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Bacterial taxa associated with the hematophagous mite *Dermanyssus gallinae* detected by 16S rRNA PCR amplification and TTGE fingerprinting

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Abstract

Dermanyssus gallinae (Arthropoda, Mesostigmata) is suspected to be involved in the transmission of a wide variety of pathogens, but nothing is known about its associated non-pathogenic bacterial community. To address this question, we examined the composition of bacterial communities in *D. gallinae* collected from standard poultry farms in Brittany, France. Genetic fingerprints of bacterial communities were generated by temporal temperature gradient gel electrophoresis (TTGE) separation of individual polymerase chain reaction (PCR)-amplified 16S rRNA gene fragments, followed by DNA sequence analysis. Most of the sequences belonged to the Proteobacteria and Firmicute phyla, with a majority of sequences corresponding to the Enterobacteriales order and the *Staphylococcus* genus. By using statistical analysis, we showed differences in biodiversity between poultry farms. We also determined the major phylotypes that compose the characteristic microbiota associated with *D. gallinae*. Saprophytes, opportunistic pathogens and pathogenic agents such as *Pasteurella multocida*, *Erysipelothrix rhusiopathiae* and sequences close to the genus *Aerococcus* were identified. Endosymbionts such as *Schineria* sp., *Spiroplasma* sp. Anistosticta, ''*Candidatus* Cardinium hertigii'' and *Rickettsiella* sp. were also present in the subdominant bacterial community. Identification of potential targets within the symbiont community may be considered in the future as a means of ectoparasite control.

Keywords: Ectoparasite; Bacterial community; Symbionts; Molecular fingerprints

1. Introduction

The poultry red mite *Dermanyssus gallinae* is a hematophagous mite frequently present in breeding facilities, but especially in laying hen facilities. This arthropod can be responsible for anemia, dermatitis, weight loss and a decrease in egg production [15]. It is difficult to eradicate due to its particular biological characteristics in poultry farms; short life cycle, high resistance to starvation, numerous available hiding places and suspected resistance to pesticides [5]. In addition to poultry species, this mite is able to parasitize a wide range of other birds and can also bite mammals such as horses, rodents and humans [2]. This hematophagous ectoparasite is also involved in transmission of many pathogenic agents responsible for serious diseases in both animals and humans [27,28]. Unfortunately, studies dealing with the vectorial role of Dermanyssoidea are few and their scope has been limited, which

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could lead to an underestimation of the importance of this agent in the transmission of pathogens. Consequently, the poultry red mite is a real emerging problem which must not be ignored.

The interaction between arthropod hosts, particularly acari and their non-symbiotic microbiota, has not been widely studied, although they could play a role both in mite physiology and in turkey health status. In ticks, it has been demonstrated that endosymbionts can establish a relationship with pathogenic bacteria [18], and manipulation of endosymbionts has been proposed as a novel means of preventing insects from vectoring diseases [1]. Consequently, knowledge of the bacterial community associated with *D. gallinae* and the roles that the different components of this community play in the biology of the mite could lead to new biotechnological approaches and targets for biological control.

Most studies have examined insect microbiota by isolating and culturing its constituent organisms. However, only a small percentage of gut microbes can be detected by cultivation, as already observed with the human gut [25]. To gain access to the fraction that cannot be cultured, molecular methods such as denaturating gradient gel electrophoresis (DGGE) and temporal temperature gel electrophoresis (TTGE) have been developed using amplification by polymerase chain reaction (PCR) of the 16S rRNA gene. These methods have already proven to provide sufficient and reproducible information about changes in the community structure as a whole [17] and have been used on various environmental and food samples in recent years [12,20]. While very useful, they do not provide information on community composition, unless combined with sequence analysis.

Given the status of *D. gallinae* as a pest in poultry production and the potential for manipulating bacterial communities as a form of pest management, knowledge of the bacterial taxa associated with the mite is of importance in several basic and applied fields. The goal of this study was to provide an overview of bacteria in *D. gallinae* and to determine the most common bacterial community associated with mites originating from different standard poultry farms.

2. Materials and methods

2.1. Mite samples

The populations of *D. gallinae* were collected on metallic structures of cages from 16 standard laying hen-breeding facilities in the Brittany region (France) that corresponded to standardized industrial practices. All farms were constantly monitored by veterinarians and had not undergone recent episodes of disease outbreaks of more than 2% spontaneous mortality. The mites were kept in plastic tubes until there was no sign of continuing digestion and then manipulated with a paint brush and a suction pump attached to a collector. Only adult mites were used for analysis. For each farm, 15 individual mites and 5 pools of 10 mites were randomly selected for analysis. Surface disinfection of mite cuticles was first performed to ensure subsequent detection of the internal bacterial community only [28].

2.2. DNA extraction from D. gallinae

To extract genomic DNA, mites were mechanically crushed with sterile piston pellets. Individual mites and pooled mites were crushed in 20 and 40 μL PBS, respectively and DNA was extracted from homogenates as already described [8], with the Nucleospin extraction kit II (Macherey Nagel, Hoerdt, France) following tissue protocol provided by the manufacturer, but with some adjustments due to the weight of a single *D. gallinae* (about 11 mg). The amounts of DNA obtained from individual animals was monitored and normalized prior to but not after PCR.

2.3. PCR amplification of 16S rRNA fragments

A fragment of approximately 180 bp of the bacterial conserved V3 region of 16S rRNA was amplified with a broad-range bacterial primer set 350F-GC (5′- CGCCCGCCGCG CGCGGCGGGCGGGGCGGGGGCCGGGGGGCTCCTACG GGAGGCAGCAGT-3′) and PC535 (5′-GTATTACCGCGGC TGCTGGCA-3′) from all DNA extracts.

PCR reactions were performed in 50 μL volumes containing 5 μL of 10× buffer (Invitrogen, Carlsbad, CA, USA), 2 mM of $MgCl_2$ (Invitrogen), 0.8 pmol/μL of each nucleotide primer (Invitrogen), 0.2 mM of each dNTP (Invitrogen), 0.5 μL of 100× bovine serum albumin (Promega, Charbonnières-Les-Bains, France), 2 U of recombinant Taq polymerase (Invitrogen) and 5 μL of the DNA extract, and made up to 50 μL with sterile water. PCR amplifications were performed in an MWG thermal cycler (Biotech, Ebersberg, Germany), with a cycling program that consisted of an initial denaturation step (10 min, 95 °C) followed by 30 denaturation cycles (1 min each, 95 °C), annealing (1 min, 54.9 °C) and extension (1 min, 72 °C) and a final extension cycle (10 min, 72 °C). Negative and positive controls were added.

2.4. Temporal temperature gel electrophoresis analysis

16S rRNA fingerprints of the bacterial community present in sample mites were performed by TTGE using the Dcode universal mutation detection system (Bio-Rad, Marnes-la-Coquette, France) with gels of 16 cm × 16 cm × 1 mm. Polyacrylamide (9% w/vol) gels were prepared and run with 1× TAE running buffer (Eppendorf, Hamburg, Germany) and a final urea concentration of 7 M. Running conditions were at constant voltage for 16 h with temperature gradient from 63 to 70.5 °C at a constant temperature increment of 0.4 °C/h. The TTGE gels were loaded with 10 μL of PCR products obtained from V3 region amplification. After completing electrophoresis, gels were incubated using the sensitive SYBR green nucleic acid gel staining method (Roche Diagnostics, Mannheim, Germany).

To allow standardization of band migration, 2 μL of 16S rRNA PCR products derived from seven different bacterial species were pooled together and the total sample was used as a marker for TTGE analyses. This external marker consisted of both pathogenic bacteria and bacteria with specific GC

contents: *Borrelia garinii, Pasteurella multocida*, *E. rhusiopathiae*, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serovar Enteritidis and *Mycobacterium avium*.

2.5. Sequencing of TTGE fragments and sequence analyses

TTGE bands were excised directly from the gels with a sterile scalpel. They were allowed to diffuse overnight at 4 °C in 100 µL of sterile water. Three microliters of eluate from individual bands were used to reamplify PCR products using the same bacterial primer set, except that primer 350F did not contain the GC clamp. Reaction conditions were the same as those described above. PCR products were directly purified using a Nucleospin extract II kit (Macherey Nagel, Hoerdt, France) and sent to a commercial sequencing facility (Genoscreen, Lille, France). Sequences were compared with known sequences listed in the GenBank nucleotide sequence databases. The BLASTN (basic local alignment search tool) search option of the National Center for Biotechnology Information (NCBI) internet site (http://www.ncbi.nlm.nih.gov) was used to identify close evolutionary relatives in the GenBank database. The sequences obtained in this study have been assigned accession numbers EF674483–EF674516 in the database.

Different categories were defined for classifying bacteria detected by TTGE. ''Saprophytic bacteria'' are those for which no species in the genus have been described as being pathogens other than opportunistic pathogens. The term ''strict pathogen'' is applied to bacteria for which most of the species belonging to the genus are pathogens for animals. Bacteria for which most of the species in the genus cause a disease in a compromised host which typically would not occur in a healthy host are considered ''opportunistic pathogens''. The last category used was that of arthropod ''symbionts'', e.g. bacteria which cannot survive outside the arthropod host.

2.6. Analysis of TTGE banding patterns and statistical analyses

Temporal temperature gradient gel electrophoresis banding patterns were analyzed using Quantity One software (Bio-Rad, Marnes-la-Coquette, France). Once the bands in the individual TTGE lanes were aligned, the ''front rate'' was calculated for each band. This parameter is defined as the distance of the fragment to the well, divided by the distance of the slowest bacterial fragment of the marker (*M. avium*) to the well. This parameter was used to generate a table corresponding to the presence (1) or absence (0) of each band in a sample and was performed for each farm [10] (data not showed). The peak surface of each band (n_i) and the sum of all peak surfaces of all bands (N) were then used to calculate community biodiversity using two indices: the Shannon index (H) calculated using the formula $H = -\sum(n_i/N)\ln(n_i/N)$ and the Dominance index (c) calculated using the formula $c = \sum(n_i/N)^2$ [19]. These diversity indices were calculated to describe possible differences between bacterial species [10]. The data were then processed by analysis of variance using the MIXED procedure of SAS v8 (SAS Inst. Inc., Cary, USA). The statistical model included, first, poultry farms and individual mites, and second, poultry farms and pooled mites. Pooled mites or individual mites were considered as a random effect for analysis. Effects were declared significant at $P < 0.05$. Multivariate analysis methods were then used to analyze the TTGE banding patterns table only with data of presence–absence obtained from pooled mites. Principal component analysis (PCA) and between-group analysis (BGA) were used to evidence differences between poultry farms [7,9,29]. Within group analysis (WGA) was then used to remove the mean differences between farms and to extract common dominant bacterial taxa [4,9]. Combined with cluster analysis (complete link), WGA enabled selection of the dominant and common bacterial communities among the different farms analyzed.

3. Results

3.1. Identification of internal bacterial community associated with D. gallinae

DNA was isolated from 320 *D. gallinae* mite samples collected from 16 standard poultry farms and representing 240 individual mites and 80 pools each containing 10 mites. The 16S rRNA genes in these samples were amplified by PCR and the amplicons were analyzed by TTGE. The profiles were normalized using an external marker. For phylogenetic identification, sequences of bacteria found in *D. gallinae* were compared with 16S rRNA sequence information from known bacteria listed in the GenBank databases and classified according to their phyla and family. No chimera sequences were detected using the RDP database project, possibly due to targeting of a short DNA fragment. Out of a total of 58 distinct bands obtained from the 320 profiles analyzed, 37 bands could be assigned to an operational taxonomic unit (OTU) from the results obtained with DNA sequencing (Table 1). Percent identity values between the sequences retrieved from mite samples and sequences already present in GenBank ranged from 88% to 100%. Among these sequences, some contained more than one genotype as very different gene sequences may, upon denaturation, migrate to the same position [20]. This was observed for bands (rf 0.09), (rf 0.13), (rf 0.19), (rf 0.31), rf (0.35), (rf 0.43), (rf 0.53), (rf 0.57) and (rf 0.61). Overall, a total number of 49 OTUs were associated with the 37 sequenced bands. Identified bacteria belonged to the Proteobacteria (44.2%), Firmicutes (42.4%), Actinobacteria (9.6%) and Bacteroidetes (3.8%) phyla, revealing the presence of a variety of different lines of descent. Most of the Proteobacteria detected were Gammaproteobacteria (74%).

According to the bacterial categories defined in Section 2, bacteria were classified as 24 saprophytes, 16 opportunistic pathogens, 4 strict pathogens and 5 arthropod symbionts. Among the strict pathogens, *Aerococcus viridans* (100%), *Aerococcus urinaequi* (100%), *P. multocida* (98%) and *E. rhusiopathiae* (100%) were identified. The saprophytic

Table 1
Phylogenetic affiliation of partial 16S rRNA gene coding sequences of bacteria detected in *D. gallinae* to sequences of closest bacterial relatives listed in the GenBank nucleotide sequence database.

	Phylum		Rf	Bacterial categories	Common bacterial community
Proteobacteria	Alphaproteobacteria	Rhizobiales (99%)	0.61b	sap.	
		Rhizobiales (99%)	0.73	sap.	
		Devosia sp. (100%)	0.75	sap.	x
	Betaproteobacteria	*Alcaligenes faecalis* (100%)	0.34	op. path.	
		Polaromonas aquatica (98%)	0.68	sap.	
	Gammaproteobacteria	*Pseudomonas* sp. (97%)	0.04	op. path.	
		Schineria sp. (98%)	0.09a	symb.	
		Proteus mirabilis, Proteus vulgaris (100%)	0.13b	op. path.	x
		Pasteurella multocida (100%)	0.15	strict path.	x
		Psychrobacter pulmonis, *Psychrobacter psychrophilus* (100%)	0.30	sap.	x
		Proteus mirabilis, Proteus vulgaris (100%)	0.41	op. path.	x
		Proteus mirabilis (99%)	0.43a	op. path.	
		Acinetobacter sp. (98%)	0.43b	sap.	
		Pseudomonas putida, *Pseudomonas japonica* (97%)	0.43c	sap.	
		Rickettsiella-like isolated from *D. gallinae* (91%)	0.46	symb.	
		Delftia acidovorans (98%)	0.47	sap.	
		Rickettsiella endosymbiont of *Folsomia candida* (97%)	0.53b	symb.	
		Escherichia coli (97%)	0.55	op. path.	
		Bacillus sp. (93%)	0.57a	sap.	
		Uncultured soilγProteobacterium (96%)	0.57b	sap.	
		Bacillus sp. (88%)	0.59	sap.	x
		Providencia sp. (99%)	0.62	sap.	
Firmicutes		*Staphylococcus equorum* (100%)	0.03	sap.	x
		Staphylococcus sp. (98%)	0.05	op. path.	
		Vagococus lutrae (98%)	0.08	sap.	x
		Aerococcus viridans (100%)	0.11	strict path.	x
		Aerococcus viridans, *Aerococcus urinaequi* (100%)	0.13a	strict path.	x
		Lactobacillus johnsonii, *Lactobacillus gasseri* (98%)	0.13c	sap.	x
		Spiroplasma sp. Anisosticta (98%)	0.14	symb.	
		Staphylococcus lentus, Staphylococcus pulvereri (98%)	0.16	op. path.	
		Staphylococcus sp. (96%)	0.17	op. path.	
		Staphylococcus equorum (100%)	0.19a	sap.	
		Staphylococcus xylosus, *Staphylococcus saprophyticus* (99%)	0.19b	op. path.	
		Staphylococcus equorum (99%)	0.21	sap.	x
		Staphylococcus xylosus, *Staphylococcus saprophyticus* (100%)	0.23	op. path.	x
		Staphylococcus cohnii, *Staphylococcus nepalensis* (100%)	0.26	op. path.	x
		Erysipelothrix rhusiopathiae (100%)	0.28	strict path.	x
		Enterococcus faecalis (100%)	0.31a	op. path.	x
		Bacillus psychrodurans, *Bacillus psychrophilus* (96%)	0.31b	sap.	x
		Jeotgalicoccus sp. (98%)	0.31c	sap.	x
		Weissella thailandensis (99%)	0.38	sap.	x
		Bacillus benzoevorans (99%)	0.53a	sap.	
		Bacillus sp. (94%)	0.61a	sap.	
		Staphylococcus pseudointermedius, *Staphylococcus intermedius* (100%)	0.81	op. path.	
Actinobacteria		Intrasporangiaceae (99%)	0.07	sap.	
		Streptomyces (*phaeochromogenes*, *fumigatiseleroticus*) (97%)	0.09b	sap.	
		Brevibacterium sanguinis, *Brevibacterium iodinum* (100%)	0.35a	sap.	
		Tsukamurella tyrosinosolvens, *Tsukamurella inchonensis* (100%)	0.88	op. path.	x
		Streptomyces tricolor, *Streptomyces griseosporeus* (100%)	0.92	sap.	
Bacteroidetes		*Myroides odoratimimus (100%)*	0.32	op. path.	x
		Candidatus Cardinium hertigii (97%)	0.35b	symb.	

Bacteria were classified according to their biological characteristics: strict path., strict pathogens; op. path., opportunistic pathogens; sap., saprophytes; and symb., symbionts. Bacterial taxa belonging to the common bacterial community, determined after statistical analysis are specified in the last column.

bacteria originated from various environments such as soil, plants, water and air. Sequences related to arthropod symbionts corresponded to *Spiroplasma* sp. *Anistosticta* (98%), "*Candidatus* Cardinium hertigii" (97%), *Schineria* sp. (98%) and the *Rickettsiella* endosymbiont of the microarthropod *Folsomia candida* (97%). *Rickettsiella*-like bacteria associated with *D. gallinae* were also identified, although identity was only 91%.

3.2. Determination of characteristic microbiota associated with D. gallinae

TTGE profiles were described by assigning to each detected band a relative position (rf) with respect to the external marker. Thus, detectable TTGE profiles of the *D. gallinae* bacterial community varied from 10 to 25 bands per mite and 6 to 23 bands per pool of mites. The most complex TTGE pattern obtained from a single *D. gallinae* showed 25 visible bands, indicating higher diversity in bacterial microbiota in the mite, whereas the fingerprint of a sample derived from a pool of mites taken from another farm showed no more than six bands. The number of bands corresponds to the number of predominant members in the microbial communities of *D. gallinae*. The lower concentration at which a given band could be detected was estimated at less than 1%. Therefore some minority bands were probably not recovered. As shown in Fig. 1, comparison of the fingerprints from each poultry farm revealed similarities in the positions of several fingerprints for some TGGE bands. Analysis of PCR-TTGE banding patterns using the biodiversity indices were performed for individual mites as well as for pooled mites to measure the richness and evenness of the mite communities based on the number and intensity of the PCR-TTGE bands. Using analysis of variance, the community diversity in the mite populations was significantly different ($P < 0.05$) between poultry farms, indicating that there existed a poultry farm effect for both indices calculated. This result suggests that the number of phylotypes and their relative abundance are different between farms.

Principal component analysis (PCA) enabled us to highlight differences in banding pattern profiles between poultry farms. The Monte Carlo test of BGA (between-group analysis) showed that these differences were statistically significant, but no clear biological interpretation of the differences could be given. Consequently, WGA (within group analysis) was used to remove the between-farm differences followed by the use of cluster analysis (complete link) to select 19 OTUs (Fig. 2A, B). By comparing results obtained with the data available on the presence–absence of each band in each farm, the OTU that corresponded to rf 0.39 was rejected, as it was only present in two pools of mites from a single farm. Finally, 18 OTUs were considered dominant and common to all poultry farms (Table 1).

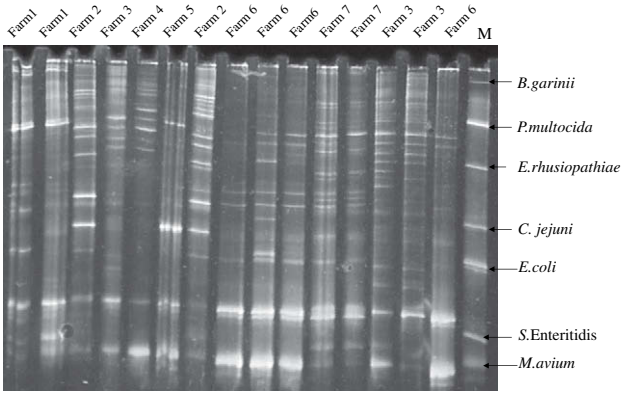

Fig. 1. Example of TTGE bacterial community profile of individual *D. gallinae* samples isolated from seven different poultry farms (lanes 1 and 2, farm 1; lanes 3 and 7, farm 2; lanes 4, 13 and 14, farm 3; lane 5, farm 4; lane 6, farm 5; lanes 8, 9, 10, 15, farm 6; lanes 11, 12, farm 7; lane 16, TTGE marker).

4. Discussion

As specified by Sorum and Sunde [24], the microflora present at any site in a healthy animal is collectively referred to as the normal flora. In general, bacteria in the normal flora can be divided into symbionts or commensals and opportunistic pathogens that do not seem to be of any benefit to the host but may produce disease under certain circumstances. Wray [30] defines opportunistic pathogens as those that can be isolated from an apparently disease-free animal. As previously stated, we classified the bacteria isolated from *D. gallinae* into four categories: saprophytes, symbiont, opportunistic pathogens and strict pathogens. In ticks, it has been previously shown that the Rickettsiales order is the most frequently represented group, followed by the Gammaproteobacteria, including, in particular, the Coxiellaceae family [12,22]. Benson et al. [3] reported, from a survey on a community of bacteria associated with tick nymphs of *Ixodes scapularis,* that Gram-negative bacteria were dominant, which they explained by the sensitivity of ixodid ticks to desiccation. Concerning *D. gallinae*, Gram-positive bacteria were slightly more diverse than Gram-negative bacteria and several of the sequences were associated with Firmicutes and, in particular, with bacteria of the genus Staphylococcus. Bands belonging to the Gammaproteobacteria were also particularly numerous, with a majority of Enterobacteriales corresponding to bacteria associated with the intestinal tract (*E. coli*, *Proteus* sp., *Providencia* sp.). Other taxa have been recovered from both *D. gallinae* and ticks, such as *Staphylococcus* sp., *Bacillus* sp., *E. coli*, *Acinetobacter* sp., *Delftia* sp., *Spiroplasma* sp. and *Pseudomonas* sp. *Pseudomonas* spp. are an important biological component of agricultural soils, and since many metabolic features of these bacteria are beneficial to plants, the possibility exists that these bacteria could provide ticks with beneficial molecules, even though some of them are potential opportunistic pathogens [16]. It was no surprise to detect *Acinetobacter* sp. or *Staphylococcus aureus* and *Staphylococcus xylosus* from *D. gallinae,* because these taxa are bacteria of the normal intestinal microbiota of fowl and bacteria localized on their skin, respectively. Moreover, with other mite species, Seniczak and Stefaniak [23] using a culture approach, showed that the bacterial communities associated with the *Oppia nitens* mite varied depending on the host species, the age of the specimens, the habitats from which they were isolated and the

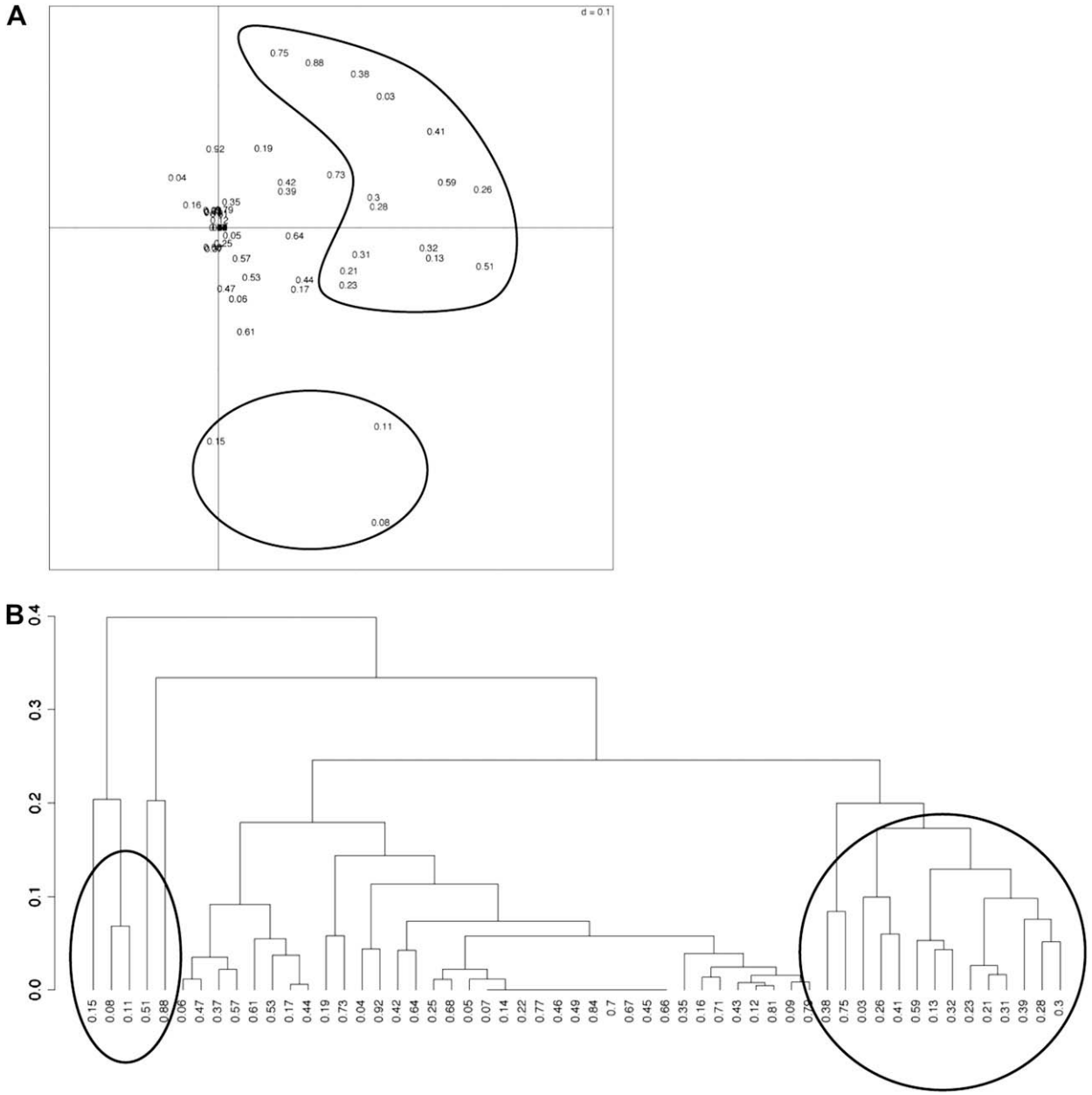

Fig. 2. (A) WGA factor map. Phylotypes close to the center corresponded to phylotypes rare or present only on some farms. The phylotypes most distant from the origin represented the most dominant and common at poultry farms. (B) Cluster analysis (complete link). Two clusters were identified and reported on the WGA factor map, enabling selection of the 18 OTU characterizing the dominant microbiota associated with *D. gallinae*.

substrates on which they fed. Another study focused on bacteria associated with *Psoroptes ovis*, the sheep scab mite, revealed the presence of bacteria associated with skin lesions, dermatitis and otitis media caused by *P. ovis* [13].

A variety of symbiotic bacteria have already been detected in the tick *I. scapularis*. These include members of *Rickettsiaceae* and *Anaplasmataceae*, bacteria belonging to the *Wolbachia persica* group [18]. In our study, *D. gallinae* was found to be associated with endosymbiotic bacteria such as *Spiroplasma* sp. (98% identity), a particularly interesting bacterium that may be responsible for sexual determination in insects. Tinsley and Majerus [26] recently demonstrated that *Spiroplasma* sp. are male-killing bacteria causing female-biased offspring ratio in female ladybirds *Anisosticta novemdecimpunctata*. As this bacteria was also identified from *D. gallinae*, it would be interesting to further evaluate its potential role in sex determination in *D. gallinae*. Although *Spiroplasma* sp. are usually considered to be pathogens, they have also been reported to be symbionts in some insects and the potential role of mosquito spiroplasmas as vector control agents has been discussed [14]. This bacterium could also be considered a symbiont of hematophagous arthropods, as it has been recovered from both *D. gallinae* and ticks. Bacteria corresponding to *Candidatus* Cardinium hertigii were also identified in *D. gallinae* (97% identity). This bacterium has been implicated in the parthenogenesis of parasitoid wasps and has also been recently

considered to be a symbiont of the phytophagous mite, *Tetranychus puerariicola* [11,31]. Recognized bacterial symbionts such as *Wolbachia* sp., which cause reproductive anomalies in a wide range of arthropods, have not been detected in *D. gallinae,* although they are generally described as symbionts of insects or arthropods. Nevertheless, it would be interesting to confirm their absence by using more specific and sensitive tools such as directly targeted PCR detection.

Two diversity indices were first calculated to provide information on the number of phylotypes and their relative abundance at poultry farms. Shannon's index, used to characterize diversity in a community, accounts for both abundance and evenness, while the Dominance index allows highlighting of dominant taxa. Statistical analysis showed a poultry farm effect indicating differences in bacterial communities between the poultry farms analyzed. At the start of the study, poultry farms were chosen for their global homogeneity, i.e. they were all in the same geographical region and used the same breeding system, very common and standardized in this type of production (zootechnics, feeding mode, etc.). Consequently, one explanation for this result is almost certainly the difference in the environmental bacterial taxa present at each poultry farm. To remove the between-farms differences, as confirmed by both these results and PCA and BGA analyses, and in order to determine characteristic microbiota associated with *D. gallinae*, a statistical method for data analysis, WGA, was used combined with cluster analysis (complete link). In the end, 18 OTU were selected to define the characteristic microbiota of *D. gallinae,* corresponding mostly to saprophytic bacteria. Two pathogens, already isolated from *D. gallinae* in previous studies, were also included: *P. multocida* and *E. rhusiopathiae* (six farms, respectively, including two with more than 50% of individuals harboring the pathogen) [6,21]. The detection of such pathogens as part of the normal microbiota of an organism is not a rare event, as an analogous situation has already been described in human biology with *S. aureus*, the most common cause of staph infections, which is frequently found living on the skin or in the nose of healthy individuals. Moreover, it was not possible to reach a conclusion on the pathogenic status of the identified taxa, as techniques led to identification of the fraction of bacterial taxa not yet cultured.

In conclusion, this is the first report on bacterial composition of *D. gallinae*. A characteristic microbiota was identified for mites collected from standard poultry farms in Brittany, France. It would be interesting to further compare *D. gallinae* populations at farms in different geographical regions or from different typologies, particularly organic farms. Both pathogens and symbionts were identified in the poultry red mite. The next step of the study would be to analyze how diversity of microbiota within mites may influence the transmission of pathogens. As a consequence, identification of potential targets within the symbiont community could be considered in the future as a means for ectoparasite control by studying their influence on reproduction of arthropods and their population dynamics. It is therefore possible to foresee monitoring and control of bacterial communities by altering feeding and rearing practices, thereby reducing the risk of bacterial disease transmission.

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