Gut and oral bacterial diversity of the lizard Diploderma splendidum investigated using metagenomic analysis

Zhige Tian
Yibin University
xiaoliang hu
Yibin University https://orcid.org/0000-0003-4237-1195
Dandan Gou
Yibin University
Yayong Wu
Yibin University
Peng Guo (ybguop@163.com)
Yibin University

Research article

Keywords: Diploderma splendidum, microbiota, metagenomic analysis, diversity

DOI: https://doi.org/10.21203/rs.3.rs-128688/v1

Abstract

Background

Gut and oral microbial communities are complex and play a key role in their co-evolution with their hosts. However, little is understood about the bacterial community in lizards. In this study, we first investigated the gut and oral bacterial community in *Diploderma splendidum* from Sichuan Province, China. Metagenomic analysis of feces and oral cavity samples showed distinct differences between *Diploderma splendidum* and *Liolaemus parvus*, and *L. ruibali* and *Phymaturus williamsi* species.

Results

Bacteridetes, *Firmicutes*, *Proteobacteria* and *Fusobacteria* were the most abundant phyla in fecal samples. However, the composition of the gut bacterial community of insectivorous lizards (*Diploderma splendidum*) exhibited unique abundance of phyla *Proteobacteria* and *Chlamydiae* when compared with *L. parvus*, *L. ruibali* and *P. williamsi*. Furthermore, *Proteobacteria* were abundant in oral cavity samples, followed by *Actinobacteria*, *Chlamydiae* and *Firmicutes*. Most striking was that the phylum *Chlamydiae* was most common in the oral cavity of *Diploderma splendidum*, when compared with a carnivorous lizard (*Varanus komodoensis*). In addition, more than 26 bacterial species were detected in the gut and/or oral cavity that were identified as potential human pathogens.

Conclusions

In this study, metagenomic analysis was carried out to reveal the gut and oral microbiomes, which brought new insight into the complex bacterial community and ecology in *Diploderma splendidum*.

Background

Diploderma splendidum, which is also called the Chinese Tree Dragon, and is the largest species among the 17 known lizard species, is widely distributed in and endemic to China. It mainly occurs in the Yangtze River Basin of southwestern China, including Yunnan, Sichuan, Chongqing and Hubei. It is frequently found at the forest edge and shrub or beside gravel piles and is good at climbing, strongly arboreal and active. They are exclusively insectivorous [1].

Vertebrates and invertebrates maintain complex relationships with microbial communities living within their gastrointestinal tracts and oral cavities [2, 3]. These gut microbes can affect behavior [4, 5], immune training [6], nutrition [7] and reproductive isolation [8], and influence the ecology and evolution of their host. At present, there are two important issues to investigate: 1) the microbial ecology of gut bacterial diversity, and 2) the way in which diet, altitude, physiology and genetics determine microbial population

structure [9–11]. The microbes of the oral cavity also coevolve with their hosts and adapt to the diverse conditions for colonization resistance [12].

In the present study, to analyze and clarify the gut and oral bacterial diversity of *Diploderma splendidum*, metagenomic analysis was carried out and typical microbial community features are mentioned.

Methods

Description of samples

During the Second Tibetan Plateau Scientific Expedition and Research, one of theme is that gut and oral cavity bacterial diversity of reptiles. For this, individuals of *Diploderma splendidum* were collected using lassos from Quebrada, located in the Laojun mountains, 110 km from Yibin city, Sichuan province in the summer of 2020. To collect wild fecal and oral samples, animals were placed individually in sterilized tubs. Sterile swabs were placed in RNase-free tubes, and transported with dry ice to the OE Biotech Co., Ltd. (Shanghai, China). Then, the lizards were released to the wild field.

DNA isolation and library construction

Total DNA was isolated from the samples using a QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA concentration and integrity were assessed by a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. DNA was fragmented by S220 Focused-ultrasonicators (Covaris, USA) and cleaned up by Agencourt AMPure XP beads (Beckman Coulter Co., USA). Subsequently, libraries were constructed using a TruSeq Nano DNA LT Sample Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The metagenome sequencing and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China).

Bioinformatic analysis

Sequences in the FastQ file were trimmed and filtered using Trimmomatic (v0.36) [13]. The post-filtered pair-end reads were aligned against the host genome using BWA (v0.7.12) [14] and the aligned reads were discarded. Metagenome assembly was performed using SOAPdenovo2 (v2.04) [15] after getting valid reads. Gaps inside the scaffold were used as breakpoints to interrupt the scaffold into new contigs (Scaftig), and new Scaftigs with length > 200 bp (or 500 bp) were retained. Open reading frame (ORF) prediction of assembled scaffolds was performed using prodigal (v2.6.3) [16] and translated into amino acid sequences. The nonredundant gene sets were built for all predicted genes using CDHIT (v4.6.7) [17]. The clustering parameters were 95% identity and 90% coverage. The longest gene was selected as the representative sequence of each gene set. Clean reads of each sample were aligned against the nonredundant gene set (95% identity) use Bowtie2 (v2.2.9) [18], and the abundance of the gene in the corresponding sample was counted. The gene set representative sequence (amino acid sequence) was annotated with the KEGG database with an e-value of 1e−5. The taxonomy of the species was obtained

as a result of the corresponding taxonomy database of the NR Library, and the abundance of the species was calculated using the corresponding abundance of the genes. In order to construct the abundance profile on the corresponding taxonomy level, abundance statistics were calculated at each level of Domain, Kingdom, Phylum, Class, Order, Family, Genus and Species.

Results

Description of the sequencing data

Fecal (DNA12_12) and oral (S30_1) samples were collected from five *Diploderma splendidum* individuals. We retrieved 80 632 436 (DNA12_12) and 102 079 564 (S30_1) total reads, respectively. After quality-filtering, 220 828 (DNA12_12) and 253 812 (S30_1) ORF reads with an average of 867.55 bp (DNA12_12) and 365.61 bp (S30_1) in length were obtained for the following analysis (Table 1).

Gut microbial diversity and community composition

The 10 most abundant phyla, families and genera in the fecal samples are shown in Figure 1 and 3. Four bacterial phyla (99.0% of the observed phyla) and 15 bacterial genera (89.8% of the observed genera) were significantly abundant in the lizard *Diploderma splendidum*. *Bacteroides* (63.7%) was the most predominant phylum in the fecal samples, followed by *Firmicutes* (23.3%), *Fusobacteria* (1.72%) and *Proteobacteria* (1.43%).

Interestingly, given their different diet, reproductive status and physiological parameters, it should be noted that the insectivorous species (*Diploderma splendidum*) exhibited unique abundance of the phyla *Proteobacteria* and *Chlamydiae* and genera *Clostridium*, *Odoribacter*, *Alistipes*, *Fusobacterium*, *Escherichia*, *Hungatella*, *Mycobacterium*, *Prevotella*, and *Chlamydia* when compared with *Liolaemus. parvus*, *L. ruibali* and *P. williamsi*.

Oral cavity microbial diversity and community composition

The bacterial composition at the phylum level is illustrated in Figure 2 and 3, which shows that *Proteobacteria* (4.43%) was the most predominant phylum in the oral samples, followed by *Actinobacteria* (1.08%), *Chlamydiae* (0.55%), *Firmicutes* (0.43%) and *Bacteroidetes* (0.13%).

Additionally, a carnivorous lizard (Komodo dragon) exhibited the phyla *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes* and *Fusobacteria*, and genera *Abiotrophia*, *Alicyclobacillus*, *Staphylococcus*, *Streptococcus*, *Clostridium*, *Bacillus* (phylum *Firmicutes*), *Corynebacterium* (phylum *Actinobacteria*), *Acinetobacter*, *Basilea*, *Escherichia*, *Klebsiella*, *Proteus*, *Providencia*, *Pseudomonas*, *Serratia* (phylum *Proteobacteria*), *Bacteroides*, *Parabacteroides* (phylum *Bacteroidetes*), and *Fusobacterium* (phylum *Fusobacteria*). These findings suggested that there was considerable diversity of bacterial species between *Diploderma splendidum* and *Varanus komodoensis*.

Interestingly, there was a remarkable difference in abundance between the gut and oral cavity with regard to the microbial composition. In the gut microbiome, *Bacteroidetes*, *Firmicutes*, *Fusobacteria* and *Proteobacteria* were the dominant phyla. However, oral bacterial composition was mainly aggregated in *Proteobacteria*, *Actinobacteria*, *Chlamydiae* and *Firmicutes* (Figure 4).

Potential human pathogenic bacteria

Notably, a total of 26 species in the gut and/or oral cavity were identified as potential human pathogenic bacteria. These have not been reported previously in wild lizards (Table 2).

Clostridium perfringens, *Clostridium baratii*, *Clostridium botulinum*, *Fusobacterium mortiferum*, *Streptococcus pneumoniae*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Chlamydia trachomatis* and *Mycoplasma pneumoniae* were found in both gut and oral cavity.

Fourteen kinds of pathogen, *Fusobacterium russii*, *Prevotella denticola*, *Prevotella melaninogenica*, *Prevotella loescheii*, *Enterococcus durans*, *Streptococcus minor*, *Streptobacillus moniliformis*, *Lactococcus garvieae*, *Comamonas testosterone*, *Enterococcus cecorum*, *Desulfovibrio desulfuricans*, *Bartonella rattaustraliani*, *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*, were found only in the gut.

Discussion

Reptiles are an ancient group with more than 10,000 species, over 60% of which belong to the clade Sauria, also known as lizards [19]. Lizards have a variety of ecological, physiological and behavioral characteristics, which may influence the ecology of their microbial communities in the gut and oral cavity [20]. However, few investigations are conducted on the microbial community of reptiles, which has been greatly ignored [21]. In this study, the microbial community in the gut and oral cavity of a lizard (*Diploderma splendidum*) was described using metagenomic analysis.

Diploderma splendidum live in the Yangtze River Basin of southwestern China, and only exist in China; they are widely kept as pets and are insectivorous. However, there is a lack of basic research on the microbial ecology of the lizard gut and oral cavity. In order to expand our knowledge of the gut and oral cavity microbial diversity of lizards, five individual lizards (*Diploderma splendidum*) were captured in the Laojun mountains of Yibin, Sichuan province. The five fecal samples and oral swabs were collected and pooled.

Previous studies of a variety of species have shown that various factors have a significant influence on the microbiota, including the host species, sex, and season [22]. As expected, diet has a significant effect on the bacterial community, with differentiation observed between insectivores (*Diploderma splendidum*), omnivores (*L. parvus* and *L. ruibali*) and a herbivore (*P. williamsi*). Given the differences in geography,

species and diet, there were marked differences in the bacterial communities in insectivorous, omnivorous and herbivorous species [22].

In the present study, *Bacteroidetes* (63%) and *Firmicutes* (23%) were the dominant phyla in fecal samples, accounting for 86% of the sequences. However, *Firmicutes* (46–53%) and *Bacteroidetes* (35–39%) in *L. parvus* and *L. ruibali*, and *Firmicutes* (62–74%) and *Bacteroidetes* (11–15%) in *P. williamsi* were found in fecal samples. Bacteroidetes are abundant in many mammalian gut communities, but a low abundance of Bacteroidetes are found in insectivorous mammals such as hedgehogs and *Suncus murinus* [23, 24]. The reason for the high relative abundance of Bacteroidetes in the insectivorous lizard needs further investigation.

The phyla *Proteobacteria* and *Chlamydiae* were present in the insectivorous lizard (*Diploderma splendidum*), but absent from the omnivores (*L. parvus* and *L. ruibali*) and herbivore (*P. williamsi*). In addition, *Oscillospira*, *Ruminococcus* and *Desulfovibrio* were present in *Diploderma splendidum*, which suggested that these bacteria possibly contribute to fiber digestion and anaerobic fermentation [25, 26]. This suggested that insectivorous *Diploderma splendidum* may occasionally be herbivorous.

Diploderma splendidum and *Iguana* species are popular pets in China and the United States, which affords them with opportunities to interact with humans. Therefore, there is a risk of transmission of pathogenic bacteria to humans. As previously described, two pathogens, *Enterobacter* and *Salmonella*, were present in a herbivorous lizard species (*P. williamsi*) [27]. In this study, 26 kinds of pathogens were found in *Diploderma splendidum*, which were potentially pathogenic to reptiles and may undergo zoonotic transmission to humans. These observations indicated that keeping *Diploderma splendidum* could lead to a high risk of potential spread of emerging infectious diseases through bite wounds, or water or food contaminated with feces.

In human and rat oropharyngeal samples, *Firmicutes* is the most common phylum [28]. Very few researchers have investigated the bacterial composition of the oral cavity of lizards. However, previous studies have isolated bacterial clones from oral and saliva samples using aerobic and anaerobic cultures [29–31]. A few cases have been reported of lizards biting humans, resulting in *Staphylococcus aureus* and *Serratia marcescens* infections [32, 33]. This study investigated the bacterial communities of the oral cavity of *Diploderma splendidum*. *Proteobacteria*, *Actinobacteria*, *Chlamydiae* and *Firmicutes* were the four dominant phyla in the oral cavity. The results suggested a greater difference in oropharyngeal bacterial composition than between humans and rats, in accordance with the host tropism of microbiota. Importantly, when compared with the *Varanus komodoensis*, all bacterial phyla were common, except phylum *Chlamydiae*, which was only present in *Diploderma splendidum*. *Chlamydiae* were found in the oral cavity; they can cause human diseases and are potentially a zoonosis. Given that metagenomic analysis is more sensitive than aerobic and anaerobic cultures, a greater variety of phyla and genera of bacteria were found in this study.

In summary: 1) the composition of bacterial community of the gut of an insectivorous lizard (*Diploderma splendidum*) exhibited unique phyla when compared with *L. parvus*, *L. ruibali* and *P. williamsi*, 2) more

than twenty-six pathogenic bacteria were found in *Diploderma splendidum*, and 3) phylum *Chlamydiae* and genus *Chlamydia* were only present in *Diploderma splendidum*. All of these findings suggested that metagenomic analysis is a useful tool for revealing gut and oral microbiomes, and it has brought new insight into the complex bacterial community and ecology in *Diploderma splendidum*, allowing development of a basic database for further investigation.

Abbreviations

L. parvus: *Liolaemus parvus*; *L. ruibali*: *Liolaemus ruibali*. ORF: open reading frame

Declarations

Ethics approval and consent to participate

All applicable international, national and institutional guidelines for animal care and use were observed. All the procedures were conducted according to the Animal Ethics Procedures and Guidelines of Experiment Animals Committee affiliated to Yibin University, Yibin, China.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Doctor Launch Project of Yibin University (No. 2019QD09 and No. 2019QD10) and partly supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA 20050201) and the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (2019QZKK05010105)

Authors' contributions

TZG, HXL and GP conceived the project and GP, TZG and HXL obtained the funding. TZG and HXL wrote the paper. TZG and GDD performed the experiments and data analysis. WYY performed sample collection. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to WYY for his participation in the collection of samples.

References

1. Wang Guang-li GZ-f, Yu Ping-jing,ZHao Er-mi: **Behaviour of Japalura splendida under Artificial Feeding Condition**. *Sichuan Journal of Zoology* 2007, **04**:834-836+985.
2. McFall-Ngai M, Hadfield MG, Bosch TC, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF *et al*: **Animals in a bacterial world, a new imperative for the life sciences**. *Proceedings of the National Academy of Sciences of the United States of America* 2013, **110**(9):3229-3236.
3. Kuramitsu HK, He X, Lux R, Anderson MH, Shi W: **Interspecies interactions within oral microbial communities**. *Microbiology and molecular biology reviews : MMBR* 2007, **71**(4):653-670.
4. Archie EA, Theis KR: **Animal behaviour meets microbial ecology**. *Animal Behaviour* 2011, **82**(3):425-436.
5. Ezenwa VO, Gerardo NM, Inouye DW, Medina M, Xavier JB: **Microbiology. Animal behavior and the microbiome**. *Science (New York, NY)* 2012, **338**(6104):198-199.
6. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI: **Molecular analysis of commensal host-microbial relationships in the intestine**. *Science (New York, NY)* 2001, **291**(5505):881-884.
7. Mackie RI: **Mutualistic fermentative digestion in the gastrointestinal tract: diversity and evolution**. *Integrative and comparative biology* 2002, **42**(2):319-326.
8. Shropshire JD, Bordenstein SR: **Speciation by Symbiosis: the Microbiome and Behavior**. *mBio* 2016, **7**(2):e01785.
9. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA *et al*: **Diet rapidly and reproducibly alters the human gut microbiome**. *Nature* 2014, **505**(7484):559-563.
10. McKenney EA, Williamson L, Yoder AD, Rawls JF, Bilbo SD, Parker W: **Alteration of the rat cecal microbiome during colonization with the helminth Hymenolepis diminuta**. *Gut microbes* 2015, **6**(3):182-193.
11. Moeller AH, Li Y, Mpoudi Ngole E, Ahuka-Mundeke S, Lonsdorf EV, Pusey AE, Peeters M, Hahn BH, Ochman H: **Rapid changes in the gut microbiome during human evolution**. *Proceedings of the National Academy of Sciences of the United States of America* 2014, **111**(46):16431-16435.
12. He X, McLean JS, Guo L, Lux R, Shi W: **The social structure of microbial community involved in colonization resistance**. *The ISME journal* 2014, **8**(3):564-574.
13. Bolger AM, Lohse M, Usadel B: **Trimmomatic: a flexible trimmer for Illumina sequence data**. *Bioinformatics (Oxford, England)* 2014, **30**(15):2114-2120.

14. Li H, Durbin R: **Fast and accurate long-read alignment with Burrows-Wheeler transform**. *Bioinformatics (Oxford, England)* 2010, **26**(5):589-595.
15. Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y *et al*: **SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler**. *GigaScience* 2012, **1**(1):18.
16. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ: **Prodigal: prokaryotic gene recognition and translation initiation site identification**. *BMC bioinformatics* 2010, **11**:119.
17. Li W, Jaroszewski L, Godzik A: **Clustering of highly homologous sequences to reduce the size of large protein databases**. *Bioinformatics (Oxford, England)* 2001, **17**(3):282-283.
18. Langmead B, Salzberg SL: **Fast gapped-read alignment with Bowtie 2**. *Nature methods* 2012, **9**(4):357-359.
19. Uetz P HJ: **The Reptile Database.** . 2016.
20. Pianka ER VL: **Lizards: windows to the evolution of diversity** 2003.
21. Colston TJ, Jackson CR: **Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown**. *Molecular ecology* 2016, **25**(16):3776-3800.
22. Kohl KD, Brun A, Magallanes M, Brinkerhoff J, Laspiur A, Acosta JC, Caviedes-Vidal E, Bordenstein SR: **Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission**. *Molecular ecology* 2017, **26**(4):1175-1189.
23. Shinohara A, Nohara M, Kondo Y, Jogahara T, Nagura-Kato GA, Izawa M, Koshimoto C: **Comparison of the gut microbiotas of laboratory and wild Asian house shrews (Suncus murinus) based on cloned 16S rRNA sequences**. *Experimental animals* 2019, **68**(4):531-539.
24. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R *et al*: **Evolution of mammals and their gut microbes**. *Science (New York, NY)* 2008, **320**(5883):1647-1651.
25. Mackie RI, Aminov RI, Hu W, Klieve AV, Ouwerkerk D, Sundset MA, Kamagata Y: **Ecology of uncultivated Oscillospira species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches**. *Applied and environmental microbiology* 2003, **69**(11):6808-6815.
26. Hong PY, Wheeler E, Cann IK, Mackie RI: **Phylogenetic analysis of the fecal microbial community in herbivorous land and marine iguanas of the Galápagos Islands using 16S rRNA-based pyrosequencing**. *The ISME journal* 2011, **5**(9):1461-1470.
27. Schumacher J: **Selected Infectious Diseases of Wild Reptiles and Amphibians**. *Journal of Exotic Pet Medicine* 2006, **15**(1):18-24.
28. Gong H, Shi Y, Zhou X, Wu C, Cao P, Xu C, Hou D, Wang Y, Zhou L: **Microbiota in the Throat and Risk Factors for Laryngeal Carcinoma**. *Applied and environmental microbiology* 2014, **80**(23):7356-7363.
29. Goldstein EJ, Tyrrell KL, Citron DM, Cox CR, Recchio IM, Okimoto B, Bryja J, Fry BG: **Anaerobic and aerobic bacteriology of the saliva and gingiva from 16 captive Komodo dragons (Varanus**

komodoensis): new implications for the "bacteria as venom" model. *Journal of zoo and wildlife medicine : official publication of the American Association of Zoo Veterinarians* 2013, **44**(2):262-272.

30. Montgomery JM, Gillespie D, Sastrawan P, Fredeking TM, Stewart GL: **Aerobic salivary bacteria in wild and captive Komodo dragons**. *Journal of wildlife diseases* 2002, **38**(3):545-551.
31. Fry BG, Wroe S, Teeuwisse W, van Osch MJ, Moreno K, Ingle J, McHenry C, Ferrara T, Clausen P, Scheib H *et al*: **A central role for venom in predation by Varanus komodoensis (Komodo Dragon) and the extinct giant Varanus (Megalania) priscus**. *Proceedings of the National Academy of Sciences of the United States of America* 2009, **106**(22):8969-8974.
32. Hsieh S, Babl FE: **Serratia marcescens cellulitis following an iguana bite**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 1999, **28**(5):1181-1182.
33. Grim KD, Doherty C, Rosen T: **Serratia marcescens bullous cellulitis after iguana bites**. *Journal of the American Academy of Dermatology* 2010, **62**(6):1075-1076.

Tables

Table 1 Sequencing data of samples

Samples	Total_reads	Dehost_reads	ORF_Num	Mean_Len	GC_Content
DNA12_12	80632436	63351756	220828	867.55	44.64%
S30_1	102079564	62876978	253812	365.61	45.68%

Table 2. potential human pathogenic bacterial species in faecal and oral cavity samples

Faecal samples	Oral cavity samples
Clostridium perfringens	*Clostridium perfringens*
Clostridium baratii	*Clostridium baratii*
Clostridium botulinum	*Clostridium botulinum*
Fusobacterium russii	
Fusobacterium mortiferum	*Fusobacterium mortiferum*
Prevotella denticola	
Prevotella melaninogenica	
Prevotella loescheii	
Enterococcus durans	
Streptococcus minor	
Streptococcus pneumoniae	*Streptococcus pneumoniae*
Streptobacillus moniliformis	
Citrobacter freundii	*Citrobacter freundii*
Lactococcus garvieae	
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*
Comamonas testosteroni	
Enterococcus cecorum	
Staphylococcus aureus	*Staphylococcus aureus*
Mycobacterium tuberculosis	*Mycobacterium tuberculosis*
Listeria monocytogenes	*Listeria monocytogenes*
Chlamydia trachomatis	*Chlamydia trachomatis*
Desulfovibrio desulfuricans	
Mycoplasma pneumoniae	*Mycoplasma pneumoniae*
Bartonella rattaustraliani	
Yersinia pseudotuberculosis	
Yersinia enterocolitica	