Microsensors for the characterization of the insect gut environment

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Abstract. Microorganisms establish a wide range of symbiotic associations with their insect host, encompassing mutualistic, parasitic and commensal relationships. Insect gut microbiota is involved in many essential aspects of the host biology, physiology and behaviour. Factors such as pH, oxygen availability, retention time of the food and host innate immune system are important determinants of the gut symbionts' colonization. Deciphering the interactions that take place and shape the symbiosis is an intriguing topic that cannot exclude the characterization of the physicochemical conditions occurring in the insect gut niche. Microsensors and microelectrodes can be usefully employed to characterize the physicochemical conditions occurring in the insect intestine, allowing to gain a better knowledge of the gut microenvironment in which the gut microbiota thrives.

1. Introduction

Microbial symbionts are ubiquitous in animals, including mammals and insects, where they contribute to the host health. Particularly, insects had an extraordinary evolutionary success that, partly due to their microbial symbionts, led to their adaptation to very diverse terrestrial and aquatic habitats, including those niches with nutritionally imbalanced or limited regimes e.g. wood, phloem sap or blood [1].

Insect symbionts are classified in different categories, according to the host dependence and the evolutionary age of the association [2]. Primary or obligate symbionts are essential for the host survival; they are generally involved in the nutritional supplementation of the insect and commonly associated with hosts that rely on nutrient-deficient diets. They are vertically transmitted and housed in specialized cells. They typically own a reduced genome and show a congruent evolution with their hosts [2]. Secondary or facultative symbionts are not essential for the host survival, but contribute to the host fitness, for instance by increasing the host resistance against environmental stressors [2]. Within this category, a particular group is represented by reproductive manipulators, the best-known example of which is the Alpha-Proteobacterium Wolbachia, responsible for several host reproductive alterations [3]. Finally, gut symbionts are inhabitants of the digestive systems, usually acquired with the diet and subjected to the selection of the intestine physicochemical and biological conditions [4, 5]. Evidences are revealing that they can critically affect the host physiology and biology, influencing larval development [6, 7], host weight gain [8], immune system [9] or mate choice [10]. In order to obtain a better knowledge of the microenvironment in which the microbiota thrives and where the host-microbe interactions that shape the symbiosis take place, a detailed characterization of the physicochemical conditions occurring in the peculiar niche represented by the insect gut is required.
2. Factors shaping the microbial colonization of the insect gut

The insect gut is a challenging niche for the microbial inhabitants. Factors such as pH, oxygen availability, retention time of the food and host innate immune system are important determinants of the gut symbionts’ colonization [4]. For instance, the gut pH is finely regulated and can vary from alkaline (e.g. midguts of lepidopteran larvae), to acidic (e.g. crops of mosquitoes of bees) or neutral values according to the considered compartments. Variations of pH can be also found within a single compartmentalized gut, as in the case of some soil-feeding species with gut pH ranging from 5 to >12 [11, 12]. By the release of fermentation products, gut symbionts can also affect the gut pH and, ultimately, the microbial colonization [4]. Often indicated as a factor that can shape the gut microbiota and drive the microbial colonization, oxygen has been experimentally measured in few examples [8, 11, 13-17]. Oxygen is delivered to the insect gut cells (and microbiota) by the respiratory system, which is based on cuticle-lined tracheae organized in a dense branched apparatus [18]. Considering the restrictions imposed by the diffusive transport of oxygen through epithelia, it has been estimated that the insect gut, in spite of the efficient oxygen consumption by the epithelial cells (in a fraction of a millimeter), is oxic or microoxic [19]. Hence, the insect gut anaerobic niches result from the high microbial oxygen-consuming activity or from particular chemical processes, such as the one occurring in the highly alkaline P1 segment of soil-feeding termites [8, 11, 13-17, 19].

Insect gut has been also indicated as an unstable habitat for microorganisms considering the development-related modifications of the digestive system. Before the adult stage, insects molt several times eliminating, with the foregut and hindgut exoskeletal layers, also the attached microbial populations. Furthermore, the midgut secretes the peritrophic membrane (to surround the bolus) and continually sheds it, discarding in this way the associated microorganisms that generally do not cross it. Finally, in holometabolous insects the metamorphosis results in the remodeling of the gut and other organs with consequences for the microbial partners attached to the gut mucosa [4].

3. Microsensors and microelectrodes to measure physicochemical parameters

Microsensors and microelectrodes have been used to characterize the physicochemical conditions, e.g. pH, oxygen and hydrogen concentrations and redox potential, occurring in the different compartments of the termite gut (e.g. Nasutitermes corniger or Thoracotermes macrothorax) [11, 16, 20]. For instance, oxygen has been profiled along the gut, evidencing strong and peculiar variations of the oxygenation conditions and highlighting that only the dilated gut regions show anoxic condition in the center [11, 16, 20]. The high microbial oxygen-reducing activity throughout the gut is responsible for the maintenance of anoxic conditions in the gut center [20].

Recently, hypoxic/anoxic gut compartments have been also found in honeybees [8] and mosquitoes [17] (Figure 1). In both cases the use of germ-free insects allowed the detection of higher amounts of oxygen in the germ-free intestines rather than in the ones dissected from conventional insects [8, 17]. Authors have also demonstrated that the hypoxic conditions created by the gut microbiota are essential for the host development [17]. Besides microsensors and microelectrodes, other solutions could be adopted to characterize the physicochemical parameters occurring in the insect intestine. For instance, proper kits have been used to reveal hypoxic conditions of the mosquito gut sections [17] or specific dyes have been administered to the insects (through diet) to detect the pH values of the different Drosophila gut compartments [21].

Figure 1. Example of a honeybee gut embedded in agarose gel for oxygen measurement. Courtesy of Matteo Callegari, King Abdullah University of Science and Technology, Saudi Arabia.
4. Conclusions
The composition and structure of the insect gut microbiota is influenced by many factors, such as host diet, genetics and developmental stage. In the last years researchers have devoted many efforts to elucidate their impact on the variation of the host gut microbiota [22-23]. Understanding the drivers that shape the microbial diversity in the insect gut is pivotal to comprehend elucidate their impact on the diet, genetics and developmental stage. In the last years researchers have devoted many efforts to have to be paralleled with the gut microbiota investigations to disentangle the host-microbe interactions.

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