The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

ISOLATION AND CHARACTERIZATION OF THE BACTERIAL FLORA IN THE GUANO OF TAPHOZOUS MELANOPOGAN (TEMMINCK, 1841) IN THE TIRUNELVELI DISTRICT

VIJI S SAKTHI *[1], JEYAPRABA *[2] and ADDLINE ESTHER PUSHPARANI [3]

[1, 2, 3] Zoology Department and Research Centre, Sarah Tucker College (Autonomous), Affiliated to Manonmaniam Sundaranar University, Tirunelveli.
*Corresponding Authors Email: vijisakthi22111993@gmail.com, jayaprabha2009@gmail.com
Orcid Id: [1] 0000-0002-4817-4435, [2] 0000-0002-1675-0741, [3] 0000-0001-6301-5865

Abstract

The ecological relevance of this particular bat species must be determined in light of the paucity of studies on the microbial ecology of bats. The *Taphozous melanopogan* is a widespread insectivorous bat species of south Asia they inhabit a variety of areas from the rainforests to woodlands. They are often found in many places such as caverns, caves, abandoned buildings near water bodies and also in Temple. They play a valuable role in the ecosystem by consume millions of insects every year, helping to stop the spread of disease and limit crop damage. In an agroecosystem, bat guano plays a significant role in supplying microbial variety and has the capacity to contain diseases. In the present study, bacterial organisms were isolated by serial dilution, plating techniques, gram staining and 16s sequencing. *Pseudomonas aeruginosa* and *Bacillus wiedmannii* was identified in the fresh pellet of *Taphozous melanopogan.* This research is an attempt to determine the bacterial loads in the guano of the bat.

Keywords: Microbes, Bat Guano, *Taphozous melanopogan, Pseudomonas aeruginosa* and *Bacillus wiedmannii*

INTRODUCTION

Microbes play a vital role in our ecosystem, contributing to biogeochemical cycles, soil development, climate regulation, and even atmospheric composition. Their unique functions make them essential components of our environment [1].

They are a source of nutrients at the base of all ecological food chains and webs. Microbes are invisible creatures and include viruses, bacteria, algae, fungi and protozoa. Microorganisms are generally categorized as beneficial and harmful to mankind. Among them some are harmless but when opportunity arises they can harm the mankind [2].

Typically, the brain tissues, muscles, and blood of living organisms are free from microorganisms, while surface tissues that come into contact with environmental organisms can be colonized by specific microbial species. The microorganisms regularly present in a specific anatomical location are referred to as the normal flora [3].

Normal flora refer to microorganisms that live in a mutually beneficial relationship with their host. They are well-suited to the specific conditions found in different areas of the body. In most animals, the largest concentration of normal flora is in the large intestine, where they play an important role in digestion and nutrient production [4] [5].

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

The microorganisms present in the intestinal tract break down food and internal sugars to produce short-chain fatty acids that supply energy to the gut lining. Additionally, they synthesize B vitamins, convert nitrogenous substances into ammonia, and generate microbial protein [6].

A marvellous symbiosis exists between these microorganisms and animals. Mammals have a wide range of microorganisms that inhabit their intestinal tracts, which includes bacteria, fungi, protozoa, and viruses [7].

The flying mammal, bat is not an exception. Among the world's mammals' bats make up 25% of the total number with 1116 recorded species [8].

As a nocturnal creature, this animal typically seeks out dark and varied habitats to roost during the daytime, such as caves, crevices, tree holes, and man-made structures. The choice of roosting site depends on factors like the availability of suitable habitats, the risk of predation, the distribution of food resources, the size of the bat, and the physical environment. [9].

India is known for its architecture – temples, towers and sculptures. These places act as a very good roosting site for many species of bats. Bats are divided into two primary suborders based on their dietary preferences: "Megachiroptera", which comprises fruit-eating bats, and "Microchiroptera", which includes insect-eating bats. Both suborders belong to the order Chiroptera, which refers to hand-winged mammals.

Microchiropterans play incredibly important roles as a primary predator of the night sky. The most important night swarming insect pests and other destructive insects are kept under check at no cost. Wildlife is widely recognized as a significant reservoir of potentially zoonotic microorganisms [10] and [11].

In general, 1,415 microbes are infectious for humans. Of these, 868 are considered to be zoonotic [12].

Zoonotic pathogens in human populations are due to increased contact between humans and wildlife [13]. The lack of understanding about the biological and ecological importance of bats is a significant obstacle to their conservation.

The specific dietary preferences of each bat species highlight their crucial role in maintaining biodiversity in both agricultural and forest ecosystems. Unfortunately, many of the 119 bat species found in India are under threat due to habitat loss and overhunting.

Figure - 1 depicts the Schematic diagram showing the steps involved in isolation and identification of Bacterial flora in the guano of *Taphozous melanopogan* [14] in Tirunelveli District

ISSN 1533-9211

DOI 10.17605/OSF.IO/ACXKW

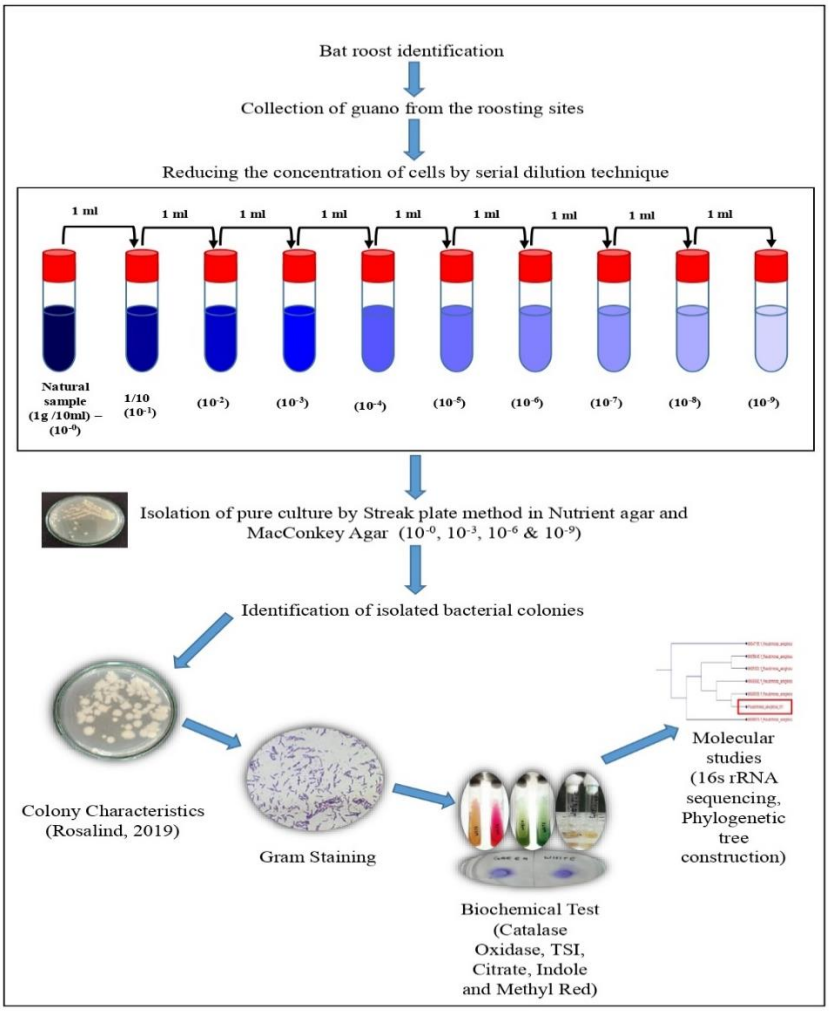

Figure 1: Schematic diagram showing the steps involved in isolation and identification of Bacterial flora in the guano of *Taphozous melanopogan* (Temminck, 1841) in Tirunelveli District

Based on the investigation from literature review, an attempt has been made to isolate and identify the faecal microbes from *Taphozous melanopogan* by using colony morphology, gram staining techniques, biochemical and 16s sequencing.

REVIEW OF LITERATURE

Bats make up the second largest mammalian order, with around 1,200 species accounting for almost 257 of the 5,000 mammalian species. The chiroptera order, which is divided into two suborders, is where bats are classified. The majority of the microbat families are represented by the yangochiroptera, formerly known as the microchiroptera, and the yinpterochiroptera, formerly known as the megachiroptera, which encompasses the Megabats. Bat species occur

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

on every continent [15].

Microorganisms play an essential role in our ecosystem, as they often have unique functions in biogeochemical cycles, soil formation, climate regulation, and the control of atmospheric composition [1]. They are a source of nutrients at the base of all ecological food chains and webs. Microbes are invisible creatures and include viruses, bacteria, algae, fungi [16]. There is a remarkable symbiotic relationship between microorganisms and animals. The selection of a roosting site by animals, such as bats, depends on factors such as the availability of suitable habitats, the risk of predation, the distribution of food resources, the size of the animal, and the physical environment [9]. India is known for its architecture – temples, towers and sculptures. These places act as a very good roosting site for many species of bats.

Microchiropteran bats are recognized as highly effective bio-control agents. Serial dilution techniques are commonly employed in various fields, including "hospitals", "public health", "virology", "immunology", "microbiology", "pharmaceuticals", and the "food industry" [17], [18], [19] and [20] for estimating the concentration (number of colonies, organisms, bacteria or viruses) by counting the number of colonies that can grow on bacteriological media [21]. The identification of colonies relies heavily on the study of colony characteristics, followed by various staining and biochemical analyses to effectively identify the microorganisms present [22]. The Gram stain is a widely used method for classifying bacteria into two groups, Gram-positive and Gram-negative, based on their permeability to organic solvents in their cell walls and membranes [23]. Biochemical tests that investigate the enzymatic activities of cells are also powerful tools for the identification of bacteria [24].

The creation and use of molecular technologies for recognising microorganisms and examining their activities has exploded recently [25]. When examining instruments designed to detect microorganisms and their activity, it is possible to distinguish between approaches that focus on measuring the activity of whole cells and those that are based on nucleic acids and other macromolecules. Nucleic acid-based technologies, such as PCR amplification and ex situ or in situ hybridization with DNA, RNA, or even peptide nucleic acid probes, are more commonly used due to their high throughput potential. These techniques entail looking at microbial DNA [26] and [27].

MATERIALS & METHOD

Study area and Study animal:

The study was conducted on the Least concern (LC) microchiropteran bat *Taphozous melanopogan,* Temmink 1841 ((black-bearded tomb bat) in November, 2019. The bats species was collected from two localities such as Site – I: Arulmigu Nellaiappar Temple, Tirunelveli town and Site – II: Arulmigu Jeganatha Perumal Temple, Shenbagaramanallur, Nanguneri taluk, 33kms south to Tirunelveli, Tamil Nadu, South India by using mist nets for morphometric measurements and released. The roosting site of *Taphozous melanopogan* is depicted in Figure – 2.

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

Figure 2: Study area (Site – I: Arulmigu Nellaiappar Temple, Tirunelveli town and Site – II: Arulmigu Jeganatha Perumal Temple, Shenbagaramanallur, Nanguneri taluk) and Study Animal (*Taphozous melanopogon)*

Isolation of bacteria:

The faecal matters was collected by spreading the white paper on the ground in the day roost. Then the guano was stored in a sterile zipper bag and transferred to Research lab at Zoology department, Sarah Tucker College (Autonomous), Tirunelveli. The collected guano was subjected to morphological and microbial studies. For the morphological study the guano was examined under binocular microscope (Nikon Eclipse 50i) and for the microbial study 1gm of guano is dissolved in 10ml of Double distilled water in a sterile test tubes.

Isolation of pure culture by serial dilution technique:

First, get a sterile micropipette and draw 1 ml of a well-mixed culture into it. Add this sample to a tube containing 9 ml of double distilled water. This will make the volume of the tube 10 ml and create an initial dilution of 10–1. Mix the dilution thoroughly several times. Use a new pipette and draw 1 ml of the 10–1 dilution and place it in another tube containing 9 ml of diluent. Mix the dilution well to create a 10–2 dilution. Discard the pipette in disinfectant. To make further dilutions, take 1 ml from the previous dilution and add it to 9 ml of diluent [28] and [29]. And this method was followed by the streak plate technique for isolation of bacteria at 10^{-0}, 10^{-3}, 10^{-6} and 10^{-9} in Nutrient agar and in Macconkey agar.

Identification of isolated bacterial colonies:

1. Colony characteristics [30]**:**

Bacteria grow on solid media as colonies are examined for shape, size, and formation of edges, chromogenesis, opacity, elevation, surface, consistency and texture. Different species of bacteria produce very different colonies. From the merging colonies, a well-isolated colony was picked up for sub-culturing.

2. Gram staining:

The Gram staining technique can be used to examine the characteristics of bacterial isolates by following a specific procedure. To perform phenotypic examination of bacterial isolates using Gram staining, start by placing a loop of sterile distilled water on a microscope slide, and then

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

touch an isolated colony with an inoculating loop and stir it in the water droplet. After letting the smear air dry at room temperature, heat fix it by waving the slide over a flame, being careful not to overheat it. Next, flood the slide with crystal violet and allow it to sit for one minute. Then, briefly wash the slide with cold water, and flood it with Gram's iodine for one minute, followed by washing it off with water. Decolorize the slide until the solvent flows colorlessly, and then flood it with safranine and let it sit for 30 seconds before washing it off. Finally, blot the slide dry and examine it under a Magnus MLX-B plus binocular microscope with a 100x oil-immersion objective to observe the Gram reaction.

3. Biochemical and Molecular study (16s sequencing):

Both the biochemical and 16srRNA sequencing was carried out at Yaazh Xenomics, Coimbatore.

4. Storage of identified microbes:

To preserve bacterial strains, choose a pure, well-isolated colony and stab it into two tubes of 1.2% TSA. These will serve as stock cultures. You can store your stock cultures in the refrigerator or at room temperature within the range of 80-120C until they are needed, but make sure to use them within 6 months. Another method is to preserve the bacterial cultures in nutrient broth with 20% glycerol and keep them at -80°C for up to 2 years. It is recommended to code or label the stocks based on the source of the samples, date of collection, color of the colony, and whether or not the colony emits light, for easy identification in further studies [31]. The bacterial cultures can be stored in a lyophilized state for a period of 1 year.

RESULT AND DISCUSSION

The main objective of this study was to isolate the bacteria from the faeces of T*aphozous melanopogan.* Two bacterial colonies were observed in streak plates which were done after serial dilution technique and given in Figure – 3. Streak plate method was more effective for primary identification and the pore plate for a pure culture of bacteria [28].

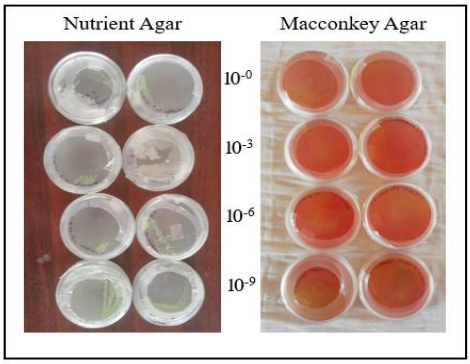

Figure 3: Identified bacterial colonies in the Streak Plate Method (Nutrient agar and Mac Conkey Agar)

ISSN 1533-9211

DOI 10.17605/OSF.IO/ACXKW

The isolated strains were identified based on the morphology, Biochemical – Staining, and also by 16s rRNA sequencing. The Morphological studies of two colonies were observed the first colony was in fluorescence green colour and the second colony was white. The surface was set to be fluorescence green irregular for sample-1 and white circular for sample-2. The texture was said to be moist for both samples. Elevation was observed at the side view of colony 2.

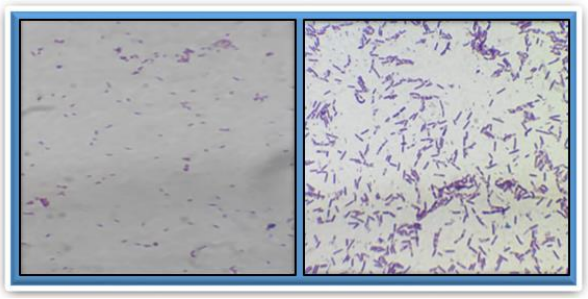

Figure 4: Identification of bacteria by Gram Staining

A biochemical test was performed for the identification and morphological studies by using gram staining and observed under MAGNUS MLX-B plus binocular microscope. From the gram staining results, (Figure – 4) the first sample showed pink coloured rods which is bacillus and it indicates gram-negative, and sample – II showed violet in colour, which indicates gram-positive. The biochemical test results were given in Figure – 5 & Table – 1. Both the strains show positive results for catalase, oxidase, and TSI test. Only the second strain showed positive results for citrate and both the strains have an absence of results for the indole and Methyl red test.

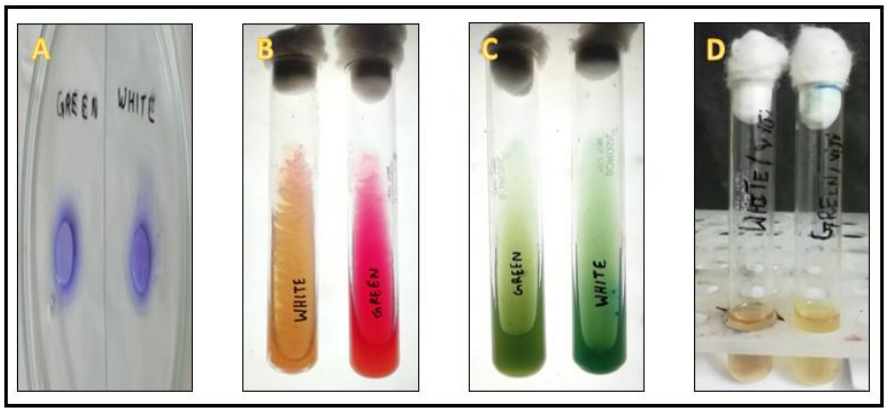

Figure 5: Routine biochemical test results of the samples (A) Oxidase test. (B) TSI, (C) Citrate Test, (D) Indole Test

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

Table 1: Identification of bacteria by Biochemical analysis

Test	Organisms	
	Colony – 1	Colony – 2
Catalase	+	+
Oxidase	+	+
TSI	+ Gas	+ Gas
Citrate	-	+
Indole	-	-
Methyl Red	-	-

Since molecular biology plays a vital role in recent days, the sequences need to be studied [32] and the results show the presence of *Pseudomonas aeruginosa* and *Bacillus wiedmannii*. Both strains are facultative anaerobic bacteria. Sixteen species of bacteria belonging to five different species in the oral and intestinal region of *T. melanopogan* was observed [16]. *P. aeruginosa* was the second most frequent nosocomial infection [33]. When concentrating on the diet of the *T. melanopogan*, it is insectivores [15]. This can be done by studying the prey preference, flight pattern, guano analysis, and also by the remnants present in the site. They are often found in many places such as caverns, caves, abandoned buildings near water bodies, and also in temples [34]. They play a valuable role in the ecosystem by consuming millions of insects every year, helping to stop the spread of disease and limit crop damage [35] and [36]. The diet of this bat species was previously studied [37] and according to their findings of the dietary composition is predominated by coleopteran, homoptera, lepidoptera, orthopteran, heiptera, odonata in the forest and in semi-urban ecosystems Lepidoptera, Coleoptera, Diptera, Orthoptera, Odonata, Hemiptera, Araneidae, and Homoptera. The predominance of lepidopterans, coleopterans, dipterans, and orthopterans in the diet of the Black-bearded Tomb Bat. This indicates the importance of in controlling important insect pests near human habitation and agroecosystems and this was also proved by [38]. The bacterial cultures are stored in a lyophilized state and sub-cultured every six months.

DATA AVAILABILITY

The 16S ribosomal RNA gene, partial sequence of *P. aeruginosa* and *B. wiedmannii* was deposited in GenBank under the accession numbers are OP925910 and OP925911.

SUMMARY AND CONCLUSION

Bats are small mammals that have the ability of powered flight. Microchiropterans play a vital role in agriculture. Megachiropterans are noted for pollination and seed dispersal. The presence of *Pseudomonas aeruginosa* and *Bacillus wiedmannii* was knotted. *P. aeruginosa* is a rod shaped gram negative, non-capsulated bacteria and it is known to cause various infections both in the respiratory tract and gastrointestinal tract in humans and *B. wiedmannii* is Gram positive bacteria and cause infection in gastrointestinal illness both in humans and insects. *P. aeruginosa* colony can be easily identified by the fluorescent green colour. The microbial flora in bats helps us to focus on their feeding habits, disproves the myths, will highlight their impact on our

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

ecosystem, and make it feasible for the government to take measures for their conservation.

Acknowledge

The authors sincere thanks to DST-CURIE (DST/CURIE-PG/2022/91 (G)) and DST-FIST (SR/FST/College-2018-423(C)) for providing infrastructure facilities at "Zoology Department and Research Centre, Sarah Tucker College (Autonomous), Tirunelveli-7".

Rferences

1) Rogers and Whitman (1991). Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halmethanes. American society for Microbiology, Washington, D. C.

2) David, G, Richard. C.B. Slack, John F. Peutherer, (1992). Medical Microbiology, 14th edition. ELBS with Churchill Livingstone.

3) Todar, K. (2002). The Bacterial flora of Humans. Todar's Online Text book of Bacteriology. Kenneth Todar University of Wisconsin-Madison Department of Bateriology.

4) Tortora, G.J., Funkle, B.R., and Case, C.L., (1995). Microbiology. An Introduction Fifth Edition. The Benjamin/Cummings Publihing, Co., Inc., Redwood City, CA, pp. 366 – 369

5) Black, J.G. 1996. Microbiological principles and Applications, Third Edition. Prentice Hall. Upper Saddle River, New Jersey. Pp.392-394,395-397.

6) Stevens, c and Ian D, H. (1998). Contributions of Microbes in Vertebrate Gastrointestinal Tract to Production and Conservation of Nutrients. Physiol Rev 78: 393-427

7) Ryan, M. J., Schloter, M., Berg, G., Kostic, T., Kinkel, L. L., Eversole, K., et al. (2020). Development of Microbiome Biobanks - Challenges and Opportunities. Trends Microbiol. S0966-842X (20), 30188–30188. doi: 10.1016/j.tim.2020.06.009

8) Simmons, M. (2005). Chiroptera. In Walker's mammels of the world. A taxonomic and Geographic References. 3rd Edition. (ed. D.E Wilson and D.A. Reader), John Hopkins Univer. Press. Baltimore.

9) Kunz, T.N. (1982). Roosting ecology of bats. In Kunz, T.H (ed) Ecology of bats, pp 1-55. New York. Plenium Press.

10) Simpson, V.R., (2002). Wild animals as reservoirs of infectious diseases in the U.K Vet J. 163: 128-146

11) Frohlich K, Thiede S, Kozikowski T, Jakob W (2002) A review of mutual transmission of important infectious diseases between livestock and wildlife in Europe. Ann N Y Acad Sci 969:4–13.

12) Taylor, L. H., Latham, S. M., and Woolhouse, M.E, (2001). Risk Factors for human disease emergence. Philos Trans R Soc Lond B Bio Sci. 356:983-989

13) Daszak P, Cunningham AA, Hyatt AD. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Trop. 78(2):103-16.

14) Temminck, C. J. (1841). "Monographies de mammalogie". 2. G. Dufour et E. d'Ocagne, 1841: 287-289.

15) Donaldson S. G., J. Vanoostdam, C. Tikhonov, M. Feeley, B. Armstrong, P. Ayotte (2010). Environmental contaminants and human health in the Canadian Arctic. Sci Total Environ. Pp. 5165-5234.

16) Addline Esther Pushparani, (2011). Identification of intestinal bacterial flora and their correlation with the food selection in three species of temple bats.

17) American Public Health Association, 2005. Standard methods for the examination of water and wastewater. American Public Health Association, Washington DC

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

18) Hollinger, F.B, (Ed), 1993. AIDS Clinical Trials Group Virology Manual for HIV Laboratories. NIH (NIAID Divison of AIDS), Rockville MD, pp. MIC-1-MIC-5.

19) Taswell. C. (1984). Limiting dilution assays for the determination of immunocompetent cell frequencies.III. Validity tests for the single-hit Poisson model.J. Immunol. Methods, 72 (1984), pp. 29-40.

20) Lin, L.I., Stephenson, W.R., 1998. Validating an assay of viral contamination. In: Peck, R., Haugh, L.D., Goodman, A. (Eds.), Statistical Case Studies: A Collaboration Between Academe and Industry. SIAM, Philadelphia, PA.

21) Avishai Ben-David, Charles E. Davidson (2014). Estimation method for serial dilution experiments. Journal of Microbiological Methods. Volume 107, Pages: 214-221.

22) Cappuccino JG, Sherman N, (1983). Microbiology a Laboratory Manual. Advison-Wesley Pub. Comp, Inc., America, Canada, pp. 315-320.

23) Steinbach WJ, Shetty AK 2001. Use of the diagnostic bacteriology laboratory a practical review for the clinician. Postgrad Med J 77,148-156

24) Petti C. A., Polage C. R., Schreckenberger P. (2005). The role of v16 S rrna gene sequencing in identification of microorganisms misidentified by conventional methods. J. Clin. Microbiol. 43, 6123–6125.

25) Ben Amor. K, Vaughan EE, de Vod WM., 2007, Advanced molecular tools for the identification of lactic acid bacteria. The journal of nutrition, (3 Suppl 2):741S-7S. doi: 10.1093/jn/137.3.741S.

26) Carrillo-Araujo, M., Taş, N., Alcántara-Hernández, R. J., Gaona, O., Schondube, J. E., Medellín, R. A., et al. (2015). Phyllostomid bat microbiome composition is associated to host phylogeny and feeding strategies. Front. Microbiol. 6:447.

27) Borda D.R., R.M. Naˇ stase-Bucur, M. Spıˆnu, R. Uricariu, and J. Mulec (2014). Aerosolized microbes from organic rich materials: case study of bat guano from caves in Romania. Journal of Cave and Karst Studies, v. 76, no. 2, p. 114–126.

28) A. A. Van Soestbergen and Ching Ho Lee, (1969). Pour Plates or Streak Plates? Department of Pathology, College of Medicine, Ohio State University, Columbus, Ohio 43210, Applied Microbiology, p. 1092-1093.

29) Basic Practical Microbiology a Manual, (2016). Microbiology Society, Charles Darwin House, Roger Street, London.

30) Rosalind J Allen and Bartłomiej Waclaw, (2019). Bacterial growth: a statistical physicist's guide, Rep Prog Phys. 2019 Jan;82(1):016601. doi: 10.1088/1361-6633/aae546. Epub 2018 Oct 1. PMID: 30270850; PMCID: PMC6330087.

31) Lila Ruangpan, & Tendencia, E. A. (2004). Bacterial isolation, identification and storage. In Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment (pp. 3–11).

32) Schell MA, Kaemirantzou M, Snel B, Vilanova D, Berger B, Pessi G, Zwahlen Mc, Desire F, Bork P, Delley M, Pridmore RD, Arigoni F. (2002). The genome sequence of Bifidobacterium longum reflects its adaptation to the human gastrointestinal tract. Proc Natl Acad Sci U S A 99, 14422-14427

33) Joana G. C. Rodrigues, Harisree P. Nair, Christopher O'Kane, Caeay a. Walker (2021). Prevalence of multidrug resistance in Pseudomonas spp. isolated from wild bird feces in an urban aquatic environment, Ecology and EvolutionVolume 11, Issue 20 p. 14303-14311.

34) Jeyaprabha. L, (2008). ''Diversity, Morphology and Roosting Ecology of a Few Bat Species in Kalakad Mundanthurai Tiger Reserve, southern Western Ghats''. Ph D Thesis submitted to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

The Seybold Report
ISSN 1533-9211
DOI 10.17605/OSF.IO/ACXKW

35) Juliet Vanitharani, (2007). Status of Bats in Tamil Nadu and Integration of Bat Conservation in Management work plan. J. Tgeor. And Expt. Biol.;3(4): 177 – 190.

36) Vadamalai Elangovan, Virendra Mathur, Mukesh Kuar and Yuvana Satya Priya, (2018). Diversity and conservation of chiropteran fauna, Pp:73.

37) Bhargavi Srinivasulu and C. Srinivasulu, (2005). Diet of the black-bearded tomb bat Thaphozous melanopogan Temminck, (1841). (Chiroptera: Emballonuridae) in India, Zoos' Print Journal 20(8): 1935-1938.

38) Elizabeth A Beilke, Joy M O'Keefe (2022), Bats reduce insect density and defoliation in temperate forests: An exclusion experiment. Ecology. 2022 Oct 30: e3903. doi: 10.1002/ecy.3903. Epub ahead of print. PMID: 36310413.