Hermetia illucens (Diptera: Stratiomyidae) larvae in waste valorization and diet-based shifts in their gut microbiome

T. Klammsteiner[1], A. Walter[1], C. Heussler[2], B. C. Schlick-Steiner[2], F. M. Steiner[2], H. Insam[1]

[1]Department of Microbiology, University of Innsbruck, Innsbruck, 6020 Austria
[2]Department of Ecology, University of Innsbruck, Innsbruck, 6020 Austria
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Presenting author email: thomasklammsteiner@hotmail.com

Fast-moving consumerism of food products in industrial states requires constant adaptations to fulfill the market's demands. Negative side effects of such developments include an increase of municipal biodegradable waste - in the EU from 14 M tons in 1995 to 40 M tons in 2016 (Eurostat, 2018). Aside from conventional methods like anaerobic fermentation and composting, people are constantly on the lookout for economic and ecologically worthwhile ways to valorize and process organic waste in an efficient manner. Some insects are infamous for their unquenchable hunger either in clearing out vast areas of agricultural crop land (e.g. locusts, van Huis *et al*, 2007) or in the disintegration of housing (e.g. termites, Querner, 2015). This appetite can be utilized to give the insects a remarkable role in valorizing organic wastes and providing a basis for novel varieties of feedstuff, as the Black Soldier Fly (BSF, *Hermetia illucens*) proves (Wang and Shelomi, 2017).

Although the fly is not native to temperate zones, an increasing number of European companies are discovering its potential for waste treatment and for the production of animal feedstuff and are tying in with the industrialization of insect farming otherwise happening mostly in south-east Asia. However, the transition from lab-scale experiments to smoothly working large-scale applications still is not methodologically sound and holds various stumbling blocks that have yet to be overcome by gaining knowledge in process management and fly biology (Rafieenia, 2016). While figuring out the optimal rearing conditions for a maximum yield in larval protein with a minimum amount of residues is crucial, it is also important to target interactions with associated microbial communities (Bienenstock *et al*, 2018).

In a series of feeding experiments, we investigated the effect of organic-waste related diets on the development and mass balance of BSF larvae and analyzed via next generation sequencing the involved impact on the microbial gut communities in larvae. We hypothesized that (i) although BSF larvae won't show the same biomass gain when offered organic wastes as with a diet based on balanced chickenfeed, they will still be able to efficiently degrade the offered substrates. (ii) Depending on the diet, the composition of microbial gut communities will be shaped by the available feed.

Material & Methods

Chickenfeed (CF; 3:2 with water), fruit/vegetable mix (FV; 0.5:1:1:1 cucumber, tomato, orange, apple) and grass cuttings (GC; collected from lawn mower) were used as diets. Water content was equalized based on dry weight. Feed amounts were adapted to 10, 100 and 130 mg larva^{-1} day^{-1} for CF, FV and GC, respectively, based on the organic content of the feed and the number of larvae, and were supplied every three days. Each of the three treatments was applied to 200 BSF larvae (age six days, henceforth referred to as day$_0$) held in non-transparent plastic boxes (25×10×5 cm; four replicates per treatment) covered with nets for aeriation. Environmental conditions were stable at 27 °C and 60 % relative humidity. Samples consisting of 20 larvae each were collected in intervals of three days to determine fresh weight, dry and organic dry matter until transition to prepupal stage. Guts were extracted at four time points (days$_{0,9,15,21}$) from surface-sterilized (70 % EtOH) larvae via scalpel and forceps, and DNA was extracted using the NucleoSpin® Soil-Kit (Macherey-Nagel GmbH, Germany) following the user manual. DNA extracts were sent to Microsynth AG (Balgach, Switzerland) for 16S rRNA Illumina MiSeq sequencing (2x250 bp, V4). Raw sequence reads were analyzed in mothur (Schloss et al., 2009), and statistical analysis was conducted in R (R Development Core Team, 2008).

Results

Conversion to larval biomass was lower, and substrate degradation over time was slower for FV and GC than for CF (WRI approx. 2.0 and ECD over 50% with CF), but higher proportions of substrate (10%) were metabolized by larvae raised on waste related substrates (mean values in Tab. 1). A nearly threefold gain in larval biomass compared with mean biomass

Tab. 1. Mean larval mass balance from diets based on organic wastes. The WRI describes the relation between overall degradation and time (higher is better). The EDC explains how efficiently larvae transform the offered feed into biomass (higher is better)

	Organic wastes (fruit, vegetables, grass cuttings)	
	Mean	SE
Residue	31.0%	10.0%
Biomass	7.6%	2.4%
Metabolism	61.5%	1.3%
Waste Reduction Index (WRI)	1.4	0.1
Efficiency of Conversion of Digested Feed (ECD)	21.0%	1.8%

gain from organic wastes was observed in larvae fed with CF. However, the amounts of residues were similar in all substrates. The earliest onset of prepupal stage was observed in CF larvae at day_{12} with a mean of 62.75 ± 10.85 pupae. Single pupae (1.75 ± 0.83) were also found in GC treated boxes. The slowest transition to prepupal stage was recorded in FV starting with day_{18}.

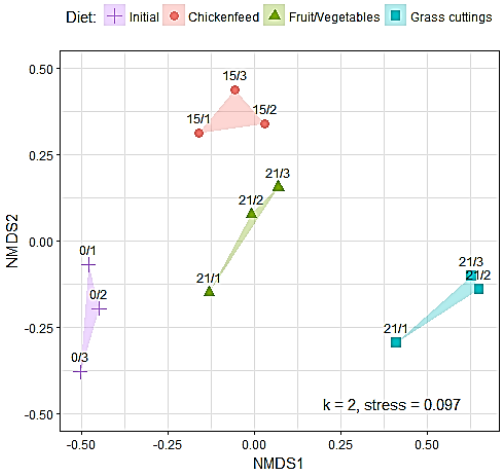

Fig. 1. NMDS from OTU data showing distances in gut communities between initial composition and compositions after exposure to different diets (X/Y = X...sampling day, Y...replicate no.)

Non-metric multidimensional scaling (NMDS) based on operational taxonomic unit (OTU) data from Illumina MiSeq sequencing resulted in samples clustering according to different diets and indicated shifts in community composition happening between day_0 and the end of treatments (day_{15}: CF and day_{21}: FV, GC; Fig. 1). CF also expressed a different community composition at the end of the experiment compared with the initial population but was monitored only until day_{15} because of faster transition to prepupal stage.

Discussion

BSF larvae show great capability in metabolizing organic substances as they were able to degrade all offered diets with similar amounts of residues left behind. However, conversion efficiency of organic wastes to biomass could not keep up with balanced CF because the diets were not designed to yield a maximum increase in animal biomass. The development from larval to prepupal stage varied among diets depending on the digestibility of the substrate. Low-energy feeds like FV and GC retarded larval development compared with CF by approx. 3-4 days. The gut microbiota from the initial population raised on CF shifted over to different compositions depending on the diet. Moreover, the age of larvae played a role in the microbial community composition as guts sampled on day_{15} diverge from guts coming from the initial population. Actinobacteria, Proteobacteria, and Bacteroidea accounted for high abundances in all guts, while the respective ratios thereof and diet-specific representative genera (e.g. *Devosia* sp. in GC fed guts) were responsible for the particular community development during the experiments (Fig. 1).

Conclusions

Our results contribute to the process of finding optimal ratios of different organic wastes typical for domestic households that maximize degradation and metabolization by larvae together with the highest possible increase in larval biomass and the minimum of substrate residues. The degradation of substrates with varying contents of organic matter resulted in similar amounts of residues, highlighting the Black Soldier Fly as an efficient all-round degrader. By simultaneously investigating the microbial gut communities, we assessed to which degree different diets influence the predominant microbiota and concluded on their role in larval activity.

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