



Review

Microbial ecology-based methods to characterize the bacterial communities of non-model insects☆



Erica M. Prosdocimi^a, Francesca Mapelli^a, Elena Gonella^b, Sara Borin^a, Elena Crotti^{a,*}

^a Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Università degli Studi di Milano, Milano, Italy

^b Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di Torino, Grugliasco, Italy

ARTICLE INFO

Article history:

Received 15 July 2015

Received in revised form 7 October 2015

Accepted 9 October 2015

Available online 22 October 2015

Keywords:

Insect microbiome

Bacterial symbiont cultivation

Molecular methods

Non-model insects

Bacterial community

Microbial ecology

ABSTRACT

Among the animals of the Kingdom Animalia, insects are unparalleled for their widespread diffusion, diversity and number of occupied ecological niches. In recent years they have raised researcher interest not only because of their importance as human and agricultural pests, disease vectors and as useful breeding species (e.g. honey-bee and silkworm), but also because of their suitability as animal models. It is now fully recognized that microorganisms form symbiotic relationships with insects, influencing their survival, fitness, development, mating habits and the immune system and other aspects of the biology and ecology of the insect host. Thus, any research aimed at deepening the knowledge of any given insect species (perhaps species of applied interest or species emerging as novel pests or vectors) must consider the characterization of the associated microbiome. The present review critically examines the microbiology and molecular ecology techniques that can be applied to the taxonomical and functional analysis of the microbiome of non-model insects. Our goal is to provide an overview of current approaches and methods addressing the ecology and functions of microorganisms and microbiomes associated with insects. Our focus is on operational details, aiming to provide a concise guide to currently available advanced techniques, in an effort to extend insect microbiome research beyond simple descriptions of microbial communities.

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☆ Sponsor: Daniele Daffonchio, King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia.

* Corresponding author.

E-mail addresses: erica.prosdocimi@unimi.it (E.M. Prosdocimi), francesca.mapelli@unimi.it (F. Mapelli), elena.gonella@unito.it (E. Gonella), sara.borin@unimi.it (S. Borin), elena.crotti@unimi.it (E. Crotti).

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1. Introduction

There is increasing awareness of the importance of microbial symbionts for animal physiology, and the number of studies focusing on non-pathogenic bacteria hosted by a wide range of organisms is also on the increase. However, for decades, research has been focused primarily on pathogenic bacteria, where most of the animal-associated microorganisms are most likely harmless, variable and acquired from the environment (Turnbaugh et al., 2007). In any case, a number of beneficial bacteria have been found, and among these is a group of essential, vertically transmitted endosymbionts that, with their host, form an inseparable holobiont (Moran et al., 2008).

Insects have been investigated by microbiologists not only as models for human-microbiome interactions, but also for their importance as pests and disease vectors. The most studied bacteria-insect associations fall roughly into two groups, i.e. heritable symbionts (including primary and secondary symbionts) (Moran et al., 2008), and gut symbionts. Primary (P) symbionts are bacteria necessary for insect survival and/or reproduction, and they inhabit highly specialized cells – bacteriocytes – that lie dispersed in the gut epithelium or be grouped within specialized organs called bacteriomes. They typically have a reduced genome, and share a long evolutionary history with the host as they propagate only through maternal transmission. This kind of symbiosis is common in insects with poor diets, such as aphids, which feed exclusively on phloem sap and host a gammaproteobacterium of the genus *Buchnera* for the synthesis of the amino acids lacking in their diet (Akman Gündüz and Douglas, 2009). Instead secondary (S) symbionts are not essential for host survival, although they can improve host fitness. They colonize various cells and organs, including hemolymph, and are able to infect new hosts, establishing stable associations with them through maternal transmission (Favia et al., 2007). This category also includes reproductive

manipulators with maternal transmission that spread throughout the population, promoting the reproduction of infected females through daughters. This is accomplished through cytoplasmic incompatibility, parthenogenesis, male feminization and son-killing (Stouthamer et al., 1999).

Gut symbionts have been extensively reviewed by Engel and Moran (2013), and it has been found that most are commensals that reside in the gut, being neither clearly harmful nor beneficial to the host (Dillon and Dillon, 2004). Our use of the term “commensals” refers to a broad range of microorganisms that vary greatly, even among members of the same species. These bacteria are generally acquired from both the environment and the diet, their selection depending on the chemical and physical conditions inside the gut, such as pH, oxygen availability, and retention time of the food bolus. Furthermore, the host immune system plays an active role in bacteria selection as it is elicited by specific bacterial features, e.g. the excretion of uracil (Lee et al., 2013). Despite the extreme variability of this type of microbial consortium, there is increasing evidence that commensals can critically affect the host physiology, acting on the immune system (Lee et al., 2013), on larval development (Shin et al., 2011) and even on the choice of mate (Sharon et al., 2010). However, these effects, though sometimes important, cannot clearly classify a microorganism as “mutualistic” or “pathogenic” (Dillon and Dillon, 2004). For example, in many cases just the presence of commensal microflora can prevent pathogen colonization (Ryu et al., 2008): in fact, any alteration in the bacterial community due to pathogen colonization can lead to “dysbiosis”, which is detrimental for the host (Hamdi et al., 2011). Moreover, there are known cases of specialized gut symbionts where the relationship with the host resembles primary symbiosis (genome shrinkage, strict heritability) (Hosokawa et al., 2006). Indeed, in Hemiptera, the vertical transmission of gut symbionts smeared on the eggs or encased in symbiont capsules, is well studied

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