Bacterial community of a spider, *Marpiss magister* (Salticidae)

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Abstract Arthropods are associated with various microorganisms which confer benefits to their hosts. Recently, research has been conducted on bacterial communities of insects to provide an insight into the potential interactions of the symbiotic bacteria and their hosts. Spiders are interesting to study as they are perceived to be natural enemies of pests. The effect of endosymbionts on spiders has been reported, but little is known about the overall bacterial communities present in spiders. Here, we report on the characterization of bacterial communities present in the whole body of the spider *Marpiss magister* using Illumina sequencing of 16S rRNA amplicons. Our study shows that the most abundant phyla of bacteria included Proteobacteria, Tenericutes, Bacteroidetes and Actinobacteria. At the genus level, the most abundant genera included *Rickettsia*, *Wolbachia*, *Spiroplasma*, and *Cardinium*. Besides these dominant endosymbionts, our study also showed the existence of bacteria in the genera *Arthrobacter*, *Novosphingobium*, *Acinetobacter*, *Pseudomonas*, *Aquabacterium* and *Sphingomonas* at an abundance ranging from 0.65 to 0.84%, and the existence of bacterial in genera *Lactobacillus*, *Sphingobium*, *Methylobacterium*, *Bradyrhizobium*, *Propionibacterium*, *Brevundimonas*, *Achromobacter*, *Microbacterium*, *Corynebacterium*, and *Flavobacterium* at a slightly lower

abundance ranging from 0.1 to 0.5%. Therefore, our finding indicates that endosymbionts are not the only microbiota present in the spider *M. magister*, and other bacterial taxa also exist in its bacterial community.

Keywords Bacterial communities · Endosymbionts · *Marpiss magister* · Illumina sequencing

Introduction

Research on insects and their bacterial communities has been undertaken to gain insights into the potential interaction of the hosts and their symbiotic bacteria (Bili et al. 2016; Monteiro et al. 2016; Fryxell and DeBruyn 2016). Studies have shown that symbiotic bacteria affected the fitness of host or conferred benefits to their hosts in most of the insects and members of Arachnoidea (Gotoh et al. 2007; Brownlie et al. 2009; Himler et al. 2011). Spiders are regarded as natural enemies of agricultural pests, and studies have shown that bacteria in members of the genera *Wolbachia*, *Rickettsia*, *Cardinium*, and *Spiroplasma* were found with high incidence in spiders (Goodacre et al. 2006; Duron et al. 2008; Martin and Goodacre 2009). These studies have focused mainly on the host's reproductive abnormalities after they were infected with the dominant endosymbionts. For instance, endosymbiont bacteria, such as *Wolbachia* and *Rickettsia* could alter sex ration through male-killing in the dwarf spider *Oedothorax retusus* (Erigoninae) (Vanthournout et al. 2014). Parthenogenesis was observed in the spider *Theotima minutissimus* (Ochyroceratidae) that were infected with *Wolbachia* (Edwards et al. 2003), and cytoplasmic incompatibility was also observed in *Habronattus pugillis* (Salticidae) (Masta and Maddison 2002). The studies by Vanthournout and

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Hendrickx (2015) on the dwarf spider *Oedothorax gibbosus*, showed that the bacterial community was dominated by four endosymbionts, which included members of the genera *Rhabdochlamydia*, *Cardinium*, *Wolbachia*, and *Rickettsia*, and that gut microbiota and environmental bacteria were absent in this spider.

Because of the short cycle, low-cost, high throughput and high accuracy, next-generation sequencing (NGS) techniques has become a popular tool for studies on microbial diversity (Caporaso et al. 2012; Kozich et al. 2013). Basing on the little insight into bacterial communities of spiders, we have used NGS to study the bacterial communities present in a field-collected adult spider, *Marpiss magister*, and we used our data to illustrate an overview of bacterial communities in spiders.

Materials and methods

Samples

Adult spider samples of *M. magister* were collected in Huazhong Agricultural University, Wuhan, China. After sampling, all samples were transported to the laboratory and were identified by morphological characteristics, and stored at -80°C until analyzed. *M. magister* is identified as a non-endangered and non-protected species.

DNA extraction and 16S rRNA gene amplicon sequencing

Each sample was cleaned using an ultrasonic cleaner (FRQ-1004T) filled with 75% alcoholic solution for 1–2 min to remove surface bacteria and pollutants, followed by three washes with sterile ultrapure water. Total DNA from each sample (the whole body of the spider) was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. The DNA concentration of each sample was determined using a Nano Photometer (NanoPhotometer NP80 Touch, Impln GmbH). Equimolar amount of DNA from two individuals of the same species were combined to create DNA pools. The mixed DNA was stored at -20°C until used.

The V3–V4 high variable region of the 16S rRNA gene was amplified using the primers (338F: 5'-ACTCCTACGG-GAGGCAGCA-3' and 806R: 5'-GGACTACHVGGGTWTC-TAAT-3'). The samples were sequenced in an Illumina MiSeq platform by BioMarKer Technologies, Beijing, China.

Bioinformatic processing and statistical analysis

Reads were merged using FLASH (Magoč and Salzberg 2011). Spliced sequences were filtered under specified screening

conditions by Trimmomatic (Bolger et al. 2014). Chimeras were removed using UCHIME (Edgar et al. 2011). The remaining sequences were clustered into Operational Taxonomic Units (OTUs) based on a 97% similarity using UCLUST (Edgar 2010). Venn Graph was drawn to show the common as well as the different OTUs from each sample. Taxonomy was assigned using ribosomal database project (RDP) classifier (Cole et al. 2009) via comparing representative sequences of OTU and reference sequences selected in Silva (Quast et al. 2013). Richness estimators and diversity indexes of microbiota associated with each sample were calculated.

Results

The microbial diversity in *M. magister* hosts

All Clean-Tags were classified into 208 unique OTUs at 97% sequence similarity. The Venn graph showed that there were 46 common OTUs amongst the three samples of *M. magister* studied (N1, N2 and N3) (Fig. 1). Each sample had its own OTUs, and the OTU number of N1, N2 and N3 were 26, 30 and 50, respectively. The OTU number of three spider samples ranged from 102 to 139, and the Alpha-diversity indices showed that the bacterial communities of *M. magister* had a high diversity (Table 1).

The composition of bacterial community in *M. magister*

All OTUs belonged to 13 phyla, 20 classes, 42 orders, 77 families and 117 genera. The most abundant phyla of

Fig. 1 The Venn graph of OTUs in *M. magister*. Each color represents one sample, the overlap-area represents common OTUs, the non overlap-area represents specific OTUs

Table 1 Richness estimators and diversity indexes of microbiota associated with each sample

Sample ID ^a	OTU	ACE	Chao1	Simpson	Shannon
N1	102	105.95	107.62	0.86	0.47
N2	115	118.93	120.00	0.28	1.59
N3	139	143.71	148.00	0.16	2.95

^aN1, N2 and N3 indicate three DNA pools **Fig. 2** The relative abundance of dominant bacterial taxa at different level in *M. magister*

bacteria found in *M. magister* included Proteobacteria (54.71%), Tenericutes (32.08%), Bacteroidetes (9.18%), and Actinobacteria (2.95%), together accounting for 98.92% of the total reads (Fig. 2a). Alphaproteobacteria (46.25%), Betaproteobacteria (5.99%) and Gammaproteobacteria (2.41%) were the most abundant classes among Phylum Proteobacteria (Fig. 2b). Mollicutes (32.08%), Cytophagia (8.73%) and Actinobacteria (2.91%) were also the dominated classes in Tenericutes, Bacteroidetes and Actinobacteria respectively (Fig. 2b). At a family level, the most abundant family was Spiroplasmataceae with a relative abundance of 32.08% of the total reads, followed by Rickettsiaceae (16.84%), Anaplasmataceae (15.63%), and Flammeovirgaceae (8.73%) (Fig. 2c). The other bacterial families with the relative abundance of more than 1% were Comamonadaceae (5.49%),

Sphingomonadaceae (1.79%), Rhodobacteraceae (1.12%), Moraxellaceae (1.07%) and Micrococcaceae (1.02%). The dominant bacterial groups detected at a genus level included *Spiroplasma* (32.08%), *Rickettsia* (16.84%), *Wolbachia* (15.63%) and *Cardinium* (8.73%) (Fig. 2d). Besides endosymbionts, 6 bacterial genera, including *Arthrobacter* (0.84%), *Novosphingobium* (0.82%), *Acinetobacter* (0.70%), *Pseudomonas* (0.68%), *Aquabacterium* (0.65%) and *Sphingomonas* (0.65%) were found in the bacterial communities of *M. magister* (see Table 2), and 21 other genera, which included *Lactobacillus*, *Sphingobium*, *Methylobacterium*, *Bradyrhizobium*, *Propionibacterium*, *Brevundimonas*, *Achromobacter*, *Microbacterium*, *Corynebacterium*, *Flavobacterium* were also found in *M. magister* with a relative abundance ranging from 0.1 to 0.5% (see Table 2).

Table 2 The other bacterial (except dominated endosymbionts) taxa and their relative abundance in *M. magister*

Phylum	Class	Family	Genus	Relative abundance
Proteobacteria	Alphaproteobacteria	Sphingomonadaceae	<i>Novosphingobium</i>	0.82%
			<i>Sphingomonas</i>	0.65%
			<i>Sphingobium</i>	0.33%
		Methylobacteriaceae	<i>Methylobacterium</i>	0.23%
		Bradyrhizobiaceae	<i>Bradyrhizobium</i>	0.17%
		Caulobacteraceae	<i>Brevundimonas</i>	0.11%
	Betaproteobacteria	Comamonadaceae	<i>Aquabacterium</i>	0.65%
			<i>Variovorax</i>	0.11%
		Alcaligenaceae	<i>Achromobacter</i>	0.24%
		Methylophilaceae	<i>Methylothera</i>	0.11%
	Gammaproteobacteria	Moraxellaceae	<i>Acinetobacter</i>	0.70%
			<i>Perluclidibaca</i>	0.30%
		Pseudomonadaceae	<i>Pseudomonas</i>	0.68%
		Nevskiaceae	<i>Hydrocarboniphaga</i>	0.14%
Actinobacteria	Actinobacteria	Micrococcaceae	<i>Arthrobacter</i>	0.84%
			<i>Kocuria</i>	0.15%
		Propionibacteriaceae	<i>Propionibacterium</i>	0.32%
		Geodermatophilaceae	<i>Modestobacter</i>	0.27%
		Microbacteriaceae	<i>Microbacterium</i>	0.21%
		Corynebacteriaceae	<i>Corynebacterium</i>	0.16%
		Intrasporangiaceae	<i>Terrabacter</i>	0.13%
		Tsukamurellaceae	<i>Tsukamurella</i>	0.13%
		Phycisphaeraceae	<i>SM1A02</i>	0.24%
Planctomyces	Phycisphaerae	Phycisphaeraceae	<i>SM1A02</i>	0.24%
Firmicutes	Bacilli	Staphylococcaceae	<i>Staphylococcus</i>	0.20%
		Lactobacillaceae	<i>Lactobacillus</i>	0.15%
Bacteroidetes	Flavobacteriia	Flavobacteriales	<i>Cloacibacterium</i>	0.17%
			<i>Flavobacterium</i>	0.12%

The dominant OTU types across four kind of endosymbionts

Six different OTUs was detected for *Wolbachia*, among which, OTU182 was the most dominant OTU, with its relative abundance of total sequences being 13.70%. Followed by OTU182, OTU113629 was with a relative abundance of 1.92%. For the *Spiroplasma* endosymbiont, OTU678954 was the most dominant with a relative abundance of 32.06%, and OTU125206 presented a relative abundance of 0.02%. In addition, OTU62246 and OTU87695 present were the main OTU types in *Rickettsia* symbionts and *Cardinium*, respectively, exhibiting the high relative abundance of 16.84 and 8.73% (Table 3). The relative abundance of other OTU types in Table 2 was less than 0.01%.

Discussion

In our studies, the bacterial communities in the body of the spider *M. magister* were analyzed with the method of high throughput sequencing. The results showed Proteobacteria,

Tenericutes, Bacteroidetes, and Actinobacteria were the dominant phyla in *M. magister*, and endosymbionts (i.e., *Wolbachia*, *Cardinium*, *Spiroplasma* and *Rickettsia*) dominated the bacterial communities. In addition, we have also found other bacteria suspected of gut microbiota and environmental bacteria in the body of *M. magister*, such as *Novosphingobium*, *Sphingomonas*, *Arthrobacter*, *Pseudomonas*, *Acinetobacter*, *Lactobacillus*, *Sphingobium*, *Methylobacterium*, *Bradyrhizobium*, *Propionibacterium*, *Brevundimonas*, *Achromobacter*, *Microbacterium*, *Corynebacterium*, and *Flavobacterium* (see Table 2). Although the relative abundance of these bacteria was lower than that of the dominated bacteria, our experiments indicated that besides endosymbionts, other bacteria including gut microbiota and environmental bacteria also existed in spiders.

It has been reported that *Cardinium*, *Spiroplasma*, *Rickettsia* and *Wolbachia* were detected in spiders from family Agelenidae, Linyphiidae, Araneidae, and Tetragnathidae, and endosymbionts such as *Wolbachia* and *Cardinium* were also detected in spiders of the family

Table 3 The OTU types and their relative abundance in four symbiotic bacteria

<i>Wolbachia</i>	<i>Spiroplasma</i>	<i>Rickettsia</i>	<i>Cardinium</i>
OTU182 (13.70%)			
OTU28984 (< 0.01%)	OTU67894 (32.06%)	OTU62246 (16.84%)	OTU87695 (8.73%)
OTU45118 (< 0.01%)			
OTU86859 (< 0.01%)			
OTU113629 (1.92%)	OTU125206 (0.02%)	OTU7005 (< 0.01%)	
OTU117532 (< 0.01%)			

The number in parentheses indicates the relative abundance among all sequences for each OTU

Salticidae (Goodacre et al. 2006; Martin and Goodacre 2009; Vanthournout and Hendrickx 2015). In our studies, *Spiroplasma*, *Rickettsia*, *Wolbachia* and *Cardinium* were dominated groups in *M. magister* at a genus level. Besides *Wolbachia* and *Cardinium*, *Rickettsia* and *Spiroplasma* were also detected in spiders of family Salticidae in this study, suggesting that endosymbionts (such as *Cardinium*, *Spiroplasma*, *Rickettsia*, and *Wolbachia*) were the dominant bacteria in most taxa of spiders, and the co-infection of endosymbionts in spiders is common.

Other than the four dominated endosymbionts, other bacteria also existed in the bacterial community of *M. magister* (Table 2). Our results showed that the relative abundance of *Arthrobacter*, *Novosphingobium*, *Acinetobacter*, *Pseudomonas*, *Aquabacterium* and *Sphingomonas* in the bacterial community was in the range of 0.65–0.84%, and the abundance of *Lactobacillus*, *Sphingobium*, *Methylobacterium*, *Bradyrhizobium*, *Propionibacterium*, *Brevundimonas*, *Achromobacter*, *Microbacterium*, *Corynebacterium*, and *Flavobacterium* were in the range of 0.1–0.5%. *Novosphingobium*, *Pseudomonas*, *Corynebacterium*, *Acinetobacter*, *Arthrobacter*, *Lactobacillus*, *Sphingobium*, *Bradyrhizobium*, *Brevundimonas*, *Propionibacterium* and *Microbacterium* have been detected in the gut of some insects (Wang et al. 2011; Gupta et al. 2014; Snyman et al. 2016; Anjum et al. 2017). Although the relative abundance of these bacteria was extremely less than those of the dominant endosymbionts in *M. magister*, they were verified to be existing in the bacterial community of a spider in our studies. Gut microbiota of insects potentially provide many beneficial services to their hosts, such as upgrading nutrient—poor diet, aiding digestion, protecting from predators, parasites and pathogens, and governing mating and reproductive systems (Engel and Moran 2013). Our experiments have not dissected the digested tract and tested the gut microbiota of *M. magister*, but our results suggested that gut bacteria were the pivotal constituent part in the microbiota of spider. Therefore, detecting gut microbiota in spiders, and seeking the relationships between gut bacteria and their

spider hosts, will be a vital portion of symbionts research in spiders.

Although several endosymbionts have been widely distributed in spider species, little information was available about the interaction between endosymbionts and the spider hosts. The variation of reproductive phenotypes, such as cytoplasmic incompatibility, parthenogenesis and sex ratio distortion has been reported in some species of Araneae (Martin and Goodacre 2009). Among these, primary sex ratio distortion was found in the families of Linyphiidae, Eresidae and Theridiidae, and parthenogenesis was found in the families of Dysderidae, Ochyroceratidae and Amaurobiidae, and the cytoplasmic incompatibility was only found in *Habronattus pugillus*, a spider belongs to the family of Salticidae. Absence of males or strongly biased sex ratio was recorded in Symphytognathidae and Araneidae. As to the mechanism of the reproductive phenotype, only Edwards et al. (2003) reported that it was possible that *Wolbachia* caused the parthenogenesis in *Theotima minutissimus* (Ochyroceratidae). Therefore, maybe the connection between reproductive phenotypes and symbionts infection in spiders would be a way to reveal the mechanism of spider's reproductive abnormalities. In our study, *Spiroplasma*, *Rickettsia*, *Wolbachia* and *Cardinium* were the dominated groups in *M. magister*. However, the variation of reproductive phenotype in *M. magister* remains unknown, which may be studied in future research. It can be believed that the study of the relationship between reproductive phenotypes and endosymbionts co-infection in spiders would strengthen our current understanding of the importance of endosymbionts to the life of arthropods.

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Author contributions Yueli Yun and Guimin Zhang designed the experiments. Lihua Zhang, Yueli Yun and Yu Peng conducted the experiments and data analysis. Yueli Yun and Lihua Zhang wrote the manuscript.

Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest in the publication.

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