Detection and comparative analysis of cutaneous bacterial communities of farmed and wild *Rana dybowskii* (Amphibia: Anura)

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Abstract

*Rana dybowskii* Gunther, 1876 is a dominant amphibian species in northeast China. In order to understand the composition and structure of the cutaneous bacterial communities of farmed and wild *R. dybowskii*, two experimental groups (farmed and wild) were investigated in this study. Following DNA extraction, the V3-V4 hypervariable region of the 16S rRNA gene was targeted and analyzed by high-throughput sequencing. The cutaneous bacterial community diversity was investigated and compared through analysis of alpha and beta diversity. A total of 524,852 valid sequences and 1,023 operational taxonomic units (OTUs) were obtained from these two experimental groups. The number of shared OTUs was 333, while there were 603 unique OTUs in the farmed group and 87 unique OTUs in the wild group. The Chao, ACE and Shannon indices of the farmed group were significantly higher than those of the wild group (*p* < 0.05). The composition and abundance of the dominant bacteria at the phylum and genus levels were different. The dominant phyla in the farmed group were Firmicutes, Proteobacteria, Actinobacteria, Chloroflexi, Fusobacteria, Bacteroidetes, and Cyanobacteria. The dominant phyla of the wild group were Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria. Furthermore, the alpha and beta diversities of the cutaneous bacterial communities of farmed and wild *R. dybowskii* exhibited significant differences. This study provides a theoretical basis for comprehensively understanding the composition and abundance of bacteria on the skin of farmed and wild *R. dybowskii*, which helps to develop its breeding industry and gain economic benefits.

Keywords: *Rana dybowskii*, high-throughput sequencing, cutaneous, bacterial community, compare

Introduction

In recent decades, amphibians have become the most endangered group of vertebrates with their numbers sharply decreasing throughout the world. The major causes of the decline in amphibians are global warming, habitat loss, diseases and various human factors, including extermination and use of pesticides. Almost one-third of amphibian species are threatened with extinction worldwide, and the conservation of amphibians is necessary (Ochoa-Ochoa et al. 2009). Chytridiomycosis, which is caused by a fungus named *Batrachochytrium dendrobatidis* (*Bd*), has received increasing attention among the factors that are causing the decline of amphibians. Chytridiomycosis can affect the function of amphibian skin and cause a large number of amphibian deaths in a short time (Ryan et al. 2008; Van Rooij et al. 2015). Chemicals released by *Bd* can cause host pathology, even in the absence of infection (Mcmahon et al. 2013).

*Rana dybowskii* is taxonomically classified as Amphibia, Anura, Ranidae, *Rana*. *Rana dybowskii* is one of the dominant amphibian species in northeast China and is mainly found in the northeastern provinces of Heilongjiang, Jilin, Liaoning and Inner Mongolia. *Rana dybowskii* has been classified as a near-threatened species on the Red List of China’s Vertebrates. In recent years, a large number of *R. dybowskii* have been killed, as their oviducts are used in expensive traditional Chinese medicine, thus, they have a very high economic value. In order to protect wild *R. dybowskii* while still gaining economic benefits, artificial breeding of *R. dybowskii* is gradually
emerging as an industry. Artificial breeding is an important method for preventing the extinction of wild animals; however, there are many difficulties with this process, such as a reduced survival rate and susceptibility to disease. One possible cause of these difficulties is the change of resident microorganisms. It has been experimentally shown that the cutaneous bacterial communities of wild species change after artificial breeding (Becker et al. 2014).

An animal’s skin is the first level of protection against potential environmental pathogens. The function of the skin is more than a physical obstacle. Over the years, skin has been proven to have a critical immunological role, not only being a passive protective barrier but also a highly sophisticated network of effector cells and molecular mediators known as the “skin immune system” (SIS). Some microorganisms on the surface of an animal’s skin can enhance host immunity, whereas some are opportunistic pathogens that are harmful to the body. Microorganisms that inhabit the skin of vertebrates dynamically change throughout the host’s life cycle. Metabolites produce by these microorganisms combine with active compounds secreted by the host’s glands to form a chemical defense (Sabino-Pinto et al. 2016). For amphibians, the skin provides a protective barrier against pathogens and plays an important role in the immune response of the host. In addition, the amphibian’s skin has other functions. It is rich in glands (mainly mucous and granular glands) that secrete different bioactive compounds, as alkaloids and antimicrobial peptides, to enhance defense (Huang et al. 2016).

Amphibians have a large number of bacteria on their skin. In addition to participating in the physiological processes of the host, symbiotic bacteria also play an important role in disease resistance and can directly defend against pathogen invasion (Familiar López et al. 2017). Symbiotic bacteria can increase protection by competing with pathogens for resources, supporting the host immune system, or through secretions (Bates et al. 2018). Studies have identified *Janthinobacterium lividum* and *Serratia marcescens* on the skin of Atelopus zeteki, both of which secrete antibacterial active substances that increase the host’s ability to resist pathogens (Woodhams et al. 2018). The study of Malagasy frogs found that skin-associated bacteria mainly belong to Actinobacteria, followed by Proteobacteria and Bacteroidetes, with many able to inhibit *Bd* (Bletz et al. 2017). The living environment will, to some extent, affect the bacterial community structure in amphibians (Harris et al. 2006, 2009; Becker et al. 2009). Medina et al. (2017) demonstrated that the bacterial community on the skin of amphibians is altered in direct relation to the environment (Medina et al. 2017). Becker et al. (2014) found that the species diversity, phylogenetic diversity and the structure of cutaneous bacterial communities were significantly different between captive and wild Panamanian golden frogs (Becker et al. 2014). Furthermore, changes in diet have a great impact on the bacterial community of amphibians’ skin (Antwis et al. 2014).

Parasitic bacteria on the skin are closely related to the health of *R. dybowskii*; however, some bacteria may play a role in resistance to external pathogens. Therefore, it is important to know the structure of the bacterial community on the skin of *R. dybowskii*. The community of parasitic bacteria on the skin of *R. dybowskii* is subject to many environmental factors such as humidity, temperature, and pH. As farmed and wild *R. dybowskii* live in different environments, the skin parasitic bacteria change accordingly. In addition, the lack of natural environmental bacterial reservoirs can also alter the composition of the bacterial community associated with the host. In order to get a better understanding of differences in bacterial communities on the skin of *R. dybowskii* from different environments, wild and farmed *R. dybowskii* in north-eastern China were sampled. In this paper, the 16S rRNA gene of cutaneous bacteria was subjected to high-throughput sequencing to determine the composition and abundance of cutaneous bacterial communities on farmed and wild *R. dybowskii*. This study focused on alpha diversity and comparative analysis between the two groups, whereby the community composition and relative abundances were measured to understand the differences in these cutaneous bacteria communities.

**Materials and methods**

**Sample collection**

Two groups of *R. dybowskii* were collected from north-eastern China. The wild individuals (*n* = 7; weight, 4.89 ± 0.24 g) were collected from a natural *R. dybowskii* habitat in Luobei County (47°65′24″N, 130°46′24″E; 98 m alt) in June 2017. The farmed individuals (*n* = 7; weight, 5.14 ± 0.35 g) were collected from a farm in Huanan County (46°44′54″N, 130°69′32″E; 80 m alt) in July 2017. The individuals in both groups were sampled on the same day and at the same location. They were sacrificed immediately upon arriving in the laboratory to prevent changes to the cutaneous bacterial community due to the laboratory environment.

Cultivating conditions: The young frogs used for this experiment were robust second instar *R. dybowskii*,
which end hibernation in early May. The culture density was forty individuals per m² and the *R. dybowskii* was fed mealworms. The frogs were housed in a greenhouse (40 m length × 8 m width) with sparse and low vegetation planted on the ground. Water spray equipment and shading nets were installed in the greenhouse. The ground humidity was 25%–35% and there was usually no accumulation of water. The soil temperature was basically the same as the temperature in the greenhouse, but there was a certain degree of hysteresis. Iodophors were sprayed every 3–5 days in the greenhouse for disinfection.

For this study, seven individuals per group were randomly collected from the same area to reduce pseudo-replicates. Exploratory analyses of our dataset did not yield differences in bacterial community composition or diversity between sexes (Becker et al. 2014; Kueneman et al. 2014; Rebollar et al. 2016); therefore, data from male and female individuals were combined prior to analysis. All individuals were sacrificed and the skin of the back, venter, and limbs were peeled off and collected. The skin samples were loaded into 5 mL sterile tubes and cryopreserved at −80°C.

Ethical approval: Before sample collection, all animal protocols were approved by the Institutional Animal Care and Use Committee of the Northeast Agricultural University, China. All experiments were performed in accordance with the approved guidelines and regulations. IACUC#2015-035.

**DNA extraction and PCR amplification**

Genomic DNA extraction was performed using the FastDNA® Spin Kit for Soil (MP Biomedicals, U.S.), based on the manufacturers’ protocol. For each sample, the V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCRs were performed in triplicate on a thermocycler (GeneAmp 9700, ABI, USA) using the following program: 3 m denaturation at 95°C; 27 cycles of 30 s at 95°C, 30 s at 55°C and 45 s at 72°C; and a final extension at 72°C for 10 minutes. Reaction mixtures (20 μL) contained 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, 0.2 μL of BSA (20 mg/mL) and 10 ng of template DNA. The resulting PCR products were visualized on a 2% agarose gel, purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and then quantified using QuantiFluor™-ST (Promega, USA), according to the manufacturers’ protocol.

**Illumina MiSeq sequencing**

Purified amplicons were pooled in equimolar concentrations and paired-end sequenced (2 × 300) on the Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols.

Raw fastq files were demultiplexed, quality-filtered using Trimmomatic and merged with FLASH as follows: (i) reads were truncated at any site that received an average quality score < 20 over a 50 base pairs (bp) sliding window; (ii) primers were exactly matched allowing 2 nucleotide mismatching and reads containing ambiguous bases were removed; and (iii) sequences overlapping by < 10 bp were merged accordingly.

OTUs were clustered at a 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/). Chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed with the RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the SILVA 16S rRNA gene database (Release128 http://www.arb-silva.de) using a confidence threshold of 70% (Li et al. 2015).

**Statistical analysis**

MOTHUR (version v.1.30.1 http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity) was used to create rarefaction data and alpha diversity indices. Wilcoxon rank-sum tests were used to analyze the difference between alpha diversity indices and the relative abundance of phyla and genera in these two groups. Bray Curtis was used for the analysis of similarities (ANOSIM). Weighted and unweighted Unifrac distance matrices were used to calculate beta diversity and were visualized by principal coordinates analysis (PCoA) (Rebollar et al. 2016). The linear discriminant analysis (LDA) effect size (LEfSe) method was used to determine the bacterial taxa that most likely explained differences between the two groups. Following the precedent of previous studies, phyla and genera with LDA scores > 3.5 were considered informative (Lokesh & Kiron 2016).

**Results**

In this study, the V3-V4 hypervariable region of the bacterial 16S rRNA gene was sequenced to determine the composition and abundance of cutaneous bacteria of *Rana dybowskii*...
**R. dybowskii** bacterial communities using high-throughput sequencing.

### Sample sequencing data

A total of 14 samples were sequenced for the two study groups, resulting in 524,852 valid sequences (average sequence length = 446) and 233,963,272 bases after sequence filtration and double-stitched splicing. After clustering to 97% similarity, 1,023 total OTUs were obtained, which belonged to 700 species, 471 genera, 218 families, 113 orders, 52 classes, and 26 phyla. The rarefaction curve tended to be flat (Supplementary material 1), indicating that the samples were sequenced to a reasonable amount and reflected the vast majority of the species in the samples.

### Alpha diversity

With respect to microbial community ecology, alpha diversity can reflect the abundance and diversity of microbial communities, including a series of statistical indices to estimate species abundance and diversity within the community. Sobs, which represents the actual number of observed OTUs in each sample, confirms that the species richness was higher in the farmed group (Table I). The coverage index estimates how well the sequenced community represents the actual microbial community of the sample. The experimental results show that the coverage index of the samples was greater than 99% (Table I), indicating that the sequencing results were credible. The Chao and ACE indices, which estimate the total number of species and reflect bacterial abundance, were significantly higher ($p < 0.01$; Chao: $p = 0.0022$; ACE: $p = 0.0022$) for the farmed group than for the wild group (farmed group: $\text{Chao} = 672.33 \pm 52.32$, $\text{ACE} = 661.65 \pm 50.57$; wild group: $\text{Chao} = 253.40 \pm 31.14$, $\text{ACE} = 247.66 \pm 25.74$). The Shannon (H) and Simpson (D) indices reflect the diversity of the bacterial community. The higher the Shannon index, the higher the community diversity; whereas the higher the Simpson index, the lower the community diversity. The diversity of the farmed group was significantly higher ($p < 0.01$; $H$: $p = 0.0022$; $D$: $p = 0.0049$) than that of the wild group (farmed group: $\text{H} = 3.5540 \pm 0.8557$, $\text{D} = 0.1434 \pm 0.1168$; wild group: $\text{H} = 1.5773 \pm 0.3887$, $\text{D} = 0.3872 \pm 0.0961$).

### Bacterial community structure and distribution at the phylum and genus levels

The main phyla (relative abundance > 1.0%) of the samples collected in this study were Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Chloroflexi, Fusobacteria, Cyanobacteria and Deinococcus-Thermus (Supplementary material 2). In the wild group, there were four major phyla accounting for 99.73% of the community, namely Proteobacteria (77.91% average relative abundance), Bacteroidetes (17.06%), Firmicutes (3.30%) and Actinobacteria (1.47%). Most of the sequences in the farmed group (98.46% of the community) belonged to the following seven phyla: Firmicutes (35.93%), Proteobacteria (35.28%), Actinobacteria (19.39%), Chloroflexi (2.91%), Fusobacteria (1.76%), Bacteroidetes (1.66%) and Cyanobacteria (1.53%).

A total of 471 genera were observed in both groups, of which 37 genera had an average relative abundance greater than 1.0% (Supplementary material 3). The majority of farmed group sequences (74.04%) belonged to Pseudomonas (21.26%), Staphylococcus (13.41%), Salinicoccus (10.50%), Nesterenkonia (4.90%), Lactococcus (3.74%), Citrobacter (3.62%), Glutamicibacter (2.58%), norank_o__JG30-KF-CM45 (2.45%), Rhodococcus (2.33%), Enterococcus (1.84%), Cetobacterium (1.76%), Bacillus (1.70%), Vagococcus (1.38%), Ralstonia (1.37%) and Leuconobacter (1.20%). Most wild group sequences (90.87%) belonged to Pseudomonas (55.65%), Sphaerotilus (15.98%), Chryseobacterium (8.57%), unclassified_f__Flavobacteriaceae (6.60%), Bacillus (1.57%), Ralstonia (1.32%) and Flavobacterium (1.12%).

Of the 15 most dominant bacteria, 14 phyla exhibited significant differences in the relative abundances between the wild and farmed R. dybowskii (Wilcoxon rank-sum test, two-tailed test, FDR correction, $p < 0.05$; Figure 1(a)), 13 that were extremely significant ($p < 0.01$). At the genus level, there were significant differences in the relative abundances between the two groups for 12 of the genera.

| Groups  | Sequence number | Sequence length | sobs     | Coverage%   |
|---------|----------------|----------------|----------|-------------|
| Farmed  | $37039 \pm 4636$ | $442 \pm 3$   | $585 \pm 70$ | $99.61 \pm 0.07$ |
| Wild    | $37940 \pm 3707$ | $448 \pm 0$   | $197 \pm 29$  | $99.81 \pm 0.04$ |
shared OTUs and beta diversity analysis

To determine the differences in bacterial communities between the farmed and wild groups, a heatmap was drawn using the relative abundances of the top 30 species (Figure 2). The heatmap shows that the relative abundance of most bacteria was different, although there are similarities between some of the samples in the two groups.

There were 333 shared OTUs within the farmed and wild groups. Although these OTUs were present in
most samples, their abundance was different between the groups. In contrast, there were 603 unique OTUs in the farmed group and 87 unique OTUs in the wild group. The shared OTUs accounted for 35.58% of the OTUs in the farmed group and 79.29% in the wild group. There were 13 shared OTUs with a high abundance (> 1% of the total OTUs), belonging to Firmicutes (n = 4 OTUs), Bacteroidetes (n = 2), Proteobacteria (n = 3), Actinobacteria (n = 3) and Fusobacteria (n = 1).

The beta diversity using both unweighted (Figure 3(a)) and weighted (Figure 3(b)) UniFrac analyses showed that the communities of the farmed and wild groups were phylogenetically distant from each other. The weighted method takes into account not only the presence/absence of each OTU but also the abundance. Clustering was performed by principal coordinates analysis (PCoA) of the UniFrac distance matrix, with the farmed and the wild groups separating along with the principal coordinates. Furthermore, ANOSIM showed that the difference between the groups was greater than the difference within each group and that the difference was significant ($R = 0.771$, $p < 0.05$).

Figure 2. Heat map of the top 30 taxa. The lower and the right side of the heat map are the sample and genus names, respectively. The left side is the species clustering tree. The depth of color in the heat map represents the abundance of the genus.

Figure 3. PCoA plot. PCoA plot depicting the clustering of bacterial communities in the two groups and the differences in phylogenetic diversities using unweighted (a) and weighted (b) UniFrac distance matrices. The yellow rectangle encompasses the farmed samples and the blue rectangle encompasses the wild samples.
**Linear discriminant analysis (LDA) effect size**

The LEfSe method (Rebollar et al. 2016) was used to identify bacteria that best explained the differences between the two groups (Figure 4). LEfSe identified eight distinct phyla (Firmicutes, Actinobacteria, Chloroflexi, Fusobacteria, Cyanobacteria, Deinococcus__Thermus, Proteobacteria, and Bacteroidetes) with significant differences in abundance. Among them, Bacteroidetes and Proteobacteria were associated with the wild group and the rest were associated with the farmed group. The largest number of species with significant differences was Actinobacteria. Alphaproteobacteria, which belongs to Proteobacteria, was a significantly different class of the farmed group. Simultaneously, Proteobacteria was the most abundant phyla in the wild group whose dominance was driven by Betaproteobacteria and Gammaproteobacteria, as demonstrated with higher LDA scores. All significant species identified in Actinobacteria and Firmicutes belonged to the farmed group. The number of significantly different bacteria at the genus to the phylum level in the farmed group was far more than that in the wild group.

**Discussion**

In recent years, a great deal of attention has been given to cutaneous bacterial communities in animals because it is thought to be a key factor affecting the animal status, including health, growth, and disease (Mckenzie et al. 2012; Walke et al. 2014; Avena et al. 2016). The study of microorganisms has become relatively facilitated due to the latest advances in high-throughput sequencing, enabling culture-independent analysis. Thus, high-throughput sequencing was used in this study to explore the composition and structure of cutaneous bacterial communities of farmed and wild *R. dybowskii*. The results show that the bacterial community structures between the two groups living in different environments were significantly different.

The dominant phyla of the farmed group were Firmicutes, Proteobacteria, Actinobacteria, Chloroflexi, Fusobacteria, Bacteroidetes, and Cyanobacteria. The dominant phyla of the wild group were Proteobacteria, Bacteroidetes, and Actinobacteria. When comparing the two groups, there was a similarity in bacterial composition at the phylum level, but the abundances were quite different. The abundance of Firmicutes and Actinobacteria were significantly higher ($p < 0.05$) in the farmed group compared with the wild group. Actinobacteria have been detected in soil samples from many places (Elbendary et al. 2018) and they are also common bacteria on *R. dybowskii* skin (Kueneman et al. 2014). They can produce antibiotics (Barka et al. 2015) and some of them can be used to produce enzyme preparations (Le Roes-Hill et al. 2011). The abundance of Proteobacteria in the wild group was...
higher than in the farmed group. Proteobacteria are more competitive than other microorganisms because of their morphological and physiological diversity (Shin et al. 2015). Additionally, they have the capacity to adapt to harsh conditions of high temperature, and many of them are opportunistic pathogens (Jardine et al. 2017). Bacteroidetes was highly dominant in the wild group, second only to Proteobacteria. Compared to the wild group, the abundance of Bacteroidetes in the farmed group was very low (average relative abundance < 1%). Furthermore, the abundance of Firmicutes in the wild group was lower than that in the farmed group. Studies have shown that Firmicutes can survive under harsh environmental conditions, which may be related to their ability to produce endospores that are resistant to desiccation (Gomez-Montano et al. 2013). Bacillus is often used as a probiotic on farms to prevent disease; therefore, the high abundance of Firmicutes in the farmed group may depend on human factors. Bacteroidetes and Firmicutes are predominant bacteria on the skin of many amphibians (Abarca et al. 2017; Bates et al. 2018). The relative abundances of the phyla Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria in the two groups were significantly different, both of which belonged to the phylum level in LDA.

There were differences at the genus level between the farmed and the wild groups. The relative abundances of Pseudomonas, unclassified_f_Flavobacteriaceae, and Staphylococcus were significantly higher than those in the farmed group (p < 0.05). In contrast, the relative abundances of Sphaerotilus, Salinicoccus, Nesterenkonia, Citrobacter, Lactococcus, Rhodococcus, Glutamicibacter, norank_o__JG30-KF-CM45 and Enterococcus were significantly higher in the farmed group (p < 0.05). Pseudomonas was abundant in both the wild and farmed groups. Pseudomonas is a common opportunistic pathogen (Meynet et al. 2018) widely found in air, skin, water and soil ecosystems. The genus Pseudomonas is ubiquitous in moist environments and some are pathogenic to animals and humans (Moradali et al. 2017). It is also a resident bacterium on the skin of amphibians and studies have shown that Pseudomonas isolated from Hemidactylus scutatum has an inhibitory effect on Bd in vitro (Harris et al. 2006). The abundance of Ralstonia and Bacillus was similarly low in both groups. The relative abundances of twelve dominant bacteria in the farmed group were less than 1% in the wild group, while four of the dominant genera in the wild group had an average relative abundance of less than 1% in the farmed group. The experimental results showed that the average relative abundance of genera belonging to R. dybowskii grown in two contrasting environments was quite different.

Most OTUs were shared between the two groups. Nevertheless, the PCoA plot showed little overlap between the samples. Furthermore, the beta diversity between the bacterial communities was significantly different. The 603 unique OTUs observed in the farmed group, which was much higher than that of the wild group, may explain why the Shannon diversity index of the farmed group was higher than the wild group. The ANOSIM showed that the cutaneous bacterial communities were significantly different between the farmed and wild groups.

There are many factors that can affect the cutaneous bacterial community structure of amphibians, such as the living environment, different body parts, host species, temperature, the effects of pathogens, the developmental stage of the host, seasons and Bd infections (Longo et al. 2015; Van Rooij et al. 2015). Many other factors that affect the cutaneous bacterial communities remain to be explored. In this study, the living environment may primarily explain the changes in the data sets. Thus, among the many influencing factors, the cutaneous bacterial community structures may be affected most by the living environment.

Wild R. dybowskii prefers to inhabit vegetated environments with tall trees, shrubs and humid air, such as broad-leaved or conifer-broadleaf forests. Although the R. dybowskii farm is located in the natural distribution area of R. dybowskii, the farm environment is affected by human factors and many other differences compared with the natural environment. Studies have shown that after approximately about eight years of living in captivity, the offspring of captive golden frogs still shared 70% of their microbial communities with wild frogs. Host-related microbiial communities changed significantly during captive management, but most community members can be retained (Becker et al. 2014). However, differences in cutaneous bacterial community structure, species richness, and diversity between the wild and farmed R. dybowskii were observed in this research. Similar results have been reported in previous studies on other amphibians. For example, the cutaneous bacterial communities were significantly different between wild and captive Cynops pyrrhogaster (newts), with the wild individuals exhibiting a higher alpha diversity (Sabino-Pinto et al. 2016). Conversely, this study found that the alpha diversity of the farmed bacterial community significantly increased (p < 0.05), which is inconsistent with the previous studies. For most
animals, wild individuals live in a complex environment, while cultured individuals live in a single environment (Sabino-Pinto et al. 2016). However, the ability of R. dybowskii to adapt to the living environment is not strong. This conclusion can be drawn from the limited distribution range of R. dybowskii (mainly distributed in Northeast China). The R. dybowskii farm needs to be built within the natural habitat of R. dybowskii, so that the natural survival environment of wild and farmed R. dybowskii is similar. At the same time, the farms are disturbed by human factors, which are more complex than the natural environment. This may be the reason for the increased bacterial community diversity of the farmed R. dybowskii. A possible reason for this is the complex farm environment where pollution is more serious. Studies have shown that mice in a farmhouse harbored a significantly more diverse and richer gut microbiota, due to their exposure to unhygienic conditions, compared with mice reared in a specific pathogen-free animal room (Zhou et al. 2016). The cultured R. dybowskii is susceptible to disease (Weng et al. 2016), and the treatment options are limited. If they are infected, their bacterial community will be affected. Furthermore, there is an interaction between surface and pathogenic bacteria, which is also a way for amphibians to resist pathogenic bacteria (Lam et al. 2010). Higher microbial diversity may be caused by infection, because the host may actively recruit beneficial microorganisms as a mechanism to resist infection (Longo et al. 2015). The differences in bacterial communities can also be caused by different diets. Wild R. dybowskii are free to prey upon many species while the food for farmed R. dybowskii is limited mainly to yellow mealworm (Tenebrio molitor). However, the food of wild R. dybowskii comes from their own environment, while the food of farmed R. dybowskii has been exposed to further complexities, such as transportation equipment and farm staff. Subsequently, the bacteria obtained from this environment may be the reason for the higher diversity of the bacterial community of farmed R. dybowskii (Becker et al. 2014).

Conclusion

This work can provide basic data for the study of R. dybowskii. The dominant phyla in the farmed group were Firmicutes, Proteobacteria, Actinobacteria, Chloroflexi, Fusobacteria, Bacteroidetes, and Cyanobacteria. The dominant phyla of the wild group were Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria. There were significant differences in the alpha and beta diversities of the cutaneous bacterial communities of the farmed and wild R. dybowskii. During farming, R. dybowskii is susceptible to various diseases, including red-legged syndrome, rotten skin disease, fester disease and curved worm disease. Further studies are needed on the changes in cutaneous bacterial community structures of R. dybowskii after disease and drug application. The bacteria that inhabit the skin of the host have a certain influence on the status of the disease. Thus, an understanding of the cutaneous bacterial community can provide a theoretical basis for the prevention and treatment of R. dybowskii disease.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here.

References

Abarca JG, Zuniga I, Ortiz-Morales G, Luqo A, Viquez-Cervilla M, Rodriguez-Hernandez N, Vázquez-Sánchez F, Murillo-Cruz C, Torres-Rivera EA, Pinto-Tomás AA, Godoy-Vitorino F. 2017. Characterization of the skin microbiota of the cane toad Rhinella cf. marina in Puerto Rico and Costa Rica. Frontiers in Microbiology 8:2624. DOI: 10.3389/fmicb.2017.02624.

Antwis RE, Haworth RL, Engelmoer DJ, Ogilvy V, Fidgett AL, Preziosi RF. 2014. Ex-situ diet influences the bacterial community associated with the skin of red-eyed tree frogs (Agalychnis callidryas). PLoS One 9:e85563. DOI: 10.1371 journal.pone.0085563.

Avenda CV, Parfrey LW, Leff JW, Archer HM, Frick WF, Langwig KE, Kilpatrick AM, Powers KE, Foster JT, McKenzie VJ. 2016. Deconstructing the bat skin microbiome: Influences of the host and the environment. Frontiers in Microbiology 7:1753. DOI: 10.3389/fmicb.2016.01753.

Barka EA, Vatsa P, Sanchez L, Gayeau-Vaillant N, Jacqaud C, Meier-Kolthoff JP, Klenk HP, Clement C, Ouhdouch Y, van Wezel GP. 2015. Taxonomy, physiology, and natural products of Actinobacteria. Microbiology and Molecular Biology Reviews: MMBR 80:1–43.

Bates KA, Clare FC, O’Hanlon S, Bosch J, Brookes L, Hopkins K, McLaughlin EJ, Daniel O, Garner TWJ, Fisher MC, Harrison KA. 2018. Amphibian chytridiomycosis outbreak dynamics are linked with host skin bacterial diversity.
community structure. Nature Communications 9:693. DOI: 10.1038/s41467-018-02967-w.

Becker MH, Brucker RM, Schwantes CR, Harris RN, Minbiole KP. 2009. The bacteriologically produced metabolite violacein is associated with survival of amphibians infected with a lethal fungus. Applied & Environmental Microbiology 75:6635. DOI: 10.1128/AEM.01294-09.

Becker MH, Richards-Zawacki CL, Gratwicke B, Belden LK. 2014. The effect of captivity on the cutaneous bacterial community of the critically endangered Panamanian golden frog (Atelopus zeteki). Biological Conservation 176:199–206. DOI: 10.1016/j.biocon.2014.05.029.

Bletz MC, Myers J, Woodhams DC, Rabemananjara FCE, Rakotonirina A, Weldon C, Edmonds D, Vences M, Harris RN. 2017. Estimating herd immunity to amphibian chytridiomycosis in Madagascar based on the defensive function of amphibian skin bacteria. Frontiers in Microbiology 8:1751. DOI: 10.3389/fmicb.2017.01751.

Elbendary AA, Hossain AM, El-Hariri MD, Seida AA, Moussa IM, Mubarak AS, Kabli SA, Hemeg HA, El Jakee JK. 2018. Isolation of antimicrobial producing Actinobacteria from soil samples. Saudi Journal of Biological Sciences 25:44–46. DOI: 10.1016/j.sjbs.2017.05.003.

Familiar Lópezm, Rebollar EA, Harris RN, Vredenburg VT, Hero JM. 2017. Temporal variation of the skin bacterial community and Batrachochytrium dendrobatidis infection in the terrestrial cryptic frog Phyloria loveridgei. Frontiers in Microbiology 8:2535. DOI: 10.3389/fmicb.2017.02535.

Gomez-Montano L, Junpponen A, Gonzales MA, Cusicanqui J, Valdivia C, Mostavalli PH, Herman M, Garrett KA. 2013. Do bacterial and fungal communities in soils of the Bolivian Atlapino change under shorter fallow periods? Soil Biology & Biochemistry 65:50–59. DOI: 10.1016/j.soilbio.2013.04.005.

Harris RN, Brucker RM, Walke JB, Becker MH, Schwantes CR, Flaherty DC, Lam BA, Woodhams DC, Briggs CJ, Vredenburg VT, Minbiole KP. 2009. Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. Isme Journal 3:818–824. DOI: 10.1038/ismej.2009.27.

Harris RN, James TY, Lauer A, Simon MA, Patel A. 2006. Amphibian pathogen Batrachochytrium dendrobatidis is inhibited by the cutaneous bacteria of amphibian species. EcoHealth 3:35–56. DOI: 10.1007/s10393-005-0003-1.

Huang L, Li J, Anboukaria H, Luo Z, Zhao M, Wu H. 2016. Comparative transcriptome analyses of seven anurans reveal functions and adaptations of amphibian skin. Scientific Reports UK 6:24069. DOI: 10.1038/srep24069.

Jardine JL, Abia A, Mayumungwana V, Ubomba-Jaswa E. 2017. Phylogenetic analysis and antimicrobial profiles of cultured emerging opportunistic pathogens (Phyla Actinobacteria and Proteobacteria) identified in hot springs. International Journal of Environmental Research & Public Health 14:1070. DOI: 10.3390/ijerph14091070.

Kueneman JG, Parfrey LW, Woodhams DC, Archer HM, Knight R, McKenzie VJ. 2014. The amphibian skin-associated microbiome across species, space and life history stages. Molecular Ecology 23:1238–1250. DOI: 10.1111/mec.12510.

Lam BA, Walke JB, Vredenburg VT, Harris RN. 2010. Proportion of individuals with anti-Batrachochytrium dendrobatidis skin bacteria is associated with population persistence in the frog Rana muscosa. Biological Conservation 143:529–531. DOI: 10.1016/j.biocon.2009.11.015.

Le Roes-Hill M, Rohland J, Burton S. 2011. Actinobacteria isolated from termitite guts as a source of novel oxidative enzymes. Antonie Van Leeuwenhoek 100:589–605. DOI: 10.1007/s10482-011-9614-x.

Li LT, Yan BL, Li SH, Xu JT, An XH. 2015. A comparison of bacterial community structure in seawater pond with shrimp, crab, and shellfish cultures and in non-cultured pond in Ganyu, Eastern China. Annals of Microbiology 66:1–12.

Lokesh J, Kiron V. 2016. Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. Scientific Reports UK 6:19707. DOI: 10.1038/srep19707.

Longo AV, Savage AE, Hewson I, Zamudio KR. 2013. Seasonal and ontogenetic variation of skin microbial communities and relationships to natural disease dynamics in declining amphibians. Royal Society Open Science 2:140377. DOI: 10.1098/rsos.140377.

Mckenzie VJ, Bowers RM, Fierer N, Knight R, Lauber CL. 2012. Co-habitating amphibian species harbor unique skin bacterial communities in wild populations. Isme Journal 6:588–596. DOI: 10.1038/ismej.2011.129.

Mcmahon TA, Brannelly LA, Chatfield MW, Johnson PT, Joseph MB, McKenzie VJ, Richards-Zawacki CL, Venesky MD, Rohr JR. 2013. Chytrid fungus Batrachochytrium dendrobatidis has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. Proceedings of the National Academy of Sciences of the United States of America 110:210–215. DOI: 10.1073/pnas.1200592110.

Medina D, Hughey MC, Becker MH, Walke JB, Umile TP, Bęszynski EA, Iannetta A, Minbiole KPC, Belden LK. 2017. Variation in metabolite profiles of amphibian skin bacterial communities across elevations in the Neotropics. Microbial Ecology 74:227–238. DOI: 10.1007/s00248-017-0933-y.

Meynet E, Laurin D, Lenormand JL, Camara B, Toussaint B, Le Gouëllec A. 2018. Killed but metabolically active Pseudomonas aeruginosa -based vaccine induces protective humoral- and cell-mediated immunity against Pseudomonas aeruginosa pulmonary infections. Vaccine 36:1893–1900. DOI: 10.1016/j.vaccine.2018.02.040.

Moradali MF, Ghods S, Rehm BHA. 2017. Pseudomonas aeruginosa lifestyle: A paradigm for adaptation, survival, and persistence. Frontiers in Cellular and Infection Microbiology 7:39. DOI: 10.3389/fcimb.2017.00039.

Ochoa-Ochoa L, Urbina-Cardona JN, Vázquez LB, Flores-Villela O, Beraury-Creel J. 2009. The effects of governmental protected areas and social initiatives for land protection on the conservation of Mexican amphibians. PLos One 4:e6878–e6878. DOI: 10.1371/journal.pone.0006878.

Rebollar EA, Hughey MC, Medina D, Harris RN, Ibáñez R, Belden LK. 2016. Skin bacterial diversity of Panamanian frogs is associated with host susceptibility and presence of Batrachochytrium dendrobatidis. Isme Journal 10:1682–1695. DOI: 10.1038/ismej.2015.234.

Ryan MJ, Lips KR, Eichholz MW. 2008. Decline and extirpation of an endangered Panamanian stream frog population (Craugastor punctariolus) due to an outbreak of chytridiomycosis. Biological Conservation 141:1636–1647. DOI: 10.1016/j.biocon.2008.04.014.

Sabino-Pinto J, Bletz MC, Islam MM, Shimizu N, Bhuju S, Geffers R, Jarek M, Kurabayashi A, Vences M. 2016. Composition of the cutaneous bacterial community in Japanese amphibians: Effects of captivity, host species, and body region. Microbial Ecology 72:460–469. DOI: 10.1007/s00248-016-0797-6.
Shin NR, Whon TW, Bae JW. 2015. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends in Biotechnology 33:496–503. DOI: 10.1016/j.tibtech.2015.06.011.

Van Rooij P, Martel A, Haesebrouck F, Pasmans F. 2015. Amphibian chytridiomycosis: A review with focus on fungus-host interactions. Veterinary Research 46:1–22. DOI: 10.1186/s13567-015-0266-0.

Walke JB, Becker MH, Loftus SC, House LL, Cormier G, Jensen RV, Belden LK. 2014. Amphibian skin may select for rare environmental microbes. The ISME Journal 8:2207–2217. DOI: 10.1038/ismej.2014.77.

Weng CH, Yang YJ, Wang D. 2016. Functional analysis for gut microbes of the brown tree frog (Polypedates megacephalus) in artificial hibernation. Bmc Genomics 17(13 Supplement):1024. DOI: 10.1186/s12864-016-3318-6.

Woodhams DC, LaBumbard BC, Barnhart KL, Becker MH, Bletz MC, Escobar LA, Flechas SV, Forman ME, Iannetta AA, Joyce MD, Rabemananjara F, Gratwicke B, Vences M, Minbiole KPC. 2018. Prodigiosin, violacein, and volatile organic compounds produced by widespread cutaneous bacteria of amphibians can inhibit two Batrachochytrium fungal pathogens. Microbial Ecology 75:1049–1062. DOI: 10.1007/s00248-017-1095-7.

Zhou D, Zhang H, Bai Z, Zhang A, Bai F, Luo X, Hou Y, Ding X, Sun B, Sun X, Ma N, Wang C, Dai X, Lu Z. 2016. Exposure to soil, house dust, and decaying plants increases gut microbial diversity and decreases serum immunoglobulin E levels in BALB/c mice. Environmental Microbiology 18:1326–1337. DOI: 10.1111/1462-2920.12895.