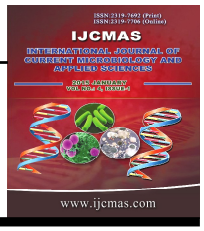
Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 143-149

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 3 (2015) pp. 143-149**
http://www.ijcmas.com



Original Research Article

Identification, Isolation and Characterization of *Enterococcus* species (Gen Bank Accession No: KF777815) from Fecal Contents of *Pteropus giganteus*

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K e y w o r d s

Gulab Bagh, *Pteropus giganteus*, Fecal contents, *Enterococcus Spp.* (KF777815), Nosocomial infections and transmission

A B S T R A C T

Gulab Bagh in Udaipur city contains around 20,000-25000 macrochiropterans and is one of the largest colony of *Pteropus giganteus* in Asiatic region. Investigations regarding bacterial pathogens with potential for mutual transmission between bats and humans are sparse. Gastrointestinal flora plays an important role in the health status of the host. In the present study *Enterococcus species* (KF777815) was identified, isolated and characterized from fecal contents of *Pteropus giganteus* inhabiting Gulab Bagh. *Enterococcus species* has been reported to be responsible for a number of debilitating conditions in humans such as infections of the urinary tract and also nosocomial infections, which could be life threatening in nature. The presence of this microorganism in the gastrointestinal tract of *Pteropus giganteus* e delineates a novel mode of its transmission, not discussed till date.

Introduction

Bats represent a highly diversified group of mammals, seen to be present all over the globe except for Arctic and Antarctic regions. Chiropterans are considered to be highly unique due to their evolutionary status, aerial habit, diverse and distinct ecological niches. However little is known about their gastrointestinal flora and fauna and reports pertaining to the presence of bacterial pathogens with a potential for mutual transmission between bats and humans are sparse (Beraud and Richard, 1988). Many chiropterans roost near field, pertaining to their role in human transmission of potential zoonotic pathogens (Nogueira, 2004; Adesiyun *et al.*, 2009). A number of reports have shown that bats serve as reservoirs and vectors of a number of viral pathogens e.g. rabies, Hendra, rubella, Lyssa, Ebola, SARS corona, Hanta, etc. (Austin, 1998; Calisher, 2006; Hasebe and Mai, 2007). Their role in transmission of bacterial pathogens e.g. *E. coli*, *Salmonella spp*, *Campylobacter spp*. has also been elucidated **(**Arata *et al.*, 1968; Wang *et al.*, 2002). Gulab Bagh in Udaipur

city contains around 20,000-25000 macrochiropterans and is one of the largest colony of *Pteropus giganteus* in Asiatic region. Hence in the presence study and effort was made to isolate, identify and characterize bacteria from fecal contents of *Pteropus giganteus* an inhabitant of Gulab Bagh, Udaipur India, so as to correlate the role of these organisms as reservoirs in transmission of bacterial pathogens.

Materials and Methods

(1) Sample collection:-

Samples of fecal matter were collected in the month of March 2013, from Gulab Bagh, in Udaipur city of Southern Rajasthan India. Care was taken to avoid contamination with environmental bacterial species, hence these were placed immediately inside sterile plastic containers and were later processed in laboratory for subsequent culture. Some portion of faeces was also preserved in laboratory at 4^0C for further utilization.

(2) Isolation of bacterial species:-

Fecal pellets were dissolved in soluble sterile buffers and cultured in nutrient medium in the laboratory using the kits which were purchased from Sisco Research Laboratory Pvt. Ltd. Mumbai, India (NM011 Nutrient Medium). Serial dilutions of saline samples were used for bacterial isolation on nutrient agar media. For isolation, each sample was separately incubated aerobically for 24 hours at 37^0C. A total of 18 isolates were obtained by random selection from different colonies. Identification of Bacterial species was done as per the technique of "Bergey's manual of Determinative Bacteriology" (Bergey, 1994) and also from "Experiments microbiology, Plant Pathology and Biotechnology Fourth Edition", (Aneja, 2003).

(3) Experimental protocol for identification of bacterial species:-

1. DNA was isolated from the culture and its quality was evaluated on 1.2% Agarose Gel where a single band of high-molecular weight DNA was observed (Fig. 1).
2. Fragment of 16S rDNA gene was amplified by PCR from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose gel (Fig. 1).
3. The PCR amplicon was purified to remove contaminants.
4. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.
5. Consensus sequence of 1287bp 16S rDNA gene was generated from forward and reverse sequence data using aligner software.
6. The 16S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI GenBank database. Based on maximum identity score, first ten sequences were selected and aligned, using multiple alignment software program, Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4.
7. The 16S rRNA gene was sequenced and the sequences were compared with the available gene sequences to NCBI website by using BLAST and the species was obtained showed more than 100% similarity with the GeneBank sequences. Sequence data were aligned

and analyzed for finding the closest homologs for the sample. Based on nucleotide homology and Phylogenetic analysis the sample was detected to be *Enterococcus Spp.* (Accession No: KF777815) nearest homolog species was found to be *Enterococcus spp.2010_Ileo_MS_XVIIIb* (Accession Number: JQ680307.1)

8. Phylogenetic tree (Fig. 2) was constructed using the 16s rRNA sequences of *Enterococcus* spp. isolated from the fecal matter *Pteropus giganteus*. The other known related species of KF777815 are being indicated in parentheses.

The evolutionary history of KF777815 was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions produced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) has been shown next to the branches (Felsenstein, 1985).

The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980). These were in the units of the number of base substitutions per site Codon position included which were 1st+2nd+3rd+Noncoding. All position containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1287 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007).

Result and Discussion

1. The isolated culture was determined to be *Enterococcus spp.* (Fig. 3) (Gen Bank Accession Number: JQ680307.1), based on nucleotide homology and phylogenetic analysis.
2. Phylogenetic tree (Fig. 2) was constructed using the 16s rRNA sequences of *Enterococcus spp*. isolated from the of fecal matter *Pteropus giganteus*. The other known eleven related species of KF777815 are being indicated in parentheses.
3. Information about other close homologs for the microbe was determined from the Alignment View (Table 1, 2).

There has been an enhanced awareness and increased scientific interest in the role of chiropteran species in transmittance of various microbial diseases. Migratory bats could act as long-distance vectors for several infectious agents (Preeti *et al.*, 2012). *Pteropus giganteus* the organism of interest in the present study colonizes Gulab Bagh region in Udaipur city. This area is near the lake Pichola from where water is supplied to the city for home consumption. Many citizens take bath in it and its water is used for human intake. In the evenings, thousands of these bats swarm over the lake and city. Their excreta may therefore contribute to the spread of zoonotic agents.

In the present study *Enterococcus spp.* (GenBank Accession Number KF777815), was isolated characterized and identified from faeces of *Pteropus giganteus* Inhabiting Gulab Bagh of Udaipur city India. Reports indicate *Enterococci* to be Gram-positive bacteria that can survive harsh adverse conditions in nature. These

can be found in soil, water, and plants. Some strains are used in the manufacture of foods, whereas others are the cause of serious human and animal infections e.g. these are known to colonize the gastrointestinal and genital tracts of humans (Pinus and Muller, 1980). These are associated with both community and hospital acquired infections. *Enterococci* can grow at a temperature range of 10 to 42°C and in environments with broad pH values. Some are known to be motile. While there are over 15 species of the *Enterococcus* genus, 80–90% of clinical isolates are *E. faecalis* (Gilmore, 2002).

Enterococci typically form short chains or are arranged in pairs. However, under certain growth conditions, they elongate and appear cocobacillary.

This bacterium has been reported to cause nosocomial infections, which could also be life threatening in nature (Nogueira, 2004; Adesiyun *et al.*, 2009). Till date their role as vectors of life threatening pathogens has not been ascertained. This study has generated a base line data which could serve as a model for futuristic experimental designs.

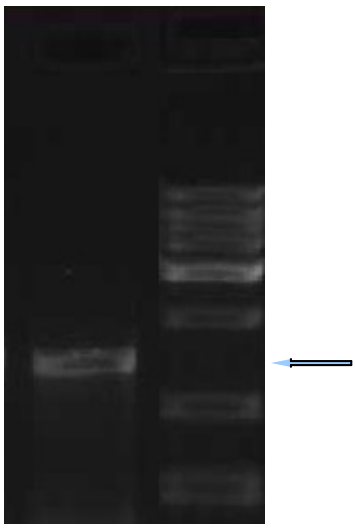
Table.1 Distance matrix based on nucleotide sequence homology of KF777815.1

Accession	Description	Max	Total	Query	E	Max
KC465417.1	*Enterococcus faecalis* strain SK23.001	2377	2377	100%	0.0	100%
JQ680311.1	*Enterococcus sp.* 2010_Ileo_MS_XVIIIf	2377	2377	100%	0.0	100%
JQ680309.1	*Enterococcus sp.* 2010_Ileo_MS_XVIIId	2377	2377	100%	0.0	100%
JQ680307.1	*Enterococcus sp.* 2010_Ileo_MS_XVIIIb	2377	2377	100%	0.0	100%
JQ680280.1	*Enterococcus sp.* 2010_Ileo_MS_XIVe	2377	2377	100%	0.0	100%
JQ680279.1	*Enterococcus sp.* 2010_Ileo_MS_XIVd	2377	2377	100%	0.0	100%
JQ680278.1	*Enterococcus sp.* 2010_Ileo_MS_XIVc	2377	2377	100%	0.0	100%
JQ680217.1	*Enterococcus sp.* 2010_Ileo_MS_IVa	2377	2377	100%	0.0	100%
JQ680265.1	*Enterococcus sp.* 2010_Ileo_MS_XIIb	2377	2377	100%	0.0	100%
JQ680264.1	*Enterococcus sp.* 2010_Ileo_MS_XIIa	2377	2377	100%	0.0	100%

Table.2 Sequences producing significant alignments distance matrix

KF777815.1	1		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.00
KC465417.1	2	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.00
JQ680311.1	3	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.00
JQ680309.1	4	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.00	0.000
JQ680307.1	5	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.00	0.000
JQ680280.1	6	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.00	0.00
JQ680279.1	7	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.00	0.00
JQ680278.1	8	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.00	0.00
JQ680217.1	9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.00	0.00
JQ680265.1	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000
JQ680264.1	11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00	

Fig.1 Gel Image of 16SrDNA amplicon



The arrow shows the presence of 1500 bp band
Lane 1:16S rRNA amplicon
Lane 2: DNA marker

Fig.2 Phylogenetic Tree showing evolutionary relationships of 11 taxa

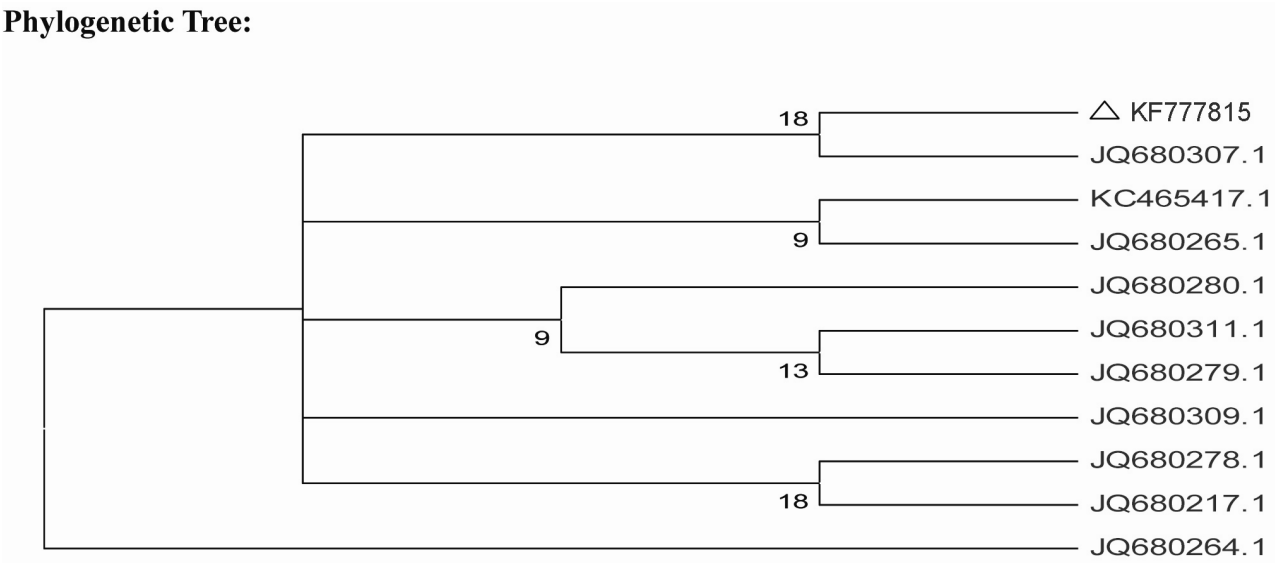
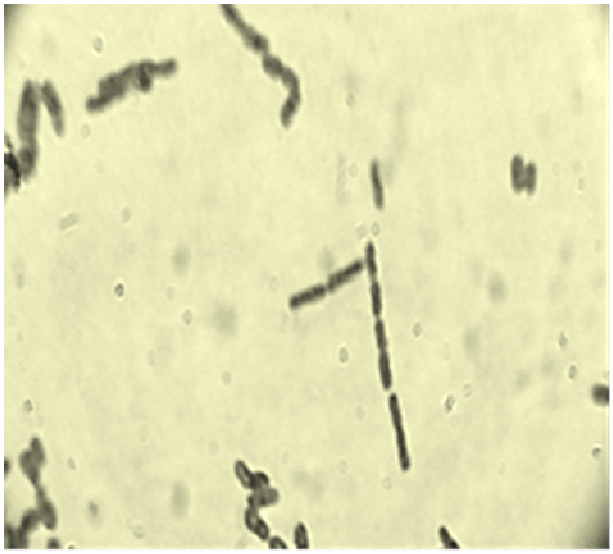


Fig.3 Slide Smear showing the presence of Enterococcus Spp.(100 X magnification)



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