International Journal of Microbiology and Biotechnology
2017; 2(3): 116-120
http://www.sciencepublishinggroup.com/j/ijmb
doi: 10.11648/j.ijmb.20170203.12

Molecular Characterization and Identification of *Burkholderia Multivorans BPSS* Isolated from Fecal Contents of *Pteropus Giganteus* in Udaipur, Rajasthan, India

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To cite this article:

Sushil Kumar Barolia, Preeti Singh, Deepak Kumar Sharma. Molecular Characterization and Identification of *Burkholderia Multivorans BPSS* Isolated from Fecal Contents of *Pteropus Giganteus* in Udaipur, Rajasthan, India. *International Journal of Microbiology and Biotechnology.* Vol. 2, No. 3, 2017, pp. 116-120. doi: 10.11648/j.ijmb.20170203.12

Received: December 30, 2016; **Accepted**: February 24, 2017; **Published**: March 21, 2017

Abstract: Previous and ongoing studies have incriminated bats as reservoirs of several emerging and re-emerging zoonoses. Most of these studies, however, have focused on viral agents and neglected important bacterial pathogens. To date, there has been no report investigating the prevalence of *Burkholderia multivorans* spp. in bats. The *Burkholderia* genus, being the largest, consists of Gram-negative, forms part of the *Burkholderia* complex, a group of Gram negative organisms which are commonly found in soil and water. And can survive for prolonged periods in moist environments. These bacteria can act as a powerful pesticide, capable of eliminating many soil-borne plant pathogens. Many species of *Burkholderia* are of considerable economic importance as these serve as insecticides, cause food poisoning, produce antibiotics etc. Hence in the present study an effort has been made to elucidate the presence of *Burkholderia multivorans BPSS* isolated, characterized and identified from the faeces of *Pteropus giganteus* from Udaipur, Rajasthan India. Its phylogenetic tree has also been derived, which showed evolutionary relationship of eleven related taxa. This is the first report from Indian subcontinent correlating the role of this megachiropteran as a carrier of *Burkholderia multivorans BPSS*.

Keywords: Pteropus Giganteus, Faeces, Burkholderia Multivorans *BPSS*, Phylogenetic Tree

1. Introduction

India is one of the world's richest countries in terms of its vast array of biological diversity and has a rich repertoire of chiropteran fauna. Chiropterans belong to order Chiroptera of class Mammalia. These are unique to their evolutionary status, aerial habit, diverse ecological habit and habitat and diversified geographical regions in which they occur. Chiropterans are classified in two suborders-the Megachiroptera which includes all the frugivorous bats and Microchiroptera that includes the insectivorous bats. This family Pteropidae of fruit- and nectar-feeding bats is found in the tropical and subtropical regions of the old world, Australia etc. [1, 11, 17, 26, 27].

All megachiropterans feed primarily on plant material that could be fruit, nectar or pollen. The remaining 16 families (around 759 species) belong to Microchiroptera [10, 14, 15, 20]. The majority of these species are insectivorous, and insectivory is widely distributed through all microchiropteran families. The megachiropterans act as seed dispersers and pollinators while microchiropterans play an important role in pest control. The various aspects related to the behavior and physiology of bats has intensively been studied [18, 19, 20]. There is relevant literature available related to the transmission of various pathogenic protozoan, viral, bacterial species through their bites (1, 8, 26, 31] however their role as vectors in transmission of microbial pathogens through their faeces is yet to be ascertained and elucidated. Parasitic diseases continue to be a cause of major concern to human and animal health in several part of the globe including India, causing high

morbidity, mortality and economic losses [6] Food, water and soil borne infection are estimated to be affecting almost half of the world population. Udaipur city of Rajasthan, India, boasts of hosting largest colonies of *Pteropus giganteus* in the world. The present study aims to investigate the presence of gut microbial parasites on the basis of fecal matter analysis and culture of microbes obtained from the fecal matter of *Pteropus giganteus* present in Udaipur region. Hence in the present research work an effort has been made to evaluate the status of *Pteropus giganteus* as a host for various microbes and to acertain its role in spread of zoonotic disorders. This study would help to generate a data base for assessing the transmission of certain microbial generated diseases.

2. Material and Methods

2.1. Sample Collection

The samples of feces were collected from Samor, Udaipur city of Southern Rajasthan India (lat_lon="24.0095 N 73.0137 E") at 5:30 PM in the month of March 2013.

The fecal Sample was collected in a special sterile box which was placed just beneath the tree. Care was taken to avoid contamination; hence the collected matter was placed immediately inside sterile plastic containers and was later processed in laboratory for subsequent culture. Some portion of faeces was also preserved in laboratory at 4°C for further utilization.

2.2. Isolation of Bacterial Species

Fecal pellets were dissolved in soluble sterile TE 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 prepared for bacterial isolation on nutrient agar media. For isolation, these were respectably incubated aerobically for 24 hours at 37°C. After assessment of the samples from all media, on the basis of Gram staining, a total of 18 isolates were obtained by random selection. Identification of Bacterial species was done as per the listed techniques [3, 4]. The isolated microorganism appeared to *Burkholderia multivorans* spp., on the basis of its morphology, and identification was further substantiated on the basis of its molecular characterization.

2.3. Identification of Bacterial Species

The bacterial isolate was identified with 16S rRNA sequence and Bioinformatics analysis. Genomic DNA was isolated from the culture as per the listed techniques [7].

2.3.1. Genomic DNA Extraction

Genomic DNA from all the isolates was extracted by the methods listed [23, 27, and 28]. Its quality was evaluated on 1.2% AgaroseGel, where as single band of high-molecular weight DNA was observed. Fragment of 16SrRNA gene was amplified by PCR from the above isolated DNA. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequence in reaction of PCR amplicon was carried out with 8F and1492RPrimers (5'TACGTAGGGTGC AAGCGTTA3') and as reverse primer (5'CATGAGCGTCAGTATTGGCC) using BDTv3.1, cycle sequencing kit on ABI 3730xl genetic analyzer. Consensus sequence of 1312 bp 16S rRNA gene was generated from forward and reverse sequence data using aligner software. The 16S rRNA gene sequence was used to carry out BLAST with the NCBI gene bank data base. Based on maximum identity score first ten sequences were selected and aligned using multiple alignments of ware program and "Clustal W. Distance" matrix was generated using RDP data base and the phylogenetic tree was constructed using the software, MEGA4 [29].

2.3.2. Molecular Phylogenetic Analysis of the Derived Species Sequence Analysis

The closest relatives of 16S rRNA sequences were determined by a search of the GeneBank DNA database using the BLAST algorithm [2]. Homology comparisons were performed using the Basic Local Alignment Search Tool (BLAST), online at the National Centre for Biotechnology Information (NCBI) homepage (http://www.ncbi.nlm.nih.gov). Identities of isolates were determined based on the highest score.

The 16S rRNA gene was sequenced were compared with the available gene sequences to NCBI website by using BLAST and following species obtained showing more than 99% 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 KT210887 was detected to be *Burkholderia multivorans BPSS* (Accession No. KT210887) the nearest homolog species was found to be *Burkholderia anthina, strain R-4183.* (GenBank Accession Number: AJ420880.1)

The evolutionary history was inferred using the Neighbor-Joining method [25]. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed [12]. The percentage of replicated resin which the associated taxa clustered to get her in the boot strap test (500 replicates) is shown next to the branches. The evolutionary distances were computed using the Kimura2-parameter method [21]. And are in the units of the number of base substitutions per site Codon positions included were 1st+2nd+3rd+Noncoding. All positions contain in 4 gaps and missing data were eliminated from the data set (Completed election option). There were a totalof1312 bp positions in the final data set. Phylogenetic analyses were conducted in MEGA4 [29].

3. Results and Discussion

The culture showed the presence of *Burkholderia multivorans BPSS* spp. based on nucleotide homology and

phylogenetic analysis its nearest homolog species was determined to be. 16S rRNA sequence of *Burkholderia multivorans BPSS* (Figure:-3) the phylogenetic analysis indicated that the present sequence occurred in the same clade of AJ420880.1 with high bootstrap value. This novel *Burkholderia* spp. was named "*Burkholderia multivorans BPSS*". A single discrete PCR amplicon band of 1312 bp was observed when resolved on Agarose Gel (Gel Image Figure:-1). GenBank accession number: KT210887 was accorded by NCBI, based on nucleotide homology and phylogenetic analysis. Phylogenetic tree was constructed using the 16S rRNA sequences of *Burkholderia multivorans BPSS* isolated from fecal matter *Pteropus giganteus*. The other known eleven related species of KT210887 are being indicated in parentheses (Figure:-2). Information about other close homologs for the microbe was determined from the Alignment View (Table: 1). this present undertaken investigation has identified and characterized *Burkholderia multivorans BPSS.* From faeces of *Pteropus giganteus* present in Udaipur city, Rajasthan, India and describes the presence and genetic characteristics of this species. From the assessment of its phylogenetic tree it can be postulated that this bacterium has a diverse distribution for it has been isolated from hot springs, sludge's, paddy fields, vermicompost and mites [5, 23, 24, 28, 31].

Table 1. *Alignment view of registered bacterial species (Burkholderia multivorans BPSS) showing Distance Matrix Based on Nucleotide Sequence Homology.*

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
KF425002.1	*Burkholderia anthina strain SPP_5*	2412	2412	100%	0.0	99%
KC241903.1	*Burkholderia anthina strain Ba8*	2412	2412	100%	0.0	99%
JX480629.1	*Burkholderia sp. enrichment culture clone t5*	2412	2412	100%	0.0	99%
JX025731.1	*Burkholderia anthina strain VP18*	2412	2412	100%	0.0	99%
JQ659867.1	*Burkholderia anthina strain R7-112*	2412	2412	100%	0.0	99%
AB252073.1	*Burkholderia cepacia gene*	2412	2412	100%	0.0	99%
AJ420880.1	*Burkholderia anthina, strain R-4183*	2412	2412	100%	0.0	99%
AB041730.1	*Burkholderia sp. CAB-02*	2412	2412	100%	0.0	99%
KF733685.1	*Burkholderia ambifaria strain ChDC B361*	2407	2407	100%	0.0	99%
KF114029.1	*Burkholderia vietnamiensis strain AU4i*	2407	2407	100%	0.0	99%
HQ236034.1	*Burkholderia cepacia strain SAT1-2*	2401	2401	100%	0.0	99%
HM461177.1	*Burkholderia sp. enrichment culture clone HSL52*	2401	2401	100%	0.0	99%
GQ383907.1	*Burkholderia cepacia strain 2EJ5*	2401	2401	100%	0.0	99%
EU597838.1	*Burkholderia cepacia strain G63*	2401	2401	100%	0.0	99%
FJ823011.1	*Burkholderia sp. gx-152*	2401	2401	100%	0.0	99%

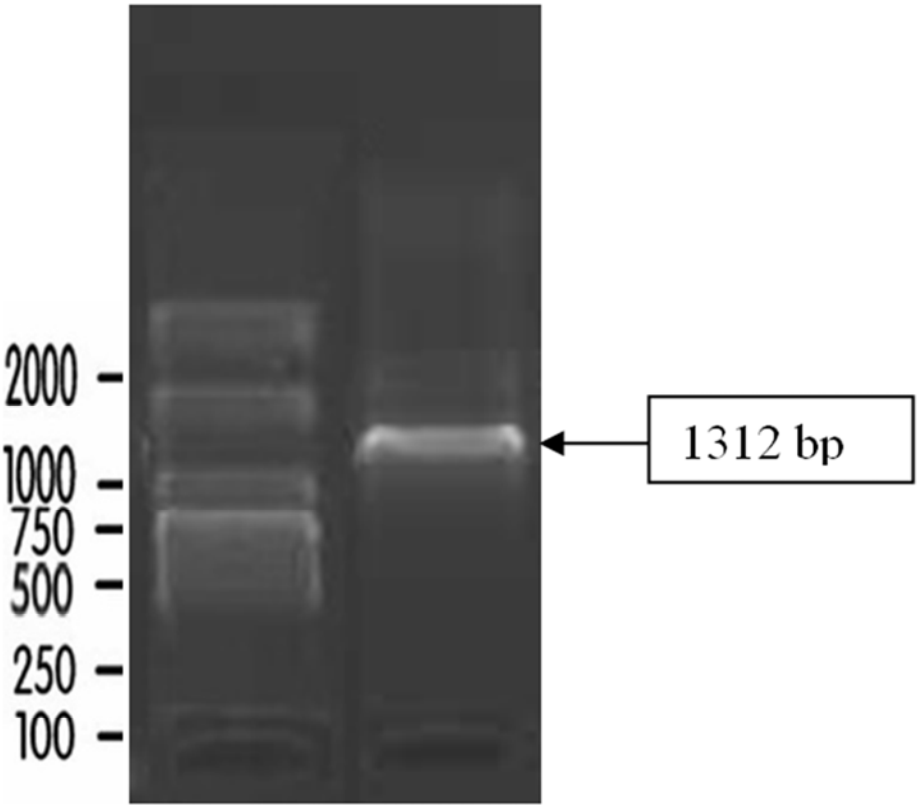

Figure 1. *Gel Image of 16S rDNA Amplicon.*

1. DNA Marker 2. *Burkholderia multivorans BPSS*.16S rDNA

[The arrow shows visualization of amplification 16S rDNA fragment isolated *Burkholderia multivorans BPSS* presence of 1312bp]

Lane 2: 16S rDNA amplicon band

Lane 1: DNA marker

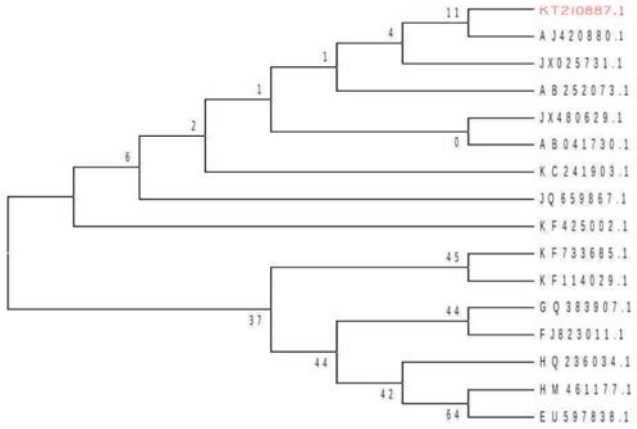

Figure 2. *Phylogenetic Tree Showing Evolutionary Relationship of 16 Taxa.*

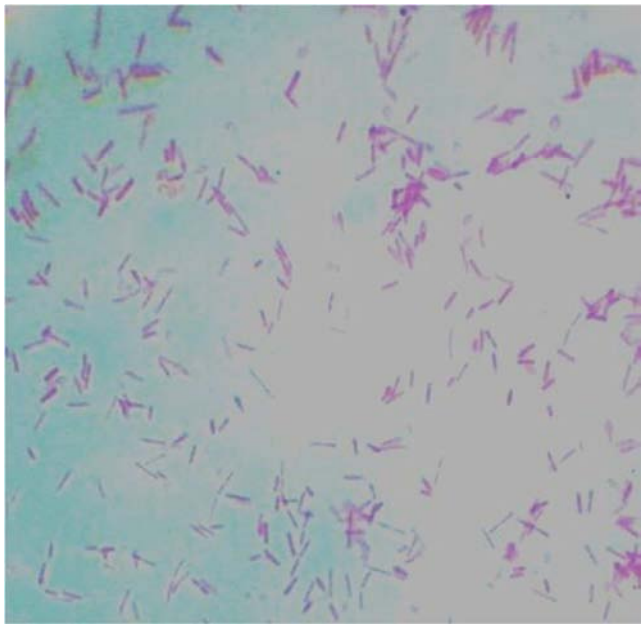

Figure 3. *Slide Smear of Burkholderia multivorans BPSS (100 X magnification).*

The smear shows the presence of *Burkholderia multivorans BPSS* in filamentous form stained with Gram's stain.

4. Conclusion

This present undertaken investigation has identified and characterized *Burkholderia multivorans BPSS* from the faeces of *Pteropus giganteus* present in Udaipur city, Rajasthan, India and describes the presence and genetic characteristics of this species. This present undertake investigation has categorically demonstrated that *Pteropus giganteus serves* as a zoonotic transmission agent for *Burkholderia multivorans BPSS* which by the construction of its phylogenetic tree, showed a worldwide distribution.

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