Isolation and identification of symbiotic bacteria from the skin, mouth, and rectum of wild and captive tree shrews

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Abstract: Endosymbionts influence many aspects of their hosts' health conditions, including physiology, development, immunity, metabolism, etc. Tree shrews (*Tupaia belangeri chinensis*) have attracted increasing attention in modeling human diseases and therapeutic responses due to their close relationship with primates. To clarify the situation of symbiotic bacteria from their body surface, oral cavity, and anus, 12 wild and 12 the third generation of captive tree shrews were examined. Based on morphological and cultural characteristics, physiological and biochemical tests, as well as the 16S rDNA full sequence analysis, 12 bacteria strains were isolated and identified from the wild tree shrews: body surface: *Bacillus subtilis* (detection rate 42%), *Pseudomonas aeruginosa* (25%), *Staphlococcus aureus* (33%), *S. Epidermidis* (75%), *Micrococcus luteus* (25%), *Kurthia gibsonii* (17%); oral cavity: *Neisseria mucosa* (58%), *Streptococcus pneumonia* (17%); anus: *Enterococcus faecalis* (17%), *Lactococus lactis* (33%), *Escherichia coli* (92%), *Salmonella typhosa* (17%); whereas, four were indentified from the third generation captive tree shrews: body surface: *S. epidermidis* (75%); oral cavity: *N.mucosa* (67%); anus: *L. lactis* (33%), *E. coli* (100%). These results indicate that *S. epidermidis, N. mucosa*, *L. lactis* and *E. coli* were major bacteria in tree shrews, whereas, *S. aureus*, *M. luteus*, *K. gibsonii, E. faecalis* and *S. typhosa* were species-specific flora. This study facilitates the future use of tree shrews as a standard experimental animal and improves our understanding of the relationship between endosymbionts and their hosts.

Keywords: Tree shrew; Microbial; Separation; Identification

Symbiotic bacteria are bacteria living in symbiosis with their hosts. They influence almost every aspect of the physiological prosess of the host, including the growth and development, physiology and biochemistry, gene expression, metabolism, immunology, etc. Recent years, tree shrews (*Tupaia belangeri chinensis*) have been rapidly and widely applied in biomedical research as a novel experimental animal model, especially in studies on human diseases (Wang et al, 2010; Huang et al, 2013; Xu et al, 2013). Domestic tree shrews of China include northern tree shrews (*Tupaia belangeri*) and six suspecies (Simpson, 1945). Currently, laboratory tree shrews are mainly from the field, and therefore their unclarified genetic background, physical condition and situations of symbiotic bacteria have brought many difficulties into their application in research into the development of novel antimicrobial medications, the bacterial diseaseas, etc. Although scientists have carried out studies on the symbiotic bacteria of healthy wild tress shrews (Gao et al, 2009; Wang et al, 1987; Wang et al, 2011; Xing et al, 2012; Zhang et al, 2009), there is still no report on symbiotic bacteria from the body surface, oral cavity and anus of captive tree shrews, especially the third generation captive tree shrews. The most commonly used method in studying symbiotic bacteria is the meta-genomic methods basing on the second generation sequencing tools, which means, the extracted DNA of flora will connect with T-vector before Sanger sequencing.

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In this study, the isolated symbiotic bacteria from the body surface, oral cavity and anus of wild and third generation captive tree shrews were identified based on their morphological, cultural, physiological and biochemical characteristics, as well as the results of 16*S* rDNA whole sequence analysis. These findings not only provide basic data in setting microorganism standard of laboratory tree shrews but also are metagenomic reference of studies on the symbiotic bacteria in tree shews.

MATERIALS AND METHODS

Experimental animals

Adult (12-month-old, body weight=130−150 g), healthy wild (n=12, six males and six females) and the third generation captive tree shrews (n=12, six males and six females) were provided by the Laboratory Animal Center of Kunming Institute of Zoology, CAS. Wild tree shrews were captured from the western suburb of Kunming, Yunnan, China, and their symbiotic bacteria were sampled on the same day of capture. The rearing environment of captive tree shrews was well ventilated, at 16−25 ℃ in temperature, 40%−80% in humidity, 12 h/12 h (0800h−2000h lights on) in light/dark cycle. Tree shrews were fed a grain premixture (main ingredients include corn meal, wheat flour, fish meal, milk powder, bone meal, sugar, salt, yeast, dregs of beans and vitamins), fruits (apples and bananas), terzebrio molitors and cooked eggs. The laborotary animal production and utilization numbers are SCXK (Yunnan) K2012-0001 and SYXK (Yunnan) K2012-0003, respectively.

Experimental reagents and facilities

Columbia blood agar bases, Salmonella Shigella agar bases and Mac Conkey agar bases were all from OXIOD (UK); Biochemical identification tubes were from Biomerieux (France); Luria Bertani (LB) agar bases were homemade.

The instruments and facitities used in this study include PCR instrument (Biorad Mycycler), CO_2 incubator (Thermo scientific Series 8000DH), thermostatical rocking plate (COS-211C), sterile operation platform (BAKER A2), automatic autoclave (THA-3560C), transmission electron microscope (HITACHI, H-7650), spectrophotometer (mode 721).

Experimental methods

Symbiotic bacteria from the body surface, oral cavity and rectum of wild and third generation captive tree shrews were sampled, cultured and isolated. The isolated bacteria stains were identified and systematically classified based on their morphological, cultural, physiological and biochemical characteristics, combining the results of 16*S* rDNA whole sequence analysis. The flowchart of experimental procedures (approve number: SYBW20110416-1) is shown in Figure 1.

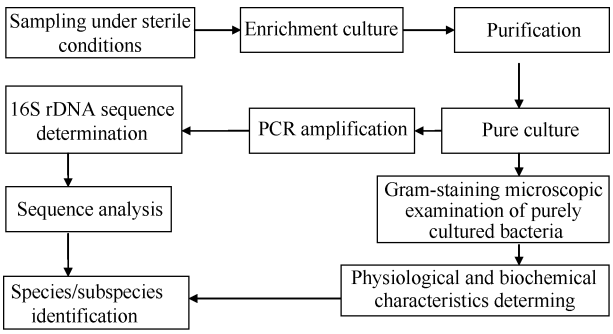


Figure 1 Flowchart of experimental procedures

Bacteria sampling and pure culture

Next to the alcohol burner inside of the sterile operation platform, symbiotic bacteria from the body surface (sampling a bit of fur by a sterile biceps), (using sterile cotton swab) and rectum (using sterile cotton swab) of 24 tree shrews were sampled. Body surface and oral specimens were inoculated onto the Columbia blood agar bases and LB agar bases, whereas, rectal specimens were inoculated onto the SS agar bases and Mac Conkey agar bases. Bacterial species can be initially and roughly identified according to their growth situations on different agar bases. Innoculated agar bases were cultured in 37 ℃ incubator. The morphology, color, hemolysis and pigments of the colonies were observed 18−72 h later. Single colony (numbered from hs1 to hs12) was picked by a sterile toothpick and placed on a fresh surface for futher indentification.

Morphological identification

Gram-staining used in this study was ammonium oxalate crystal violet staining (Katznelson et al, 1964). Specimens were fixed in 2.5% glutaraldehyde, stained with 0.1mol/L tungstophosphoric acid and then observed under a transmission electron microscope.

Biochemical characteristics identification

Main biochemical indexes of isolated bacteria strain were determined *via* the biochemical identification tubes. Strains cultured overnight at 30 ℃ were inoculated into the biochemical identification tube *via* the sterile inoculating loop.Every strain inoculation were triplicated. Negative control tubes were void of bacteria. Results were read within 12−72 h. Positive results went through

a three-generation continuous inoculation. Indentification conclusions were obtained by comparing the data against the characterics of other close bacteria strains.

Physiological characteristics identification

The physiological characters of bacteria strains mainly include growth temperature, pH tolerance range and optimal pH, as well as salt endurance.

Fresh bacteria fluid (5%) was inoculated onto the improved LB liquid agar bases, shaking cultured by thermostatical rocking plates (180 r/min) at 5, 10, 25, 30, 35, 37, 40, 42, 45 and 50 ℃, respectively. OD_{600} light obsorption values were obtained at 24, 48, 72 h, respectively, to determine the growth range at different temperatures.

To obtain the optimal pH value, fresh bacteria fluid (5%) were inoculated onto the improved LB liquid agar bases with pH at 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0, respectively, and shaking cultured at 30 ℃, 180 p/min. OD_{600} light absorption values were obtained at 24, 48, 72 h, respectively, to determine the pH tolerance range.

Fresh bacteria fluid were inoculated onto the improved LB liquid agar bases with different NaCl concentrations (0%−10%) and cultured at 30 ℃ for 48 h, then their OD_{600} light absorption values were obtained to determine their salt endurance range and the optimal salt concentration.

16S rDNA sequence analysis

The unindentified bacteria strains were under pure cultures and small scale amplifications. Universal primers were designed based on the high conservativeness of 16*S* rDNA sequence in prokaryotes and produced by Sangon Biotech (Shanghai, China) (forward: 5'-AGAGTTTGA TCCTGGCTCAG-3'; reverse: 5'-AAG GAGGTGATCC AAGCCGCA-3'). Bateria DNA were templates and the PCR products were larger than 1 400 bp. The reaction solution (50 μL) included 2.5 ng templated DNA, 10 μL forward primer, 10 μL reverse primer, 0.25 μL TaKaRa (5 U/μL), 5.0 μL 10× PCR Buffer (Mg^{2+} Free), 4.0 μL $MgCl_2$ dNTP mixture and 35.75 μL sterile distilled water. PCR reactions were pre-denaturation at 94 ℃ for 4 min, denaturation at 94 ℃ for 1 min, renaturation at 52 ℃ for 1 min, extension at 72 ℃ for 2 min, incubate at 72 ℃ for 10 min after 30 cycles and then kept at 8 ℃. PCR products were detected by 1% agarose gel electrophoresis and then sequenced by Genscript (Nanjing, China).

16S rRNA sequences were obtained via Chromas and DNASTAR.Lasergene.v7.1, and then went through the phylogenetic analysis *via* Blast (NCBI) (http: www.ncbi. nlm.nih.gov/Blast/).

Bacteria identification

The isolated bacteria stains were identified and systematically classified based on their morphological, cultural, physiological and biochemical characteristics, combining the results of 16*S* rDNA whole sequence analysis.

RESULTS

Identification results of the isolated symbiotic strains

The results of staining microscopy, physiological characteristics and 16S rDNA whole sequence analysis of the isolated symbiotic bacteria were shown in Table 1.

High homologies were found by comparing the 16*S* rDNA sequences of these isolated strains with the gene sequences of the online registed stains (http: //www.ncbi. nlm.nih.gov). When the results of phylogenetic tree show that an isolated unknown strain has more than 99% homology with a known strain, then according to its morphology, physiological and biochemical characteristics, this isolated unknown strain can be identified.

Physiological and biochemical characteristics of the isolated bacterial strains

The physiological and biochemical characteristics of the isolated bacterial strains, from hs1 to hs12, were shown in Table 2 to Table 13, respectively.

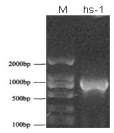
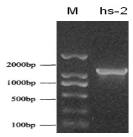
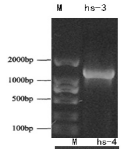
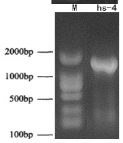
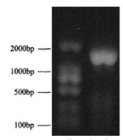
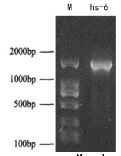
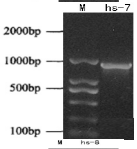
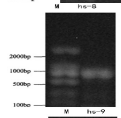
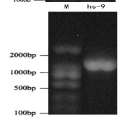
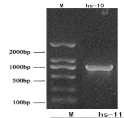
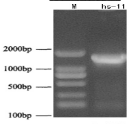
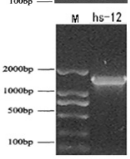
Detected bacteria from the wild (Table 14) and third generation captive tree shrews (Table 15)

In this study, 12 bacteria species were identified from the wild tree shrews, body surface: *B. subtilis* (42%), *P. aeruginosa* (25%), *S. aureus* (33%), *S. epidermidis* (75%), *M. luteus* (25%) and *K. gibsonii* (17%); oral cavity: *N. mucosa* (58%), *S. pneumoniae*, (17%); anus: *E. faecalis* (17%), *L. lactis* (33%), *E. coli* (92%) and *S. typhosa* (17%). Four bacteria specises were identified from the third generation captive tree shrews, body surface: *S. epidermidis* (75%); oral cavity: *N. mucosa* (67%); rectum: *L. lactis* (33%) and *E. coli* (100%). *S. epidermidis*, *N. mucosa*, *L. lactis* and *E. coli* were the major symbiotic bacteria strains of tree shews, whereas, *S. aureus*, *M. luteus*, *K. gibsonii*, *E. faecalis* and *S. typhosa* are species-specific bacteria strains of tree shrews.

DISCUSSION

Symbiotic bacteria affect many aspects of the host's physiological conditions, including the growth and development, gene expression, metabolism, biochemical

Table 1 Results of staining microscopy, physiological characteristics and electrophoregram of the isolated symbiotic bacteria

Bacteria	Microscopy					Temperature (℃)		pH tolerence range	NaCl agar base (%)		Electrophoregram
	Gram's stain	Morphology	Brood body	Flagellum	Capsule	Growth range	Optimal		Growth range	Optimal	
B. subtilis	+	Rhabditiform; paired or in chains; round or squre ends	Y			5−50	25−30	6.5−9.5	0.6−1.2	1.0	
E. coli	−	Brevibacterium; median sized; blunt ends; scattered or paired	N			5−40	20−30	6.5−9.5	0.1−1.0	0.6	
E. faecalis	+	Globular or oval	N	Y	N	10−45	37	4.2−4.6	0.1−6.5	4.0	
P. aeruginosa	−	Thallus slender and in various sizes; shape in rod or linear; paired or in short chains	N	Y		25−42	25−30	4.2−4.6	0.1−6.5	4.0	
K. gibsonii	+	Blunt ends; scattered	N	Y		25−42	25−30	4.2−4.6	0−6.0	4.0	
N.mucosa	−	Small thallua, reniformed; in dual rank	N	Y		25−38	30	7.0−7.5	0−6.0	4.0	
S.epidermidis	+		N	N	N	20−45	37	6.0−9.0	1.0−10.0	5.0	
S. epidermidis	+	Globular or slightly oval; aciniformed	N	N	N	25−40	37	4.5−9.8	1.0−8.5	3.0	
L. lactis	+	Globular or oval	N		N	5−40	30	6.5−9.8	0.1−7.5	2.0	
M. luteus	+	Cellular spherical; paired, in quadruplet or clustered	N			5−40	25−37	5.5−9.0	0.1−7.5	2.5	
S. pneumoniae	+	Globular or oval; paired or in chains	N	N	N	5−40	37	6.5−8.5	0.1−8.5	4.0	
S. typhosa	−	Brevibacterium; blunt ends; scattered	N		N	5−40	35	6.5−9.5	0.1−1.0	0.6	

+: Positive in Gram's stain; −: Negative in Gram's stain; Y: Presence; N: Absence.

Table 2 Physiological and biochemical characteristics of hs1

Characteristics	Results	Characteristics	Results
Catalase	+	Nitrate-reducing	+
Carbamide	−	Amylohydrolysis	+
Gelatin liquefaction	+	Citrate	+
Sucrose	+	D-Xylose	+
D-mannitol	+	D-Mannose	+/−
Sorbierite	−	Maltose	−
D-raffinose	−	L-pectinose	+

+: Positive or capable of utilizing; −: negative or incapable of utilizing; +/−: Subtle differences between strains of one genus.

Table 3 Physiological and biochemical characteristics of hs2

Characteristics	Results	Characteristics	Results
Mannite	+	V-P test	−
Lactose	+	Phaseomannite	−
Esculiw	+	D+ (−) glycogen	−
Maltose	+	Salicin	−
Glucose	+	Methyl red	+
Raffinose	+	Mushroom sugar	+
Arabinose	+	Peroxidase	+
Citrate	−	Tyrosine	−
Xylose	+	Indole	+
Carbamide	−	Melibiose	+
Phenylalanine	−	Oxidase	−
H_2S	−	Casein protein breakdown	−
Fructose	+	Amylohydrolysis	+
Sorbierite	+	Gelatin liquefaction	−

+: Positive or capable of utilizing; −: Negative or incapable of utilizing.

Table 4 Physiological and biochemical characteristics of hs3

Characteristics	Results	Characteristics	Results
Lactose	+	Hemolytic	+
Maltose	+	Catalase	−
Mannite	+	Sorbierite	+
Glucose	+	10% gail	+
Raffinose	−	40% gail	+
Xylose	−	Salicin	+
Arabinose	−	B-Galactose glucoside enzyme	+
Rhamnose	−	Oxidase	−
Amylase	−	Arginine hydrolysis	+
Esculin hydrolysis	+	Sucrose	+
Nitrate-reducing	+		

+: Positive or capable of utilizing; −: Negative or incapable of utilizing.

Table 5 Physiological and biochemical characteristics of hs4

Characteristics	Results	Characteristics	Results
Maltose	−	Glutin	+
Mannite	−	Mushroom sugar	−
Sucrose	−	Xylose	−
Galactose	−	Acetamide	+
Carbamide	+	H_2S	−
Arabinose	−	Nitrate-reducing	+
Fructose	−	Oxidase	+
Indole	−	Arginine hydrolysis	+
Glucose; acid-producing Glucose; aerogenesis	+ −	Citrate	+

+: Positive or capable of utilizing; ー: Negative or incapable of utilizing.

Table 6 Physiological and biochemical characteristics of hs5

Characteristics	Results	Characteristics	Results
Arabinose	−	Mannite	−
V-P test	−	Seminose	−
Esculin hydrolysis	−	Indole	−
Rhamnose	−	Fucose	−
Lactose	−	A-methyl-D-glucoside	−
Methyl red	−	Nitrate-reducing	−
Fructose	−	Acetate	−
Sucrose	−	Sorbose	−
Raffinose	−	Phaseomannite	−
Maltose	−	B-galactosidase	−
Galactose	−	Melibiose	−
Sorbierite	−	Glycerol	−
Glucose; acid-producing Glucose; aerogenesis	−	Citrate	−
Urease	−	Gelatin liquefaction	−
Amylase	−	Oxidase	−
Melampyrite	−	Catalase	+

+: Positive or capable of utilizing; −: Negative or incapable of utilizing.

Table 7 Physiological and biochemical characteristics of hs6

Characteristics	Results	Characteristics	Results
Sucrose	+	Nitrate	+
Glucose	+	Nitrite	+
Peroxidase	+	Oxidase	+
Maltose: acid-producing Aerogenesis	+	Indole	−
Lactose	−		

+: Positive or capable of utilizing; −: Negative or incapable of utilizing.

Table 8 Physiological and biochemical characteristics of hs7

Characteristics	Results	Characteristics	Results
Robiocina	+	Sucrose	+
Plasma-coagulase	+	lactose	+
Oxidase	+	maltose	+
Carbamide	+	glucose	+
Methyl red	+	arginine	+
Mannite	+	V-P test	Weak +
Gelatin Liquefaction	+	nitrate	+

+: Positive or capable of utilizing; −: Negative or incapable of utilizing.

Table 9 Physiological and biochemical characteristics of hs8

Characteristics	Results	Characteristics	Results
Gelatin liquefaction	+	Glucose	+
V-P test	+	Mannite	−
Urease	−	Sucrose	+
Plasma-coagulase	−	Lactose	+
H_2S	−	Maltose	+
M．R	+	Fructose	+

+: Positive or capable of utilizing; − : Negative or incapable of utilizing.

Table 10 Physiological and biochemical characteristics of hs9

Characteristics	Results	Characteristics	Results
Arginine hydrolase	+	Maltose	+
Glucose	−	Melibiose	−
Amylase	−	Galactose	+
Indole	−	Lactose	+
Methyl red	+	Ribose	+
Oxidase	−	Fructose	+
Glutin	−	Melizitose	−
Catalase	−	Raffinose	−

+: Positive or capable of utilizing; − : Negative or incapable of utilizing.

Table 11 Physiological and biochemical characteristics of hs10

Characteristics	Results	Characteristics	Results
Mannite	−	Lactose	−
Gelatin hydrolysate	+	Inorganic nitrogen agar	+
Glycerol	−	Glucose	−
Oxidase	+	Arginine	−
Catalase	+	Nitrate	−
Esculin hydrolysis	+	Citrate	−

+: Positive or capable of utilizing; −: Negative or incapable of utilizing.

Table 12 Physiological and biochemical characteristics of hs11

Characteristics	Results	Characteristics	Results
Starch	+	Synanthrin	−
Mannite	−	Lactose	−
50% gall	+	Maltose	+
Sucrose	+	Arabinose	+
Glucose	−	Sorbierite	+
Raffinose	−		

+: Positive or capable of utilizing; −: Negative or incapable of utilizing.

Table 13 Physiological and biochemical characteristics of hs12

Characteristics	Results	Characteristics	Results
Arabinose	−	Sucrose	−
Ornithine	+	Mannite: acid-producing Aerogenesis	+ +
Maltose	+	Carbamide	−
D-tartrate	−		
Lactose	−	A-methyl-D-glucoside	−
V-P test	−	Melampyrite	−
Sorbierite	−	Methyl red	+
Glucose: acid-producing Glucose: aerogenesis	+ +	Phenylalanine	−
Fructose	−	Glutin	−
Indole	−	Citrate	+

+: Positive or capable of utilizing; −: Negative or incapable of utilizing.

changes and immunity. Therefore, it is important to understand the classifications of symbiotic bacteria.

The symbiotic bacteria hosted in tree shrews are affected by the living environment of microorganisms

The symbiotic bacteria hosted in tree shrews are diversified and complicated, and are greatly affected by the living environment of microorganisms. In this study, 12 bacteria strains were isolated and identified from the wild tree shrews. *B. subtilis* widely exsits in earth and soiled organisms, and multiplies quickly in hay infusion. In this study, *B. subtilis* was identified only from the body surface of wild tree shrews, with detectable rate at 50%, indicating it is a commonly hosted bacterium in wild tree shrews. *P. aeruginosa*, which was indentified from wild tree shrews with detectable rate at 25% in this study, is one of the most commonly found bacteria in earth. *S. aureus* is highly pathogenic and exsits in air, water, dirt and excretions, whereas, *S. epidermidis* breeds on body surfaces and is a normal flora. The detectable rates of these two *Staphlococcus* bacteris, which were 33% and 75%, respectively, are probably due to their

Table 14 Detected bacteria of 12 wild tree shrews

Genus	Species	Sampling area			Numbers of the detected bacteria (*n*)		Detectable rate (%)
		Body surface	Oral	Rectal	♀	♂	
Bacillus	*B. subtilis*	●	○	○	3	2	42
Escherichia	*E. coli*	○	○	●	6	5	92
Enterococcus	*E. faecalis*	○	○	●	1	1	17
Pseudomonas	*P. aeruginosa*	●	○	○	1	2	25
Kurthia	*K. gibsonii*	●	○	○	1	1	17
Kurthia	*N. mucosa*	○	●	○	3	4	58
Staphlococcus	*S. aureus*	●	○	○	2	2	33
Staphlococcus	*S. epidermidis*	●	○	○	5	4	75
Lactococus	*L. lactis*	○	○	●	2	2	33
Micrococcus	*M. luteus*	●	○	○	1	2	25
Streptococcus	*S. pneumoniae*	○	●	○	1	1	17
Salmonella	*S. typhosa*	○	○	●	1	1	17

●: Positive; ○: Negative.

Table 15 Detected bacteria of 12 third generation captive tree shrews

Genus	Species	Sampling area			Numbers of the detected bacteria (*n*)		Detectable rate (%)
		Body surface	Oral	Rectal	♀	♂	
Escherichia	*E. coli*	○	○	●	6	6	100
Neisseria	*N. mucosa*	○	●	○	4	4	67
Staphlococcus	*S. epidermidis*	●	○	○	5	4	75
Lactococus	*L. lactis*	○	○	●	2	2	33

●: Positive; ○: Negative.

wide distrbutions in natural environments. *M. luteus* can be found in air, earth, water, as well as on the body surface of animals and plants. This conditioned pathogen may cause local or severe infections in tissues. *K. gibsonii* aften grows in animal excretions or meat products and so far, there are no reports of *K. gibsonii* as a pathogen. In this study, 17% *K. gibsonii* were detected from the body surface of wild tree shrews. The detectable rate of oral *N. mucosa* was 67%. It is mainly hosted in the oral mucosa of mammals and is a non-pathogenic bacterium. *S. pneumoniae* exists in natural environments, animal excretions and the pharynx nasalis of healthy human beings and was detected in two wild tree shrews. *E. faecalis* was only found in wild tree shrew rectums. *L. lactis* can be found in diary and plantation products. Our results show that its detectable rates in both wild and third generation tree shrew were both 33%, indicating its probiotic advantages in tree shrews. *E. coli* are widely distributed in natural environments and mostly are non-panthogenic. Although the detectable rates of *Escherichia coli* were extremely high in this study (92%−100%), we consider them as intestinal tract normal flora, because all the experimental tree shrews were healthy and did not show any clinical disorders. *S. typhosa* is one of the bacteria that can easily cause infections and exists in almost every natural environment, including the air, water, food, vegetable, dirt and animal excretions. We only found *Salmonella typhosa* in two wild tree shrews, indicating it is a rare strain in tree shrews.

The influences of artificial environments to the symbiotic bacteria hosted in tree shrews

The succeful artifical reproductions of tree shrews require several aspects the artifical environments, including the facilities, nutritions, as well as the proper managing and reproducing methods (Li et al, 2009; Jiang et al, 2011; Zhao et al, 2013). The long-term and frequent application of sterilization measures determined that less bacteria exist in artifical environments than in wild.

In this study, four bacteria strains were isolated and identified from the third generation of captive tree shrews, suggesting that the living environments are significantly correlated with the species of microorganisms.

As a novel animal to study human diseases and from the angle of evolution, to understand the similarities and differences of symbiotic bacteria from the same boby part of human and tree shrewsis necessary. Now, Human Microbiome Project (HMP) has carried out detailed investigations on the bacteria flora of human skin, oral cavities and intestinal tracts to determine the factors influencing the growth of symbiotic bacteria, such as gender, race, geography distribution, diet and body weight.

CONCLUSIONS

The tree shrews used in this study were all in good healthy condition at time of sampling and 30 days after sampling. Therefore, we assume that the indentified bacteria are major parasitic bacteria of tree shrews. The high detectable rate indicates that *E.coli* is the major normal parasitic bacteria, which is consistant with the study of Gao et al (2009). In other conventional laboratory animals, *Staphlococcus* bacteria is rare; however, in both wild and third generation captive tree shrews, its detectable rates were high (75%). Our results also show a high occurrence of pathogens in wild tree shrews (17%−33%), whereas no pathogens were found in the third generation captive tree shrews.

As a novel laboratory animal, the applications of tree shrews are still lack of national standards (Shen et al, 2011). For example, for the conventional animal model rhesus monkeys (*Macaca mulatta*), it is clearly pointed out that laboratory rhesus monkeys have to be clean of *Salmonella* spp. *Pathogenic dermal fungi* and *Campylobaceter jejuni.* Wang et al (1987) reported that *C. jejuni* and Shigella exsit in the intestinel tract of healthy adult tree shrews. So far, no *Brucella* spp., *Leptospira* spp., *Mycobacterium tuberculosis* or *Yersinia enterocolitica* have been found in tree shrews, suggesting that bacteria strains may function species-specifically in different parts of different animals.

In recent years, the advantages of tree shrews as a novel animal model have become apparent, because they are close to primates, they have been widely applied in studies on virus (Li et al, 2011; Wang et al, 2012b), neuronal peptide Y (Dong et al, 2011), disbetes (Wu et al, 2013), depression (Wang et al, 2012a), cerebral ischemia (He et al, 2011), etc. Therefore, it is nessassary and to set standards to normalize tree shrews as a laboratory animal model.

Based on the enriched tree shrew resources of Yunnan Province, our lab for the first time has successfully isolated and identified the symbiotic bacteria of the third generation captive tree shrews. These findings not only provide basic data in setting the microorganism standard of laboratory tree shrews but also are metagenomic reference of studies on the symbiotic bacteria in tree shews.

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