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VISIONS AND PERSPECTIVES

Insect-symbiont: the key relationship to get in-depth insight on the host choice of bacteria

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Abstract

Insects are extremely successful animals in view of their great adaptability to a wide range of terrestrial niches. Symbiotic bacteria gave a precious contribution to such a success playing crucial roles in different contexts such as nutrition, development, reproduction, immunity, defense against natural enemies and speciation.

Recently, the study of symbiosis furnished precious data not only on insect evolution, but also on the mechanisms involved in the bacterial host choice giving us new perspectives to study this process that was poorly understood up to date through the study of pathogenic interactions.

Key Words: symbiosis; host choice; symbiont genome degeneration; insect-bacteria interaction

Introduction

Insects are undoubtedly one of the most successful animal group in nature in view of the high number of species and the high number of individuals observed in insect population.

The success of insects is due to their remarkable adaptability to a vast array of terrestrial habitats, including those that are strongly limited or imbalanced in nutrients and to their ability to face pathogens (such as bacteria). Nevertheless, insect success is also due to the collaboration with bacteria in term of symbiosis since bacteria play crucial roles in the biology and life cycle of most insects species, affecting nutrition, development, reproduction, immunity, defense against natural enemies and speciation (Buchner, 1965; Moran and Baumann, 2000; Moran, 2001; Moran, 2006).

Up to date several papers faced the relationship between bacteria and insects in term of insect defense so that most of the attention has been put on insect pathogens and antimicrobial peptides synthesized by insects or onto other strategies that they set up to avoid bacterial infection (Mandrioli *et al*., 2003; Brivio *et al*., 2005; Schmidt *et al*., 2005; Brown and Hancock, 2006; Lemaitre and Hoffmann, 2007; Lazzaro, 2008; Müller *et al*., 2008).

However, in the last years the interaction between insects and bacteria has been studied with particular attention to symbiosis and, interestingly, the study of mutualism and symbiosis is furnishing several intriguing evidences about the host choice giving us new data about this process that has been poorly understood up to date through the study of pathogenic interactions (Mandel *et al*., 2009).

Symbioses are categorized according to the extent of dependence between the host and the symbionts, which generally depends on evolutionary antiquity of the symbiosis (Moran and Baumann, 2000; Moran, 2001). While obligate primary symbionts are essential for the host survival and/or reproduction, secondary are facultative and thought to be of more recent acquisition, even though they can contribute to the fitness of the host, e.g., conferring resistance to parasites (Moran and Baumann, 2000; Moran, 2001). Most primary symbionts are vertically transmitted to the progeny with a process starting at early stages of oogenesis or embryogenesis. Vertical transmission is common also in secondary symbionts, but they can also colonize novel hosts through horizontal transmission among host individuals belonging to the same or different species (Dale and Moran, 2006).

Sequencing of bacterial genomes is facilitating our understanding of the relationships between insects and their symbionts bringing to a better comprehension of the genome interdependence that occurs between host and bacteria (Zientz *et al*.,

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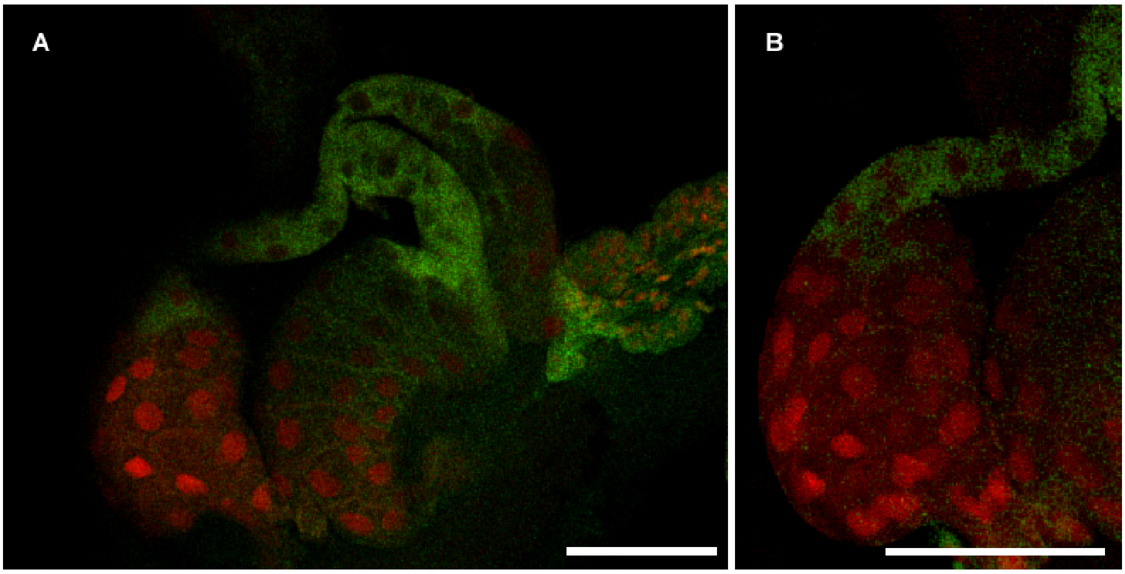

Fig. 1 Whole mount *in situ* hybridization: analysis of the distribution of FITC-labeled (green) *Asaia* bacteria in *Anopheles gambiae* salivary glands stained with propidium iodide (red) observed by confocal microscopy. Bar = 100 μm (**A**). Magnification of a portion of the left salivary gland showing the presence of *Asaia* in the gland duct. Bar = 100 μm (**B**).

2001; Feldhaar and Gross, 2009). In particular, symbiosis results in a genome reduction in endosymbiotic bacterial lineages that loose preferentially genes involved in catabolic pathways, since these functions may be played by the insect metabolism (Zientz *et al*., 2001; Feldhaar and Gross, 2009). Genome reduction may also affect the anabolic pathways if symbionts succeed in recruiting metabolic precursors from the host cell metabolisms bringing to a further rationalization of the symbiont's genome (Andersson and Kurland, 1998; Andresson and Andersson, 1999; Goebel and Gross, 2001; Moran and Mira, 2001).

Interestingly, genome degeneration could be a key aspect not only in the study of symbiont genome evolution, but in the understanding of the host choice, since genome degeneration can not affect genes that are essential for the interaction with the host, neither genes that serve to avoid the exposition of bacteria to the host's immune system. Therefore, the occurrence of smaller genomes can make symbionts perfect experimental models to test the role of different genes in the host-bacteria interaction. Examples include three genomes of *Buchnera aphidicola* strains from different aphid hosts, two of *Candidatus* Blochmannia species from ants, one of *Wigglesworthia glossinidia* from tsetse flies, and one each of *Candidatus* Baumannia cicadellinicola and *Candidatus* Sulcia muelleri from leafhoppers (Dale and Moran, 2006; McCutcheon and Moran, 2007). Their genomes are below one megabase in size and are known to encode as few as 500 genes. An extreme case is that of *Carsonella ruddii*, a primary symbiont of psillids, that has a genome of 160 kb, the smallest bacterial genome described so far (Nakabachi *et al*., 2006). In contrast, *Escherichia coli* and other free-living relatives in this group have genomes of about four to five megabases encoding some 5000 genes.

Despite these advances, however, the mechanisms by which host-symbiont specificity develops in animal-bacterial interactions are not clear. Many animals, including humans, are born devoid of symbionts and must recruit their microbiota from the environment and the process by which hosts and symbionts find each other to initiate a mutualism must be sensitive enough to identify the correct partner even when the symbiont is a minority constituent of the microbial community, and specific enough to exclude interlopers from gaining access to the host (Mandel *et al*., 2009). The species specificity is also poorly understood for pathogenic interactions and at present is very difficult to explain why similar congeneric bacteria have distinct host ranges as reported, for example, in *Salmonella* and *Brucella* species (Edwards *et al*., 2002; Rajashekara *et al*., 2004).

Attempts to understand the molecular basis of host specificity have been unsuccessful in many pathogen-host animal interactions, including humans. *Salmonella enterica* serovar Typhi, for instance, can infect humans only, whereas serovar Typhimurium has a broad range of hosts that includes mice, although the genomes of these two strains are over 97 % identical (Edwards *et al*., 2002). Similarly, different *Brucella* species share over 98 % identity across 90 % of their genes, but exhibit strict host specificity (Rajashekara *et al*., 2004). In contrast, the study of mutualism is providing insights into how specificity develops. For instance, works from many laboratories has established nitrogen-fixing, nodulating rhizobia as the best-understood system for the development and evolution of host specificity in plant-associated bacteria (Long, 2001).

A strong confirmation of the hypothesis that symbionts may favour our understanding of the mechanisms involved in host choice better than pathogens has been recently published by Mandel and colleagues (2009) showing that a single regulatory gene is sufficient to alter host range in an animal-bacterial mutualism, suggesting that the same could be true in the host-pathogen interaction. Despite the relevance of this paper, however, the fundamental biological questions on how animal-bacterial partnerships are established is still difficult to access and it is still impossible to define when bacteria passed the thin line that separates patogenicity and mutualism/symbiosis (Gilmore and Ferretti, 2003).

In insects, some good candidates for taking a glance into the mechanisms involved in host choice are already present and in particular bacteria of the genus *Asaia* could be perfect experimental models since they are cultivable *in vitro* (that is not a common feature for symbionts), can be manipulated at a chromosomal level in order to obtain stable transgenic strains and can be used for study of colonization of the insect body (Favia *et al*., 2007).

Asaia belong to the group of the acetic acid bacteria that can be identified in virtue of their ability to oxidize ethanol into acetic acid even if *Asaia* differentiates because it does not (or weakly) oxidize ethanol to acetic acid. Besides tropical plants, where it was originally isolated (Malimas *et al*., 2008), *Asaia* has thus far been found associated to the insects *Scaphoideus titanus*, the leafhopper vector of the phytoplasma causing flavescence dorée, a severe disease of grapevine (Marzorati *et al*., 2006), and three mosquito vectors of malaria, *Anopheles stephensi*, *Anopheles maculipennis* and *Anopheles gambiae*. In particular *Asaia* has been found stably associated with larvae and adults of *A. stephensi*, dominating the microbiota of the mosquito (Favia *et al*., 2007).

The distribution of *Asaia* in the body of *A. stephensi* has been investigated by the use of a strain, previously isolated from the mosquito, after genetic modification to express a green fluorescent protein (Gfp). The Gfp-tagged strain efficiently colonized the gut, salivary glands, and male and female reproductive organs. It is noteworthy that *Asaia*, after assumption with a sugar-based diet by females, was detected in the gut and then in the salivary glands of the insect (Fig. 1), crucial organs for the development of the cycle of the malaria parasites *Plasmodium* spp. (Favia *et al*., 2007). By using fluorescent strains it was shown that in *A. stephensi*, *Asaia* is vertically transmitted from the mother to offspring (Favia *et al*., 2007), but also undergoes paternal transmission to the progeny, by the way of venereal transfer from male to female during mating (Damiani *et al.,* 2008).

The efficient capacity of *Asaia* of colonizing adults and larvae of *A. stephensi* and the discovery of this bacterium in other insect vectors (i.e., other *Anopheles* species and *Scaphoideus titanus*) rise the question of whether this bacterium can cross-colonize different insect hosts. The reply to this question could be very useful not only in order to better understand *Asaia* biology, but also to verify which *Asaia* genes may be involved in the host choice that is necessary for establishing a symbiotic interaction.

Finally, it is important to underline that the investigation of the basis of host-symbiont interaction could be very useful also from an applicative point of view since *Asaia* represents a promising bacterial species for the development of *Asaia*-based symbiotic control approaches to block parasite transmission by insect vectors (Favia *et al*., 2008). The symbiotic control approach would utilize bacteria capable of colonizing the insect body to produce effector molecules (natural or transgenic in the paratransgenic models) that kill or inhibit the causative agent of the disease or interfere with the survival of parasitic insects (Beard *et al*., 2001). Considering the localization in the insect body, the capability of colonizing very different hosts the culturability and the genetic transformability, *Asaia* may be also accounted as potential interesting agents for natural or paratransgenic symbiotic control opening new perspectives also from an applicative point of view.

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