

Symbiont Genomic Features and Localization in the Bean Beetle *Callosobruchus Maculatus*

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Abstract

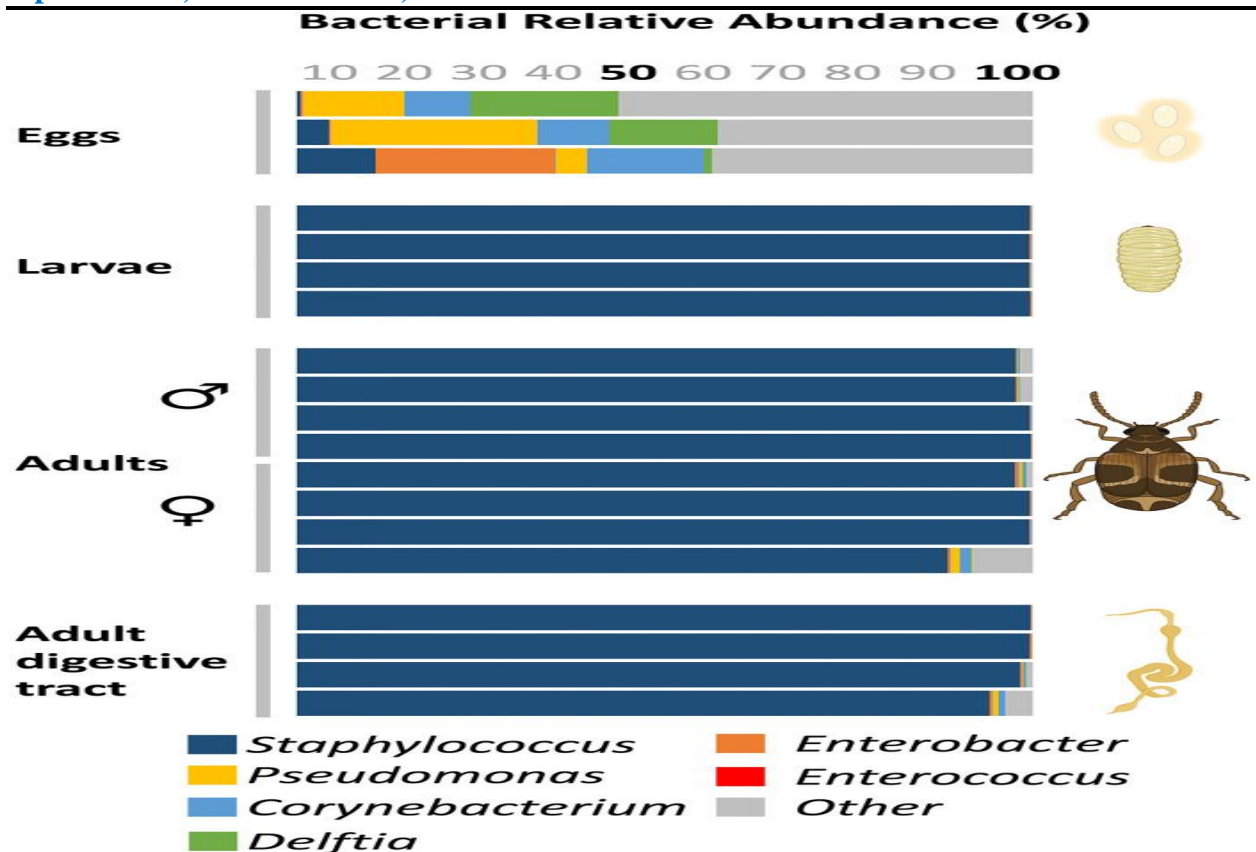
A pervasive pest of stored leguminous products, the bean beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) associates with a simple bacterial community during adulthood. Despite its economic importance, little is known about the compositional stability, heritability, localization, and metabolic potential of the bacterial symbionts of *C. maculatus*. In this study, we applied community profiling using 16S rRNA gene sequencing to reveal a highly conserved bacterial assembly shared between larvae and adults. Dominated by Firmicutes and Proteobacteria, this community is localized extracellularly along the epithelial lining of the bean beetle's digestive tract. Our analysis revealed that only one species, *Staphylococcus gallinarum* (phylum Firmicutes), is shared across all developmental stages. Isolation and whole-genome sequencing of *S. gallinarum* from the beetle gut yielded a circular chromosome (2.8 Mb) and one plasmid (45 kb). The strain encodes complete biosynthetic pathways for the production of B vitamins and amino acids, including tyrosine, which is increasingly recognized as an important symbiont-supplemented precursor for cuticle biosynthesis in beetles. A carbohydrate-active enzyme search revealed that the genome codes for a number of digestive enzymes, reflecting the nutritional ecology of *C. maculatus*. The ontogenic conservation of the gut microbiota in the bean beetle, featuring a "core" community composed of *S. gallinarum*, may be indicative of an adaptive role for the host. In clarifying symbiont localization and metabolic potential, we further our understanding and study of a costly pest of stored products.

Keywords: Host-microbe, symbiosis, bean beetle, insect, herbivory.

Introduction

The bean beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchidae) is a prolific pest of stored leguminous grains. Originating in the Old World, members of the *Callosobruchus* genus presently inhabit every continent owing to a global dispersal network that is largely facilitated by agricultural commerce. Exploiting leguminous seeds throughout

cultivation, storage, and transport, *C. maculatus* drastically limits grain yields in both industrial and backyard cropping systems. Numerous grain varieties belonging to the Fabaceae are susceptible to infestations by *C. maculatus*, including mung (*Vigna radiata*), adzuki (*Vigna angularis*), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), and hyacinth beans (*Lablab purpureus*). Similar to other bruchid species, *C. maculatus* females oviposit on grains by exuding a protective secretion to fixate and attach the egg onto the seed coat. Upon hatching, emerging larvae burrow into the seed and complete their development entirely within the grain, consuming ~11% of its overall mass. With a life cycle spanning 4 weeks, breakouts of *C. maculatus* in granaries can halve the overall weight of stored products within 3 to 5 months. Beyond compromising the commercial value of pulses, the preference of *Callosobruchus* larvae for seeds' cotyledons drastically arrests germination, contributing to thousands of tons of spoiled produce annually. Reflecting the pervasiveness of *C. maculatus* and the experimental tractability afforded from short generation times under laboratory settings, numerous aspects of the beetle's reproductive cycle, developmental biology, and behavioral ecology have been elucidated. The resident microbiome of *C. maculatus*, in contrast, remains understudied. *Callosobruchus maculatus* harbors a simple, heritable bacterial community throughout development. The bacterial community of the bean beetle *C. maculatus* was characterized throughout development using Illumina amplicon sequencing of the bacterial 16S rRNA gene. A total of 480,536 high-quality sequences were generated across 20 samples representing eggs, larvae, and adults. These sequences were subsequently binned to 425 amplicon sequence variants (ASVs) (100% sequence similarity). After taxonomic assignment, 42 ASVs were removed because they were classified as chloroplasts or Archaea. The 5 ASVs assigned to Archaea belonged to the YLA114 phylum and were detected in just one egg sample, comprising 5% of all reads within the sample. The rarity of these microbes within bean beetles, in addition to their presence in nonscarabaeoid beetles being unusual, suggests that Archaea may be a contaminant in this sample. The remaining 383 ASVs contained an average of 24,129 reads per sample, representing 95.4% of the original sequences (see Table S1 in the supplemental material). These ASVs were assigned to 91 bacterial species spanning 81 genera (97% and 95% ASV similarities, respectively). Eggs more commonly associate with Proteobacteria, followed by Actinobacteria, Firmicutes, and Bacteroidetes, whereas larvae and adults largely harbor Firmicutes and, to a lesser extent, Proteobacteria (Table S2). The bacterial communities of larvae and adults, compositionally simple, are predominantly represented by *Staphylococcus*, *Enterococcus*, and *Enterobacter* (Fig. 1). The high relative abundance of *Staphylococcus* in larvae and adults is especially notable, ranging between 99.5% and 97.6% of the total sequences per sample, respectively (Fig. 1). This is consistent with culture-dependent approaches pointing to *Staphylococcus* as the most common bacterial genus consistently isolated from *C. maculatus*.



Figur 1.C. maculatus harbors a simple gut microbial assembly that is representative of the beetle’s overall bacterial community and is largely stable throughout development. (A) Genus-level gut bacterial community composition of C. maculatus across different developmental stages, sexes, and body sites. Each bar corresponds to one sample with pooled individuals. “Adult” refers to whole adults, including the digestive tract.

In summary, we show that the simple bacterial community previously described for C. maculatus is stable across development and that the microbial assembly along the insect’s digestive tract is representative of the insect’s microbiome as a whole. In characterizing the metabolic features of its sole core member, S. gallinarum, we highlight several putatively symbiotic functions toward upgrading the physiology and nutritional ecology of its herbivorous host. Granivorous insects rely on a multitude of metabolic functions from their obligate symbionts, ranging from nutritional supplementation to the degradation of recalcitrant or toxic plant compounds. The annotation of a complete biosynthetic pathway for the production of tyrosine by S. gallinarum is notable given recent findings implicating the symbiont-produced amino acid as an important feature defining numerous beetle-bacterial symbioses, including grain-feeding species belonging to the Silvanidae and Curculionidae families. As a precursor for the biosynthesis of both melanin and catecholamines, tyrosine is central to the tanning and hardening of beetle cuticles.

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