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Isolation and Identification of Skin Microbiota from the Fruit Bat Indian flying fox, *Pteropus giganteus*

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Abstract

In mammals, the knowledge on microbiota composition is scarce, especially in poorly studied organisms such as bats. Besides playing a key role in various ecological services like pollination, seed dispersion and insect-pest control, bats are known to be the reservoirs of various zoonosis. To date, there are only a handful of records on microbiota from bats with isolates originating from fecal matter or different parts of the gastrointestinal tract, namely the stomach, intestine and rectum. Studies on the distribution and diversity of skin microbiota found in bats from the south India is scarce. However, such information is important not only for a better understanding on the biology of bats, but also in the screening of any potential pathogens present in the bats which commonly serve as a natural reservoir for pathogens. Therefore, the present study is an attempt to identify the microbial communities associated with skin samples collected from different bat species using biochemical tests and 16S rRNA gene sequence analysis. A total of 10 isolates from the skin of two *Pteropus giganteus* were retrieved. The estimation of viable bacteria present in the skin ranges from $2.35x10^6$ to $4.62x10^8$ CFU/ml. All isolates will be subjected to various chromogenic media, biochemical tests and 16S rRNA sequencing for bacterial identification. Considering the scarcity of literature related to microbial communities of bat skin microbiota, this study can direct future microbial diversity studies in south Indian bats.

Keywords: Microbiota, *Pteropus giganteus*, biochemical tests, 16S rRNA.

Introduction

In mammals, the knowledge on microbiota composition is scarce, especially in poorly studied organisms such as bats. Besides playing a key role in various ecological services like pollination, seed dispersion and insect-pest control, bats are known to be the reservoirs of various zoonoses (Calisher et al. 2006). To date, there are only a handful of records on microbiota from bats with isolates originating from faecal matter or different parts of the gastrointestinal tract, namely the stomach, intestine and rectum. Studies on the distribution and diversity of skin microbiota found in bats from the south India is scarce (Ley et al. 2008).

However, such information is important not only for a better understanding on the biology of bats, but also in the screening any potential pathogens present in the bats which commonly serve as a natural reservoir for pathogens (klite 1965; Graves et al. 1988; Heard et al. 1997; Bella et al. 2003; Anand and Sripathi 2004; Whitaker et al. 2004; Gloriana, 2006; Mühldorfer et al. 2010; Apun et al. 2011).

Bats are cosmopolitan in distribution and their microbial community play role in influencing their growth, ecology and evolution (Avena et al. 2016). Animals are the residing places for bacteria which

influence their metabolism and physiology. Recent studies reveal that the social behaviour influences their microbial patterns and chemical signals produced by microbes provoke the host's central nervous system (Li et al. 2013). All the microbes cannot be transmitted via social routes. Depending on the physiological health of hosts, bacterial transfer happens. Limited studies are available to know about the life cycle of bacterial transmission (Tung et al. 2015).

Studies reveal that bats are widespread all over the world with different microbial populations. Diet plays a major role in sharpening their microbiota. They may be insectivorous, carnivorous, and omnivorous and nectarivorous. Based on their feeding habits the evolutionary patterns change (Fenton et al. 1996). They are economically important species, ecologically diverse which enhance the pollination of fruit crops and act as insect pest control. By understanding the microbiome of bats helps to overcome from habitat destruction and the dangerous levels can be identified (Quay, 1970). Influence of microbiome based on social behaviour have been reported in primates, chimpanzees, humans reside in same place have same microbial community (Aprill et al. 2014). These organisms share similar microbes because of exposure to same environmental conditions. Social behaviour and microbial influence is a common process but still it is not completely understood (Tung et al. 2015).

Socially integrated organisms share same environmental exposure and their behavioural patterns show similar ecological niche within their microbial patterns because they consume same diets. This is long-term co evolution process but the influence of bacterial community in host's physiology is still unclear. Both beneficial and harmful bacteria find different transmission routes. Finding the suitable host by bacteria is not clearly understood (Ezenwa et al. 2012). Analysing the origin of evolution the host-microbiome signalling interactions can give the connection between the function of microbial communities involved in producing olfactory signals. The establishment of connection between microbes and signalling odors and understanding the odors that provoke response in receivers (Archie et al. 2011).

Microorganisms harbouring in bats have the ability to influence the health of their hosts and also provide defence against the invading pathogens (McGuire et al. 2008). However, information on the skin and fur bacterial microbiota on bats and the factors influencing the structure of these communities is very sparse. South Indian region is an excellent site to study the external bat bacterial microbiota due to the presence of diverse bat species and variety of abiotic and biotic factors that may govern bat bacterial microbiota communities (Ezenwa et al. 2014). With this idea, we framed the objectives for my Master's dissertation

Therefore, I studied the isolation and identification of skin microbiota from the fruit bat Indian flying fox, *Pteropus giganteus* with the following objectives.

Objectives

1. To isolate and identify the common and specific bacterial communities from skin of fruit bat Indian flying fox, *Pteropus giganteus*.
2. To identify the diversity of microbiome influenced by different habitats and
3. To identify the behavioural patterns influenced by skin microbiome.

Materials and Methods

Materials:

Experimental Animal

In this study, we used fruit-eating (Megachiroptera) and insect- eating (Microchiroptera) bats as study animal. Bats species present in Madurai (Tamil Nadu) are listed in the **Table 1.**

Equipments

Microbiology and molecular equipments used in this study are listed in the **Table 2.**

Buffers, Solutions and Reagents:

1 M Tris (pH 8.0)

121.1 Tris base was dissolved in 1000 ml distilled water, autoclaved and stored at room temperature.

0.5 M EDTA (pH 8.0)

EDTA	- 186.1 g
$2H_2O$	- 800 ml

Stirred vigorously on a magnetic stirrer and the pH was adjusted to 8.0 with NaOH. Made up to 1000 ml, dispensed into aliquots and sterilized by autoclaving.

PBS

NaCl - 8.00 g
KCl - 0.20 g
Na_2HPO_4 - 1.15 g
KH_2PO_4 - 0.20 g

Made up to 1000 ml using double distilled water and autoclaved.

Table 1. Bat species used in this study for skin microbiome

S. No.	Scientific Name	Common Name
1	*Pteropus giganteus*	Indian flying fox

Total Bat Species: In the World-1331; In India-123; In Tamil Nadu-16

Table 2. Microbiology and molecular equipments used in this study.

- Mist Nets (Avinet-Dryden, USA)
- Torch Lights
- Hoop Net
- Bat Cage (small and big)
- Metal Poles
- Spring Balance
- Canon camera with Tripod
- Medical Syringe
- Leather Gloves
- Sterile Collection Tubes (1.5 ml)
- Tarsons PCR tubes
- PCR Machine (Eppendorf)
- Petri Plates
- Test tubes
- Incubator
- Laminar Air Flow
- Conical flasks
- Petri plates
- L-rod
- Rotating table
- Micropipettes

Solutions for Gel Electrophoresis

Running buffer: 50X TAE

Tris-base -242 g
Glacial acetic acid -57.1 ml
0.5 M EDTA (pH 8.0) -100 ml
Made up to 1000 ml. 50X TAE was diluted to 1X prior to use.

EtBr (Ethidium Bromide)

Prepared as 10 mg/ml stock solution in distilled water and stored in a bottle wrapped with thin foil at 4 °C. This was added to the gel at 0.5ug/ml before casting the gel.

DNA loading dye (6X)

Glycerol - 50 %
Bromophenol blue - 0.25 %
Xylene cyanol - 0.25 %
Dissolved in autoclaved distilled water.

Media preparations for Microbiology

Luria-Bertani (LB) Broth

Peptone - 10 g
Yeast Extract - 5 g
NaCl - 10 g
Made up to 1000 ml using distilled water and autoclaved.

Luria-Bertani (LB) Agar

Peptone - 10 g
Yeast Extract - 5 g
NaCl - 10 g
Agar - 15 g
Made up to 1000 ml using distilled water and autoclaved.

Methodology: Fruit-Eating Bats Capture

The fruit eating bats (Megachiropterans) were captured in and around Madurai, Tamil Nadu and South India (lat: 9° 58’ N; long: 78° 10’ E). Nylon mist nets of 9 m x 2.6 m with a mesh size of 38 mm (Avinet-Dryden, New York, USA) have been used. The mist nets were placed away from illuminated areas to avoid the visual detection by bats. Mist nets were set up at 4 m above ground level. They were tied about half an hour before sunset and removed at 0600 h. The bats, which were trapped in the mist net (Figure 4.1), were removed immediately with gloved hands and placed in cloth bags.

After capturing bats were taken and handled with sterile hand gloves (Avena et al. 2016). Sterile swab protocol was followed. The swabs were autoclaved to prevent cross contamination. Bacterial samples from skin were collected using sterile cotton swab. Gentle press was given on the surface of skin and samples were collected by rubbing the cotton swabs gently on the surface of skin (wings and neck).

Figure 1. Fruit bats captured from MKU Botanical Garden using a mist net (a) bat first caught in the net (b) bat hanging in the net.

Culturing of Bacteria:

LB medium was prepared for 50 ml in a conical flask and sterilized at 121°C for 15 min. After sterilization, medium was taken out and allowed to cool. Cotton swabs were gently rubbed on the surface of skin and then inoculated inside the broth then medium was incubated at 37 °C for 24 hours.

Serial Dilution:

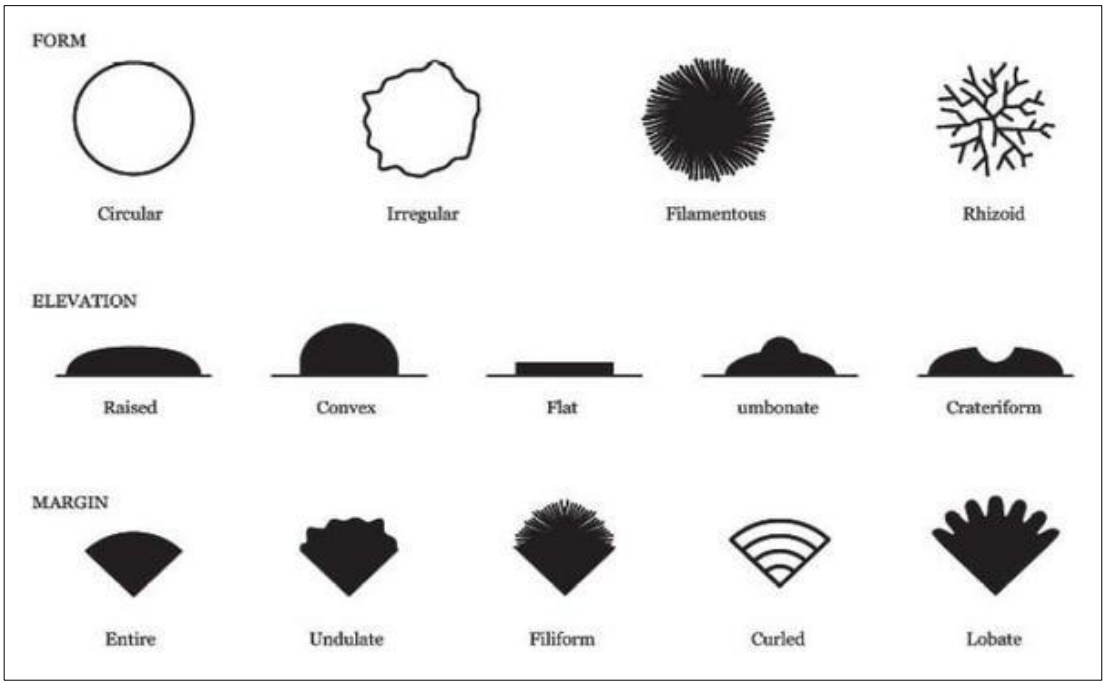
To reduce the microbial load, serial dilution was carried out. Overnight cultures were serially diluted in 0.85 % saline. Saline was sterilized at 121 °C for 15 minutes. Set of 10 test tubes were taken and marked as 10^{-1}-10^{-10} dilutions. Each tube was filled with 9 ml of saline and kept in sterile condition. First tube was marked as 10^{-1} dilution and next tube as 10^{-2} dilution and so on. Up to 10^{-10} dilutions were marked. In 10^{-1} dilution tube 1ml of overnight culture (mother culture) was transferred and mixed well. After that 1ml of sample was serially diluted. 1ml of culture from 10^{-1} dilution tube transferred to 10^{-2} dilution tube and mixed well using micropipette. Up to 10^{-10} dilution tube 1ml of sample was serially diluted. Finally 1ml of sample from 10^{-10} tube was discarded (Bergey and Holt 1994).

Spread Plate Technique:

LB agar plates and medium were prepared and sterilized at 121°C for 15 minutes. After sterilization, media was poured in sterile Petri plates and allowed to solidify. After solidification spread plate technique was carried out using L-rod. 0.1ml cultures from 10^{-1} to 10^{-10} tubes were spread on respective plates. Plates were kept on rotating table and cultures were spread using L-Rod. Plates were incubated at 37 °C for 24 hours. By spread plate tecnique viable colonies, can be obtained.

Characterization of Bacteria

Morphology, texture and colour of the bacterial colonies were noted and colonies with different texture and morphology were streaked again in LB medium plates.



Texture and margin of the bacterial colonies identified from the picture. Each bacterium has unique texture and morphology. It helps in identify the bacterial colonies (mcmccrindle.weebly.com)

Crowded Plate Technique:

Viable colonies were streaked on three different medium:

1) **LB medium**: to identify the morphology and texture, LB agar medium was prepared and sterilized at 121 °C for 15 minutes. After sterilization medium was poured on sterile petri plates and allowed to solidify. After solidification, bacterial samples were streaked to get isolated colonies. Since LB medium is enriched with carbon, nitrogen and salt sources bacteria could utilize all the sources in the medium and differentiate.

2) **Eosin Methylene Blue agar:** A selective medium for gram negative bacteria. Eosin and methylene blue dyes enhance the growth of gram negative bacteria and suppress the growth of gram positive bacteria.

3) **MacConkey medium:** Being both selective and differential medium it is used to identify gram negative bacteria. Lactose fermenting colonies present in the medium indicates that gram negative bacteria present. Crystal violet and bile salts in the medium inhibit the growth of gram positive bacteria and allows the growth of gram negative bacteria (Voigt et al. 2015).

4) **Blood agar medium:** To check whether the bacterial isolates are pathogen or non-pathogen blood agar is used.5% of sheep blood is amended in the medium which is used to isolate beta-hemolytic colonies. Beta hemolytic colonies indicate that bacteria belong to pathogenic group.

Biochemical Characterization:

Following tests were carried out to identify the genus of bacteria. Test was carried out used HiMedia biochemical test kit. Each test has unique characteristic. Based on the substrate utilization and colour change at regular time intervals the genus level bacteria were identified. After 48 hours of incubation the results vary. Following chart helps to identify the bacterial samples (Aslanzadeh 2006).

S.No	Biochemical test
1	CNPG
2	lysine
3	Ornihtine
4	Urease
5	Phenyl alanine
6	Nitrate reduction
7	Hydrogen sulfide
8	Citrate utilization
9	Voges proskauer
10	Methyl red
11	Indole production
12	Malonate utilisation
13	Esculin hydrolysis
14	Arabinose
15	Xylose
16	Adonitol
17	Rhamnose
18	Cellobiose
19	Melibiose
20	Saccharose
21	Raffinose
22	Trehalose
23	Glucose
24	Lactose
25	oxidase
26	Triple sugar iron
27	Starch hydrolysis
28	Casein hydrolysis

DNA isolation:

DNA from bacterial samples was isolated manually. Colonies (overnight) were picked from LB plates and mixed with 20 µl of distilled water taken in Eppendorf tubes. Tubes were incubated at 90 °C for 20 minutes. Then centrifuged at 10,000 rpm for 10 minutes. Supernatant was taken and used as template for PCR.

Primers used:

Universal primers were used:

27 Forward: (AGATTTGATCMTGGCTCAG)
1492 Reverse: (CCGTTACCTTGTTACGATT)

PCR amplification:

PCR reaction was carried out in 25 µl reaction containing of template DNA, 12.5 µl of 2X PCR Master Mix, 1 µl of 20 µm of each 27F and 1492R primer, and 9.5µl of H_2O. DNA amplifications were performed using an Eppendorf PCR machine, with cycling conditions including initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 40 sec, annealing at 58 °C for 1 min extension at 72 °C for 1min and final extension at 72 °C for 10 min. Electrophoresis was done for PCR Products on 1% agarose gel.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal matrix of agarose with 1X TAE buffer. The amplified PCR products were loaded on 1.2 % agarose gel along with a size standard and stained with ethidium bromide (0.5µg/ml). Electrophoresis was performed at 80V until the run was complete. After completion of electrophoresis, the gel was visualized with a UV transilluminator and photographed with a gel documentation system (Biorad, USA, Model 2000).

Gel Elution

The amplified products that were run on gel were eluted by QIAquick gel extraction kit The elution procedure is as follows. The excised gel of approximately 100 mg containing the amplified fragment was placed into a 1.5 ml polypropylene micro centrifuge tube. 3-volumes Gel Solubilization Buffer (QG) was added for every 1 volume of gel. These tubes were incubated at 50 °C in the water bath or heat block for 10 minutes or until the gel dissolves completely. The samples were mixed well and preceded to purify the DNA using a centrifuge. 1-gel volume of isopropanol was added and mixed well. The contents were transferred onto the center of a QIAquick Gel Extraction Column inside a wash tube and centrifuged for 1 min at 13,000 rpm.

The flow-through was discarded. 500 µl of QG buffer was added to the column again and centrifuged form 1 min at 13,000 rpm. The flow-through was discarded. To it, 500 µl Wash Buffer (W1), containing ethanol was added to the QIAquick Gel Extraction Column. The solution was centrifuged at 13,000 rpm for 1 min.

The flow-through was discarded and the column was replaced into the wash tube. The column was centrifuged again at maximum speed for 1–2 min to remove any residual wash buffer and ethanol. The wash tube was discarded and the QIAquick Gel Extraction Column was placed into a recovery tube. 30 µl of Elution buffer was added at the centre of the column. The column was incubated for 5 min at room temperature. The column was then centrifuged at 13,000 rpm for 1 min. The DNA was collected in the recovery tube. The Quick Gel Extraction Column was discarded. The purified DNA was stored at -20 °C for further analysis.

Sequence Analysis:

The sequenced data was blasted in the NCBI website (https://blast.ncbi.nlm.nih.gov/) and looked for similarity between other bacteria.

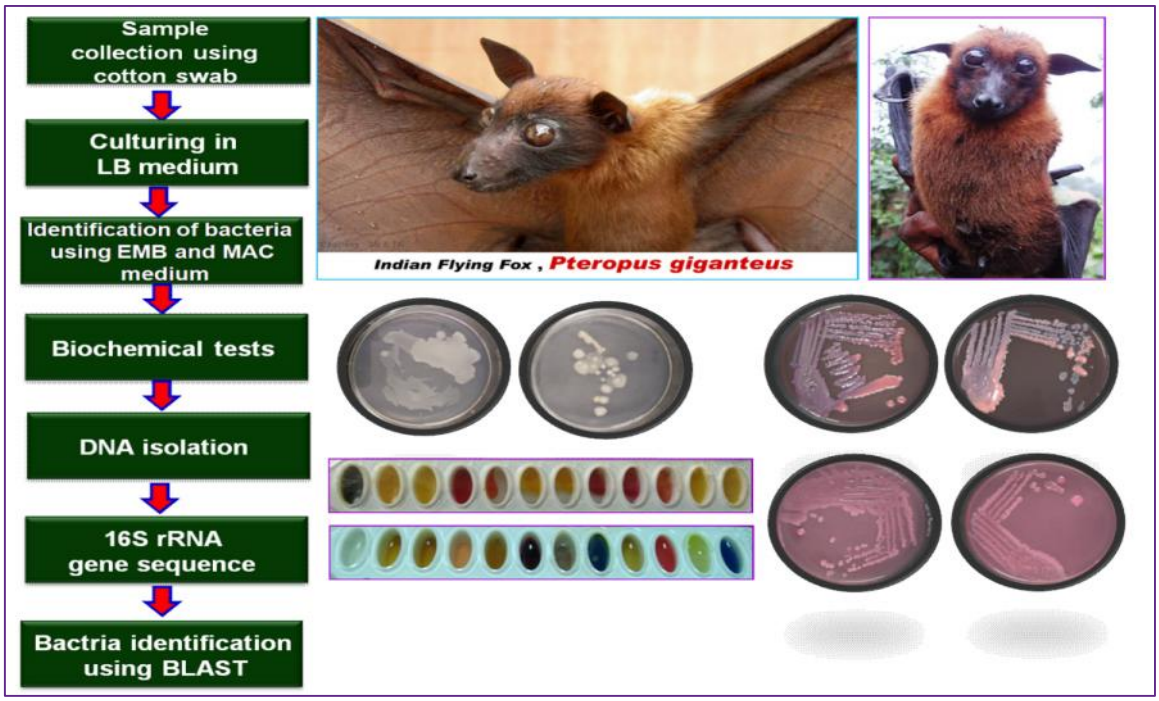


Figure 2. Flow chart of bats capture, sample collection, bacterial identification, biochemical tests, DNA isolation, PCR, 16S rRNA sequencing and analysis.

Results

Total Viable Bacteria Count in the Bat Skin:

The total viable bacteria in the skin of *P. giganteus* were successfully enumerated for all the 10 samples. The estimation of viable bacteria present in the in the skin ranges from $2.35x10^{-6}$ to $4.62x10^{-8}$ CFU/ml.

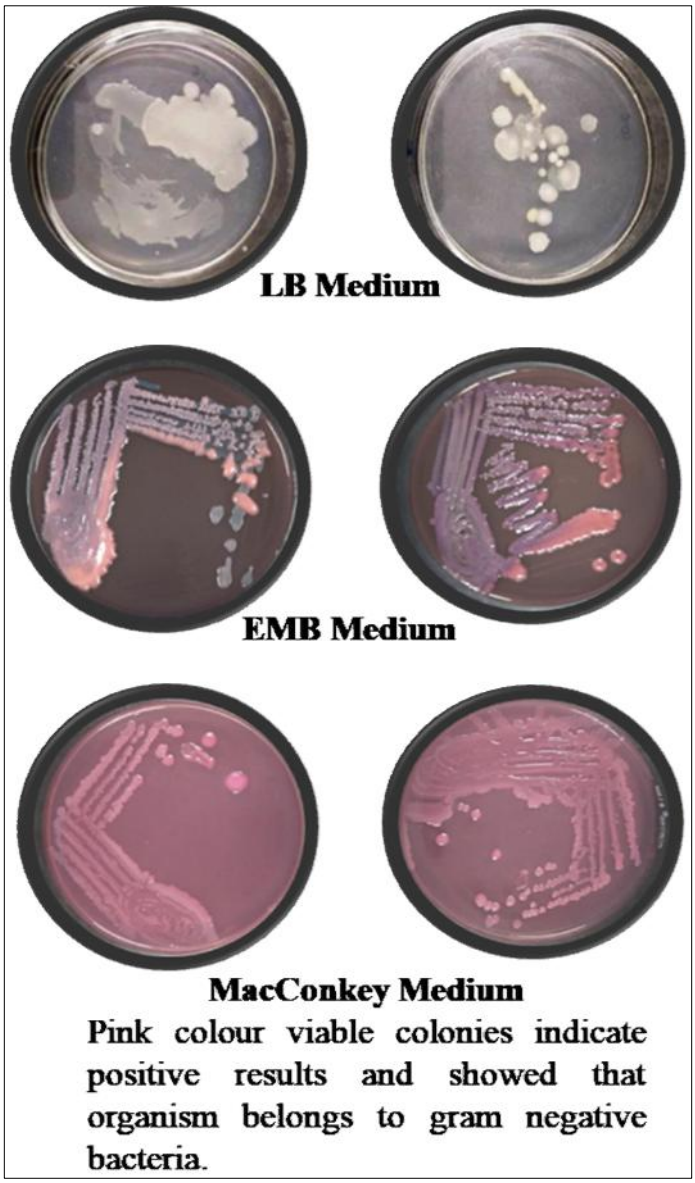
S.No	Biochemical test	Result
1	ONPG	Negative
2	lysine	Negative
3	Ornihtine	Negative
4	Urease	Negative
5	Phenyl alanine	Positive
6	Nitrate reduction	Positive
7	Hydrogen sulfide	Negative
8	Citrate utilization	Positive
9	Voges proskauer	Negative
10	Methyl red	Positive
11	Indole production	Negative
12	Malonate utilisation	Positive
13	Esculin hydrolysis	Positive
14	Arabinose	Positive
15	Xylose	Positive
16	Adonitol	Negative
17	Rhamnose	Negative
18	Cellobiose	Positive
19	Melibiose	Positive
20	Saccharose	Negative
21	Raffinose	Negative
22	Trehalose	Negative
23	Glucose	Positive
24	Lactose	Positive
25	oxidase	Negative
26	Triple sugar iron	Positive
27	Starch hydrolysis	Negative
28	Casein hydrolysis	Negative

Table 1: Biochemical tests performed on bat wing isolates according to gram type.

Identification of Bacteria using Biochemical Tests:

A total of 10 bacterial isolates were recovered from the bat skin of *P. giganteus.* Various biochemical tests were performed for bacterial identification up to genus level (Table 1).

In summary, A total of 3 genera of bacteria were identified from the skin using biochemical tests; *Acinetobacter, Alkanindiges* and *Pseudomonas.*



Identification of Bacteria using 16S rRNA Gene Sequence Analysis:

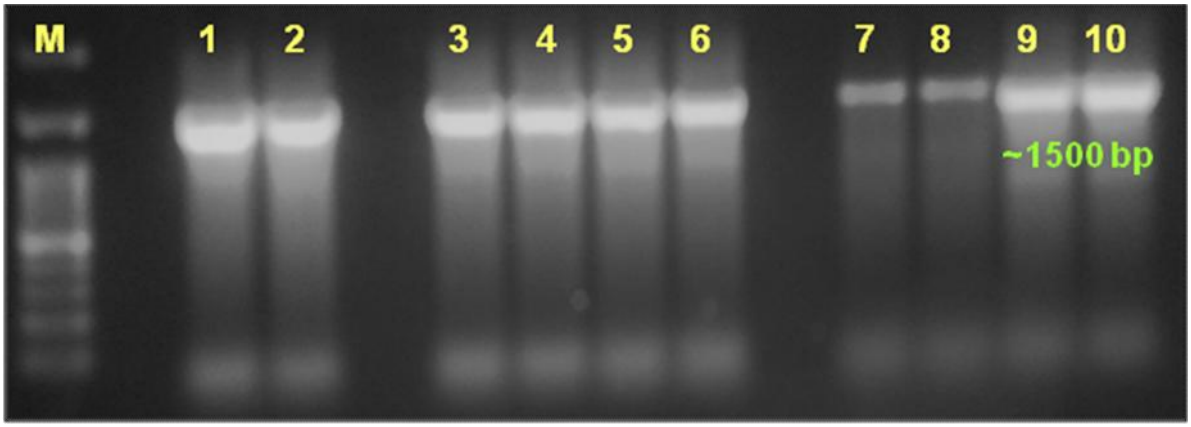


Figure 3. 16SrRNA gene (~1500 bp) from genomic DNA of 10 isolates were obtained by PCR using the primer pair (27F1 and 1492R). PCR products were purified and sent to sequencing for bidirectional DNA sequencing using the forward and reverse primers.

Analysis of Sequence and Bacterial Identification:

DNA sequencing results were aligned using ApE and manually corrected using the FASTA format. These aligned sequences were then compared in the BLAST (NCBI) database based on the homology sharing percentage for bacteria identification. A total of 10 isolates (each sequences 1500 bps) were identified based on their percentage of similarity in the BLAST results (Table 2). Eight isolates had 99 % matching identities whereas 1 isolate had 94% matching identities based on the closest match of GenBank sequences.

A similarity rate of ≥99% was applied for species level identification while ≥97% was used for genus level identification. All samples determined by phylogenetic positioning were correctly classified, however not all of these results tallied with the genus identification using biochemical tests.

List of microbes identified in skin of *P. giganteus* based on gene sequence analysis using BLAST:

1. *Acinetobacter sp,*
2. *Acinetobacter baumannii*
3. *Acinetobacter calcoaceticus*
4. *Acinetobacter pittii*
5. *Acinetobacter dijkshoorniae*
6. *Acinetobacter lactucae*
7. *Acinetobacter oleivorans*
8. *Acinetobacter junii and*
9. *Alkanindiges illinoisensis*

Table 2. Identification of bats wing isolates based on gene sequence analysis using BLAST

Sequence ID	Closest Match from GenBank	Similarity (%)	Accession Number
PG1F	*Acinetobacter baumanii*	99.68%	HQ632003.1
PG1R	*Acinetobacter sp.*	99.58%	JN696656.1
	Acinetobacter baumanii	99.58%	MK044849.1
PG2F	*Acinetobacter calcoaceticus*	100.0%	KY596056.1
	Acinetobacter pittii	100.0%	KY941129.1
PG2R	*Acinetobacter calcoaceticus*	99.79%	LT984792.1
	Acinetobacter dijkshoorniae	99.79%	MH880847.1
PG3F	*Acinetobacter baumanii*	99.37%	HQ631961.1
PG3R	*Acinetobacter baumanii*	99.58%	GQ916532.1
PG5F	*Acinetobacter sp.*	99.08%	KF908839.1
	Acinetobacter baumanii	99.08%	JF751054.1
PG5R	*Acinetobacter baumanii*	99.48%	MG234437.1
PG6F	*Acinetobacter baumanii*	99.25%	LC437022.1
PG6R	*Acinetobacter sp.*	99.37%	KJ921622.1
PG7F	*Acinetobacter oleivarans*	99.58%	MF521562.1
PG7R	*Acinetobacter calcoaceticus*	98.23%	JN934383.1
	Acinetobacter dijkshoorniae	98.23%	MH880845.1
PG8F	*Acinetobacter sp.*	99.59%	LC437022.1
	Acinetobacter baumanii	99.48%	HQ632001.1
PG8R	*Acinetobacter sp.*	99.96%	FJ53401.1

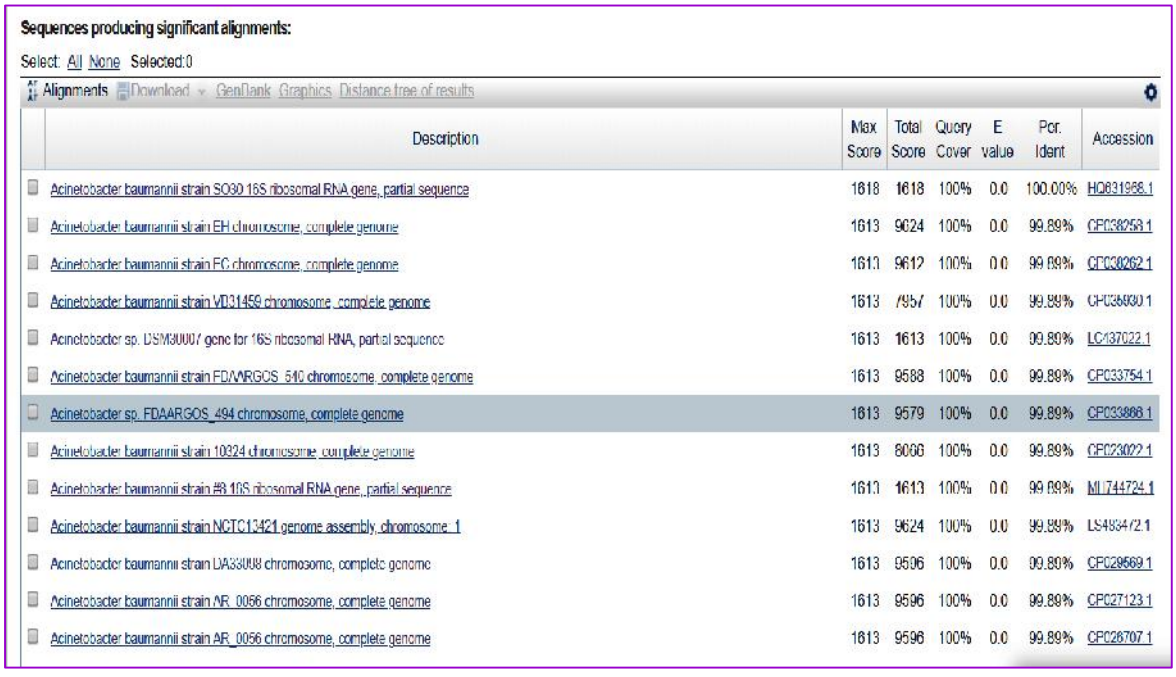


Figure 4. Blast analysis shows that bat wing isolates bactriea *Acinetobacter baumanii* share similar identity with other study *Acinetobacter baumanii* bacterial species.

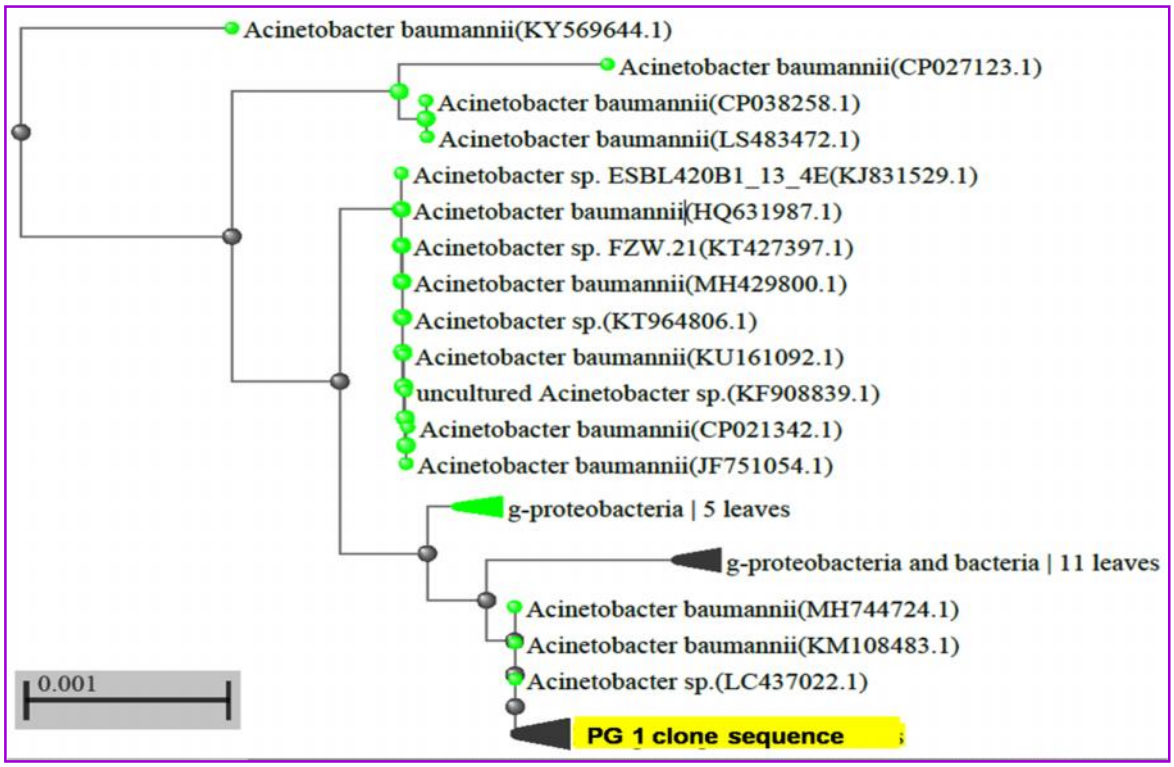


Figure 5. Phylogenetic relationship between the bat wing isolates from this study and the selected DNA sequences obtained from the GenBank. Neighbor-joining tree of bacteria isolated in this study reconstructed using Kimura 2-parameter model with 1000 bootstrap replications. The yellow highlighted box is the query sequence which is newly sequenced bat wing isolate.

In this present study, the genus *Acinetobacter* was dominant in terms of number of isolates obtained from the bat wing of *P. giganteus*. Seeing that 16S rRNA gene sequence analysis has higher bacterial identification accuracy.

Identification of Pathogenic Bacteria

To check whether the bacterial isolates are pathogen or non-pathogen blood agar is used and a total of 9 bacterial colonies were patched on the blood agar medium and incubated at 37 °C for 24 hours. No zone of growth observed after incubation period. Since the colonies did not show beta haemolytic properties it confirmed that bacteria belong to non-pathogenic bacteria.

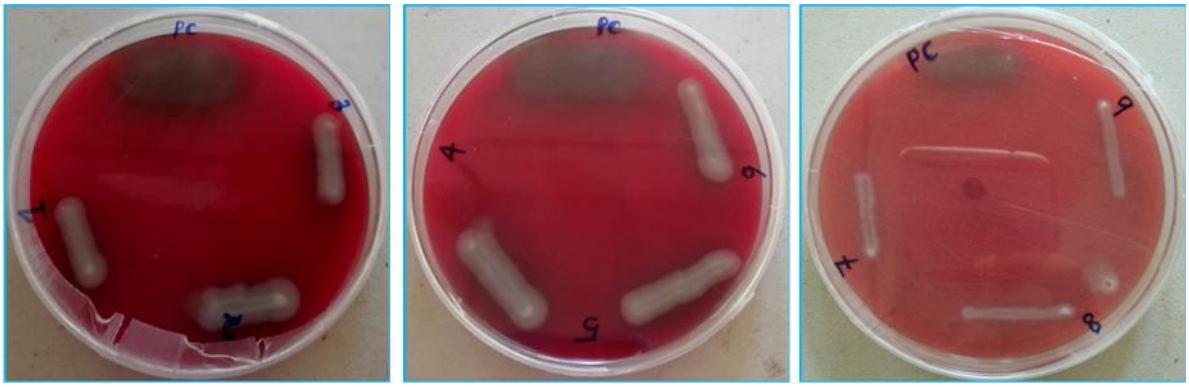


Figure 6. A total of 9 bacterial colonies were patched on the blood agar medium. No zone of growth was observed our bat wing isolates (1-9) and growth zone was observed in the positive control: *Pseudomonas aeruginosa (PC).*

Discussion

Microbiota plays an important role in enhancing host's health, physiology and behaviour. Influence of this microbiota may alter even the reproduction pattern of its host (Avena et al. 2016). Bats are the only mammals with flying capacity. Their social behaviour is diverse. They dwell in various places such as caves, trees, buildings and temples etc., So far more reports are present about their gut microbiota. Only handful of records is present in skin microbiota of bats. Since, skin acts as barrier between the host and environment the microflora is more diverse (Avena et al. 2016). So far not much works have done in skin microbiota of south indian bats. So far 15 species of bacteria reported in *Cynopterus brachyotis* (*E. faecalis, K. pneumonia, K. oxytoca, B. cereus, B. thuringiensis, Escherichia coli, E. hermannii, P. aeruginosa, E. cloacae, E. hormaechei, E. aerogenes, E. amnigenus, E. cancerogenus, S. marcescens and P. agglomerans*) have been reported as opportunistic pathogens to humans (Fischetti and Ryan, 2008).

We have isolated 9 species of Acinetobacter bacteria from the skin of fruit bat (*Pteropus giganteus*). The results of the study reveals bacteria count $2.35X10^{-6}$ to $4.62X10^{-8}$ CFU/ml .The bacterial count may be influenced by environmental factors (Temperature, humidity etc.,), host genetics and geographic location (Kingston et al. 2006). 9 species of *Acinetobacter* species were isolated which belongs to Gram negative bacteria group. *Acinetobacter sp, Acinetobacter baumannii, Acinetobacter calcoaceticus, Acinetobacter pitii, Acinetobacter dijkshoorniae, Acinetobacter lactucae, Acinetobacter oleivorans, Acinetobacter junii, Alkanindiges illinoisensis.*

Biochemical tests and 16srRNA sequencing were used to identify the bacteria at genus level. So far only the bacterial community was identified. According to my study it is non-pathogen as did not produce any beta haemolytic colonies in blood agar medium. It may play a beneficial role like host defence mechanism in bats. By isolating and identifying the skin microbiota from frugivorous and insectivorous bats we could provide insights on the behavioural patterns of bats (Kingston et al. 2006).

To date, there are only a handful of records on microbiota from bats with isolates originating from fecal matter or different parts of the gastrointestinal tract, namely the stomach, intestine and rectum. Studies on the distribution and diversity of skin microbiota found in bats from the south India is scarce. However, such information is important not only for a better understanding on the biology of bats, but also in the screening of any potential pathogens present in the bats which commonly serve as a natural reservoir for pathogens (klite 1965; Graves et al. 1988; Heard et al. 1997; Bella et al. 2003; Anand and Sripathi, 2004; Whitaker et al. 2004).

The skin microbiota of bats has just begun to be understood. Understanding the host-microbiome relationship is an important goal of evolutionary biology. Bats are the only mammals which have the capacity to fly. They are cosmopolitan distribution so they can play as a better model organism to study the skin microbiota. Recent studies reveal that microbes present in animals might modulate their behavioural patterns. Social behaviour of bats greatly affects their metabolism and physiology. Recent studies reveals that the connection between microbiome and social behaviour of an individual organism influence physiology, health and evolution (Voigt et al. 2002). Two main strategies have been found. First one is when the animals share common habitat there are more chances of spreading microbe from one animal to another either by direct physical contact or by environment. Second one is the metabolism of microbiome may release volatile compounds which may influence their communication process. For example pheromone like compounds involves in olfaction and some chemical signal may inhibit the nervous system of hosts. These are intimate links to study about the influence of microbiome in social behaviour of bats (Ezenwa et al. 2014).

In great-sac winged bat *Saccopteryx bilineata* the skin microbiota plays role in olfaction and courtship. Male bats have scent organs in which bacteria secrets peculiar odor to attract females while mating. They have distinct skin microflora. Microbial degradation in scents can greatly enhance their courtship. The bacteriome secrete some volatile compounds like indole derivatives and amino-acetophan (Voigt et al. 2002). Even the presence of different groups of microflora (gram positive and gram negative) the pattern of behaviour differs. Diverse dwelling behaviour greatly influences its behaviour. Exact microflora which enhances these chemicals is still not clearly understood.

The relationship, between odor secreting bacteria in chemical signalling of bats which enhance their behavioural patterns will be studied in future. Host defence mechanism could not be studied because of lack of knowledge in skin microbiota . Finding the suitable host by bacteria is not clearly understood (Ezwana et al. 2012). Hypothesis can be made applied in both laboratory and field settings. Analysing the origin of evolution the host-microbiome signalling interactions can give the connection between the function of microbial communities involved in producing olfactory signals. The establishment of connection between microbes and signalling odors and understanding the odors that provoke response in receivers. By combining molecular techniques and informatics tools helps to analyse the signalling pathways. Current knowledge on microbial signals in bats helps to understand the signalling process in a better way.

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