

A hungry need for knowledge on the black soldier fly digestive system

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EDITORIAL

Abstract

The interest towards the black soldier fly (BSF), *Hermetia illucens*, has grown impressively in the last few years, fostered by the legislative changes in the European landscape that have lifted the ban regarding the use of BSF larvae as feedstuff. In addition, bioconversion mediated by the larvae of the BSF is viewed as one of the most promising technologies for organic waste processing and valorisation. Finally, new, alternative applications to exploit various larval products such as lipids, chitin, antimicrobial peptides, and frass are being explored. However, this positive trend, confirmed by the increasing number of companies that deal with BSF mass rearing and processing, is in sharp contrast with the limited information on the biology of this insect, in particular on aspects related to its digestive features. This lack of knowledge needs to be carefully considered and filled in coming years, as a deep characterisation of the morphology, physiology, transcriptomics, and proteomics of the digestive system of the insect, as well as a fine dissection of related aspects as gut microbiota and pathogens, is a prerequisite to improve the amazing bioconversion capabilities of this dipteran. So far, the larval stages received the most attention in research, but there might still be a lot to win by focusing more on the adult stage. Further expanding the basic knowledge on both the larval and the adult gut could lead to unexpected findings and open new perspectives to produce value-added bioproducts.

Keywords: *Hermetia illucens*, insect gut, microbiota, applications

1. Introduction

The digestive system plays a key role in insects since it is situated at the intersection of different processes such as nutrient ingestion, digestion, absorption and storage, metabolism, immunity, and defence against biotic and abiotic factors (Caccia *et al.*, 2019). Moreover, recent insights into the roles of the associated microbiota have widened our view on the functions of this system (Engel and Moran, 2013). The statement ‘It seems that Nature has taken pleasure in varying the same mechanism in an infinity of different ways’ by the French philosopher Denis Diderot (1753) could perfectly describe the process that has driven the evolution of the digestive system in these animals. In fact, this multifaceted taxon, that comprises more than one million of species, is characterised by a bewildering array of digestive systems to exploit an enormous number of biological niches that are largely

unavailable to other animals. So, although the insect gut basically consists of a continuous tube running from the mouth to the anus, a marked morphological and functional sub-regionalisation of the canal, local pH variations, as well as the presence of peculiar cell types in specific districts have significantly shaped the gut in different species according to the mechanical properties of the diet they feed on (solid or liquid form) and its nutrient composition (plant or animal origin) (Dow, 1986). This phenomenon is greatly emphasised in saprophages, such as some dipteran larvae, since they need to exploit different food sources.

2. The larval digestive system: morphofunctional features and its microbiota

Saprophagous dipteran larvae have recently attracted the attention of researchers since they can be profitably used for the reduction and valorisation of waste and by-products.

Among them, *Hermetia illucens* (Diptera: Stratiomyidae) (known as black soldier fly, BSF) is probably the most promising species for the bioconversion of low-quality biomass (Pinotti and Ottoboni, 2021) into sustainable products such as proteins for animal feed, pet food, and bioplastics (Barbi *et al.*, 2019; Bosch and Swanson, 2021; Dörper *et al.*, 2021; Liland *et al.*, 2021; Veldkamp and Vernooij, 2021), lipids for biodiesel (Zheng *et al.*, 2012), and bioactive compounds with biotechnological potential (e.g. chitin and antimicrobial peptides) (Di Somma *et al.*, 2022; Vogel *et al.*, 2018; Złotko *et al.*, 2021), contributing to the development of innovative and integrated circular economy supply chains (Cappelozza *et al.*, 2019). Despite the great interest on this insect from an applicative point of view, information on its physiology is still limited. This aspect requires great attention since only an in-depth knowledge about the morphofunctional features of organs and systems, their regulatory mechanisms, and the functional role of gut microbiota can promote the development of biotechnological approaches that will improve and expand applications of *H. illucens* larvae and their bioconversion capability.

Among the physiological aspects completely ignored for a long time, there is the larval digestive system, despite the fact that it represents the core of the bioconversion processes. A simple search on Scopus of articles dealing with the alimentary canal of BSF clearly shows an increase of publications in the last 10 years that reflects the interest

in this topic, although they still represent a small fraction of articles dealing with *H. illucens* in general (Figure 1). As in other insects, the alimentary canal of BSF larvae consists of three regions, namely the foregut, the midgut, and the hindgut, and only recently the larval midgut (i.e. the region of the gut which is involved in the digestion and absorption of nutrients) has been characterised in depth (Bonelli *et al.*, 2019). The midgut is a very complex organ organised in three distinct tracts, each having peculiar chemical and morphofunctional features. Indeed, the anterior midgut, whose lumen has a pH around 6, is characterised by columnar cells with secretory activity and a significant amylase and lipase activity. The lumen of the middle midgut is highly acidic (pH around 2) due to the action of copper cells, a peculiar cell type that is present only in the midgut epithelium of brachycerous Diptera. This midgut tract is not devoted to digestive processes, but the high activity of lysozyme and the strong acidic pH of the lumen could confer an important role to this gut portion in killing pathogens ingested with the diet and shaping the microbiota composition and density along the midgut (Bruno *et al.*, 2019b). In the posterior midgut, whose lumen is alkaline (pH around 8), further digestion of lipids and polysaccharides occurs, although the peculiar functional property of this tract is the digestion of proteins, ensured by endopeptidases (mainly serine proteases) and exopeptidases. Moreover, the presence of microvilli in columnar cells that are longer than in other regions suggests a main role of this tract in nutrient absorption.

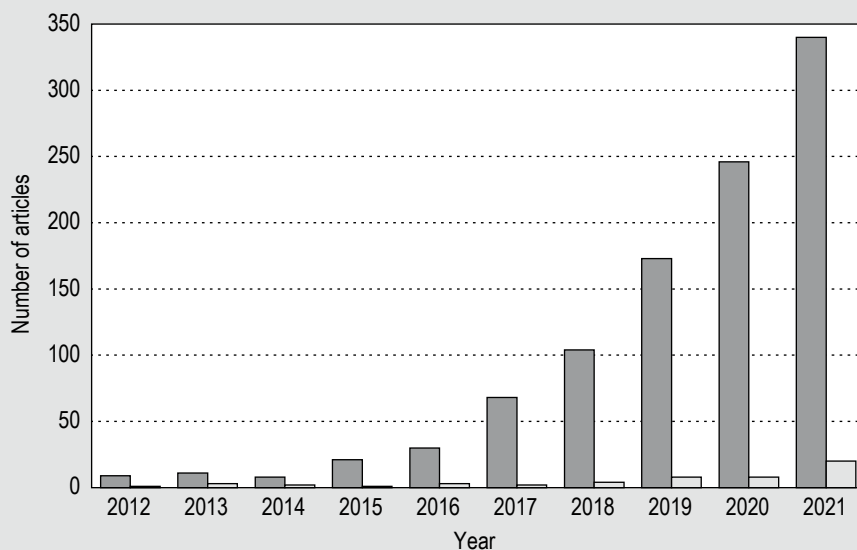


Figure 1. Evolution of the number of publications related to black soldier fly and BSF gut retrieved from the Scopus database. Publications on *H. illucens* (dark grey bars) were searched using the terms ‘*Hermetia illucens*’ (Article title, Abstract, and Keywords) OR ‘black soldier fly’ (Article title, Abstract, and Keywords), considering original articles published in the time span from 01-01-2012 to 31-12-2021. Publications on BSF gut (light grey bars) were selected manually from the database, considering original articles published in the same time span dealing with all aspects related to the larval and adult digestive system (i.e. physiology and digestion, morphology, genomics, transcriptomics, proteomics, microbiota, pathogens, and mouthparts).

The ability of BSF larvae to grow on a variety of industrially relevant substrates and side streams (e.g. municipal organic waste, fruit and vegetable waste, brewery spent grains, by-products of coffee beans roasting, manure) is surely correlated to the morphological and functional complexity of the midgut, but a key aspect that contributes to this ability is the functional plasticity of this organ. Indeed, the larvae can finely and profitably regulate digestive processes and nutrient absorption thanks to post-ingestion mechanisms to match their nutritional requirements and they easily adapt to rearing substrates with low nutritional content (Bonelli *et al.*, 2020). In particular, digestion is optimised through a sophisticated modulation at transcriptional level of genes coding for digestive enzymes, and absorption is regulated by increasing the length of microvilli in the posterior midgut, thus maximising the absorbing surface. The molecular mechanisms underlying midgut plasticity, which may involve peptides secreted by midgut endocrine cells and regulatory molecules released by the fat body and nervous system, as well as the role of the gut microbiota in supporting the ability of the larvae to adapt to different substrates, are topics that need to be addressed to fully understand the amazing functional properties and plasticity of this organ, opening new possibilities for the exploitation of BSF bioconversion capabilities. Another interesting aspect coming from a recent study (Bruno *et al.*, 2020) is related to the functional morphology of mouthparts. A well developed and peculiar buccal apparatus gives the larvae the ability to process and ingest semisolid substrates of different texture and moisture content. This aspect is noteworthy since the nutritional value of a diet not only depends on its composition but also on its texture and microstructure, which can impact on nutrient bioaccessibility (Turgeon and Rioux, 2011), and the functional features of BSF larvae mouthparts are surely another characteristic that ensure the best exploitation of the rearing substrate.

As mentioned before, the gut microbiota has to be considered as an integral part of the digestive system, enabling the larva to feed on a range of substrates. While culture-dependent plating techniques also have their merits, microbiome research of BSF larvae is merely based on next generation sequencing technologies. DNA-based amplicon sequencing techniques allow to list individual bacterial or fungal 'operational taxonomic units', or more recently termed 'amplicon sequence variants', to determine their relative abundance in the community and to study their diversity. RNA-based sequencing (transcriptomics), proteomic approaches, and other techniques such as phenotype microarrays allow to study the functions of the microbiome. Research involving sequencing of the gut microbiome in BSF larvae is growing tremendously. Most of the research papers investigating the gut microbiome composition also tackle the question whether there is a 'core microbiome' in BSF larvae. The term 'core microbiome' is generally considered to cover those bacterial genera

that are always present in the larvae, regardless of rearing conditions and other factors that may affect microbial composition. While a clear and usable definition of the term entails a discussion on itself, the existence and the members of the core microbiome are heavily debated. In the same way as already done several times for traditional livestock, recently a first meta-analysis of all publications containing sequencing data on the *H. illucens* larvae microbiome was performed (Ijdema *et al.*, 2022). The raw sequence data were retrieved and processed through a uniform bioinformatic pipeline in order to make general conclusions over the 11 data sets included. From this work, the genera *Enterococcus*, *Klebsiella*, *Morganella*, *Providencia*, and *Scrofulimicrobium* appeared to be core members over all datasets. In addition, the factors 'study' (relating to specific aspects on how larvae were grown and how DNA was extracted, processed and sequenced), 'larval instar', and 'substrate' (including the chemical as well as microbiological composition of the substrate) significantly affected the composition of the microbiota. The focus on identifying the core members of the BSF larval gut microbiome can be useful because it can be the first step in the development of probiotics for substrate conversion and biomass production by this insect. Nevertheless, the 'quest' related to the core microbiome should not result in neglecting the non-core part, which is obviously more diverse, may contain useful microorganisms, and may also be exchanged between the substrate microbiota and the larval microbiota (horizontal transmission).

The research on the microbiome should be taken one step further and consider its functions. Among others, Engel and Moran (2013) reviewed the possible functions of the gut microbiome for insects in general. More specifically, gut microorganisms may be involved in providing protection from predators, parasites, and pathogens via, e.g. nutrient competition, niche occupation, and antagonistic interactions. They also may be involved in intestinal cell renewal, they may aid digestion, provide microbial fermentation, stimulate overall growth and development of the insect by influencing hormone signalling, be digested themselves and hence serve as nutrient, synthesise essential nutrients such as vitamins or amino acids, produce molecules for communication with other individuals and in this way control mating and oviposition, and increase tolerance to abiotic stress such as heat and desiccation. Many of these functions can be assumed to be exerted by specific microorganisms in BSF larvae as well. The first publications on this aspect appeared in the last few years (e.g. Greenwood *et al.*, 2021; Jiang *et al.*, 2019; Klammsteiner *et al.*, 2021; Kooienga *et al.*, 2020; Tegtmeier *et al.*, 2021; Yang *et al.*, 2021; Zhang *et al.*, 2021), but likely they have uncovered only the top of the iceberg of possible roles that microorganisms can have in the BSF larval gut. For instance, while it may be tempting as a first step to make predictions or draw conclusions on functions of specific

genera (metabolic phenotypes, for instance determined by Greenwood *et al.* (2021) and Yang *et al.* (2021)) based on their abundance in different substrates, Klammsteiner *et al.* (2021) noticed that shifts in diets induce clear shifts in the microbiome composition, but they do not modify the main metabolic pathways, as they remain relatively stable.

3. The adult digestive system: need for revision of the assumption that the fly does not feed

For a long time, there has been no interest towards the digestive capabilities of adult *Hermetia* since it was a common belief that, due to its short life span, feeding at this developmental stage was unnecessary. It was thought that the fly could largely rely on the energy provided by the reserves accumulated at larval stage (Sheppard *et al.*, 2002; Tomberlin and Sheppard, 2002). Thus, the quantity and quality of food provided to the larva appeared to be solely responsible for the adult performance and only water intake by the adults seemed essential to prevent dehydration, keep them vigorous, as well live and mate for longer (Gobbi *et al.*, 2013; Sheppard *et al.*, 2002; Tomberlin *et al.*, 2002). The scenario is more complex, however, and recent studies have completely overturned this vision. In fact, thanks to a multilevel approach, it has been clearly demonstrated that the fly possesses a fully functional alimentary canal endowed with food transit and digestive capabilities. Most importantly, the production of digestive enzymes can be modulated at mRNA level to adapt to different diets, suggesting the existence of post-ingestion capabilities also at the adult stage (Bruno *et al.*, 2019a). The functional evidence on the digestive system of the adult insect is reflected in the possibility of modulating the fly performance through the nourishment provided at the adult stage. In fact, it has been shown that adult longevity, oviposition, and even egg hatchability, can be significantly modified by giving sugar or protein feeding substrates to the flies (Bertinetti *et al.*, 2019; Bruno *et al.*, 2019a; Nakamura *et al.*, 2016). Moreover, it seems likely that while preoviposition period – a short, fixed phase during which mating occurs – depends on fat reserves stored during larval stage, oviposition is strongly affected by the feeding conditions of the fly (Bertinetti *et al.*, 2019), opening new perspectives for the optimisation of the breeding procedures for BSF.

Due to the misconception that the adult BSF does not need to eat or is even incapable of doing it, the gut microbiota of the fly has been completely overlooked, too, and no information is available on this aspect. This is a significant gap of knowledge since, similarly to the larva, gut bacteria could play a significant contribution to the digestive processes of the fly. Actually, an accurate investigation of this aspect must necessarily take into account the profound changes that, at metamorphosis, lead to the generation of the adult midgut starting from the larval gut. Indeed, the complete remodelling process of the alimentary canal

typical for holometabolous insects not only drives the formation of a new alimentary canal with peculiar, adult-specific morphofunctional features (Bruno *et al.*, 2019a), but it can also decouple the gut microbiota between the larval and adult stages, reshaping it according to the digestive needs of the fly. Insight in the latter aspect is in turn important to assess the risk of transmission of pathogens (insect, animal, or human pathogens) over consecutive generations (vertical transmission), affecting production yield and microbiological safety of BSF larvae production.

4. Knowledge about the BSF digestive system: an essential prerequisite to improve applications

As mentioned above, a solid platform of knowledge about the digestive system of BSF can promote the exploitation of its bioconversion capabilities and foster the development of new industrial sectors dealing with insect farming and processing. Some relevant aspects that can benefit from this information are discussed below.

Genetic and genomic tools (Eriksson and Picard, 2021), such as the CRISPR/Cas9 approach recently developed in BSF larvae (Zhan *et al.*, 2020), can be exploited to select/produce strains with traits that can improve the insect performance on specific substrates, the bioconversion process, and the quality of the insect biomass or derived bioproducts. High survivability, disease resistance, high fecundity, and fast development are only some of the desired traits that can be selected/obtained using these tools. These aspects take advantage from knowledge on digestion and absorption processes and their modulation, as well as on the functional characterisation of the microbiota.

Unpredictable or variable growth performance of *H. illucens* larvae on different by-products and organic waste is an issue that can negatively affect their use in bioconversion at an industrial scale. A significant support can be derived from *in vitro* digestion models, which are able to partially predict the outcomes of *in vivo* feeding experiments (Gold *et al.*, 2020). Information on digestive properties of the larval midgut, as well as its chemical and physical properties (e.g. luminal pH, redox conditions, temperature), is essential to develop reliable *in vitro* simulations, thus rapidly and easily estimating *in vivo* reduction of the rearing substrates and their exploitation by the larvae.

Post-ingestion mechanisms and adaptation to substrates with low nutritional content are costly in terms of energy and resources allocated by the insect and potentially affecting larval performance. Therefore, morphological, physiological, and molecular parameters related to insect digestion processes could be suitable diagnostic markers to monitor larval health and development during mass rearing.

Sequencing of the BSF larval gut microbiome can lead to the identification of genes coding for enzymes that can be exploited to degrade challenging waste. Similarly, microbial consortia composed of different types of microorganisms with possible metabolic complementarities can be identified and used for the same purpose.

As the alimentary canal represents the main route of infection in insects, this organ counteracts microbial colonisation thanks to its physical and physiological properties, the secretion of lysozyme, and the local production of AMPs and ROS. Secondly, soluble signals can emanate from the gut of infected insects and reach the haemocytes and fat body, giving rise to an inter-tissue signalling pathway that triggers the systemic immune response. Thirdly, it has been shown that systemic immunity is influenced by the diet. All these aspects together highlight the intimate relationship between the gut and the defence mechanisms of the insect, and raise the intriguing prospect to increase BSF resistance to pathogens and optimise health status during mass rearing by acting selectively on the gut.

The unexpected evidence on the feeding capabilities of the fly prompts the search for nutrient requirements of adult insects bred in captivity, that cannot have free access to nutrients as in nature, in order to maximise egg production and oviposition in mass rearing processes. On the other hand, aspects such as the possible escape of opportunistic pathogens from the gut into the insect hemocoel during the metamorphic remodelling of this organ (Johnston and Rolf, 2015), regurgitation and defecation processes in the fly, as well as transstadial transmission of pathogens must be carefully considered in view of a safe use of this insect for feed production purposes.

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