, and *Lariskella* within the Rickettsiales (Alphaproteobacteria). *Wolbachia* is a common symbiont of arthropods [14], residing in somatic and germline tissues. Whilst traditionally viewed as intracellular, some evidence exists for an extracellular, gut-associated lifestyle [15, 16]. *Rickettsia* exhibits a diversity of host-dependent lifestyles [17, 18], and like *Wolbachia*, some are vertically transmitted symbionts of invertebrates [19]. Despite such transmission, both *Wolbachia* and *Rickettsia* can move horizontally between arthropod species [20, 21 22]. Several strains of these symbionts can also manipulate arthropod reproduction [17, 23]. Prior findings suggest *Wolbachia* could aid the use of plant-based diets by providing B-vitamins [24, 25]. Nutritional, digestive, and detoxification capacities of *Rickettsia* are largely unknown, as are those of *Lariskella* – another vertically transferred facultative symbiont of arthropods, including stinkbugs and weevils [26, 27].

Among these Rickettsiales we detected differences in abundance/activity across the three pollen-beetle species: *Wolbachia*, *Rickettsia* and *Lariskella*, dominated in *B. aeneus*, *M. atratus* and *B. matronalis*, respectively, and were rare or inactive in the other species (**Fig. 1**). Since Rickettsiales are common insect symbionts and since some may assist phytophagy [28], we further explored their presence in DNA from 71 separately collected Meligethinae specimens through diagnostic PCR (**Supplementary Methods**). The generated data largely validated initial findings (**Table 1**), supporting the confinement of *Lariskella* to *B. matronalis*, and the occurrence of *Rickettsia* in the two generalist species, most notably *M. atratus*. *Wolbachia* was more widespread than expected from our initial study, suggesting fluctuating prevalence or environmental modulation of *Wolbachia* activity.

**Table 1** Diagnostic PCR-assessed prevalence of three abundant/active Rickettsiales in 2018-2019 field-collected Meligethinae
Beyond the Rickettsiales, we identified five other abundant/active 97% OTUs (i.e. 6, 17, 34, 40 and 99) in our 16S rRNA metabarcoding dataset, hailing from the Gammaproteobacteria and comprising 36.4% of our reads (Fig. 1; Supplementary data) - four were Enterobacteriales (32.32%), while the fifth belonged to the Pseudomonadales (4.08%). Like the Rickettsiales, Enterobacteriales exhibited differential activity patterns across the three Meligethinae. For instance, *Sodalis*, a common facultative symbiont [29], was only active in the two generalist species. In other hosts, *Sodalis* provision nutrients such as tyrosine, lysine, biotin and riboflavin [30], suggesting the potential for beneficial impacts. Another bacterium, *Morganella* - found in other insects and beetles [31, 32] - was also active in the two generalist species (Fig. 1). While several functions, and even commensalism, are possible, *Sodalis* and *Morganella*, like other Enterobacteriales, could aid the breakdown of plant cell wall compounds, including pectin [33].

Based on common plant-association for their closest relatives, we speculate that two other Enterobacteriales OTUs (i.e. 6 and 46), *Pantoea/Erwinia* and *Pseudomonas*, represent transiently acquired bacteria, noting that they accordingly showed higher activity/abundance in mid- and hind-gut containing tissues of *B. matronalis* (Fig. 1). Related bacteria are, however, known symbionts of insects. Based on prior findings, these microbes could shape pollen cell wall breakdown [34, 35], nutrient provisioning or recycling [36, 37], or diet detoxification [38].

In conclusion, three pollen-feeding beetles from the Meligethinae harbor variable active microbiomes. Close relatives of abundantly active bacteria encode functions of possible use in a plant-based diet, with potential to influence utilization of a sometimes nitrogen-rich [39], yet often nutrient-imbalanced [40], well-defended tissue [41]. Yet belonging to two different genera, the two generalist species shared more symbionts (Fig. 1; Table 1). Future studies on other pollen-beetles are needed to weigh the combined influence of host-plant use and phylogeny in structuring symbiotic communities.

### Declarations

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Conflicts of interest

The author declares no bias nor conflicts of interest in this research.

Availability of data and material

The short-read DNA sequences have been deposited in the Sequence Read Archive (SRA) database of NCBI (http://www.ncbi.nlm.nih.gov/sra) under bioproject number (requested, not yet available).

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Figures
Relative abundance (="activity") of bacterial genera across the three Meligethinae species (polyphagous or oligophagous = B. aeneus and M. atratus; monophagous = B. matronalis) on V4 amplicon sequencing of 16S rRNA from 2015-collected specimens. Bar graphs for each library (one column = pooled tissues from all field-caught beetles; AB = abdomen, HE = head) show the percentage of denoised and quality-controlled Illumina sequence reads classified to genera. Rare bacteria (never exceeding a relative
abundance of 3% in any of our n=6 sequence libraries) were pooled into the single category ‘Others’. Bacteria from different genera are represented by distinct colors.

**Supplementary Files**

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