ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Isolation, Characterization and Identification of *Bacillaceae bacterium Spp.* (GenBank Accession No: KF777817) from Fecal Contents of *Pteropus giganteus* in Udaipur, Rajasthan, India.

P.Singh[1], S.K.Barolia[1], D.K, Sharma[2]

1. Cytogenetic & Endocrinology Research laboratory Department of Zoology, College of Science, M. L. Sukhadia University, Udaipur Rajasthan, India 313001
2. Regional Animal Disease Diagnostic Center, Department of Animal Husbandry, Veterinary polyclinic campus Chetak Circle Udaipur, Rajasthan, India.313001

Manuscript Info

Manuscript History:

Received: 15 June 2015
Final Accepted: 29 July 2015
Published Online: August 2015

Key words:

Pteropus giganteus, faeces, Bacillaceae bacterium spp**.** (KF777817), phylogenetic tree

Corresponding Author

P.Singh

Abstract

The present study is a primary report, illustrating the presence of *Bacillaceae bacterium spp.* (KF777817) isolated, characterized and identified from the faeces of *Pteropus giganteus* from Udaipur, Rajasthan India. Its phylogenetic tree was derived and showed evolutionary relationship of eleven related taxa. This is a first report from Indian subcontinent correlating the role of this megachiropteran as a carrier of *Bacillaceae bacterium spp.* (KF777817).

INTRODUCTION

The *Bacillus* genus was first described and classified by Ferdinand Cohn (1872).This genus is the largest containing gram-positive, endospore-forming, chemoheterotrophic rods that are usually motile and peritrichously flagellated. Many species of *Bacillus* are of considerable economic importance. e.g *Bacillus thuringiensis is* a known insecticide, *Bacillus cereus* causes food poisoning and can infect humans, various members of the genus *Bacillus* are known to *produce* antibiotics viz. bacitracin, gramicidin, and polymyxin. *Bacillus anthracis* similarly, has been reported to be the causative agent of the disease anthrax, which affects farm animals. Chiropterans mediated zoonoses has to be investigated for those mammals which can serve as major reservoirs for transmission of zoonotic agents to domestic, wild animals and humans (Van Der Poel et al.,2006). In prior investigations chiropterans have been considered to be important vectors of a number of infectious diseases as they can transmit these diseases to human through direct or indirect contact (Constantine,1988; Hasebe and Mai,2007;Wong et al.,2007; Singh et al., 2012,2015).Hence the present report has been an effort to elucidate the role of *Pteropus giganteus* as a carrier of zoonotic diseases.

MATERIAL AND METHODS

(1) SAMPLE COLLECTION

Samples of fecal matter were collected in the month of March 2013, from Udaipur city of Southern Rajasthan India. Care was taken to avoid contamination, 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 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 37^0C.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 techniques described in Bergey's manual (Bergey, 1939) and by (Aneja, 2003)

(3) IDENTIFICATION OF BACTERIAL SPECIES

GENOMIC DNA EXTRACTION

The bacterial isolate was identified with 16S rRNA sequence and Bioinformatics analysis. Genomic DNA was isolated from the culture as per the technique of (Clare et al.,2009). Genomic DNA from all the isolates was extracted by the Following method:

Bacteria were cultured overnight in peptone broth and 1ml of culture was harvested in TE buffer (1M Tris, 0.5 M, EDTA, pH 8) by centrifugation at 6000 rpm for 5 minutes. Pellets were re suspended in 440 µl of buffer (pH 8), lysis was done by adding 100 µl of 4mg/ml lysozyme and incubating at 37^oC for 30 minutes, 40 µl of 10% sodium dodecyl sulphate (SDS) was added and mixed gently. The mixture was further incubated at 45^oC for 1 hour in water bath. Cell debris and protein were separated by using 500 µl of phenol and centrifuged at 8000 rpm for 5 minutes at 4^oC. The aqueous phase was transferred to a new tube, 250µl of phenol and chloroform was added to it and mixed well. Aqueous phase was transferred to new tube; 500µl of chloroform was added and mixed well. Centrifugation was done at 8000 rpm for 5 minutes at 4^oC. Aqueous phase was transferred to a new tube and 1/10 volume of cold sodium acetate (3 M) and twice volumes of absolute ethanol were added .Solution was mixed by inverting the tubes, incubated at -20^oC for 12 hours. Centrifugation was done at 10000 rpm for 10 minutes and supernatant was discarded gently, pellet was washed by using 70% ethanol, air dried and dissolved in 100 µl of TE buffer. It was stored at -20^oC for further use.

16 s r RNA GENE AMPLIFICATION

PCR amplifications were carried out in a 100 µl volume using a master cycler pro (Eppendorf, Germany). The reaction mixture contained 10X PCR TE buffer, 0.25 mm dNTPs, 2U Taq DNA polymerase, 0.2 µM of each primer, and 20 ng of DNA template.

The cycling program was as follows: 94 °C for 5 min followed by 35 cycles of 94 °C for 1minute, 53 °C for 55s, 72 °C for 1minute, and 1 cycle at 72 °C for 10 minutes. The 16s rRNA gene sequence of LAB was amplified using 16s rRNA gene sequence as forward primer (5'AGA GTT TTGA TCC TGG CTC AG 3') and as reverse primer (5'AGG AGG TGA TCC AGC CGC A 3'). The PCR conditions were standardized as follows. Initial denaturation at 94^oC for 5 minutes 35 cycles of denaturation at 94^oC for 1 minute, annealing at 55^oC for 45 sec and extension at 72^oC for 1 minutes the final extension at 72^oC for 10 minutes. PCR products were quantified by electrophoresis on 1% (w/v) agarose gel in 0.5× TBE buffer (45mM Tris–HCl, 45 mM boric acid and 1 mM EDTA pH 8.0) and visualized by ethidium bromide staining and UV light.

MOLECULAR PHYLOGENETIC ANALYSIS OF THE DERIVED SPECIES

SEQUENCE ANALYSIS:

The closest relatives of 16S rRNA sequences were determined by a search in the GeneBank DNA database using the BLAST algorithm (Altschul et al., 1990). Homology comparisons were performed using the Basic Local Alignment Search Tool (BLAST), online at the National Centre for Biotechnology Information (NCBI). Identities of isolates were determined based on the highest score.

The 16S rRNA gene was sequenced and the sequences were compared with the available gene sequences to NCBI website by using BLAST and following species obtained showing 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 B3 was detected to be *Bacillaceae bacterium Spp. (*Accession No.KF777817) nearest homolog species was found to be *Bacillaceae bacterium spp. SPMd_8* (GenBank Accession Number: JN392833.1)

PHYLOGENETIC TREE EVOLUTIONARY RELATIONSHIP OF 11 TAXA

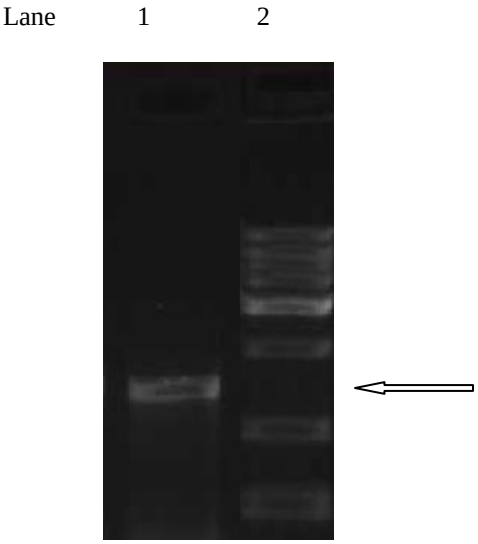
The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than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

next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura,1980) and are in the units of the number of base substitutions per site Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option).There were a total of 1333 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

RESULTS

The isolated culture was determined to be *Bacillaceae bacterium spp*. (Fig:-3) and was accorded GenBank accession number: JN392833.1, by NCBI, based on nucleotide homology and phylogenetic analysis. Phylogenetic tree (Fig:-2) was constructed using the 16s rRNA sequences of *Bacillaceae bacterium spp*. isolated from the of fecal matter *Pteropus giganteus* .The other known eleven related species of KF777817 are being indicated in parentheses. Information about other close homologs for the microbe was determined from the Alignment View showing Distance Matrix Based on Nucleotide Sequence Homology of *Bacillaceae bacterium spp.* (KF777817) in (BLAST).

Fig.1. Gel Image of 16SrDNA Amplicon



[The arrow shows the presence of 1500bp]

Lane 1:16S rDNA amplicon band
Lane 2: DNA marker

Fig.2 .Phylogenetic Tree Showing Evolutionary Relationship of 11 Taxa

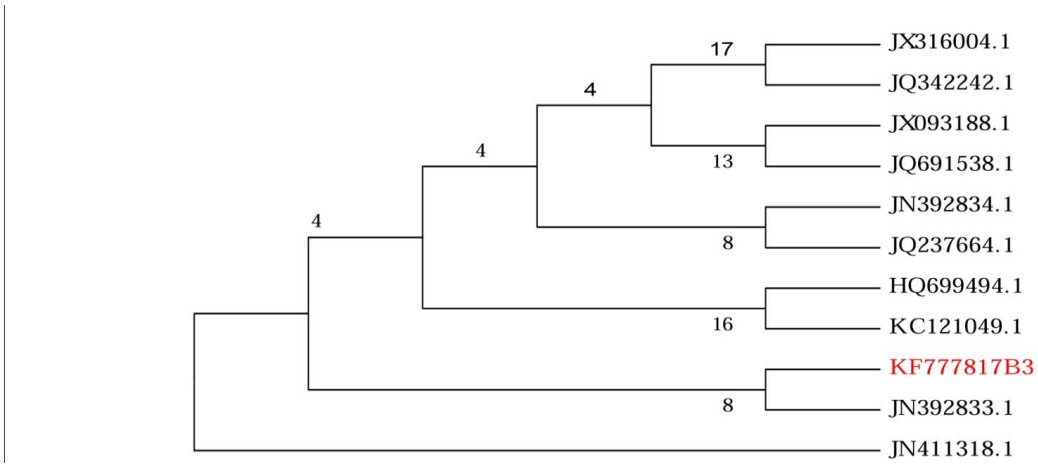
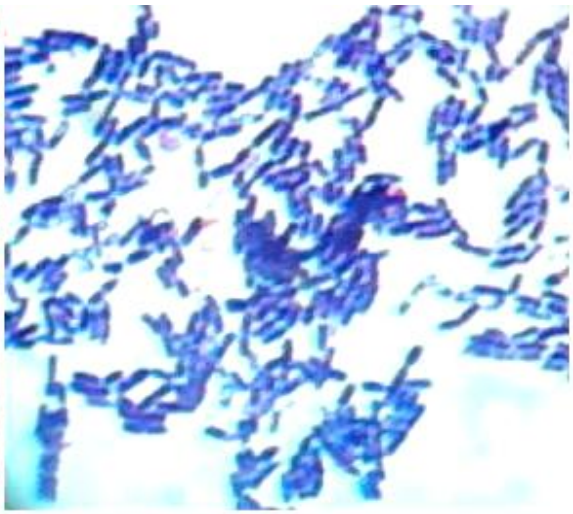


Fig: - 3 Slide Smear Showing the Presence of *Bacillus Spp. (100 X magnification)*



DISCUSSION

This present undertaken investigation has identified and characterized *Bacillaceae bacterium spp.* from faeces of *Pteropus giganteus* present in Udaipur city, Rajasthan, India and describes the prevalence 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(Henault-Ethier, mites (Hubert, Hamudun Tang, B. and Wang. 2012)etc. However all the samples collected and assessed for the presence of this species all were not positive, and tested negative for the bacteria thereby indicating that even in Udaipur city all the megachiropterans were not potential carriers of this species**.**

REFERENCES

Aneja, K.R., (2003): Experiments in microbiology, plant pathology and Biotechnology. New age international Publication.4th Revised Ed. New Delhi, India.

Bergey's, D.H., (1994): Bergey's manual of Determinative Bacteriology.

Calisher, C.H., (2006): Recent recognition of bats as reservoir hosts of emerging. Journal of Infection.26:149–155.

Clare, E.L., Fraser, E.E.,Braid., H.E. Fenton, M.B. and Herbert, P.D.N. (2009): Species on the menu of a generalist predator, the eastern red bat (Lasiurus borealis): using a molecular approach to detect arthropod prey. Molecular Ecology 18: 2532-2542

Constantine, D.G. (1988): Transmission of pathogenic organisms by vampire bats. Natural history of vampire bats, 167-189. Boca Raton, Florida, USA, CRC Press.

Felsenstein, J. (1985): Confidence limits on phylogenies an approach using the bootstrap. Evolution.39:783-791.

Hasebe, F. Mai. (2007): Surveillance of bats as reservoir host of emerging zoonotic in Vietnam.Tropical Medicine and Health. 35: 51-53.

Kimura, M. (1980): A Simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution.16:111-120.
Pinus, M., and Muller, H.E. (1980): Enterobacteria of bat (Chiroptera, Zentralbl, Bakteriol. 247(3):315 -322.

Preeti, S., Barolia, S.K., Deora, K., Mogra, P., Bano, H. and Javeria, S. (2012): Studies on Gut Microbial Parasites from Faecal Contents of *Pteropus giganteus*: Short Review. World Journal of Environmental Biosciences 1, Issue 1,5-8.

Saitou, N., and Nei, M. (1987): The neighbor-joining method a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Singh, P., Barolia, S.K., and Sharma, D.K. (2015): 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 ISSN*: 2319-7706 ISSN: 2319-7706* Volume 4 Number 3:143-149

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0: Molecular Biology and Evolution 24:1596-1599.

Van der Poel, W.H., Lina, P.H. and Kramps, J.A. (2006): Public health awareness of emerging zoonotic viruses of bats: a European perspective. Vector Borne Zoonotic Diseases. 6:315–24 10

Wong, S., Lau, S., Woo, P. and Yuen, K.Y. (2007): Bats as a continuing source of emerging infections in humans. Rev Med Virol. 17:67-91.