



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

Sushil Kumar Barolia^{1,*}, Preeti Singh¹, Deepak Kumar Sharma²

¹Cytogenetic & Endocrinology Research Laboratory Department of Zoology, College of Science, M. L. Sukhadia University, Udaipur Rajasthan, India

²Department of Microbiology C. V. A. S. Navaniya, Vallabhanagar, Udaipur, Rajasthan, India

Email address:

harshitasushil@yahoo.com (S. K. Barolia)

*Corresponding author

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 ascertain 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 and 1492RPrimers (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	<i>Burkholderia anthina</i> strain SPP_5	2412	2412	100%	0.0	99%
KC241903.1	<i>Burkholderia anthina</i> strain Ba8	2412	2412	100%	0.0	99%
JX480629.1	<i>Burkholderia</i> sp. enrichment culture clone t5	2412	2412	100%	0.0	99%
JX025731.1	<i>Burkholderia anthina</i> strain VP18	2412	2412	100%	0.0	99%
JQ659867.1	<i>Burkholderia anthina</i> strain R7-112	2412	2412	100%	0.0	99%
AB252073.1	<i>Burkholderia cepacia</i> gene	2412	2412	100%	0.0	99%
AJ420880.1	<i>Burkholderia anthina</i> , strain R-4183	2412	2412	100%	0.0	99%
AB041730.1	<i>Burkholderia</i> sp. CAB-02	2412	2412	100%	0.0	99%
KF733685.1	<i>Burkholderia ambifaria</i> strain ChDC B361	2407	2407	100%	0.0	99%
KF114029.1	<i>Burkholderia vietnamiensis</i> strain AU4i	2407	2407	100%	0.0	99%
HQ236034.1	<i>Burkholderia cepacia</i> strain SAT1-2	2401	2401	100%	0.0	99%
HM461177.1	<i>Burkholderia</i> sp. enrichment culture clone HSL52	2401	2401	100%	0.0	99%
GQ383907.1	<i>Burkholderia cepacia</i> strain 2EJ5	2401	2401	100%	0.0	99%
EU597838.1	<i>Burkholderia cepacia</i> strain G63	2401	2401	100%	0.0	99%
FJ823011.1	<i>Burkholderia</i> sp. gx-152	2401	2401	100%	0.0	99%

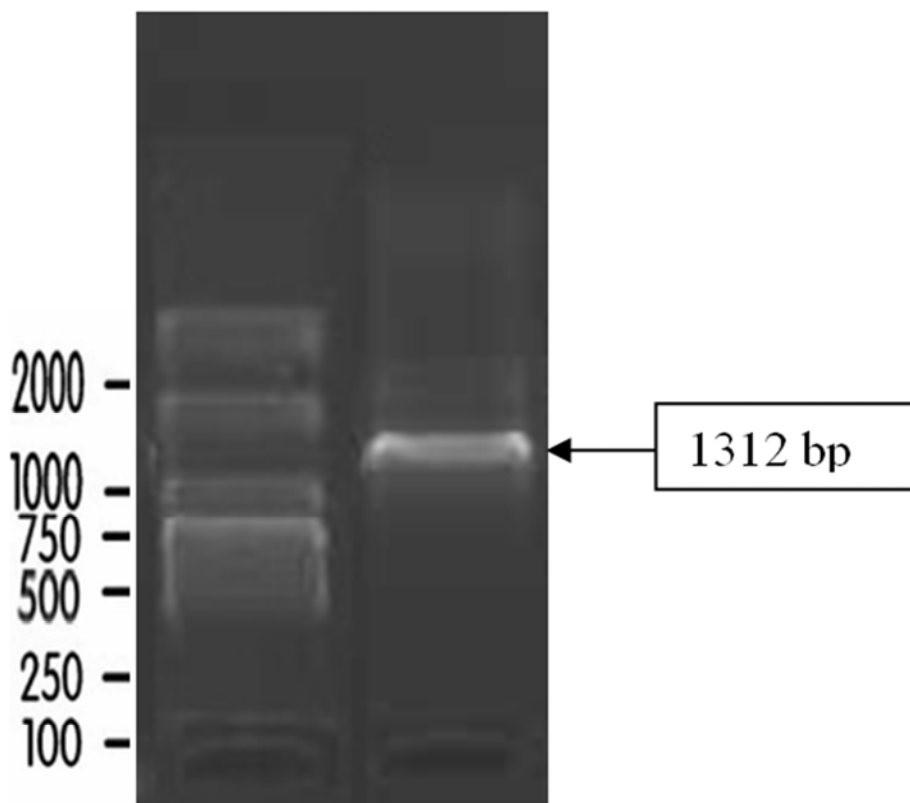


Figure 1. Gel Image of 16S rDNA Amplicon.

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

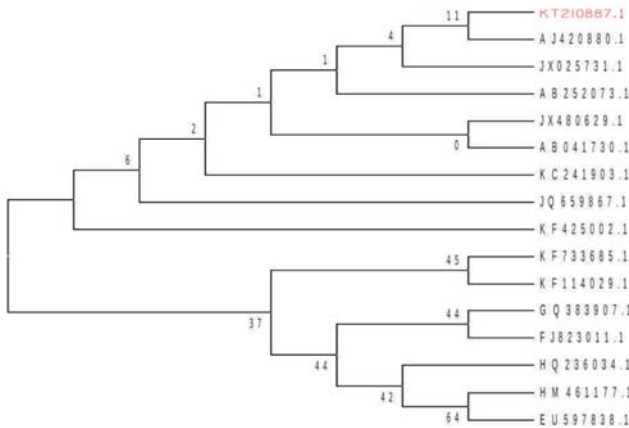


Figure 2. Phylogenetic Tree Showing Evolutionary Relationship of 16 Taxa.

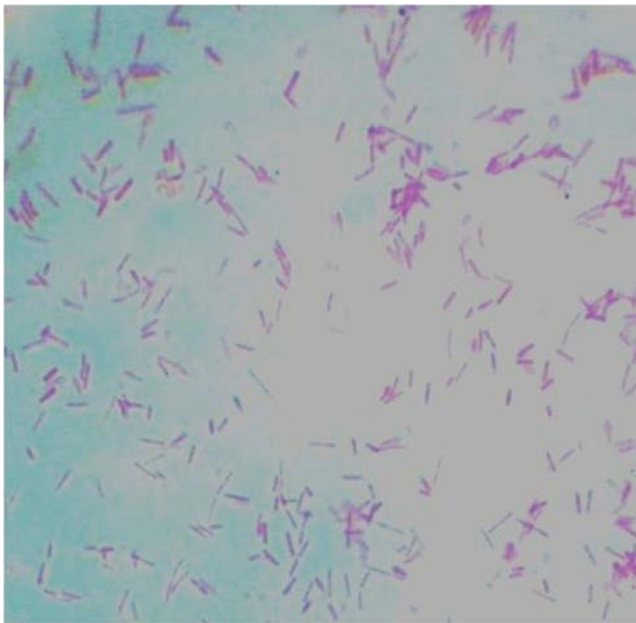


Figure 3. Slide Smear of *Burkholderia multivorans* BPSS (100 X magnification).

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.

References

- [1] Adesiyun, A. A. (2009) Isolation of enteric pathogens from bats in Trinidad. *Journal of Wildlife Diseases*, 45 (4): 952–961.
- [2] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*. 1990; 5; 215 (3):403–410.
- [3] Aneja KR. Experiments in microbiology, plant pathology and Biotechnology. New age international publication. (4th) Edition New Delhi, India; 2003.
- [4] Bergey's DH. Bergey's manual of Determinative Bacteriology 1105-1139; 1939.
- [5] Calisher CH. recent recognition of bats as reservoir hosts of emerging. *Journal of Infection*. 2006; 26:149-155.
- [6] Chhabra, M. B. and Pathak, K. M. L. (2008) *Journal of parasiticdisease*. 3 (2): 77-86.
- [7] Clare EL, Fraser EE, Braid HE, Fenton MB, Herbert PDN. Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. *Molecular Ecology* (2009); (18): 2532-2542.
- [8] Constantine DG. Transmission of pathogenic organisms by vampire bats. Natural history of vampire bats, Boca Raton, Florida, USA, CRC Press; 1988; 167-189.
- [9] Constantine, D. G., Green hall, A. M., Schmidt, U. (1988) Transmission of pathogenic microorganisms by vampire bats. Natural History of Vampire Bats Descriptors: rabies, reviews, vectors, transmission, parasites, 167-189.
- [10] Cox, P. A., Elmqvist, T., Pierson, E. D. and Rainey, W. E. (1991) Flying foxes as strong inter actors in south Pacific island ecosystems: a conservation hypothesis. *Conservation Biology*, 5 (4): 448-454.
- [11] Darryl, J. H. Jennifer., Young, L. D., Barbara Goodyear, B. S., and Georgeann, A. Ellis., B. S. (1997) Comparative rectal bacterial flora of four species of flying fox (*Pteropus* sp.). *Journal of Zoo and Wildlife Medicine*, 28 (4): 471-475.
- [12] Felsenstein J. Confidence limits on phylogenies an approach using the bootstrap. *Evolution* 1985; 39: 783-791.
- [13] Ferdinand JC. Studies on bacteria. Contributions to the biology of plant No. 2, 1872; 1: 127-224.
- [14] Fleming, T. H. (1986) Opportunism versus specialization: the evolution of feeding strategies in frugivorous bats. 105-118.
- [15] Fleming, T. H. (1988) The short-tailed fruit bat: A study in plant animal interactions. University of Chicago Press, Chicago, Illinois.
- [16] Hasebe F Mai. Surveillance of bats as reservoir host of emerging zoonotic in Vietnam. *Tropical Medicine and Health*. 2007; 35: 51-53.

- [17] Hill, J. E. and Smith, J. D. (1984) Bats: a natural history. University of Texas press, Austin, Texas, pp.243.
- [18] Klite, P. D. (1965) Intestinal bacterial flora and transit time of three Neotropical bat species. *J Bacteriol.* 90: 375-379.
- [19] Klite, P. D. (1965) Isolation of *Salmonella* from a Neotropical bat. *J Bacteriol.*, 90: 831-138
- [20] Klite, P. D. and Dierks, F. H. (1965) Histoplasma capsulatum in fecal contents and organs of bat in the canal Zone- Amer. *J trop Med Hyg.*, 14: 433-439.
- [21] Kimura M. A Simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution.* 1980; 16: 111-120.
- [22] Pierson, E. D. and Rainey, W. E. (1992) the biology of flying foxes of the genus *Pteropus*: a review. Pacific Island flying foxes: proceedings of an international conservation conference. U.S. 90: 1-72.
- [23] Pinus M, Muller HE. Enterobacteria of bat (Chiroptera, Zentralbl, Bakteriologie A. 1980; 247 (3): 315 -322.
- [24] Preeti S, Barolia SK, Deora K, Mogra P, Bano H, Javeria S. Studies on gut microbial parasites from faecal contents of *Pteropus giganteus*: short review. *World Journal of Environmental Biosciences* 2012; 1 (1): 5-8.
- [25] Saitou N, Nei M. The neighbor-joining method a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 1987; (4): 406-425.
- [26] Servat, A., Herr, J., Picard-Meyer, E., Schley, L., Harbusch, C., Michaux, C., Pir, J., Robardet, E., Engel, E., Cliquet, F. and Meyer Frist. (2015) isolation of Rabid Bat infected with European Bat lyssa virus in Luxembourg.
- [27] Singh P, Barolia SK, Sharma DK. Identification, isolation and characterization of *Enterococcus* species (GenBank accession no: KF777815) from fecal contents of *Pteropus giganteus* International Journal of Current Microbiology and Applied Sciences 2015; 4 (3): 143-149.
- [28] Singh P, Barolia SK, Sharma DK. Isolation, Characterization and Identification of *Bacillaceae bacterium* spp. (GenBank Accession No: KF777817) from Fecal Contents of *Pteropus giganteus* in Udaipur, Rajasthan, India. *International Journal of Advanced Research* 2015; 3: (8): 331-335.
- [29] Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0: *Molecular Biology and Evolution* 2007; (24): 1596-1599.
- [30] Turnbull P, Kramer J, Melling. J *Bacillus* In: Topley and Wilson Principles of Bacteriology, Virology and Immunity 8th ed Edward Arnold, London pp. 1990; 185-210.
- [31] Wong S, Lau S, Woo, P, Yuen KY. Bats as a continuing source of emerging infections in humans. (*Rev Med Viro*) 2007; (17): 67-91.