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Metagenetic analysis of the bacterial communities of edible insects from diverse production cycles at industrial rearing companies

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Abstract

Despite the continuing development of new insect-derived food products, microbial research on edible insects and insect-based foods is still very limited. The goal of this study was to increase the knowledge on the microbial quality of edible insects by comparing the bacterial community composition of mealworms (*Tenebrio molitor*) and crickets (*Acheta domesticus* and *Gryllodes sigillatus*) from several production cycles and rearing companies. Remarkable differences in the bacterial community composition were found between different mealworm rearing companies and mealworm production cycles from the same company. In comparison with mealworms, the bacterial community composition of the investigated crickets was more similar among different companies, and was highly similar between both cricket species investigated. Mealworm communities were dominated by *Spiroplasma* and *Erwinia* species, while crickets were abundantly colonised by (*Para*)*bacteroides* species. With respect to food safety, only a few operational taxonomic units could be associated with potential human pathogens such as *Cronobacter* or spoilage bacteria such as *Pseudomonas*. In summary, our results implicate that at least for cricket rearing, production cycles of constant and good quality in terms of bacterial composition can be obtained by different rearing companies. For mealworms however, more variation in terms of microbial quality occurs between companies.

Key words

Acheta domesticus, bacterial community composition, edible insects, *Gryllodes sigillatus*, Illumina MiSeq sequencing, *Tenebrio molitor*.

1 Introduction

Although consumer acceptance of edible insects and insect-derived foods is still limited (Caparros Megido et al., 2014; House, 2016; Lensvelt and Steenbekkers, 2014; Verbeke, 2015; Yen, 2009), insect-based products are increasingly being investigated (Tan et al., 2017) as well as developed (Cadesky, 2017; Stoops et al., 2017) and insects are getting progressively more attention as food source in Western countries (Mlcek et al., 2014). While insect products are entering the market - despite the Novel Food status of insects and their derived products as from 2018 (Regulation 2015/2283) - the microbial quality of the insects is still not fully revealed. Some studies have already assessed the microbial quality of fresh edible insects (Klunder et al., 2012; Stoops et al., 2016; Vandeweyer et al., 2017a) and/or insect-derived products (Caparros Megido et al., 2017; Garofalo et al., 2017; Grabowski and Klein, 2016; Stoops et al., 2017). However, except for Vandeweyer et al. (2017a), these studies did not compare different production cycles and rearing companies. Furthermore, most studies only used culture-dependent methods for microbial analysis, leading to an observed microbial diversity which may be incomplete and/or biased (Justé et al., 2008). Garofalo et al. (2017) and Stoops et al. (2016) recently investigated the bacterial community composition of respectively processed and fresh edible insects using culture-independent 454 pyrosequencing of partial 16S ribosomal RNA (rRNA) genes. These studies revealed that some potential food pathogen and spoilage genera can be present, which could not be proved on this taxonomic level by general culture-dependent counts alone. Both the edible insect sector and the legislative authorities (ANSES, 2015; EFSA Scientific Committee, 2015; SHC and FASFC, 2014) are highly interested in additional microbiological (and other) data from different sources. The data are also useful for insect rearing and processing companies to gain further

insight into insects as a food matrix and to complete the Novel Food dossiers they are currently preparing.

The purpose of this study was to assess and compare the bacterial communities of fresh mealworms (*Tenebrio molitor*) and crickets (*Acheta domesticus* and *Gryllodes sigillatus*) from different production cycles, produced at industrial rearing companies in Belgium and The Netherlands. In both countries, crickets and mealworms are produced intensively for human consumption, but fresh crickets have never been investigated with next-generation sequencing techniques before and fresh mealworms only once on a small scale in a preliminary study (Stoops et al., 2016). In addition to the intrinsic properties and the traditional culture-dependent microbial counts previously determined and described in Vandeweyer et al. (2017a), this study reports on the metagenetic data obtained for the samples collected in the aforementioned study.

2 Material and methods

2.1 Sample preparation and DNA extraction

Concurrent with the culture-dependent analyses performed in Vandeweyer et al. (2017a), DNA extractions were executed on samples collected in that study (Table 1). A 30 g subsample of living insects from each production cycle was pulverised as described earlier (Stoops et al., 2016; Vandeweyer et al., 2017a) and used to execute two extractions using 2 g starting material (manufacturer's protocol, Power Soil DNA Elution Accessory Kit, Mo Bio laboratories, Carlsbad, CA, USA). DNA samples were stored at -80°C until further use.

2.2 Metagenetic analysis

To perform the metagenetic analysis, a tenfold dilution of each DNA extract was amplified in twofold by PCR targeting the V4 region of the 16S rRNA gene using sample-specific barcode-labelled versions of primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 806R (5'-GGA CTA CHV GGG TWT CTA AT-3') (Caporaso et al., 2011; dual-index sequencing strategy, Kozich et al., 2013; Table S1, Supporting Information). Each 20 µl PCR reaction contained 1x Titanium Taq PCR buffer, 150 µM of each dNTP, 0.5 µM of each primer, 1x Titanium Taq DNA polymerase (Clontech, Saint-Germain-en-Laye, France) and 1 µl 10-times diluted DNA. The reaction was initiated by denaturation at 95 °C for 120 s, followed by 30 cycles of denaturation at 95 °C for 45 s, annealing at 60 °C for 45 s and elongation at 72 °C for 45 s. Replicate amplification products were combined and loaded on an agarose gel. Next, visible bands of the expected size were excised and the DNA was purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). After quantification of all purified DNA amplicons (Qubit High Sensitivity Fluorometer kit, Invitrogen, Carlsbad, CA, USA), DNA samples were equimolarily combined into a library and purified once again (Agencourt AMPure XP kit, Beckman Coulter Life Sciences, Indianapolis, IN, USA). The library was diluted to 2 nM and sequenced at the Centre of Medical Genetics Antwerp (University of Antwerp, Antwerp, Belgium), using an Illumina MiSeq sequencer with v2 500 cycle reagent kit (Illumina, San Diego, CA, USA).

Resulting sequences were received as a de-multiplexed FASTQ file (data deposited in a Sequence Read Archive; BioProject accession PRJNA390238). Paired-end reads were merged using USEARCH (v.8.1) to form consensus sequences (Edgar, 2013) with no more than 10 mismatches allowed in the overlap region. Subsequently, sequences were truncated at the 250th base. Shorter reads or reads with a total expected error threshold above 1.0 for all the bases were discarded. Due to uneven sequencing depth, the number of sequences was rarefied

to 54,000 sequences per sample. Remaining sequences with a minimum abundance of two were grouped into operational taxonomic units (OTUs) based on a 3% sequence dissimilarity cut-off using the UPARSE greedy algorithm in USEARCH, during which chimeric sequences were also removed (Edgar, 2013). Global singletons (i.e. OTUs represented by only a single sequence in the entire dataset) were not taken into account to minimize the risk of retaining sequences from sequencing errors (Brown et al., 2015; Waud et al., 2014). Subsequently, OTUs were assigned taxonomic identities using the "classify.seqs" command in Mothur (v. 1.36.1) (Schloss et al., 2009) against the Silva taxonomy database v1.23 (Quast et al., 2013). With Mothur's "remove.lineage" command, OTUs originating from chloroplasts or mitochondria were deleted.

The taxonomic origin of each OTU was determined with the SINTAX algorithm implemented in USEARCH, (Edgar, 2016) based on the Silva Living Tree Project v123 (LTP v123) database. Taxonomic assignments were considered reliable when bootstrap confidence values exceeded 0.80. Additionally, OTU representative sequences (selected by UPARSE) were subjected to a BLAST (Altschul et al., 1990) search against GenBank (Benson et al., 2013), excluding uncultured/environmental entries. Principally, assignments were based on SINTAX results, but BLAST results were used when SINTAX assignment was inconclusive or produced assignment scores below 0.80. Finally, nonmetric multidimensional scaling (NMDS) and Chao1 and Shannon-Wiener diversity indices calculations were performed on the microbial communities of the samples using R-packages (R Development Core Team, 2013) Vegan (v.2.41) and Phyloseq (v. 1.19.0).

2.3 Statistical analyses

Statistical analyses were conducted using SPSS Statistics (version 23, IBM, New York, NY, USA). Chao1 and Shannon-Wiener diversity indices, as well as observed richness and coverage were compared between production cycles per company, between rearing companies per insect species and between insect species. To this end, independent samples T-tests were used when comparing two conditions, while ANOVA was used in all other cases. When necessary, pairwise comparison was performed using Tukey's post hoc test. All tests considered a 0.05 significance level.

3 Results and discussion

3.1 Comparison of the mealworm bacterial community composition between rearing companies and production cycles

Differences in bacterial community composition between samples from different production cycles as well as between rearing companies are visualised through NMDS in Fig.1. Both DNA extracts per sample are displayed with the same icon. A near position of these icons illustrates that the bacterial communities in both extracts were highly similar. Furthermore, it is clear from these results that the bacterial community in/on mealworms is mainly driven by the rearing company rather than by the production cycle (Fig. 1A).

The mean bacterial community composition with OTUs in a relative abundance of at least 2% (for an overview of all bacterial OTUs, see Table S2 and Table S3, Supporting Information) for the different mealworm companies is exposed in Fig. 2. These results suggest that mealworms from different rearing locations have at least a part of their bacterial community composition in common. However, important differences can be designated as well. In all mealworm samples, a bacterium related to a *Spiroplasma* species (OTU 1; Table S3, Supporting Information) and a bacterium assigned as *Erwinia oleae* (OTU 2; Table S2,

Supporting Information) were abundantly present. For rearing company 1, together they comprised over 50% of all sequences recovered, while for companies 2 and 3, both OTUs only accounted for approximately 35% of all sequences recovered, with especially OTU 1 being less abundant. Furthermore, samples from rearing company 1 also harboured substantial quantities of *Citrobacter koseri* (OTU 904; Table S2, Supporting Information) and a *Brevibacillus* species (OTU 10; Table S3, Supporting Information), while these OTUs were scarcely present in the other rearing companies. The high abundance of the *Brevibacillus* OTU, a spore-forming bacterium mainly present in cycle MW 1.2, can most probably explain the high aerobic endospore count (5.0 log cfu/g) for this sample reported in Vandeweyer et al. (2017a). Company 2, on the other hand, clearly produced larvae with a high abundance of an Enterobacteriaceae (OTU 14; Table S2, Supporting Information) and *Lactococcus* (OTU 5; Table S3, Supporting Information) species. Production cycles from company 3 contained higher amounts of two other Enterobacteriaceae OTUs (OTU 979 and 46; Table S2, Supporting Information) and *Pseudomonas deceptionensis* (OTU 4; Table S2, Supporting Information). The diversity indices (Table 2) based on these community compositions also show highly significant differences between the mealworm rearing companies. Because of the high abundance of two predominant OTUs, the mean observed richness of company 1 was rather low, but covered the estimated richness (Chao1 index) for 75%. Also the Shannon-Wiener index, which denotes the diversity based on both richness and abundance, was lower for company 1 than for the other mealworm rearing companies. It was only significantly different ($p = 0.003$) from rearing company 2, which shows the highest amount of OTUs (richness) and the highest general diversity.

On Fig. 2, the error bars representing the standard deviation are an indication of the differences between production cycles from the same company. In accordance with the

culture-dependent approach in Vandeweyer et al. (2017a), standard deviations for the most abundant OTUs from company 2 were the smallest (all below 11%), indicating that the different production cycles for this company deliver insects with a comparable microbial community. Unlike company 2, standard deviations from company 1 (up to 15%) and especially from company 3 (up to 20%) were large. Consequently, striking differences could be found between different production cycles (see also Fig. S1, Supporting Information). For company 1, cycle 2 was the most divergent, because it was the only cycle that contained a *Brevibacillus* OTU (OTU 10), even in an abundance of approximately 28%. For company 3, all cycles can be considered as very different from each other. The community of cycle 1 was for over 80% composed of the *Spiroplasma* (OTU 1) and *E. oleae* OTUs (OTU 2), while cycle 2 and 3 contained only around 30% and 5% of these OTUs, respectively. Sample MW 3.2 (cycle 2) contained an Enterobacteriaceae OTU (OTU 979; 28%) instead, and sample MW 3.3 (cycle 3) harboured large quantities of *P. deceptionensis* (OTU 4; 39%), *Pseudomonas antarctica* (OTU 23; 11%; Table S2, Supporting Information) and an Enterobacteriaceae species (OTU 46; 21%). When comparing these findings with the diversity indices in Table 2, the only sample showing a significant difference was MW 3.2, which had a larger diversity (Shannon-Wiener index, $p = 0{,}010$) compared to the other production cycles from company 3. Obviously, this implies that samples with a similar diversity level can still differ in terms of microorganisms present.

3.2 Comparison of the cricket bacterial community composition between rearing companies and production cycles

Concerning crickets, Fig. 1B visually shows the distinction between the studied samples of each rearing company and Fig. 3 shows the mean bacterial community

composition per cricket rearing company. Many dominant (abundance > 2%) OTUs were found in all three rearing companies and the bacterial communities seem to be less different from each other than for mealworm companies. Correspondingly, the diversity indices (Table 2) demonstrate the smaller variety between cricket rearing companies in comparison with the mealworm rearing companies. Based on the diversity indices, samples from cricket company 5 harboured a significantly (p = 0,000) lower amount of OTUs and show to be the least diverse. The most abundant OTUs for company 5 corresponded to *Buttiauxella agrestis* (OTU 3; 8%; Table S2, Supporting Information), a bacterium related to *Parabacteroides* (OTU 8; 8%; Table S3, Supporting Information) and a species from the family of Pseudomonadaceae (OTU 6; 8%; Table S2, Supporting Information). Company 6 and 7 were dominated by another *Parabacteroides*-related bacterium (OTU 9; 9% and 14%, respectively; Table S3, Supporting Information) and a species related to the genera *Photorhabdus* (OTU 12; 5%; Table S3, Supporting Information) and *Bacteroides* (OTU 11; 5%; Table S3, Supporting Information), respectively. In this respect, the cricket bacterial communities of company 6 and 7 were the most similar (Fig. 2), and this confirms the culture-dependent findings in Vandeweyer et al (2017a).

Again, the error bars shown in Fig. 3 represent the variety between different production cycles within the same company. While company 5 shows the highest standard deviations on the mean OTU abundance (up to 6%, while below 3% for company 6 and 7) and therefore produced the most variable cricket samples (see also Fig. S2, Supporting Information), the variability between samples from all cricket companies was small and not statistically significant. This small variation between samples can also be seen on Fig. 1B. In contrast to the mealworms, reared crickets appear to contain a more uniform bacterial

community composition over different cycles and can be delivered with a more constant bacterial quality.

3.3 Comparison of the bacterial community composition between mealworm and cricket species

Although they belong to different genera, the house cricket (*A. domesticus*, rearing companies 5 and 6) and the banded cricket (*G. sigillatus*, rearing company 7) possess a highly similar mean bacterial community composition, as shown in Fig. 3 and 4. Likewise, the calculated diversity indices (Table 2) deliver no significant differences between both species. These results are in agreement with the culture-dependent microbial counts presented earlier in Vandeweyer et al. (2017a), and can most probably be explained by similar intrinsic parameters such as pH and water activity (a_w). It is reasonable to believe that a comparable diet and rearing process for both cricket species contributes to the development of a similar microbial community composition (Yun et al., 2014).

To allow further comparison between the bacterial community composition of mealworms and crickets as two different insect types, the community compositions of both house crickets and banded crickets were combined (Fig. 4 and 5). Regarding phylum level taxonomy (Fig. 4), mealworms showed a larger relative abundance of Proteobacteria (57%) and Tenericutes (23%), while the cricket bacterial community was dominated by Bacteroidetes (43%) and Firmicutes (35%). When comparing these results to those obtained by Stoops et al. (2016) and Garofalo et al. (2017), mealworms were dominated by Proteobacteria in both studies, but the second most abundant phylum Tenericutes was not reported in Stoops et al. (2016). Since this latter study was executed using the same sequencing approach as Garofalo et al. (2017), the reason for the absence of Tenericutes

remains unclear. For crickets, Garofalo et al. (2017) reported a similar distribution of phylum abundance.

Focussing on a lower taxonomic level, it can be seen that the mean cricket bacterial community was more diverse than the mean mealworm bacterial community (Fig. 5). In particular, for crickets all OTUs had a relative abundance below 10%, and on average 60% of the mean cricket bacterial community was composed of OTUs with a relative abundance below 2% (Fig. 3 and 5). In contrast, for mealworms around 85% of recovered sequences could be attributed to the 12 most dominant OTUs, with even 2 to 4 OTUs comprising half of the community (Fig. 2 and 5). From all reported OTUs from both insects, many belong to the family of Enterobacteriaceae or the order of lactic acid bacteria (Lactobacillales). Microbial counts performed on the same samples (Vandeweyer et al., 2017a) already predicted a high abundance of these bacterial groups for both mealworms and crickets. However, our sequencing approach revealed that only a few OTUs were found in both insects (when considering an abundance threshold of 0.1%), i.e. OTUs corresponding to *E. oleae* (OTU 2), *B. agrestis* (OTU 3), an *Enterococcus* species (OTU 7; Table S3, Supporting Information) and a *Lactococcus* species (OTU 5). Altogether, these results indicate strong differences in the bacterial community composition between mealworms and crickets, which is in line with the differences in microbial counts, pH, moisture content and water activity (a_w) observed previously (Vandeweyer et al., 2017a). These differences in microbial community structure can also be derived from the calculated diversity indices (Table 2). Both the observed (i.e. amount of OTUs) and predicted (Chao1 index) OTU richness were remarkably and statistically ($p = 0.000$) higher for crickets. Likewise, the Shannon-Wiener index was statistically ($p = 0.000$) higher for crickets compared to mealworms. In summary, our results clearly show a large difference between the bacterial communities of both insect types, with

the cricket bacterial community compositions being much more diverse. Not only their taxonomic distinction with *Tenebrio* belonging to the Coleoptera order and both cricket species being Orthoptera, but also their life cycle and feed source are likely to be important determinants of the insect microbial community (Colman et al., 2012; Jones et al., 2013; Yun et al., 2014).

3.4 Biological relevance of the bacteria encountered

The most dominant OTUs found on mealworms were related to a *Spiroplasma* species (Tenericutes, OTU 1) or assigned as *E. oleae* (Proteobacteria, OTU 2). *Spiroplasma* was already found in fresh (Jung et al., 2014; Wang and Zhang, 2015) and processed (Garofalo et al., 2017) mealworms and in a mealworm-based minced meat-like product (Stoops et al., 2017). While the bacterial communities may differ largely between different rearing companies and production cycles, some bacteria, such as *Spiroplasma* spp., may be typically associated with mealworms and mealworm-derived products. *Spiroplasma* spp. are typically found as endosymbiont in the insect gut, but are hard to culture *in vitro* (Madigan et al., 2009). While some *Spiroplasma* species may protect the host insect against entomopathogens (Shokal et al., 2016), other species of the genus may be pathogenic for insects (Ammar et al., 2004) or even humans (Aquilino et al., 2015). *Spiroplasma* is generally not considered as a foodborne pathogen, but further research is needed to unravel the role of *Spiroplasma* spp. or related bacteria in edible insects.

The second most abundant OTU for mealworms was assigned to the genus *Erwinia,* and more particularly to the species *E. oleae* (OTU 2). Although this OTU was assigned *E. oleae* with an assignment score of 0.86 (Table S2, Supporting Information), the short 250 bp read length of the investigated 16S rRNA gene amplicon does not ensure a 100% correct

species determination. Further research, e.g. using other genetic markers is needed to confirm its identification. *Erwinia* species are often associated with plants as phytopathogens (Madigan et al., 2009) and may infect diverse fruits and vegetables (Aremu and Babalola, 2015; Farrar et al., 2000; Moretti et al., 2011). Therefore, it is reasonable to assume that introduction of plant pathogens such as *Erwinia* species can occur via infected carrots or apples that are provided in the diet of mealworms as a source of moisture. In a previous study on the microbial communities in mealworm-derived products, *Erwinia* was also detected (Stoops et al., 2017; relative abundance of 11%). Besides plant pathogens, *Erwinia* spp. have also been reported as spoilage organisms, but so far, no reports are available about potential human health risks (Madigan et al., 2009).

When screening the list of OTUs for possible human (food) pathogens, mealworm rearing company 2 was found to harbour an abundant OTU corresponding to an Enterobacteriaceae species (OTU 14; 22%). While phylogenetic assignment via SINTAX (Table S2, Supporting Information) was not conclusive on genus level (assignment score of 0.28), BLAST analysis against GenBank (Table S3, Supporting Information) related this OTU to the genus *Cronobacter* with 96-98% sequence identity (248/253 to 250/253 bp). Since most *Cronobacter* species are human pathogens (Grim et al., 2013), the consumption of mealworms contaminated with *Cronobacter* might pose a health risk without appropriate processing. Other Enterobacteriaceae OTUs., as well as OTU 33, related to the spore-forming class of Clostridia (Table S3, Supporting Information), might correspond with pathogenic bacteria as well. OTU 33 was also present as one of the most abundant OTUs for mealworms. Spore-forming bacteria are especially important in processing edible insects and for the food industry in general as they are easily maintained after processing (Vandeweyer et al., 2017b).

The other abundant OTUs found in mealworms do not pose severe health risks as food pathogen, but some OTUs such as those associated with *Pseudomonas* might have a role in spoilage of the mealworms as food stuffs. Many *Pseudomonas* spp. are involved in the process of food spoilage (Dijk et al., 2015; Madigan et al., 2009). The fact that some *Pseudomonas* species such as *P. deceptionensis* (OTU 4) and *P. antarctica* (OTU 23) were abundantly found on mealworms and can grow at low temperatures (Carrión et al., 2011; Reddy et al., 2004) holds the risk of spoilage during storage at refrigerator temperatures. Sample MW 3.3 contained up to 50% of those psychrotrophic *Pseudomonas* OTUs, which corresponds with the high psychrotrophic count (9.1 log cfu/g) reported in Vandeweyer et al. (2017a). The results of our and the latter study prove that psychrotrophic *Pseudomonas* spp. can easily colonise mealworms. Good practices (e.g. a proper heating step early in the food production process) are therefore necessary to use insects as a safe food (or feed) ingredient. Proper thermal processing of fresh edible insects will result in a bacterial community dominated by endospores (Vandeweyer et al., 2017b). For human consumption, insect farmers seldom sell fresh (i.e. living) insects. Most of the insects sold on the market were given a blanching or boiling step and/or a (freeze) drying treatment.

While dominant OTUs were less apparent for crickets, OTUs related to *Bacteroides* or *Parabacteroides* (Bacteroidetes) were common and repeatedly present in the most abundant OTUs encountered on crickets. Both genera are commensal intestinal inhabitants of humans and other animals. They were also found in processed crickets by Garofalo et al. (2017). Despite being very dominantly present in e.g. the human large intestine, they can also act as pathogens when occurring outside the gut (Awadel-Kariem et al., 2010; Madigan et al., 2009; Wexler, 2007). Consequently, a good processing practice is necessary for fresh crickets as well before using them as a food/feed ingredient.

4 Conclusions

In this study, culture-independent microbial data were obtained to compare bacterial communities of edible insects from different rearing companies and several production cycles. The results demonstrate that different rearing companies can provide the same insect species with a different bacterial community composition, as was demonstrated especially for mealworms. Even between several production cycles from the same company, a different bacterial community composition could be seen, which was also reflected by culture-dependent microbial counts. For crickets, the bacterial community composition compared between production cycles and rearing companies differed to a much lesser extent than for mealworms. At the same time, mealworms and crickets harbour largely different bacterial communities, but different cricket species are much more alike. Concerning food safety, only a few possible risks were identified as some of the OTUs found could be related to families (e.g. Enterobacteriaceae) or larger taxonomic groups (e.g. Clostridia class) that may contain pathogenic species. Since fungi and viruses, which may have an important role in the overall microbial quality of edible insects as well, are not considered in this study, future research on this topic is desirable.

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Fig. 1 Non-metric multidimensional scaling (NMDS) ordinations composed of the bacterial community composition data for both mealworms (Fig. 1A; stress value of 0.017) and crickets (Fig. 1B; stress value of 0.041). Samples from the same rearing company are represented by the same colour, while extracts from the same production cycle are represented by ■ (cycle 1), ● (cycle 2) or ▲ (cycle 3). The distance between different points on the plot reflects their similarity level: the more similar the bacterial communities, the smaller the distance between the points. Plots were constructed based on the bacterial community data for the most abundant OTUs, altogether representing 50% of the sequence data obtained.

Fig. 2 Bacterial community composition at OTU level for mealworms from three rearing companies. Only the most abundant OTUs (i.e. with > 2% sequence abundance) are indicated. All other OTUs were grouped together in "Other OTUs". Data represent mean values of two extracts per sample from three samples per rearing company. Error bars represent standard deviations per OTU.

Fig. 3 Bacterial community composition at OTU level for crickets from three rearing companies. Only the most abundant OTUs (i.e. with > 2% sequence abundance) are indicated. All other OTUs were grouped together in "Other OTUs". Data represent mean values of two extracts per sample from two (company 5) or three samples (companies 6 and 7) per rearing company. Error bars represent standard deviations per OTU.

Fig. 4 Distribution of the relative abundance (%) of the major bacterial phyla in the investigated insect species. Data represent mean values of two extracts per sample from three

(banded crickets), five (house crickets), eight (crickets) or nine samples (mealworms) investigated. Only the most abundant phyla (i.e. with > 2% sequence abundance) are indicated. Sequences assigned to less abundantly present phyla were grouped together in "Other phyla". Sequences without reliable assignment on phylum level were grouped in "Unclassified".

Fig. 5 Bacterial community composition at OTU level for mealworms and crickets. Only the most abundant OTUs (i.e. with > 2% sequence abundance) are indicated. All other OTUs were grouped together in "Other OTUs". Data represent mean values of two extracts per sample from eight (crickets) or nine (mealworms) samples per insect type. Error bars represent standard deviations per OTU.

Tables

Table 1 Samples investigated in this study[1]

Sample ID	Rearing company	Production cycle	Sampling month (2015)	Insect type	Species	Rearing purpose (human/pet food)
MW 1.1	1	1	March	Mealworm	*T. molitor*[2]	Human food
MW 1.2	1	2	May	Mealworm	*T. molitor*	Human food
MW 1.3	1	3	September	Mealworm	*T. molitor*	Human food
MW 2.1	2	1	March	Mealworm	*T. molitor*	Human food
MW 2.2	2	2	June	Mealworm	*T. molitor*	Human food
MW 2.3	2	3	October	Mealworm	*T. molitor*	Human food
MW 3.1	3	1	May	Mealworm	*T. molitor*	Pet food
MW 3.2	3	2	July	Mealworm	*T. molitor*	Pet food
MW 3.3	3	3	November	Mealworm	*T. molitor*	Pet food
CR 1.2	5	2	June	Cricket	*A. domesticus*[3]	Human food
CR 1.3	5	3	September	Cricket	*A. domesticus*	Human food
CR 2.1	6	1	April	Cricket	*A. domesticus*	Human food
CR 2.2	6	2	July	Cricket	*A. domesticus*	Human food
CR 2.3	6	3	October	Cricket	*A. domesticus*	Human food
CR 3.1	7	1	August	Cricket	*G. sigillatus*[4]	Human food
CR 3.2	7	2	October	Cricket	*G. sigillatus*	Human food
CR 3.3	7	3	December	Cricket	*G. sigillatus*	Human food

[1]Table adjusted from Vandeweyer et al., 2017a
[2]T.: *Tenebrio*; [3]A.: *Acheta*; [4]G.: *Gryllodes*.

Table 2 Diversity indices for the mealworm and cricket samples investigated in this study[1]

	Rearing company	Sample ID	Observed richness	Chao1	Coverage (%)[2]	Shannon - Wiener
Mealworms	1	MW 1.1	56 ± 14[a]	75.85 ± 16.29[a]	73.52 ± 2.85[a]	1.82 ± 0.03[a]
		MW 1.2	68 ± 4[a]	86.67 ± 10.97[a]	78.25 ± 5.83[a]	1.91 ± 0.02[a]
		MW 1.3	74 ± 1[a]	97.80 ± 3.96[a]	75.76 ± 4.51[a]	1.84 ± 0.01[a]
		Mean	**66 ± 9[A]**	**86.77 ± 10.97[A]**	**75.84 ± 2.37[A]**	**1.86 ± 0.04[A]**
	2	MW 2.1	110 ± 19[a]	121.81 ± 22.36[a]	89.97 ± 0.84[a]	2.16 ± 0.09[a]
		MW 2.2	151 ± 3[a]	158.13 ± 5.76[a]	95.52 ± 1.69[a]	2.33 ± 0.07[a]
		MW 2.3	117 ± 4[a]	127.72 ± 8.09[a]	91.31 ± 3.01[a]	2.56 ± 0.16[a]
		Mean	**126 ± 22[B]**	**135.89 ± 19.49[B]**	**92.27 ± 2.90[B]**	**2.35 ± 0.20[B]**
	3	MW 3.1	88 ± 4[a]	97.67 ± 3.30[a]	90.23 ± 7.39[a]	1.76 ± 0.00[a]
		MW 3.2	110 ± 31[a]	120.95 ± 35.29[a]	91.07 ± 0.85[a]	2.38 ± 0.15[b]
		MW 3.3	83 ± 1[a]	95.13 ± 0.01[a]	87.25 ± 1.47[a]	1.87 ± 0.00[a]
		Mean	**94 ± 14[C]**	**104.59 ± 14.23[A]**	**89.51 ± 2.01[B]**	**2.00 ± 0.33[A]**
	Mean MW[3]		**95 ± 30**	**109.08 ± 24.86**	**85.87 ± 8.80**	**2.07 ± 0.26**
Crickets	5	CR 1.2	248 ± 4[a]	265.77 ± 0.48[a]	93.31 ± 1.76[a]	4.06 ± 0.02[a]
		CR 1.3	288 ± 18[a]	299.47 ± 12.92[a]	96.13 ± 1.99[a]	4.07 ± 0.01[a]
		Mean	**268 ± 28[A]**	**282.62 ± 23.82[A]**	**94.72 ± 1.99[A]**	**4.06 ± 0.01[A]**
	6	CR 2.1	352 ± 25[a]	362.75 ± 24.40[a]	97.02 ± 0.49[a]	4.48 ± 0.01[a]
		CR 2.2	320 ± 11[a]	328.32 ± 18.76[a]	97.53 ± 2.13[a]	4.43 ± 0.04[a]
		CR 2.3	363 ± 25[a]	378.75 ± 27.05[a]	95.72 ± 0.30[a]	4.43 ± 0.07[a]
		Mean	**345 ± 22[B]**	**356.61 ± 25.77[B]**	**96.76 ± 0.93[A]**	**4.45 ± 0.03[B]**
	7	CR 3.1	334 ± 16[a]	341.60 ± 20.94[a]	97.82 ± 1.44[a]	4.22 ± 0.01[a]
		CR 3.2	314 ± 8[a]	326.37 ± 10.97[a]	96.22 ± 0.63[a]	4.07 ± 0.05[a]
		CR 3.3	330 ± 1[a]	349.21 ± 15.67[a]	94.58 ± 3.84[a]	4.13 ± 0.10[a]
		Mean	**326 ± 11[B]**	**339.06 ± 11.63[B]**	**96.21 ± 1.62[A]**	**4.14 ± 0.07[A]**
	Mean CR[3]		**313 ± 40**	**326.10 ± 38.66**	**95.89 ± 1.05**	**4.22 ± 0.20**

[1]Data are the mean values of two analysed DNA-extracts from the same sample ± standard deviations; [a,b]Means per production cycle and per rearing company with the same superscript (small letter) within the same columns do not differ significantly ($p > 0.05$) [A,B]Means per rearing company per insect (bold) with the same superscript (capital) within the same column do not differ significantly ($p > 0.05$).

[2]Coverage = (Observed richness/Chao1 estimate) * 100.

[3]All mean indices for mealworms are strongly significantly different from those calculated for crickets ($p = 0.000$).

Figure 1

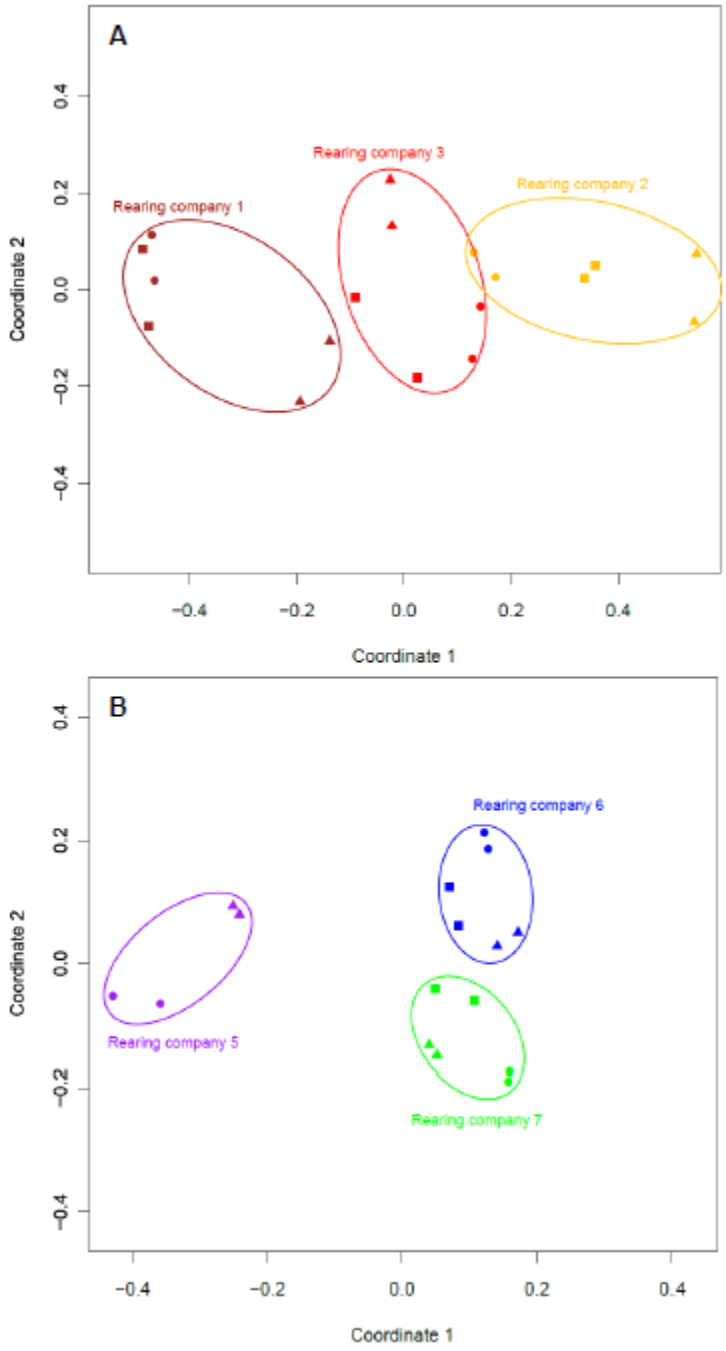


Figure 2

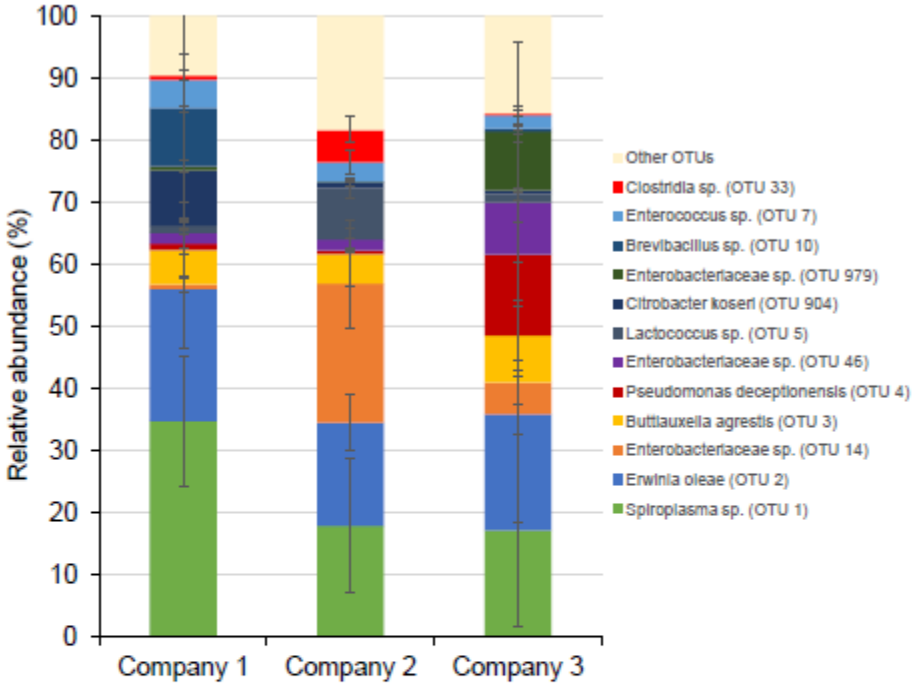


Figure 3

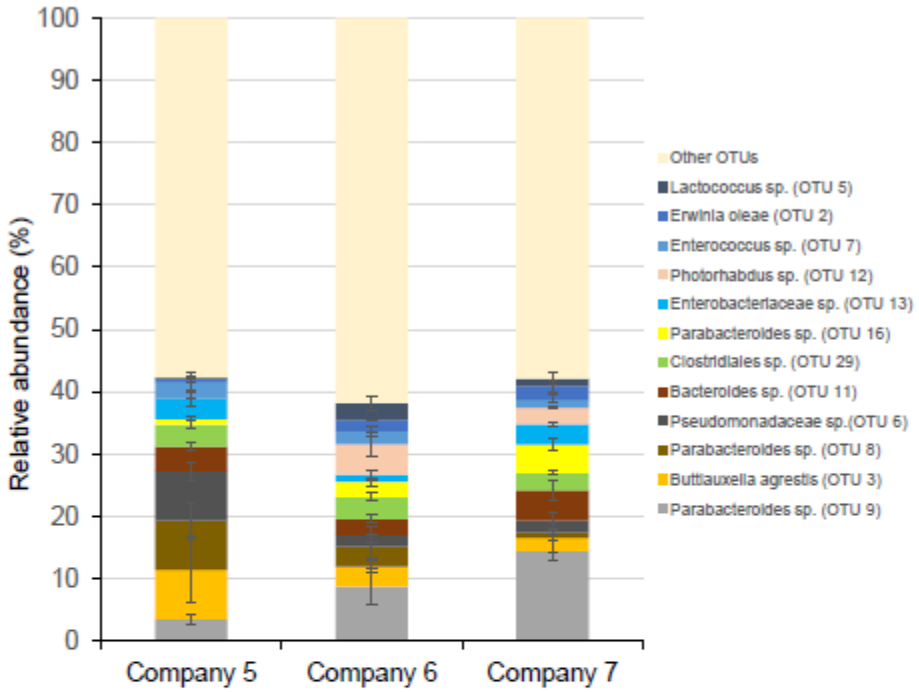


Figure 4

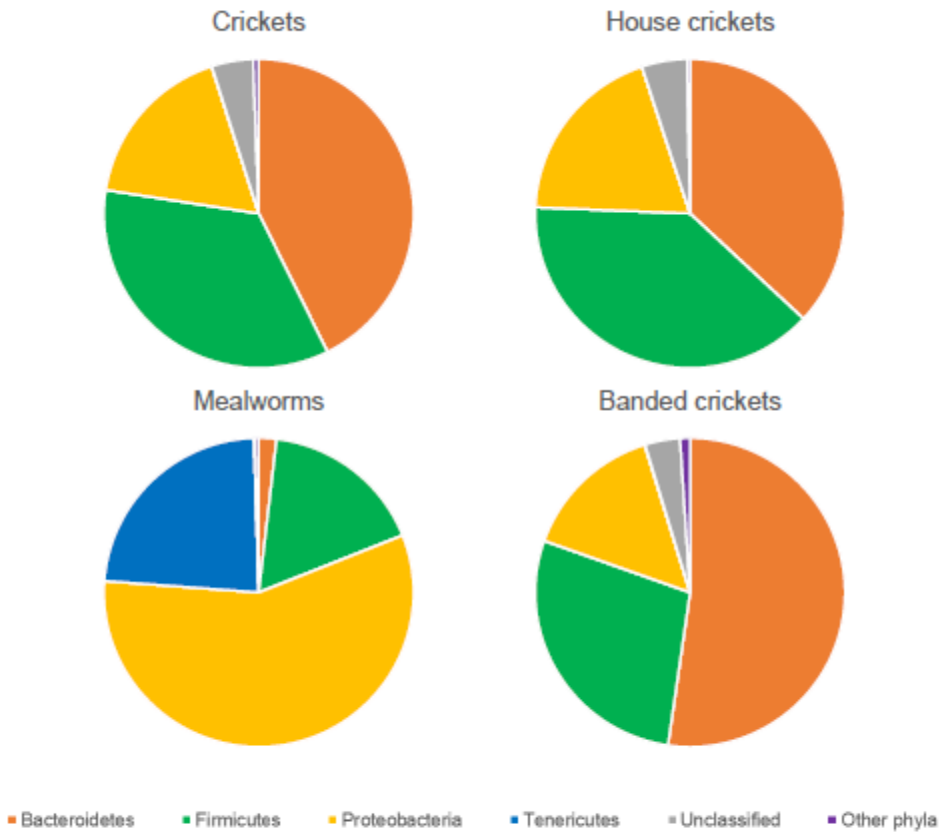
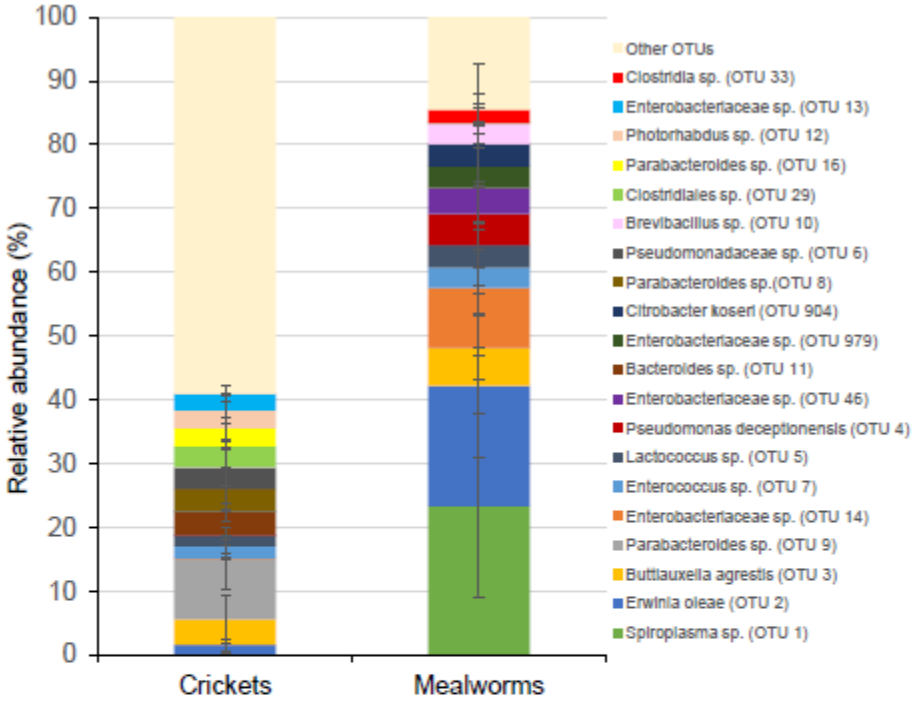


Figure 5



Highlights

- Bacterial community of 17 production cycles and 6 rearing companies was compared
- Metagenetic analysis of fresh edible crickets was performed for the first time
- Mealworm community compositions differ between companies and production cycles
- Cricket community compositions are less variable, even between different species
- Very few sequences detected correspond to potential food pathogens