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American cockro ISSN 0001 - 6209; CN 11 - 1995/Q
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Wei Fang^{1,2#}, Zemin Fa http://journals. im. ac. cn/actamicrocn
 **Phylogenetic anal
American cockro**

Wei Fang^{1,2#}, Zemin Fan

Peng^{1,2} - Yuzhi Hong^{1,2} Phylogenetic analysis of bacterial community in the gut of American cockroach (Periplaneta americana)

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n im , Zemin Fang^{1,2#}, Zhimiao Liu^{1,2}, Jing Yuan^{1,2}, Xuecheng Zhang^{1,2}, 1 1,2#
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Pei
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Peng^{1,2},
¹ School of Lift
and Biocataly
Abstract: [Commericana, as
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² Anhui Provincia²
² Anhui Provincia
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ng tl $\frac{1}{2}$ School of Life Sciences, Anhui University, ² Anhui Provincial Engineering Technology Research Center of Microorganisms

and Biocatalysis, Hefei 230601, China
 bstract: [**Objective**] The present study was to fully evalu and Biocatalysis, Hefei 230601, China
 Abstract: [**Objective**] The present
 americana, an important model to study

by culture-independent methods, invol

[**Results**] The phylotypes were affiliate
 Fusobacteria (0.6% Abstract: [Objective] The present study was to fully evaluate the intestinal bacterial community of Periplaneta The present study was to fully evaluate the intestinal bacterial community of *Periplaneta*
nodel to study insects. [**Methods**] We investigated the bacterial community of *P. americana* gut
ethods, involving constructing americana, an important model to study insects. [Methods] We investigated the bacterial community of P. americanagut an important model to study insects. **[Methods]** We investigated the bacterial community of *P. americanagut* idependent methods, involving constructing the 16S rRNA gene library and microbial diversity analysis.
The phyl by culture-independent methods, involving constructing the 16S rRNA gene library and microbial diversity analysis.

[Results] The phylotypes were affiliated with *Proteobacteria* (66.4%), *Bacteroidetes* (17.8%), *Firmicu* **[Results]** The phylotypes were affiliated with *Proteobacteria* (66.4%), Bacteroidetes (17.8%), Firmicutes (14.5%), The phylotypes were affiliated with *Proteobacteria* (66. 4%), *Bacteroidetes* (17. 8%), *Firmicutes* (14. 5%), τ (0. 6%) and unclassified bacteria (0. 6%). Phylogenetic analysis shows that 15% of the sequences ith tha *Fusobacteria* (0.6%) and unclassified bacteria (0.6%). Phylogenetic analysis shows that 15% of the sequences 0. 6%) and unclassified bacteria (0.6%) . Phylogenetic analysis shows that 15% of the sequences
that from a closely related omnivorous cockroach; and 59% clustered with that from more distantly related
ling omnivorous, h clustered with that from a closely related omnivorous cockroach;and 59% clustered with that from more distantly related
animals, including omnivorous, herbivorous, and carnivorous animals, which differ greatly in feeding h animals, including omnivorous, herbivorous, and carnivorous animals, which differ greatly in feeding habits. Moreover,
18% of the clones showed high sequence identity with potential pathogens closely related to human disea 18% of the clones showed high sequence identity with potential pathogens closely related to human diseases, which also reinforces the concept of the cockroach as a carrier of pathogens. [**Conclusion**] Due to their habits o reinforces the concept of the cockroach as a carrier of pathogens. [**Conclusion**] Due to their habits of feeding on a variety
of foodstuffs, omnivorous cockroaches harbor a large and diverse microbial community in the gut. of foodstuffs, omnivorous cockroaches harbor a large and diverse microbial community in the gut. The host phylogeny and
dietary habits might be critical for the intestinal bacterial community composition of cockroaches.
K

Keywords: American cockroach, 16S rRNA gene, microbial community

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CLC number: Q938 **Document code**: A **Article ID**: 0001-6209 (2013) 09-0984-11
There are 3500 to 4000 species of cockroaches, may be potential health prob
a American cockroach, 16S rRNA gene, microbial community

: Q938 **Document code**: A **Article ID**: 0001-6209 (

are 3500 to 4000 species of cockroaches, may be

: considered one of the most significant and neighbors

e pests CLC number: Q938 **Document code**: A **Article ID**: 0001-6209 (2013) 09-0984-11

There are 3500 to 4000 species of cockroaches, may be potential health

and they are considered one of the most significant and neighbors. In There are 3500 to 4000 species of cockroaches,
hey are considered one of the most significant and
tionable pests $^{[1]}$. Approximately 50 species of
oaches have been reported as pests in human
tecture and households worldw objectionable pests ^[1]. Approximately 50 species of
cockroaches have been reported as pests in human
architecture and households worldwide, some of which
weight and the specialized Research Fund for the Doctoral Program objectionable pests $^{[1]}$. Approximately 50 species of cockroaches have been reported as pests in human architecture and households worldwide, some of which supported by the Specialized Research Fund for the Doctoral Pro

may be problems. In China, the subtropical climate is
favorable for the growth and development of *Periplaneta*
americana, which is the dominant species in southern
China, making up 70 percent of the total ^[2].
Higher neighbors. In China, the subtropical climate is
favorable for the growth and development of *Periplaneta*
americana, which is the dominant species in southern
China, making up 70 percent of the total ^[2].
Higher Educat favorable for the growth and development of *Periplaneta*
americana, which is the dominant species in southern
China, making up 70 percent of the total $[2]$.
Higher Education of China (20093401110006), by the Natural Sci americana, which is the dominant species in southern

Supported by the Specialized Research Fund for the Doctoral Program of Higher Education of China (20093401110006), by the Natural Science
Foundation of the Department of Education of Anhui Province (KJ2010A026), by the Int Foundation of the Department of Education of Anhui Province (KJ2010A026), by the Introduction Project of Academic and Technology Leaders in
Anhui University (32030066) and by the Innovative Research Team Program of 211 Pro Anhui University (32030066) and by the Innovative Research Team Program of 211 Project in Anhui University
* Correspondence author: Tel/Fax: +86-551-63861861; E-mail: yzxiao@ahu. edu. cn
* These authors contributed equally architecture and households worldwide, some of which

Supported by the Specialized Research Fund for the Doctoral Program

Foundation of the Department of Education of Anhui Province (KJ2

Anhui University (32030066) and b architecture and households worldwide, some of which

University Supported by the Specialized Research Fund for the Doctoral Program

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Anhui University (3203 which is the dominant species in southern
 $\sin \theta$ up 70 percent of the total $^{[2]}$.

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coduction Project of Academic and Technology Leaders in

et in Anhui University China, making up 70 percent of the total $[2]$.
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26), by the Introduction Project of Academic and Technology Leaders is
am of 211 Project in Anhui Universit

Correspondence author: Tel/Fax: +86-551-63861861; E-mail: yzxiao@ ahu. edu. cn
These authors contributed equally to this work.
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| These authors contributed equally to this work.

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American cockroaches are carriers, either on the cuticle or in the gut, of a variety of bacteria, including potential pathogens; some pathogens may carry resistance to antibiotics or drugs $[3-5]$. When the cockroaches fl cuticle or in the gut, of a variety of bacteria, including
potential pathogens; some pathogens may carry
resistance to antibiotics or drugs $[3-5]$. When the
cockroaches fly, they have many opportunities to pick
up, carry potential pathogens; some pathogens may carry
resistance to antibiotics or drugs $[3-5]$. When the
cockroaches fly, they have many opportunities to pick
up, carry, and transmit various bacteria $[3]$. Although
definitive resistance to antibiotics or drugs $[3-5]$. When the cockroaches fly, they have many opportunities to pick up, carry, and transmit various bacteria $[3]$. Although definitive evidence that cockroaches are vectors for huma cockroaches fly, they have many opportunities to pick
up, carry, and transmit various bacteria^[3]. Although
definitive evidence that cockroaches are vectors for
human disease is still lacking, the prevalence of
cockroach up, carry, and transmit various bacteria $^{[3]}$. Although
definitive evidence that cockroaches are vectors for
human disease is still lacking, the prevalence of
cockroaches near human and animal wastes, human
food, and hu definitive evidence that cockroaches are vectors for
human disease is still lacking, the prevalence of
cockroaches near human and animal wastes, human
food, and human environments creates sufficient
concern about their ro human disease is still lacking, the prevalence of
cockroaches near human and animal wastes, human
food, and human environments creates sufficient
concern about their role as vectors $^{[3]}$. Therefore,
omnivorous cockroach cockroaches near human and animal wastes, human
food, and human environments creates sufficient
concern about their role as vectors $^{[3]}$. Therefore,
omnivorous cockroach *Periplaneta americana*, along
with *Blattella ge* food, and human environments creates sufficient
concern about their role as vectors $^{[3]}$. Therefore,
omnivorous cockroach *Periplaneta americana*, along
with *Blattella germanica*, *Blatta orientalis*, and *Supella*
lo concern about their role as vectors ^[3]. Therefore,
omnivorous cockroach *Periplaneta americana*, along
with *Blattella germanica*, *Blatta orientalis*, and *Supella*
longipalpa have been classified into Group I of the
 longipalpa have been classified into Group I of the "Dirty 22" by the Food and Drug Administration $^{[6]}$.

omnivorous cockroach *Periplaneta americana*, along
with *Blattella germanica*, *Blatta orientalis*, and *Supella*
longipalpa have been classified into Group I of the
"Dirty 22"by the Food and Drug Administration ^[6]. with *Blattella germanica*, *Blatta orientalis*, and *Supella*
 longipalpa have been classified into Group I of the

"Dirty 22"by the Food and Drug Administration ^[6].

Because of its ubiquity, relatively large size, by the Food and Drug Administration $^{[6]}$.
se of its ubiquity, relatively large size, and
nature, P. americana is an important
tudies of insect physiology, biochemistry,
ior $^{[7]}$. By employing various culture-
techniqu Dirty 22"by the Food and Drug Administration $^{[6]}$.
Because of its ubiquity, relatively large size, a
mnivorous nature, *P. americana* is an importa
odel for studies of insect physiology, biochemistr
nd behavior $^{[7]}$. Because of its ubiquity, relatively large size, and
vorous nature, *P. americana* is an important
1 for studies of insect physiology, biochemistry,
behavior $\left[\begin{array}{ccc} 7 \end{array}\right]$. By employing various culture-
ndent techni omnivorous nature, *P. americana* is an important
model for studies of insect physiology, biochemistry,
and behavior $\left[\begin{matrix}7\end{matrix}\right]$. By employing various culture-
dependent techniques, including scanning and
transmissi model for studies of insect physiology, biochemistry,
and behavior $\left[\begin{matrix}7\end{matrix}\right]$. By employing various culture–
dependent techniques, including scanning and
transmission electron microscopy and selective and
non-selec and behavior $^{[\7]}$. By employing various culture-
dependent techniques, including scanning and
transmission electron microscopy and selective and
non-selective medium $^{[\7-9]}$, the association and
transmission of living dependent techniques, including scanning and
transmission electron microscopy and selective and
non-selective medium $\left[\begin{array}{c} 7 & -9 \end{array}\right]$, the association and
transmission of living bacteria within the digestive tract
o non-selective medium $\left[7-9\right]$, the association and
transmission of living bacteria within the digestive tract
of *P*. *americana* have been investigated. However,
because of the high selectivity of culture-based
approa non-selective medium $\left[\begin{array}{cc} 7-9 \end{array}\right]$, the association and
transmission of living bacteria within the digestive tract
of *P*, *americana* have been investigated. However,
because of the high selectivity of culture-b of *P. americana* have been investigated. However,
because of the high selectivity of culture-based
approaches, they may not fully assess the complete
picture of bacterial community composition of *P.*
americana. Therefore of *P. americana* have been investigated. However,
because of the high selectivity of culture-based
approaches, they may not fully assess the complete
picture of bacterial community composition of *P.*
americana. Therefore approaches, they may not fully assess the complete
picture of bacterial community composition of *P*.
americana. Therefore, comprehensive cataloguing and
comparative analysis of gut bacteria from *P. americana*
are neede approaches, they may not fully assess the complete
picture of bacterial community composition of *P*.
americana. Therefore, comprehensive cataloguing and
comparative analysis of gut bacteria from *P. americana*
are needed, picture of bacterial community composition of *P.*
 americana. Therefore, comprehensive cataloguing and

comparative analysis of gut bacteria from *P. americane*

are needed, which will broaden our knowledge of the

cock americana. Therefore, comprehensive cataloguing and Therefore, comprehensive cataloguing and
analysis of gut bacteria from *P. americana*
which will broaden our knowledge of the
ssociated microbiota in American cockroach
profile the contribution of intestinal bacteria
corou comparative analysis of gut bacteria from *P. americana*
are needed, which will broaden our knowledge of the
cockroach-associated microbiota in American cockroach
and further profile the contribution of intestinal bacteria

are needed, which will broaden our knowledge of the
cockroach-associated microbiota in American cockroach
and further profile the contribution of intestinal bacteria
to the omnivorous lifestyle of the cockroach.
To better and further profile the contribution of intestinal bacteria
to the omnivorous lifestyle of the cockroach.
To better understand microbial ecology in the
American cockroach intestine, we surveyed the
intestinal bacterial com to the omnivorous lifestyle of the cockroach.

To better understand microbial ecology in the

American cockroach intestine, we surveyed the

intestinal bacterial community of P. americana by

constructing a 16S rRNA gene l To better understand microbial ecolo
American cockroach intestine, we sure
intestinal bacterial community of *P. am*
constructing a 16S rRNA gene library and re
both host phylogeny and omnivorous diet
bacterial community. The active and setting interesting ecology in the side of the inal bacterial community of *P. americana* by ructing a 16S rRNA gene library and revealed that host phylogeny and omnivorous diet shaped the rial community. Th American cockroach intestine, we surveyed the
intestinal bacterial community of *P. americana* by
constructing a 16S rRNA gene library and revealed that
both host phylogeny and omnivorous diet shaped the
bacterial communit intestinal bacterial community of *P. americana* by
constructing a 16S rRNA gene library and revealed that
both host phylogeny and omnivorous diet shaped the
bacterial community. The results also shed light on the
possible both host phylogeny and omnivorous diet shaped the
bacterial community. The results also shed light on the
possible role of *P. americana* as a reservoir for
pathogenic vectors. bacterial community. The results also shed light on the possible role of P . *americana* as a reservoir for pathogenic vectors. bacterial community. The results also shed light on the
possible role of *P*. *americana* as a reservoir for
pathogenic vectors. possible role of *P*. *americana* as a reservoir for pathogenic vectors. pathogenic vectors.

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1. 1 Sampling

. /微生物学报(2013)53(9) The insects were reared in cages at $25^{\circ}C$ and fed milled

Sampling
American cockroaches (*P*.
ded as a gift by Hefei Centers
insects were reared in cages at
and bran cob, and water.
The cockroaches were anesth
and sterilized using 75
ction. The cockroach guts were
ody was remov American cockroaches (P . *americana*) were
ded as a gift by Hefei Centers for Disease Control.
nsects were reared in cages at 25 °C and fed milled
and bran cob, and water.
The cockroaches were anesthetized using diethy The insects were reared in cages at 25°C and fed milled
corn and bran cob, and water.
The cockroaches were anesthetized using diethy
ether and sterilized using 75% alcohol before
dissection. The cockroach guts were dissec corn and bran cob, and water.

The cockroaches were anesthetized using diethyl

ether and sterilized using 75% alcohol before

dissection. The cockroach guts were dissected, and the

fat body was removed. The intestinal m corn and bran cob, and water.
The cockroaches were are ether and sterilized using
dissection. The cockroach guts
fat body was removed. The integut of the cockroaches were
modifications according to the
 $al^{[10]}$. The guts and sterilized using 75% alcohol before
ction. The cockroach guts were dissected, and the
ody was removed. The intestinal microbes from the
of the cockroaches were prepared with a few
fications according to the methods of dissection. The cockroach guts were dissected, and the
fat body was removed. The intestinal microbes from the
gut of the cockroaches were prepared with a few
modifications according to the methods of Walter *et*
 $al^{[10]}$. dissection. The cockroach guts were dissected, and the
fat body was removed. The intestinal microbes from the
gut of the cockroaches were prepared with a few
modifications according to the methods of Walter *et*
 $al^{[10]}$. gut of the cockroaches were prepared with a few
modifications according to the methods of Walter *et*
 $al^{[10]}$. The guts were minced and pooled into 50 mL
sterile phosphate-buffered saline (PBS, pH 7.4).
After vigorous sh modifications according to the methods of Walter *et* $al^{[10]}$. The guts were minced and pooled into 50 mL sterile phosphate-buffered saline (PBS, pH 7.4). After vigorous shaking under sterile conditions, the intestinal d modifications according to the methods of Walter *et* $al^{[10]}$. The guts were minced and pooled into 50 mL sterile phosphate-buffered saline (PBS, pH 7.4). After vigorous shaking under sterile conditions, the intestinal d $al^{\mathfrak{l}}$ 10]
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3 Example 1. The general saline (PBS, pH 7.4).

vigorous shaking under sterile conditions, the

inal debris was centrifuged at 4°C and 9 000 × g

min. Then, the precipitate was suspended in 40

f fresh PBS (pH 7.4) buffer a sterile phosphate-buffered saline (PBS, pH 7.4).
After vigorous shaking under sterile conditions, the
intestinal debris was centrifuged at 4°C and 9 000 $\times \beta$
for 3 min. Then, the precipitate was suspended in 4
(mL of fr After vigorous shaking under sterile conditions, the
intestinal debris was centrifuged at 4°C and 9 000 \times g
for 3 min. Then, the precipitate was suspended in 40
mL of fresh PBS (pH 7.4) buffer and centrifuged at
150 \t intestinal debris was centrifuged at 4℃ and 9 000 × g
for 3 min. Then, the precipitate was suspended in 40
mL of fresh PBS (pH 7.4) buffer and centrifuged at
150 × g and 4℃ for 5 min to remove debris. The
supernatant, whi for 3 min. Then, the precipitate was suspended in 40 mL of fresh PBS (pH 7.4) buffer and centrifuged at 150 \times g and 4°C for 5 min to remove debris. The supernatant, which contained bacterial cells, was centrifuged agai mL of fresh PBS (pH 7.4) buffer and centrifuged at 150 \times g and 4°C for 5 min to remove debris. The supernatant, which contained bacterial cells, was centrifuged again at 200 \times g for 5 min. After gradient centrifugat supernatant, which contained bacterial cells, was
centrifuged again at 200 × g for 5 min. After gradient
centrifugation, the bacterial cells were recovered by
centrifugation at 9 000 × g and 4°C for 10 min and
resuspended supernatant, which contained bacterial cells, was
centrifuged again at 200 \times g for 5 min. After gradient
centrifugation, the bacterial cells were recovered by
centrifugation at 9 000 \times g and 4°C for 10 min and
resus centrifugation, the bacterial cells were recovered by
centrifugation at 9 000 \times g and 4°C for 10 min and
resuspended in 2 mL STE buffer (0.2 mol/L NaCl,
100 mmol/L ethylenediamine tetraacetic acid, and 10
mmol/L Tris, centrifugation, the bacterial cells were recovered by
centrifugation at 9 000 \times g and 4°C for 10 min and
resuspended in 2 mL STE buffer (0.2 mol/L NaCl,
100 mmol/L ethylenediamine tetraacetic acid, and 10
mmol/L Tris,

1. 2 DNA extraction and 16S rRNA gene amplification

resuspended in 2 mL STE buffer $(0.2 \text{ mol/L NaCl},$
100 mmol/L ethylenediamine tetraacetic acid, and 10
mmol/L Tris, pH 8.0).
**1.2 DNA extraction and 16S rRNA gene
amplification**
Genomic DNA was extracted using a BacteriaGen
DN resuspended in 2 mL STE buffer (0.2 mol/L NaCl,
100 mmol/L ethylenediamine tetraacetic acid, and 10
mmol/L Tris, pH 8.0).
**1.2 DNA extraction and 16S rRNA gene
amplification**
Genomic DNA was extracted using a BacteriaGen
D 100 mmol/L ethylenediamine tetraacetic acid, and 10
mmol/L Tris, pH 8.0).
1.2 DNA extraction and 16S rRNA gene
amplification
Genomic DNA was extracted using a BacteriaGen
DNA kit (CWBIO, Beijing, China) according to t mmol/L Tris, pH 8.0).
 1.2 DNA extraction
 amplification

Genomic DNA was

DNA kit (CWBIO, Bei

manufacturer's protocol:

with proteinase K (20 µ

the cells dissociated an

column to recover genom kit (CWBIO, Beijing, China) according to the
facturer's protocol: (1) samples were incubated
proteinase K (20 μ L, 20 mg/mL) at 56°C until
rells dissociated and then transferred to a spin
nn to recover genomic DNA throu DNA kit (CWBIO, Beijing, China) according to the
manufacturer's protocol: (1) samples were incubated
with proteinase K (20 μ L, 20 mg/mL) at 56°C until
the cells dissociated and then transferred to a spin
column to reco manufacturer's protocol: (1) samples were incubated
with proteinase K (20 μ L, 20 mg/mL) at 56°C until
the cells dissociated and then transferred to a spin
column to recover genomic DNA through centrifugation
at 12000 × with proteinase K (20 μ L, 20 mg/mL) at 56°C until
the cells dissociated and then transferred to a spin
column to recover genomic DNA through centrifugation
at 12000 × g for 5 min. (2) Samples were incubated
with 20 mg/ the cells and then then then transferred to a spin-
column to recover genomic DNA through centrifugation
at 12000 \times g for 5 min. (2) Samples were incubated
with 20 mg/mL of lysozyme for 30 min at 37°C. Then,
the sample at 12000 \times g for 5 min. (2) Samples were incubated
with 20 mg/mL of lysozyme for 30 min at 37°C. Then,
the sample was heated at 56°C for another 30 min after
adding proteinase K (20 μ L, 20 mg/mL). The
genomic DNA wa at 12000 × g for 5 min. (2) Samples were incubated
with 20 mg/mL of lysozyme for 30 min at 37°C. Then,
the sample was heated at 56°C for another 30 min after
adding proteinase K (20 μL , 20 mg/mL). The
genomic DNA was r with 20 mg/mL of lysozyme for 30 min at 37℃. Then,
the sample was heated at 56℃ for another 30 min after
adding proteinase K (20 μ L, 20 mg/mL). The
genomic DNA was recovered with a spin column by
centrifugation at 1200 adding proteinase K (20 μ L, 20 mg/mL). The
genomic DNA was recovered with a spin column by
centrifugation at 12000 × g for 5 min. adding proteinase K (20 μ L, 20 mg/mL). The
genomic DNA was recovered with a spin column by
centrifugation at 12000 × g for 5 min. centrifugation at $12000 \times g$ for 5 min. centrifugation at 1200 σ

The polymerase chain reaction (PCR)

ification of 16S rRNA genes was performed using

primer pair Bact-27F (AGAGTTTGATCMTGGC

5) and Univ-1492R (GGTTACCTTGTTA

CTT) with the extracted genomic DNA as the

late. The reactio the primer pair Bact-27F (AGAGTTTGATCMTGGC
TCAG) and Univ-1492R (GGTTACCTTGTTA
CGACTT) with the extracted genomic DNA as the
template. The reaction was performed in a 50 μ L
solution containing 100 μ mol/L deoxyribonu the primer pair Bact-27F (AGAGTTTGATCMTGGC
TCAG) and Univ-1492R (GGTTACCTTGTTA
CGACTT) with the extracted genomic DNA as the
template. The reaction was performed in a 50 μ L
solution containing 100 μ mol/L deoxyribonu TCAG) and Univ-1492R (GGTTACCTTGTTA
CGACTT) with the extracted genomic DNA as the
template. The reaction was performed in a 50 μ L
solution containing 100 μ mol/L deoxyribonucleotide
triphosphates, 0.5 μ mol/L of e CGACTT) with the extracted genomic DNA as the
template. The reaction was performed in a 50 μ L
solution containing 100 μ mol/L deoxyribonucleotide
triphosphates, 0.5 μ mol/L of each primer, 2 mmol/L
MgCl₂, 10 ng D template. The reaction was performed in a 50 μ E
solution containing 100 μ mol/L deoxyribonucleotide
triphosphates, 0.5 μ mol/L of each primer, 2 mmol/L
MgCl₂, 10 ng DNA template, and 2.5 U Taq DNA
polymerase (Tra solution containing 100 μmol/L deoxyribonucleotide
triphosphates, 0.5 μmol/L of each primer, 2 mmol/L
MgCl₂, 10 ng DNA template, and 2.5 U Taq DNA
polymerase (Transgen, Beijing, China) with 1 ×
reaction buffer supplied triphosphates, 0. 5 μmol/L of each primer, 2 mmol/L
MgCl₂, 10 ng DNA template, and 2. 5 U Taq DNA
polymerase (Transgen, Beijing, China) with 1 x
reaction buffer supplied by the manufacturer. PCR was
performed with a Mas MgCl₂, 10 ng DNA template, and 2.5 U Taq DNA
polymerase (Transgen, Beijing, China) with 1 x
reaction buffer supplied by the manufacturer. PCR was
performed with a Mastercycler Personal (Eppendorf,
Germany) with the foll polymerase (Transgen, Beijing, China) with 1 ×
reaction buffer supplied by the manufacturer. PCR was
performed with a Mastercycler Personal (Eppendorf,
Germany) with the following protocol: 25 cycles of
denaturation at 94° performed with a Mastercycler Personal (Eppendorf,
Germany) with the following protocol: 25 cycles of
denaturation at 94°C for 90 s, annealing at 52°C for
30 s, and extension at 72°C for 1 min, and a final
extension at 72 performed with a Mastercycler Personal (Eppendorf,
Germany) with the following protocol: 25 cycles of
denaturation at 94°C for 90 s, annealing at 52°C for
30 s, and extension at 72°C for 1 min, and a final
extension at 72 Germany) with the following protocol: 25 cycles of
denaturation at 94°C for 90 s, annealing at 52°C for
30 s, and extension at 72°C for 1 min, and a final
extension at 72°C for 10 min. The PCR products were
analyzed by 1. denaturation at 94℃ for 90 s, annealing at 52℃ for 30 s, and extension at 72℃ for 1 min, and a final
extension at 72℃ for 10 min. The PCR products were
analyzed by 1.0% agarose gel electrophoresis, stained
with ethidium b 30 s, and extension at 72°C for 1 min, and a final
extension at 72°C for 10 min. The PCR products were
analyzed by 1.0% agarose gel electrophoresis, stained
with ethidium bromide, and visualized under UV light.
1.3 16S r 1. 3 16S rRNA gene library construction and sequencing solution containing 100 μ mol/L deoxyribonucleotide

analyzed by 1.0% agarose gel electrophoresis, stained
with ethidium bromide, and visualized under UV light.
1.3 16S rRNA gene library construction and
sequencing
After purification, the PCR products of the 16S
rRNA genes analyzed by 1.0% agarose gel electrophoresis, stained
with ethidium bromide, and visualized under UV light.
1.3 16S rRNA gene library construction and
sequencing
After purification, the PCR products of the 16S
rRNA ge with ethidium bromide, and visualized under UV light.
 1.3 16S rRNA gene library construction and
 sequencing

After purification, the PCR products of the 16S

rRNA genes were ligated into the pGEM-T vecto:

(Promega, After purification, the PCR products of the 16S

1 genes were ligated into the pGEM-T vector

mega, Madison, WI, USA) and transformed into

ically competent E. coli DH5 α cells (Transgen,

ng, China). The transformed ce (Promega, Madison, WI, USA) and transformed into
chemically competent E. coli DH5 α cells (Transgen,
Beijing, China). The transformed cells were plated on
Luria-Bertani agar containing isopropyl- β -D-
thiogalactopyran (Promega, Madison, WI, USA) and transformed into Promega, Madison, WI, USA) and transformed into
hemically competent E. coli DH5 α cells (Transgen,
eijing, China). The transformed cells were plated on
uria-Bertani agar containing isopropyl- β -D-
iogalactopyranoside chemically competent E. coli DH5 α cells (Transgen,
Beijing, China). The transformed cells were plated on
Luria-Bertani agar containing isopropyl- β -D-
thiogalactopyranoside and 5-bromo-4-chloro -3-indolyl-
 β -D-gal Beijing, China). The transformed cells were plated on
Luria-Bertani agar containing isopropyl- β -D-
thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl-
 β -D-galactopyranoside. White positive clones were
placed in 38 Luria-Bertani agar containing isopropyl-β-D-
thiogalactopyranoside and 5-bromo-4-chloro -3-indolyl-
β-D-galactopyranoside. White positive clones were
placed in 384-well plates to construct a 16S rRNA gene
library. Twenty β -D-galactopyranoside. White positive clones were
placed in 384-well plates to construct a 16S rRNA gene
library. Twenty clones were randomly selected from the
library for insert length inspection using M13F
(GTAAAACG β-D-galactopyranoside. White positive clones were aced in 384-well plates to construct a 16S rRNA gene
brary. Twenty clones were randomly selected from the
brary for insert length inspection using M13F
GTAAAACGACGCCAG) and M13R
CAGGAAACAGCTATGAC) primers specific to the Finally Euclidean I and the Hibrary. Twenty clones were randomly selected from the library for insert length inspection using M13F (CAGGAAACAGCTATGAC) and M13R (CAGGAAACAGCTATGAC) primers specific to the pGEM-T vector. Th library for insert length inspection using M13F

(GTAAAACGACGCCAG) and M13R

(CAGGAAACAGCTATGAC) primers specific to the

pGEM-T vector. The PCR conditions were as follows:

an initial denaturation at 94°C for 5 min, fol (CAGGAAACGACGCCAG) and M13R

(CAGGAAACAGCTATGAC) primers specific to the

pGEM-T vector. The PCR conditions were as follows:

an initial denaturation at 94°C for 5 min, followed by

30 cycles of denaturation at 94°C for 5 (GTAAAACGACGCCCAG) GTAAAACGACGCCAG) and M13R
AGGAAACAGCTATGAC primers specific to the
IM-T vector. The PCR conditions were as follows:
mitial denaturation at 94°C for 5 min, followed by
eycles of denaturation at 94°C for 45 s, annealing at (CAGGAAACAGCTATGAC) primers specific to the CAGGAAACAGCTATGAC) primers specific to the
GEM-T vector. The PCR conditions were as follows:
1 initial denaturation at 94°C for 5 min, followed by
9 oycles of denaturation at 94°C for 45 s, annealing at
2°C for 45 s, and pGEM-T vector. The PCR conditions were as follows:
an initial denaturation at 94°C for 5 min, followed by
30 cycles of denaturation at 94°C for 45 s, annealing at
42°C for 45 s, and extension at 72°C for 90 s and a
final e an initial denaturation at 94℃ for 5 min, followed by
30 cycles of denaturation at 94℃ for 45 s, annealing at
42℃ for 45 s, and extension at 72℃ for 90 s and a
final elongation at 72℃ for 10 min.
A total of 170 sequences

30 cycles of denaturation at 94°C for 45 s, annealing at 42°C for 45 s, and extension at 72°C for 90 s and a final elongation at 72°C for 10 min.
A total of 170 sequences were randomly selected and sequenced (Beijing Geno 42℃ for 45 s, and extension at 72℃ for 90 s and a final elongation at 72℃ for 10 min.
A total of 170 sequences were randomly selected and sequenced (Beijing Genomics Institute, Shanghai, China) with an ABI 377 genetic an A total of 170 sequences were
and sequenced (Beijing Genomics I
China) with an ABI 377 genetic
Biosystems, USA). Expedienced (Beijing Genomics Institute, Shanghai,

a) with an ABI 377 genetic analyzer (Applied stems, USA). and sequenced (Beijing Genomics Institute, Shanghai,
China) with an ABI 377 genetic analyzer (Applied
Biosystems, USA). China) with an ABI 377 genetic analyzer (Applied Biosystems, USA). Biosystems, USA).

1. 4 Taxonomic and phylogenetic analysis

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 Taxonomic and phylogenetic analysis

All of the near full-length sequences were edited

clude the flanking vector sequences and tested for

vive chimeras usin clude the flanking vector sequences and tested for
ive chimeras using the Mallard program $^{[11]}$.
nomy analysis of the 16S rRNA sequences was
rmed using a classifier program of the Ribosomal
pase Project $^{[12]}$ and mega putative chimeras using the Mallard program $^{[11]}$.
Taxonomy analysis of the 16S rRNA sequences was
performed using a classifier program of the Ribosomal
Database Project $^{[12]}$ and megaBLAST programs against
the EzTaxo putative chimeras using the Mallard program $\left[11\right]$.
Taxonomy analysis of the 16S rRNA sequences was
performed using a classifier program of the Ribosoma
Database Project $\left[12\right]$ and megaBLAST programs agains
the Ez performed using a classifier program of the Ribosomal
Database Project ^[12] and megaBLAST programs against
the EzTaxon database $^{[13]}$. All of the sequences
without chimeras were assigned as operational
taxonomic units Database Project ^[12] and megaBLAST programs against
the EzTaxon database $^{[13]}$. All of the sequences
without chimeras were assigned as operational
taxonomic units (OTUs) based on alignment using the
Mothur program, w Database Project ^[12] and megaBLAST programs against
the EzTaxon database ^[13]. All of the sequences
without chimeras were assigned as operational
taxonomic units (OTUs) based on alignment using the
Mothur program, wi the EzTaxon database ^[13]. All of the sequences
without chimeras were assigned as operational
taxonomic units (OTUs) based on alignment using the
Mothur program, with 97% sequence similarity as the
designated cutoff ^{[1} taxonomic units (OTUs) based on alignment using the
Mothur program, with 97% sequence similarity as the
designated cutoff $^{[14]}$. The representative of each OTU
was used as a query to search for similar 16S rRNA
gene seq taxonomic units (OTUs) based on alignment using the
Mothur program, with 97% sequence similarity as the
designated cutoff $^{[14]}$. The representative of each OTU
was used as a query to search for similar 16S rRNA
gene seq Mothur program, with 97% sequence similarity as the designated cutoff $^{[14]}$. The representative of each OTU was used as a query to search for similar 16S rRNA gene sequences in the National Center for Biotechnology Info designated cutoff $^{[14]}$. The representative of each OTU
was used as a query to search for similar 16S rRNA
gene sequences in the National Center for
Biotechnology Information (http://www.ncbi.nlm.
nih.gov/gorf/gorf.html gene sequences in the National Center for
Biotechnology Information (http://www.ncbi.nlm.
nih.gov/gorf/gorf.html). Sequence alignment was
performed using ClustalX 2.0 to obtain a uniform
length, and a phylogenetic tree wa Biotechnology Information (http://www.ncbi.nlm.
nih.gov/gorf/gorf.html). Sequence alignment was
performed using ClustalX 2.0 to obtain a uniform
length, and a phylogenetic tree was created using the
neighbor-joining metho Biotechnology Information (http://www.ncbi.nlm.
nih.gov/gorf/gorf.html). Sequence alignment was
performed using ClustalX 2.0 to obtain a uniform
length, and a phylogenetic tree was created using the
neighbor-joining metho nih. gov/gorf/gorf. html). Sequence alignment was
performed using ClustalX 2.0 to obtain a uniform
length, and a phylogenetic tree was created using the
neighbor-joining method with the MEGA4.0 program.
A total of 1000 bo

length, and a phylogenetic tree was created using the
neighbor-joining method with the MEGA4. 0 program.
A total of 1000 bootstrap replicates were generated.
The coverage of the 16S rRNA gene library was
calculated using length, and a phylogenetic tree was created using the
neighbor-joining method with the MEGA4. 0 program.
A total of 1000 bootstrap replicates were generated.
The coverage of the 16S rRNA gene library was
calculated using A total of 1000 bootstrap replicates were generated.
The coverage of the 16S rRNA gene library was
calculated using the formula $\begin{bmatrix} 1 & -1 \\ n/N \end{bmatrix}$, where *n*
is the number of OTUs represented by one clone, and
N is The coverage of the 16S rRNA gene library
calculated using the formula $\begin{bmatrix} 1 & -1 \\ 0 & -1 \end{bmatrix}$, where
is the number of OTUs represented by one clone,
N is the total number of clones $\begin{bmatrix} 15 \end{bmatrix}$. Bacterial diver
an lated using the formula $\left[1 - \left(n/N\right)\right]$, where *n*

e number of OTUs represented by one clone, and

the total number of clones $^{[15]}$. Bacterial diversity

richness were calculated using the Shannon-

ver index and Simp calculated using the formula $\begin{bmatrix} 1 & -\n\end{bmatrix}$ (n/N), where *n* is the number of OTUs represented by one clone, and *N* is the total number of clones $\begin{bmatrix} 151 \end{bmatrix}$. Bacterial diversity and richness were calculate is the number of OTUs represented by one clone, and
 N is the total number of clones ^[15]. Bacterial diversity

and richness were calculated using the Shannon-

Weaver index and Simpson Index, respectively ^[16].

Ra N is the total number of clones $^{[15]}$. Bacterial diversity is the total number of clones $^{[15]}$. Bacterial diversity
d richness were calculated using the Shannon-
eaver index and Simpson Index, respectively $^{[16]}$.
refaction analysis was performed using the Mothur
ogram at 97%, Weaver index and Simpson Index, respectively $[16]$.
Rarefaction analysis was performed using the Mothur
program at 97%, 95% and 90% sequence similarity
thresholds $[14]$.
1.5 Nucleotide sequence accession number
The 16 Weaver index and Simpson Index, respectively
Rarefaction analysis was performed using the M
program at 97%, 95% and 90% sequence sim
thresholds ^[14].
1.5 Nucleotide sequence accession number
The 16S rRNA genes in this st Weaver index and Simpson Index, respectively [16]. [16]
thu
arit .

1. 5 Nucleotide sequence accession number

program at 97% , 95% and 90% sequence similarity
thresholds $^{[14]}$.
1.5 Nucleotide sequence accession number
The 16S rRNA genes in this study were deposited
in the GenBank database under accession numbers
JX457 program at 97%, 95% and 90% sequence similarity
thresholds $^{[14]}$.
1.5 Nucleotide sequence accession number
The 16S rRNA genes in this study were deposited
in the GenBank database under accession numbers
JX457153-JX457 thresholds $^{[14]}$.
 1.5 Nucleoti

The $16S_1$

in the GenBa

JX457153-JX4
 2. Results

2.1 Bacteria

2

2. 1 Bacterial community in the P. americana gut

EXECUTE CONSUMED TO CONSUMERED THE 16ST THE 16ST THE 15ST THE 15ST THE 16ST RESULTS
 Results
 Resu JX457153-JX457317.

2. Results

2. 1 Bacterial community in the *P. americana* gut

To examine the total gut bacterial community, we

performed 16S rRNA gene sequencing. A total of 170

clones were randomly selected and se 2 Results
2.1 Bacterial comm
To examine the
performed 16S rRNA
clones were random
which 5 were identifi Bacteria
To examin
Free 16S
s were 1
h 5 were
ence iden To examine the total gut bacterial community, we
rmed 16S rRNA gene sequencing. A total of 170
s were randomly selected and sequenced, of
15 were identified as chimeras; 13 showed high
ence identity to the bacteria from co performance of the total content of the sequenced, of which 5 were identified as chimeras; 13 showed high sequence identity to the bacteria from cockroach fat clones were randomly selected and sequenced, of
which 5 were identified as chimeras; 13 showed high
sequence identity to the bacteria from cockroach fat which 5 were identified as chimeras; 13 showed high
sequence identity to the bacteria from cockroach fat s_{max} is the bacteria from cockroach fathering from cockroach fathering from cockroach factorial from cockroach from body and were thus excluded. Thus, 152 of the 16S

rRNA gene sequences were classified into 54 different

phylotypes with 97% sequence similarity as the

designated cutoff $^{[14]}$. Based on sequence analysis, 21

phylotyp

phylotypes with 97% sequence similarity as the
designated cutoff $^{[14]}$. Based on sequence analysis, 21
phylotypes showed < 97% sequence identity and may
be novel.
Of the 152 clones, 66.4% were affiliated with the
phylum designated cutoff $^{[14]}$. Based on sequence analysis, 21
phylotypes showed < 97% sequence identity and may
be novel.
Of the 152 clones, 66.4% were affiliated with the
phylum Proteobacteria, followed by Bacteroidetes
(17. designated cutoff $^{[14]}$. Based on sequence analysis, 21
phylotypes showed < 97% sequence identity and may
be novel.
Of the 152 clones, 66.4% were affiliated with the
phylum Proteobacteria, followed by Bacteroidetes
(17. be novel.

Of the 152 clones, 66.4% were affiliated with the

phylum *Proteobacteria*, followed by *Bacteroidetes*

(17.8%), *Firmicutes* (14.5%), *Fusobacteria*

(0.6%) and unclassified bacteria (0.6%). The

Shannon-Weav 0f tl
phylum
(17.8%)
(0.6%)
Shannon-
3.395,
rarefactio
indicating Of the 152 clones, 66. 4% were affiliated with the

um *Proteobacteria*, followed by *Bacteroidetes*

8%), *Firmicutes* (14. 5%), *Fusobacteria*

(0. 6%). The

non-Weaver index, a measure of diversity, was

5, and the Sim phylum *Proteobacteria*, followed by *Bacteroidetes*

(17.8%), *Firmicutes* (14.5%), *Fusobacteria*

(0.6%) and unclassified bacteria (0.6%). The

Shannon-Weaver index, a measure of diversity, was

3.395, and the Simpson (17.8%) , Firmicutes (14.5%) , Fusobacteria 17. 8%), Firmicutes (14. 5%), Fusobacteria

1. 6%) and unclassified bacteria (0.6%). The

1. The annon-Weaver index, a measure of diversity, was

1. The simps index was 0.06. The

1. The refaction curve was not satu (0.6%) and unclassified bacteria (0.6%) . The 0. 6%) and unclassified bacteria (0.6%) . The
hannon-Weaver index, a measure of diversity, was
395, and the Simpson index was 0.06. The
prefaction curve was not saturated at a cutoff of 97%,
dicating that the number of a Shannon-Weaver index, a measure of diversity, was
3.395, and the Simpson index was 0.06. The
rarefaction curve was not saturated at a cutoff of 97%,
indicating that the number of analyzed clones may not
be sufficient to d 3. 395, and the Simpson index was 0.06. The rarefaction curve was not saturated at a cutoff of 97%, indicating that the number of analyzed clones may not be sufficient to describe bacterial diversity within the cockroach rarefaction curve was not saturated at a cutoff of 97%,
indicating that the number of analyzed clones may not
be sufficient to describe bacterial diversity within the
cockroach gut at the species level (Figure 1).
However be sufficient to describe bacterial diversity within the cockroach gut at the species level (Figure 1).

However, a reasonable coverage of 81% was obtained,

indicating that the majority of the intestinal bacteria

were i

Figure 1. Final districts of 16S rinking gate instance
recovered from the *P. americana*. The curves were generated
based on analyses performed by the Mothur program. The
expected number of clones was calculated from the n recovered from the *P. americana.* The curves were generated
based on analyses performed by the Mothur program. The
expected number of clones was calculated from the number of
clones analyzed at the species level with 97% expected number of clones was calculated from the number of
clones analyzed at the species level with 97% sequence identity,
genus level with 95% sequence identity, and family/class level
with 90% sequence identity.
Phylo expections analyzed at the species level with 97% sequence identity,
genus level with 95% sequence identity, and family/class level
with 90% sequence identity.
Phylotypes assigned to Proteobacteria asobacteria phyla
A to clones analyzed at the species level with 97% sequence identity,
genus level with 95% sequence identity, and family/class level
with 90% sequence identity.
Phylotypes assigned to Proteobacteria an
sobacteria phyla
A to

2. 2 Phylotypes assigned to Proteobacteria and Fusobacteria phyla

genus level with 95% sequence identity, and family/class level
with 90% sequence identity.
Phylotypes assigned to *Proteobacteria* a
sobacteria phyla
A total of 101 clones belonging to 21 phyloty
re assigned to the phylu Phylotypes assisted to the phylotype of the phyla
A total of 101 clons
the assigned to the phylandra related with the contract of the phylandra related with the contract of the relationships of the contract of the contract assigned to the phylum *Proteobacteria*, and most related with the class γ -proteobacteria (Figure were assigned to the phylum *Proteobacteria*, and most
were related with the class γ -proteobacteria (Figure
2). were related with the class γ -proteobacteria (Figure 2). 2) .

. /微生物学报(2013)53(9)

1 the phylum *Proteobacte*

2) clustered with bacteria

1) related animals, such

pandas, and humans.

2d to two phylotypes clu

a from *Shelfordella latera* In the phylum *Proteobacteria*, 13 phylotypes (83
s) clustered with bacteria from the guts or feces of
ntly related animals, such as flies, mosquitoes,
pandas, and humans. In addition, 6 clones
ned to two phylotypes clust clones) clustered with bacteria from the guts or feces of
distantly related animals, such as flies, mosquitoes,
giant pandas, and humans. In addition, 6 clones
assigned to two phylotypes clustered with uncultured
bacteria distantly related animals, such as flies, mosquitoes,
giant pandas, and humans. In addition, 6 clones
assigned to two phylotypes clustered with uncultured
bacteria from *Shelfordella lateralis* $^{[17]}$.
Only CR_38, a phylo

giant pandas, and humans. In addition, 6 clones
assigned to two phylotypes clustered with uncultured
bacteria from *Shelfordella lateralis* [17].
Only CR_38, a phylotype that was represented by
only one clone, was assigned bacteria from *Shelfordella lateralis* $[17]$.

Only CR_38, a phylotype that was represented by

only one clone, was assigned to the phylum
 Fusobacterium from *S. lateralis* $[17]$ (Figure 2).
 2.3 Phylotypes assigned bacteria from *Shelfordella lateralis* $\begin{bmatrix} 1 & 7 \end{bmatrix}$.

Only CR_38, a phylotype that wa

only one clone, was assigned to
 Fusobacteria and clustered wi
 Fusobacterium from *S. lateralis* $\begin{bmatrix} 1 & 7 \end{bmatrix}$ (F
 Only CR_38, a phylotype that was represented by

one clone, was assigned to the phylum
 bacterium from *S. lateralis* $^{[17]}$ (Figure 2).
 Phylotypes assigned to the *Bacteroidetes*
 um

A total of 27 clones were cl Fusobacteria *Fusobacterium* from *S. lateralis* $[17]$ (Figure 2). Only CR_38, a phylotype that was represented by

2. 3 Phylotypes assigned to the Bacteroidetes phylum

only one clone, was assigned to the phylum
 Fusobacteria and clustered with uncultured
 Fusobacterium from *S. lateralis* ^[17] (Figure 2).
 2.3 Phylotypes assigned to the *Bacteroidetes***

phylum**

A total of 27 clon Figure 2).
 pes assigned to the Bacteroidetes

and **co** the **Bacteroidetes**

and **c** the **Bacteroidetes**

and **c** the **clusters**

and **co** 48% of the clones in the

hylotypes clustered with the sequences

in 22% 05% from *S. lateralis* $^{[17]}$ (Figure 2).
 ypes assigned to the *Bacter*

27 clones were classified in the

(Figure 3). Seven phylotype

ored with gut bacteria from *S. la*

onded to 48% of the clones

phylotypes clustere proidetes (Figure 3). Seven phylotypes (13
s) neighbored with gut bacteria from *S. lateralis*,
n corresponded to 48% of the clones in the
um. Five phylotypes clustered with the sequences
termites, with 93% -95% sequence Bacteroidetes (Figure 3). Seven phylotypes (13) Figure 3). Seven phylotypes (13
red with gut bacteria from *S. lateralis*,
ided to 48% of the clones in the
hylotypes clustered with the sequences
vith 93% - 95% sequence identity.
Example 13 assigned to the phylum *Fir*

2. 4 Phylotypes assigned to the phylum Firmicutes

clones) neighbored with gut bacteria from *S. lateralis*,
which corresponded to 48% of the clones in the
phylum. Five phylotypes clustered with the sequences
from termites, with 93% -95% sequence identity.
2.4 Phylotypes phylum. Five phylotypes clustered with the sequences
from termites, with $93\% - 95\%$ sequence identity.
2. 4 Phylotypes assigned to the phylum *Firmicutes*
A total of 22 clones were classified in the phylum
Firmicutes, phylum termites, with 93% -95% sequence identity.

2.4 Phylotypes assigned to the phylum Firmicutes

A total of 22 clones were classified in the phylum

Firmicutes, and the majority belonged to the class

Bacilli, order La from termites, with 93% - 95% sequence identity.
 2.4 Phylotypes assigned to the phylum Firmic

A total of 22 clones were classified in the phy

Firmicutes, and the majority belonged to the c

Bacilli, order Lactobacill icutes, and the majority belonged to the class
 li , order Lactobacillales (Figure 4). Under

bacillales, 8 phylotypes represented by 15 clones

ched with Enterococcus, Lactobacillus,

lactobacillus, and Lactococcus bacte Firmicutes, and the majority belonged to the class er *Lactobacillales* (Figure 4). Under

es, 8 phylotypes represented by 15 clones

with Enterococcus, *Lactobacillus*,
 illus, and *Lactococcus* bacteria, which may

1 bacteria that are involved in lactate and

action i Bacilli, order Lactobacillales (Figure 4). Under order *Lactobacillales* (Figure 4). Under
 llales, 8 phylotypes represented by 15 clones

with Enterococcus, Lactobacillus,
 bacillus, and *Lactococcus* bacteria, which may

acid bacteria that are involved in lactate Lactobacillales, 8 phylotypes represented by 15 clones th *Enterococcus*, *Lactobacillus*,
s, and *Lactococcus* bacteria, which may
acteria that are involved in lactate and
on in the *P*. *americana* gut $^{[7]}$. Two
nched with uncultured bacteria of
and *Lactobacillaceae* fam branched with *Enterococcus*, *Lactobacillus*,
 Paralactobacillus, and *Lactococcus* bacteria, which may

be lactic acid bacteria that are involved in lactate and

acetate production in the *P*. *americana* gut ^[7]. T Paralactobacillus, and Lactococcus bacteria, which may and *Lactococcus* bacteria, which may
eria that are involved in lactate and
in the *P. americana* gut $\frac{[7]}{]}$. Two
ned with uncultured bacteria of
d *Lactobacillaceae* families from the
 $\frac{[7]}{]}$, with 94% and 99% seq acetate production in the *P. americana* gut $\left[\frac{1}{2}\right]$. Two
phylotypes branched with uncultured bacteria of
Enterococcaceae and *Lactobacillaceae* families from the
S. lateralis gut $\left[\frac{1}{2}\right]$, with 94% and 99% acetate production in the *P. americana* gut $\left[\frac{7}{7}\right]$. Two
phylotypes branched with uncultured bacteria of
Enterococcaceae and *Lactobacillaceae* families from the
S. lateralis gut $\left[\frac{1}{7}\right]$, with 94% and 99% Enterococcaceae and Lactobacillaceae families from the S. *lateralis* gut $^{[17]}$, with 94% and 99% sequence

2. 5 Clones with high sequence identity with potential pathogens

Enterococcaceae and Lactobacillaceae families from the

S. lateralis gut $[17]$, with 94% and 99% sequence

identity, respectively (Figure 5).

2.5 Clones with high sequence identity with

potential pathogens

A list of t and *Lactobacillaceae* families from the

^[17], with 94% and 99% sequence

ively (Figure 5).
 with high sequence identity with
 gens

the phylotypes with high sequence

tial pathogens along with their medical

shown gut $^{[17]}$, with 94% and 99% sequence
pectively (Figure 5).
**es with high sequence identity with
thogens**
of the phylotypes with high sequence
tential pathogens along with their medical
is shown in Table 1. In this study identity, respectively (Figure 5).
 2.5 Clones with high sequelity of the set of the phylotypes

A list of the phylotypes

identity to potential pathogens alo

significance is shown in Table

phylotypes represented by 28 The set the phylotypes with their medical
ity to potential pathogens along with their medical
icance is shown in Table 1. In this study, 9
types represented by 28 clones shared high
ity with known potential pathogens. Most significance is shown in Table 1. In this study, 9
phylotypes represented by 28 clones shared high
identity with known potential pathogens. Most of them
were also identified in *P. americana*, *B. germanica*,
and *B. orien* significance is shown in Table 1. In this study, 9
phylotypes represented by 28 clones shared high
identity with known potential pathogens. Most of them
were also identified in *P. americana*, *B. germanica*,
and *B. orien* phylotentry in the booking didentity with known potential pathogens. Most of them
were also identified in *P. americana*, *B. germanica*,
and *B. orientalis* from houses and hospitals with
culture-dependent methods, except were also identified in *P. americana*, *B. germanica*,
and *B. orientalis* from houses and hospitals with
culture-dependent methods, except for *Dysgonomonas*
sp., *Myroides* sp., and *Providencia* sp. (Table 1). were also identified in *P. americana*, *B. germanica*, and *B. orientalis* from houses and hospitals with culture-dependent methods, except for *Dysgonomonas* sp., *Myroides* sp., and *Providencia* sp. (Table 1). and *B. orientalis* from houses and hospitals with
culture-dependent methods, except for *Dysgonomonas*
sp., *Myroides* sp., and *Providencia* sp. (Table 1). culture-dependent methods, except for *Dysgonomonas*
sp., *Myroides* sp., and *Providencia* sp. (Table 1). sp., Myroides sp., and Providencia sp. (Table 1).

Figure 2. Phylogenetic tree of the intestinal bacteria affiliated with phylum *Proteobactria* and Fusobacteria. Numbers at each branch points indicate
the percentage supported by bootstrap based on 1 000 replicates. Number t_{ref} based on 1 000 replication per nucleotide. Numbers in partner the sequence accession number in General sequence accession number in General Section number in General Section number in General Section 1 of $\frac{1}{$ scale bar: 0. 02 substitution per nucleotide.

Figure 3. Phylogenetic tree of the intestinal bacteria affiliated with phylum *Bacteroidetes*. Numbers at each branch points indicate the percentage
supported by bootstrap based on 1000 replicates. Numbers in parentheses

3

Discussion
To recover and analyze
crobial flora from the Als
s dissected to release To recover and
bial flora from
dissected to
ction. In our
nece identity
of *P. amer*
arations of coo bial flora from the American cockroach, the gut
dissected to release the contents for DNA
ction. In our results, 13 clones showed 99%
ence identity to *Blattabacterium* sp. from the fat
of *P. americana*, which suggested t microbial flora from the American cockroach, the gut
was dissected to release the contents for DNA
extraction. In our results, 13 clones showed 99%
sequence identity to *Blattabacterium* sp. from the fat
body of *P. americ* extraction. In our results, 13 clones showed 99%
sequence identity to *Blattabacterium* sp. from the fat
body of *P. americana*, which suggested that gut
preparations of cockroaches were easily contaminated extraction. In our results, 13 clones showed 99%
sequence identity to *Blattabacterium* sp. from the fa
body of *P. americana*, which suggested that gu
preparations of cockroaches were easily contaminated sequence identity to *Blattabacterium* sp. from the fat
body of *P. americana*, which suggested that gut
preparations of cockroaches were easily contaminated body of *P. americana*, which suggested that gut
preparations of cockroaches were easily contaminated preparations of cockroaches were easily contaminated

supported by bootstrap based on 1000 replicates. Numbers in parentheses represent the sequence accession number in GenBank. The scale bar:

0.02 substitution per nucleotide.

With fat body tissue. Nevertheless, the percent with fat body tissue. Nevertheless, the percentage of
16S rRNA gene sequences from fat body bacteria was
only 7%, which indicated that contamination of the
template from fat body bacterial symbionts was minor.
Furthermore 16S rRNA gene sequences from fat body bacteria was only 7%, which indicated that contamination of the template from fat body bacterial symbionts was minor.
Furthermore, 79% of the clones clustered with sequences derived from various gut environments, suggesting that the s Furthermore, 79% of the clones clustered with
sequences derived from various gut environments,
suggesting that the samples processed in this study were
reliable.
In a study based on published 16S rRNA gene
sequence data, C Furthermore, 79% of the clones clustered with
sequences derived from various gut environments,
suggesting that the samples processed in this study were
reliable.
In a study based on published 16S rRNA gene
sequence data, C suggesting that the samples processed in this study were
reliable.
In a study based on published 16S rRNA gene
sequence data, Colman *et al.* performed comparative

Figure 19 and the sample in the sequence $\frac{1}{2}$ of $\frac{1}{2}$ and $\frac{1}{2}$ and religions
In
sequenc In a study based on published 16S rRNA general comparative sequence data, Colman et al. performed comparative

Figure 4. Phylogenetic tree of the intestinal bacteria affiliated with phylum *Firmicutes*. Numbers at each branch points indicate the percentage
supported by bootstrap based on 1000 replicates. Numbers in parentheses rep

supported by bootstrap based on 1000 replicates. Numbers in parentheses represent the sequence accession number in GenBank. The scale bar: 0.02
substitution per nucleotide.
udies of intestinal bacterial communities from 62 substitution per nucleotide.

studies of intestinal bacterial communities from 62

insect species representing seven taxonomic orders and

nine diet types $[27]$. However, no cockroach species

were included. In the prese insect species representing seven taxonomic orders and
nine diet types $^{[27]}$. However, no cockroach species
were included. In the present study, some of our
findings validated the observations in the 62 insects. In
the mine diet types^[27]. However, no cockroach species
were included. In the present study, some of our
findings validated the observations in the 62 insects. In
the *P. americana* gut, a predominance of
Proteobacteria wa nine diet types $^{[27]}$. However, no cockroach species
were included. In the present study, some of our
findings validated the observations in the 62 insects. In
the P. americana gut, a predominance of
Proteobacteria was were included. In the present study, some of our
findings validated the observations in the 62 insects. In
the P . americana gut, a predominance of
 $\emph{Proteobacteria}$ was found, which agreed with the
findings of Colman and coll the *P. americana* gut, a predominance of
Proteobacteria was found, which agreed with the
findings of Colman and colleagues, who reported that
Proteobacteria was the predominant bacterial phylum
within the gut samples the *P. americana* gut, a predominance of
Proteobacteria was found, which agreed with the
findings of Colman and colleagues, who reported that
Proteobacteria was the predominant bacterial phylum
within the gut samples *Proteobacteria* was found, which agreed with the was found, which agreed with the
nan and colleagues, who reported that
was the predominant bacterial phylum
samples of non-termite insects $[27]$.
result was different from the results of
n, which closely resembled the hi findings of Colman and colleagues, who reported that *Proteobacteria* was the predominant bacterial phylum within the gut samples of non-termite insects $[27]$. However, this result was different from the results of *S.* Proteobacteria was the predominant bacterial phylum samples of non-termite insects $[27]$.
result was different from the results of
on, which closely resembled the hindgut
ungus-growing termites. Almost half of
om *S. lateralis* colon belonged to within the gut samples of non-termite insects $[27]$.
However, this result was different from the results of S. *lateralis* colon, which closely resembled the hindgu microbiota of fungus-growing termites. Almost half of t However, this result was different from the results of S. *lateralis* colon, which closely resembled the hindgut microbiota of fungus-growing termites. Almost half of the clones from S. *lateralis* colon belonged to S. *lateralis* colon, which closely resembled the hindgut colon, which closely resembled the hindgut
of fungus-growing termites. Almost half of
from S. *lateralis* colon belonged to the clones from S . *lateralis* colon belonged to the clones from S. lateralis colon belonged to

Bacteroidetes, followed by Firmicutes $[17]$. Moreover, followed by *Firmicutes* $^{[17]}$. Moreover,
 $16S$ rRNA gene sequences from the

n of *P. americana* revealed 54 species-

which was almost as high as that of

e second richest of the diet guilds $^{[27]}$.

d on rarefactio digestive system of *P. americana* revealed 54 species-
level OTUs, which was almost as high as that of
detritivores, the second richest of the diet guilds $[27]$.
However, based on rarefaction analysis, the number of
OTU digestive system of *P. americana* revealed 54 species-
level OTUs, which was almost as high as that of
detritivores, the second richest of the diet guilds $[27]$.
However, based on rarefaction analysis, the number of
OTU level OTUs, which was almost as high as that of
detritivores, the second richest of the diet guilds $[27]$.
However, based on rarefaction analysis, the number of
OTUs observed in *P. americana* was not fully saturated,
wh detritivores, the second richest of the diet guilds $[27]$.
However, based on rarefaction analysis, the number of OTUs observed in *P. americana* was not fully saturated which was similar to the rarefaction analysis of th However, based on rarefaction analysis, the number of OTUs observed in *P. americana* was not fully saturated, which was similar to the rarefaction analysis of the *S. lateralis* colon, whose analysis indicated that the OTUs observed in *P. americana* was not fully saturated,
which was similar to the rarefaction analysis of the *S.*
lateralis colon, whose analysis indicated that the
number of clones analyzed was not sufficient to descr which was similar to the rarefaction analysis of the *S*.
 lateralis colon, whose analysis indicated that the

number of clones analyzed was not sufficient to describe

bacterial diversity within the cockroach gut at ei *lateralis* colon, whose analysis indicated that the colon, whose analysis indicated that the

i clones analyzed was not sufficient to describe

diversity within the cockroach gut at either the

species level $^{[17]}$ (Figure 1). The low

coverage obtained in the cockroach i bacterial diversity within the cockroach gut at either the genus or species level $^{[17]}$ (Figure 1). The low sampling coverage obtained in the cockroach intestinal bacterial community analysis was also observed in the genus or species level $\begin{bmatrix} 17 \end{bmatrix}$ (Figure 1). The low sampling coverage obtained in the cockroach intestinal bacterial community analysis was also observed in the genus or species level $\left[\begin{array}{cc} [17] \\ [17] \end{array}\right]$ (Figure 1). The low sampling coverage obtained in the cockroach intestinal bacterial community analysis was also observed in the $\frac{1}{2}$ bacterial community analysis was also observed in the bacterial community analysis was also observed in the

Figure 5. Phylogenetic tree of lactic acid bacteria from *P. americana* and *S. lateralis* intestines. Numbers at each branch points indicate the percentage supported by bootstrap based on 1000 replicates. Numbers in pare

scale bar: 0. 01 substitution per nucleotide.

most diverse samples, such as termites and omnivorous

beetles $[27]$. Therefore, high intestinal microbial

diversity may be a characteristic of cockroaches.

Host phylogeny

most diverse samples, such as termites and omnivorous
beetles ^[27]. Therefore, high intestinal microbial
diversity may be a characteristic of cockroaches.
Host phylogeny is considered an important factor
influencing the beetles ^[27]. Therefore, high intestinal microbial
diversity may be a characteristic of cockroaches.
Host phylogeny is considered an important factor
influencing the diversity of the gut communities of
mammals as well a Host phylogeny is considered an important influencing the diversity of the gut commun
mammals as well as many insects $[27 - 28]$. As B
cockroaches, P. americana and S. latera
neighboring omnivorous cockroaches that exhibi The interesting the diversity of the gut communities of
mals as well as many insects $[27 - 28]$. As *Blattidae*
coaches, *P. americana* and *S. lateralis* are
boring omnivorous cockroaches that exhibit a less
ive and rela mammals as well as many insects $[27-28]$. As *Blattidae*
cockroaches, *P. americana* and *S. lateralis* are
neighboring omnivorous cockroaches that exhibit a less
invasive and relative relationship $[17]$. This relations mammals as well as many insects $[27 - 28]$. As *Blattidae*
cockroaches, *P. americana* and *S. lateralis* are
neighboring omnivorous cockroaches that exhibit a less
invasive and relative relationship $[17]$. This relation cockroaches, *P. americana* and *S. lateralis* are
neighboring omnivorous cockroaches that exhibit a less
invasive and relative relationship $[17]$. This relationship
was also reflected by the finding that 15% of the tota invasive and relative relationship $[17]$. This relationship
was also reflected by the finding that 15% of the total
clones in the *P. americana* library closely clustered
with microbiota from the *S. lateralis* gut, and was also reflected by the finding
clones in the P . *americana* lik
with microbiota from the S . *la*
were affiliated with the phylu
findings suggested that host ph
intestinal bacterial community of
has been verified by invasive and relative relationship $[17]$. This relationship 17]
ha
ary
ra t 15% of the total
closely clustered
lis gut, and most
Bacteroidetes. The
mericana, which
t regardless of host clones in the *P. americana* library closely clustered
with microbiota from the *S. lateralis* gut, and most
were affiliated with the phylum *Bacteroidetes*. The
findings suggested that host phylogeny influences the
intest clones in the *P. americana* library closely clustered
with microbiota from the *S. lateralis* gut, and most
were affiliated with the phylum *Bacteroidetes*. The
findings suggested that host phylogeny influences the
intest with microbiota from the *S. lateralis* gut, and most
were affiliated with the phylum *Bacteroidetes*. The
findings suggested that host phylogeny influences the
intestinal bacterial community of *P. americana*, which
has b were affiliated with the phylum *Bacteroidetes*. The findings suggested that host phylogeny influences the intestinal bacterial community of *P. americana*, which has been verified by observations that regardless of host intestinal bacterial community of P . americana, which
has been verified by observations that regardless of host intestinal bacterial community of P. *americana*, which
has been verified by observations that regardless of host has been verified by observations that regardless of host

percentage supplies, such as termites and omnivorous diet, the gut samples from different taxonomic order exclusively together due to host taxonomy and the section of the sequences represent the sequence of cockroaches. Th diet, the gut samples from different taxonomic orders
clustered exclusively together due to host taxonomy^[27].
Furthermore, these bacterial lineages determined by
host taxonomy may play an important role in
metabolism [[] Furthermore, these bacterial lineages determined
host taxonomy may play an important role
metabolism $^{[29]}$. For example, the potential lactic
bacteria assigned to the order *Lactobacillales*, inclu
Lactobacillus, *Lact* clustered exclusively together due to host taxonomy^[27]. [27]

b

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din . Furthermore, these bacterial lineages determined by
host taxonomy may play an important role in
metabolism $^{[29]}$. For example, the potential lactic acid
bacteria assigned to the order *Lactobacillales*, including
Lacto metabolism $[29]$. For example, the potential lactic acid
bacteria assigned to the order *Lactobacillales*, including
Lactobacillus, *Lactococcus*, and *Enterococcus*, clustered
with those from the *S. lateralis* intest metabolism $^{[29]}$. For example, the potential lactic acid
bacteria assigned to the order *Lactobacillales*, including
Lactobacillus, *Lactococcus*, and *Enterococcus*, clustered
with those from the *S. lateralis* intes bacteria assigned to the order *Lactobacillales*, including
 Lactobacillus, *Lactococcus*, and *Enterococcus*, clustered

with those from the *S. lateralis* intestine with 94% -

99% sequence identity (Figure 5). Interes Lactobacillus, Lactococcus, and Enterococcus, clustered and *Enterococcus*, clustered
 ralis intestine with 94% –

Figure 5). Interestingly,

triction fragment length
 S. lateralis gut bacteria,

roved to play an important

bacteria were also involved

oduction from end with those from the *S. lateralis* intestine with 94% -
99% sequence identity (Figure 5). Interestingly,
based on terminal restriction fragment length
polymorphism analysis of *S. lateralis* gut bacteria,
lactic acid bact 99% sequence identity (Figure 5). Interestingly,
based on terminal restriction fragment length
polymorphism analysis of *S. lateralis* gut bacteria,
lactic acid bacteria were proved to play an important
role. Similarly, l polymorphism analysis of *S. lateralis* gut bacteria,
lactic acid bacteria were proved to play an important
role. Similarly, lactic acid bacteria were also involved
in lactate and acetate production from endogenous
hexose polymorphism analysis of *S. lateralis* gut bacteria,
lactic acid bacteria were proved to play an important
role. Similarly, lactic acid bacteria were also involved
in lactate and acetate production from endogenous
hexose role. Similarly, lactic acid bacteria were also involved
in lactate and acetate production from endogenous
hexose to support the respiratory requirement of P .
americana^[6,10]. Therefore, some bacteria are role. Similarly, lactic acid bacteria were also involved
in lactate and acetate production from endogenous
hexose to support the respiratory requirement of *P*.
americana^[6,10]. Therefore, some bacteria are hexose to support the respiratory requirement of P .
americana^[6,10]. Therefore, some bacteria are hexose to support the respiratory requirement of P .
americana^[6,10]. Therefore, some bacteria are americana [[] 6,10]. Therefore, some bacteria are

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Phylotype (abundance)	Table 1 Accession number	Nearest relatives	Similarity/%	List of potential pathogenic microbes isolated from <i>Periplaneta americana</i> based on taxonomic analysis Pathogenicity
$CR_5(1)$	JX457156	Myroides odoratimimus	99.65	Nosocomial outbreak of urinary tract infection [18]
$CR_132(2)$	JX457228	Aeromonas hydrophila	99.80	Producing a wide variety of virulence factors facilitates the organism in causing both intestinal and extra-intestinal infections [19]
$CR_239(1)$	JX457307	Klebsiella pneumoniae	99.52	Opportunistic pathogens and can give rise to severe diseases such as septicemia, pneumonia, urinary tract infections, and soft tissue infections [20]
$CR_109(3)$	JX457213	Providencia rettgeri	99.26	Opportunistic pathogenic involved in ocular infections [21]
$CR_150(2)$	JX457239	Morganella morganii	99.19	Opportunistic pathogenic involved in urinary tract and wound infections [22]
$CR_171(1)$	JX457255	Serratia rubidaea	99.89	Invasive infection [23]
$CR_68(2)$	JX457190	Proteus mirabilis	99.13	Common pathogen responsible for complicated urinary tract infections and bacteremia ^[24]
$CR_95(9)$	JX457208	Citrobacter freundii	99.26	Peritonitis and tunnel infection [25]
$CR_88(7)$	JX457202	Enterobacter aerogenes	99.53	Important nosocomial pathogen [26]
		speculated to play an important role in metabolism and		other omnivorous animals and may contribute to the
thus originated from a common ancestor.				omnivorous lifestyle.
The dietary habit is another critical factor that				Cockroaches are opportunistic and omnivorous
affects the intestinal bacterial community composition of				insects that are in close contact with human beings and
		\sim 1 11 \sim 1 1 \sim 1 1 \sim 1 1		

CR_95 (9) JX457208 *Citrobacter freundii* 99. 26 Peritonitis and tunnel infection ^[25]

CR_88 (7) JX457202 *Enterobacter aerogenes* 99. 53 Important nosocomial pathogen ^[26]

speculated to play an important role in me CR_88 (7) JX457202 *Enterobacter aerogenes* 99.53 Important nosocomial pathogen [26]
speculated to play an important role in metabolism and other omnivorous ani
thus originated from a common ancestor. The dietary habit is thus originated from a common ancestor.
The dietary habit is another critical factor that
affects the intestinal bacterial community composition of
insects. Colman and colleagues found that microbial
communities were high The dietary habit is another critic
affects the intestinal bacterial community
insects. Colman and colleagues found
communities were highly similar and la
together according to feeding habits, w
verified in insects, includ is the intestinal bacterial community composition of
ts. Colman and colleagues found that microbial
nunities were highly similar and largely clustered
ner according to feeding habits, which has been
ed in insects, includi insects. Colman and colleagues found that microbial
communities were highly similar and largely clustered
together according to feeding habits, which has been
verified in insects, including detritivorous species,
carnivor communities were highly similar and largely clustered
together according to feeding habits, which has been
verified in insects, including detritivorous species,
carnivorous species, and xylophagous species $[27]$.
However together according to feeding habits, which has been
verified in insects, including detritivorous species,
carnivorous species, and xylophagous species $[27]$.
However, no such investigation has been performed for
omnivor together according to feeding habits, which has been
verified in insects, including detritivorous species,
carnivorous species, and xylophagous species $[27]$.
However, no such investigation has been performed for
omnivor verified in insects, including detritivorous species,
carnivorous species, and xylophagous species ^[27].
However, no such investigation has been performed for
omnivorous species. As omnivorous insects, houseflies
have th carnivorous species, and xylophagous species $[27]$.
However, no such investigation has been performed for
omnivorous species. As omnivorous insects, houseflies
have the ability to feed on a variety of organic
substrates, However, no such investigation has been performed for
omnivorous species. As omnivorous insects, houseflies
have the ability to feed on a variety of organic
substrates, of which many of the intestinal bacterial
phylotypes omnivorous species. As omnivorous insects, houseflies
have the ability to feed on a variety of organic
substrates, of which many of the intestinal bacterial
phylotypes exhibited high similarity with those
associated with h substrates, of which many of the intestinal bacterial
phylotypes exhibited high similarity with those
associated with humans, domestic animals, and
plants^[16]. Similarly, the majority of the gut bacteria
from *P. americ* substrates, of which many of the intestinal bacterial
phylotypes exhibited high similarity with those
associated with humans, domestic animals, and
plants^[16]. Similarly, the majority of the gut bacteria
from *P. americ* associated with humans, domestic animals, and
plants^[16]. Similarly, the majority of the gut bacteria
from *P. americana* and *S. lateralis* also clustered with
bacteria from the gut environments of more distantly
relat associated with humans, domestic animals, and
plants^[16]. Similarly, the majority of the gut bacteria
from *P. americana* and *S. lateralis* also clustered with
bacteria from the gut environments of more distantly
relat plants
from *l*
bacter
related
are al
diets.
have plants^[16]. Similarly, the majority of the gut bacteria $\begin{bmatrix} 16 \ 1 \end{bmatrix}$
a $\begin{bmatrix} 2 \ 2 \end{bmatrix}$. Similarly, the majority of the gut bacteria
 americana and *S. lateralis* also clustered with

from the gut environments of more distantly

mimals, which suggest that the intestinal

community may be influenced by die from *P. americana* and *S. lateralis* also clustered with
bacteria from the gut environments of more distantly
related animals, which suggest that the intestinal
bacterial community may be influenced by dietary habit
 \left related animals, which suggest that the intestinal
bacterial community may be influenced by dietary habit
 $\begin{bmatrix} 11 \end{bmatrix}$. Because of the habitat preferences and
unrestricted movement, the omnivorous cockroaches
are abl related animals, which suggest that the intestinal
bacterial community may be influenced by dietary habit
 $\left[\begin{array}{ccc}177\end{array}\right]$. Because of the habitat preferences and
unrestricted movement, the omnivorous cockroaches
are [17]. Because of the habitat preferences and
unrestricted movement, the omnivorous cockroaches
are able to include high-and low-fiber substrates in the
diets. Previous studies on the cockroach *P. americana*
have shown th $\left[17\right]$. 17]
unr
liet
lav estricted movement, the omnivorous cockroaches
able to include high-and low-fiber substrates in the
is. Previous studies on the cockroach *P. americana*
e shown that gut-associated bacteria played the key
in digesting any unrestricted movement, the omnivorous cockroaches
are able to include high–and low-fiber substrates in the
diets. Previous studies on the cockroach *P. americana*
have shown that gut-associated bacteria played the key
rol diets. Previous studies on the cockroach *P. americana*
have shown that gut-associated bacteria played the key
role in digesting any food available ^[17]. Thus, to meet
the requirement of digesting all types of food in
di diets. Previous studies on the cockroach *P. americana*
have shown that gut-associated bacteria played the key
role in digesting any food available $[17]$. Thus, to meet
the requirement of digesting all types of food in
d Followin that gut associated bacteria played the transporterior of digesting all types of food in different habitat, it may be necessary for the omnivorous cockroaches to possess a large and diverse gut microbial populati role in digesting any food available $[17]$. Thus, to meet
the requirement of digesting all types of food in
different habitat, it may be necessary for the
omnivorous cockroaches to possess a large and diverse
gut microbi different habitat, it may be necessary for the
omnivorous cockroaches to possess a large and diverse
gut microbial population, which is a typical feature of different habitat, it may be necessary for the
omnivorous cockroaches to possess a large and diverse
gut microbial population, which is a typical feature of gut microbial population, which is a typical feature of gut microbial population, which is a typical feature of

omnivorous lifestyle.

Cockroaches are opportunistic and omnivorous

insects that are in close contact with human beings and

can thrive under poor sanitary conditions. Based on the

16S rRNA genes of the microbiota in th Cockroaches a
insects that are in cl
can thrive under poc
16S rRNA genes of
pathogens have been
as houseflies and
revealed that 18.4
American cockroac
potential pathogen ts that are in close contact with human beings and
hrive under poor sanitary conditions. Based on the
rRNA genes of the microbiota in the gut, potential
gens have been identified in some insects, such
ouseflies and S. *la* can thrive under poor sanitary conditions. Based on the
16S rRNA genes of the microbiota in the gut, potential
pathogens have been identified in some insects, such
as houseflies and *S. lateralis*^[16-17]. Our survey
rev 16S rRNA genes of the microbiota in the gut, potential
pathogens have been identified in some insects, such
as houseflies and *S. lateralis* $^{[16-17]}$. Our survey
revealed that 18.4% of the gut bacteria from the
American 16S rRNA genes of the microbiota in the gut, potential
pathogens have been identified in some insects, such
as houseflies and *S. lateralis*^[16-17]. Our survey
revealed that 18.4% of the gut bacteria from the
American c pathogens have been identified in some insects, such
as houseflies and *S. lateralis*^[16-17]. Our survey
revealed that 18.4% of the gut bacteria from the
American cockroach exhibited high identity with
potential pathoge as houseflies and *S. lateralis*
revealed that 18.4% of the gi
American cockroach exhibited
potential pathogens, of which
Dysgonomonas, *Myroides*, and *.*
been previously been identified i
(Table 1). Therefore, more eff ^[16 - 17]
ut bac
high
ich
Provid teria from the
identity with
three genera,
encia, had not
ultured isolates
ontrol strategies
opulations are
inzato *et al.*
rial lineages in American cockroach exhibited high identity with
potential pathogens, of which three genera,
 $Dysgonomonas, Myroides,$ and $Providencia,$ had not
been previously been identified from cultured isolates
(Table 1). Therefore, more effective contr potential pathogens, of which three genera,
Dysgonomonas, Myroides, and Providencia, had not
been previously been identified from cultured isolates
(Table 1). Therefore, more effective control strategies
aimed at reducing potential pathogens, of which three genera,
Dysgonomonas, Myroides, and Providencia, had not
been previously been identified from cultured isolates
(Table 1). Therefore, more effective control strategies
aimed at reducing Dysgonomonas, Myroides, and Providencia, had not and *Providencia*, had not
fied from cultured isolates
e effective control strategies
et pest populations are
rmites, Shinzato *et al.*
pecific bacterial lineages in
to develop novel control
he metabolism of digestive
es (Table 1). Therefore, more effective control strategies
aimed at reducing insect pest populations are
important. To control termites, Shinzato *et al.*
proposed defining termite-specific bacterial lineages in
the intestin (Table 1). Therefore, more effective control strategies Table 1). Therefore, more effective control strategies
imed at reducing insect pest populations are
nportant. To control termites, Shinzato *et al.*
roposed defining termite-specific bacterial lineages in
intestine as tar important. To control termites, Shinzato *et al.*
proposed defining termite-specific bacterial lineages in
the intestine as targets to develop novel control
strategies, e.g., disturb the metabolism of digestive
symbionts important. To control termites, Shinzato *et al.*
proposed defining termite-specific bacterial lineages in
the intestine as targets to develop novel contro
strategies, e.g., disturb the metabolism of digestive
symbionts o proposed arising termine operate antitality integers in
the intestine as targets to develop novel control
strategies, e.g., disturb the metabolism of digestive
symbionts or kill key microbes ^[29]. Similarly, common
bacte strategies, e. g., disturb the metabolism of digestive
symbionts or kill key microbes $[29]$. Similarly, common
bacterial lineages in the intestine of various species of
cockroaches may be a potential target for cockroach strategies, e. g., disturb the metabolism of digestive
symbionts or kill key microbes $[29]$. Similarly, common
bacterial lineages in the intestine of various species of
cockroaches may be a potential target for cockroach symbionts or kill key microbes $[29]$. Similarly, common
bacterial lineages in the intestine of various species of
cockroaches may be a potential target for cockroach
control. However, due to the limited information about backroaches may be a potential target for cockroach
control. However, due to the limited information about
the intestinal bacterial community of different species of
cockroaches, cockroach-specific bacterial lineages
remai control. However, due to the limited information about
the intestinal bacterial community of different species of
cockroaches, cockroach-specific bacterial lineages
remain unclear and are an interesting research object.
Al control. However, due to the limited information about
the intestinal bacterial community of different species of
cockroaches, cockroach-specific bacterial lineages
remain unclear and are an interesting research object.
Al

the intertain bacterial community of different specific cockroaches, cockroach-specific bacterial lineages
remain unclear and are an interesting research object.
Along with the analysis of the bacterial community
of *S. la* cockroaches, cockroach-specific bacterial lineages
remain unclear and are an interesting research object.
Along with the analysis of the bacterial community
of *S. lateralis* colon, our findings from *P. americana*
provide Remain unclear and are an interesting research rayses.
Along with the analysis of the bacterial communit
of *S. lateralis* colon, our findings from *P. american*
provide more data on the intestinal bacteria from
cockroach Interalis colon, our findings from P. americana
de more data on the intestinal bacteria from
coach species and provide evidence that host of *S. lateralis* colon, our findings from *P. americana*
provide more data on the intestinal bacteria from
cockroach species and provide evidence that host provide evidence that host cockroach species and provide evidence that host \mathbf{r} intestinal bacterial community composition of the
insects. Furthermore, the findings presented here will
most likely contribute to identifying potential common
pathogenic bacterial lineages. Further studies are
needed to e insects. Furthermore, the findings presented here will
most likely contribute to identifying potential common
pathogenic bacterial lineages. Further studies are
needed to evaluate the potential physiological functions
of insects. Furthermore, the findings presented here will
most likely contribute to identifying potential common
pathogenic bacterial lineages. Further studies are
needed to evaluate the potential physiological functions
of pathogenic bacterial lineages. Further studies are
needed to evaluate the potential physiological functions
of the common bacterial lineages, such as culture-
based methods, which will contribute to identifying
more effec meeded to evaluate the potential physiological functions
of the common bacterial lineages, such as culture-
based methods, which will contribute to identifying
more effective methods of cockroach control.
 $\hat{\gg} \neq \hat{\pm} \hat$ of the common bacterial lineages, such as culture-
based methods, which will contribute to identifying
more effective methods of cockroach control.

 $\hat{\mathcal{B}}$ 考文献
[1] Cochran DG. Cockroaches: their biology, distribution of the common bacterial lineages, such as culture–

based methods, which will contribute to identifying

more effective methods of cockroach control.
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[1] Cochran DG. Cockroaches: their bi

参考文献

- [1] Cochran DG. Cockroaches: their biology, distribution and
- based methods, which will contribute to identifying
more effective methods of cockroach control.
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主分析
,肖亚中^{1,2*} 房伟^{1,2#},方泽民^{1,2#},刘智苗^{1,2},袁璟^{1,2},张学成^{1,2},彭惠^{1,2},洪宇植^{1,2},肖亚中 '安徽大学生命科学学院, 2安徽省微生物与生物催化工程技术研究中心, 合肥 230601

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摘要:【目的】分析美洲
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肠道得 摘要:【目的】分析美洲大蠊(Periplaneta americana)肠道微生物群落的组成。【方法】以美洲大蠊肠道微生物 基因组为模板, E Bact-27F 和 Univ-1492R 为引物, PCR 扩增 16S rRNA 基因,连接 pGEM-T 载体,构建肠道微生力
因文库,并对肠道微生物的组成及多样性进行分析。【结果】美洲大蠊肠道微生物主要包括
oteobacteria, 66.4%),拟杆菌门(Bacteroidetes, 17.8%),厚壁菌门(Firmicutes, 14.5%),梭杆
ria, 0.6%),以及未分类微生物(unclassified bacte 物 16S rRNA 基因文库,并对肠道微生物的组成及多样性进行分析。【结果】美洲大蠊肠道微生物主要包括 16S rRNA 基因文库,并对肠道微生物的组成及多样性进行分析。【结果】美洲大蠊肠道微生物主要包括
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了(Fusobacteria, 0.6%),以及未分类微生物(unclassified bacteria, 0.6%)。系统发育分析显示,15%的美
大蠊肠道微生物 16S 变形杆菌门(Proteobacteria, 66.4%), 拟杆菌门(Bacteroidetes, 17.8%), 厚壁菌门(Firmicutes, 14.5%), 梭杆 66. 4%),拟杆菌门(Bacteroidetes, 17. 8%),厚壁菌门(Firmicutes, 14. 5%),梭杆
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16S rRNA 基因, 微生物群落
文献 一支;59%的美洲大蠊肠道微生物 16S rRNA 基因序列与不同食性动物肠道微生物的 16S rRNA 基因序列聚 59% 的美洲大蠊肠道微生物 16S rRNA 基因序列与不同食性动物肠道微生物的 16S rRNA 基因序列聚

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3.美国生活方式对其肠道微生物的组成有较大影响。
3.美洲大蠊, 16S rRNA 基因, 微生物群落

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16S rRNA 基因, 微生物群落

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关键词: 美洲大蠊, 16S rRNA 基因, 微生物群落

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¹⁶S rRNA 基因, 微生物群落
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