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# BACTERIAL DIVERSITY IN SOIL FROM PERIPHERAL AREAS OF THATTEKAD BIRD SANCTUARY

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## ABSTRACT

**Microorganisms are the pioneering colonizers of this planet and have become the most adaptable organisms on Earth. This paper discusses the diversity of microbes in Western Ghats analyzed using laboratory culture methods and metagenomics method. Metagenomic approaches helped to overcome the practical difficulties in the study of microbial diversity using the traditional culturing methods. In our study the soil samples were collected from the peripheral areas of Thattekad bird sanctuary situated in Western Ghats. Soil samples from 3 different sites of the peripheral areas of Thattekad Bird sanctuary from 3 different depths were taken and analyzed. Isolation of bacteria using nutrient agar showed a total number of 42different bacterial strains which were identified using colony,morphology, biochemical tests and molecular techniques. In culturable bacteria, the most abundant species was *Bacillus cereus*. Protease, cellulase, amylase, and gelatinase enzymes were produced by many of these bacteria. In Metagenomic analysis, 20544 bacteria were identified by metagenomics among the 30391 total soil bacteria present at this site. These bacteria belong to 24 Phyla, 47 Classes, 97 Orders, 202 Families, 478 Genera and 1275 species.This result is an indicative of the assorted environment of Western Ghats which provides sufficient diversity to explore the place for various microorganisms for bio-prospecting. Further detailed studies are required to identify new species and bioactive molecules.**

**KEYWORDS:** Western Ghats, Soil Bacteria, Microbial Diversity, Metagenomics

Microbes help in the survival and fertility of soil. They underlie basic ecosystem processes such as the biogeochemical cycles and food chains, as well as maintain vital and often elegant relationships between themselves and higher organisms. Without microorganisms, all life on Earth would cease. While diversity studies of other life forms are carried out in an extensive manner meager studies are done on microbial diversity. Hence a sound void still persists in the actual information on microbial diversity in the universe. Studies indicate that the 5,000 identified species of prokaryotes represent only 1 to 10% of all bacterial species, therefore we have only a small idea of our true microbial diversity (Stanley, 2002). Microbial diversity plays a dominant role in the maintenance of ecosystem. Disrupting the normal microbial flora will results in adverse situations leading to natural imbalance of other life forms.The biological diversity of the Indian subcontinent is one of the richest in the world owing to its vast geographic area, the most important mega-diversity centeris Western Ghats. The Western Ghats are a mountain range that extends along much of the western edge of India covering Kerala, Tamil Nadu, Karnataka and Maharashtra states, and were recently designated as one of the 25 global "mega-diversity" hotspots.Covering more than 1,59,000 sq. km, the Ghats harbour different varieties of flora and fauna which has been extensively explored. Limited studies have been done on the microbial population.Thattekad Bird Sanctuary is a part of Western Ghats and have a rich biodiversity.

Our understanding on microbial diversity was changed dramatically by the introduction of metagenomics analyses. A thorough assessment of microbial diversity in forest soil using routine culturing methods is a huge task and will be heavily biased and hence culture-independent approaches have been adopted. About 1% of all bacterial and archeal species have been cultured in laboratories using the culturable methods (McInerney*et al.*, 2001). Metagenomics is a newest trend for the study of microbial diversity that helps us to reveal the information of whole microbiota including both culturable and unculturable microorganisms present in the particular area. The metagenome can be obtained directly from specific ecosystems such as forest soil which can be collected and amplified for determining the gene sequences for microbial diversity analyses. Moreover latest advancement in the area of sequencing and software technologies lead to a miraculous growth in the field of metagenomics. This study is the first metagenomic analysis of this part of Western Ghats- the peripheral areas of Thattekad bird sanctuary and an initial attempt to analyze and document the bacterial diversity of the area.

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## MATERIALS AND METHODS

### Sample Collection

Soil from 3 different sites of the peripheral areas of Thattekad Bird sanctuary was taken from 3 different depths. The soil sample from the surface was taken aseptically using a sterile spatula. Sample from 15cm and 30cm depth was taken with the help of a graduated sterile steel pipe with pointed end. The samples were collected in a properly labeled sterile zip- lock pouch (sterilized by keeping in the UV chamber for 24hrs). It was then immediately transferred to the laboratory and stored at -20°C.

### Isolation of Culturable Microorganisms

Serial dilution of the soil sample was done by Soil dilution plate method (Waksman, 1922). For isolation of culturablemicroorganism, serial dilution was done and bacteria were isolated using pour plate method.The plates were incubated at room temperature for 3-5 days and the colonies were counted. Isolated colonies were subcultured and stored for further studies.Isolated bacteriawere identified by colony morphology, Gram staining, biochemical tests and molecular analysis.

### Enumerationof Bacterial Colonies

Colony forming units (C.F.U) per 1 g of soil was determined for all samples.

### Identification of Culturable Bacteria

Bacterial colonies on nutrient agar media were identified by cultural characteristics, Gram's staining and by biochemical tests.

### Molecular Techniques

DNA was extracted using Xpress DNA Bacterial kit (MagGenome Technologies, Kochi). PCR was carried out and the amplified product was sequenced at AgriGenome Labs Pvt. Ltd., Kakkanad, Ernakulam. The sequence was further subjected to NCBI BLAST analysis.

### Enzyme Profile

Enzyme profiles of isolated bacteria were done by plate assays for proteinase, gelatinase, amylase and cellulase. Zone diameter of the enzyme producers were recorded.

### Metagenomic Analysis of Soil Microorganisms

The Soil DNA isolation was done using Macherey-Nagel kit as per the given protocol. 16S amplicon analysis was done. For this 20 ng of microbial enriched DNA was used as starting material for 16S V1-V3 Amplicon-Seq library prep. Targeted PCR amplification of the 16S V1-V3 region was performed using the universal primers contained in the kit, which contain library specific overhangs and are complementary to the conserved domains flanking the hyper-variable regions of interest. Then dilution of library was done followed by sequencing in IlluminaMiseq.The raw data obtained was analyzed using Metagenomics Rapid Annotation using Subsytems Technology (MG-RAST).The sequencing was performed at Anand Agricultural University, Ahmedabad, Gujarat.

## RESULTS

### Isolation of Culturable Bacteria

Isolated the culturable bacteria from 3 sites. Sample from Pinavoorkudi showed the highest number of bacteria in surface and 15 depth samples followed by KochuKnacheri and West of Knacheri temple samples (Table 1). In all the samples, number of bacteria decreases with increase in depth.

**Table 1: Total Count of Bacteria**

| Sl. No. | Sites | Samples | Number of Bacteria CFU / g soil |
|---|---|---|---|
| 1 | West of Knacheri temple | Surface | $3.5X10^7$ |
| | | 15 cm depth | $2.7X10^6$ |
| | | 30 cm depth | $1.6X10^5$ |
| 2 | KochuKnacheri | Surface | $5.0X10^7$ |
| | | 15 cm depth | $4.6X10^6$ |
| | | 30 cm depth | $3.3X10^5$ |
| 3 | Pinavoorkudi | Surface | $8.7X10^7$ |
| | | 15 cm depth | $8.0X10^6$ |
| | | 30 cm depth | $2.5X10^5$ |

From this, 42 organisms were selected based on the morphological difference and cultured in the laboratory for identification. Colony characters, Biochemical tests and enzyme profiles of 42 organisms were recorded (data not shown). Pale white and yellow colonies were the predominant ones. Enzyme profiles of isolated bacteria were done by plate assays for proteinase, gelatinase, amylase and cellulase. 6 organisms were not able to produce any of these enzymes out of the 42isolates. 9 of them were positive for all these enzymes. From the microbiological and biochemical analysis through ABIS online and molecular biological analysis through NCBI BLAST, the organisms were identified (Table 2). There

are 17 *Bacillus* within 42 bacteria identified, 7 of them were identified at species level, and *Bacillus cereus* being the prominent one.The molecular biological analysis through the NCBI BLAST were further confirmed by BLAST tree drawing (data not shown).

**Table 2: List of Identified Soil Bacteria from Peripheral Areas of Thattekad Bird Sanctuary**

| No. | IDENTIFIED SPECIES | No. | IDENTIFIED SPECIES |
|---|---|---|---|
| KD01 | *Acinetobacterpittii* * | KD22 | *Jeotgalicoccus sp.* |
| KD02 | *Enterobacter sp.* * | KD23 | *Jeotgalicoccus sp.* |
| KD03 | *Staphylococcus saprophyticus* * | KD24 | *Pseudomonas putida* * |
| KD04 | *Enterobacterhormaechei* * | KD25 | *Staphylococcus vitulinus* |
| KD05 | *Lysinibacillus sp.* * | KD26 | *Bacillus firmus* |
| KD06 | *Lysinibacillusfusiformis* * | KD27 | *Bacillus sp.* |
| KD07 | *Pantoeadispersa* * | KD28 | *Staphylococcus simiae* |
| KD08 | *Bacillus cereus* * | KD29 | *Bacillus sp.* |
| KD09 | *Pseudomonas sp.* * | KD30 | *Eremococcus sp.* |
| KD10 | *Bacillus cereus* * | KD31 | *Solibacillus sp.* |
| KD11 | *Enterobacterhormaechei* * | KD32 | *Bacillus cereus* * |
| KD12 | *Bacillus toyonensis* * | KD33 | *Bacillus sp.* * |
| KD13 | *Bacillus sp.* | KD34 | *Bacillus sp.* * |
| KD14 | *Bacillus cereus* * | KD35 | *Bacillus sp.* |
| KD15 | *Staphylococcus sp.* | KD36 | *Bacillus sp.* * |
| KD16 | *Staphylococcus haemolyticus* *. | KD37 | *Staphylococcus sp.* |
| KD17 | *Bacillus carboniphilus* | KD38 | *Bacillus sp.* |
| KD18 | *Lysinibacillusxylaniliticus* * | KD39 | *Bacillus sp.* |
| KD19 | *Solibacillus sp.* | KD40 | *Pantoeadispersa* * |
| KD20 | *Staphylococcus sp.* | KD41 | *Staphylococcus sp.* |
| KD21 | *Bacillus sp.* | KD42 | *Photorhabdus sp.* |

*Indicates the bacteria were identified by both biochemical and molecular biological methods. Other bacteria were identified by biochemical methods only.

**Metagenome Analysis**

The metagenome analysis for one of the sample (west of Knacheri temple) was done using MG-RAST. The input data was preprocessed by removing artificial replicate sequences and any host specific sequences (*Homo sapiens*). Reads that has five bases with quality score less than twenty also were filtered out. The quality control of the MG-RAST pipeline identified using data as ribosomal RNA. The taxonomic ranks (domain, phylum, class, order, family, genus), of the sample was reported.

Soil DNA sequencing reveals a total of 32,970,072 basepairs with an average length of 503 bps. 890 sequences (1.36%) failed to pass the QC pipeline.In metagenome analysis, 20544 bacteria were identified from a total of 30391 soil bacteria present at this site. It is now well accepted that molecular techniques are more effective in the identification of new bacteria and that exploration of their biotechnological potential is possible without the need to resort to culture (Schloss and Handelsman, 2004, 2005; Schloss*et al.*, 2004; Cowan *et al.*, 2005). Of the approximately 50 bacterial phyla, half contain only uncultured bacteria (Rappe and Giovannoni, 2003).The analysis shows the soil sample contains 24 bacterial phyla (Table 3 and Fig. 1). These bacteria belong to 47 Classes, 97 Orders, 202 Families, 478 Genera and 1275 species in this 20544 bacteria.

Phylum Actinobacteria is the predominant one, followed by Phylum Proteobacteria and Phylum Verrucomicrobia with 18%, 12% and 12% respectively (Fig. 1 and Table 4). The next predominant ones are Planctomycetes, Firmicutes, Bacteroidetes and Acidobacteria respectively of 8%, 7%, 5% and 4%. The bacteria within the remaining 17 phyla are less than 1% i.e. from 0.7% to 0.003%. Further analysis at species level reveals that the identified bacteria of 20544 belong to a total of 1275 species with varying percentages from 8 to 0.003.The analysis at species level requires further studies for conformation. Another drawback associated with measuring microbial diversity in soil is the problem of defining microbial species (Torsvik*et al.*, 1998; Trevors, 1998). There is no official definition of a bacterial species (Brose *et al.*, 2003). Moreover, Hey (2001) listed over 24 definitions of species, all of which were different. The traditional species definition was based on higher plants and animals and does not readily apply to prokaryotes or asexual organisms (Godfray, 2002).

**Table 3: Number of Bacteria Identified by Metagenome Analysis of the Soil from West of Knacheri Temple, Peripheral Area of Thattekad Bird Sanctuary**

| Bacteria | Number |
|---|---|
| Identified Bacteria | 20544 |
| Phyla | 24 |
| Class | 47 |
| Order | 97 |
| Family | 202 |
| Genus | 478 |
| Species | 1275 * |

*The analysis at species level requires further studies for confirmation.

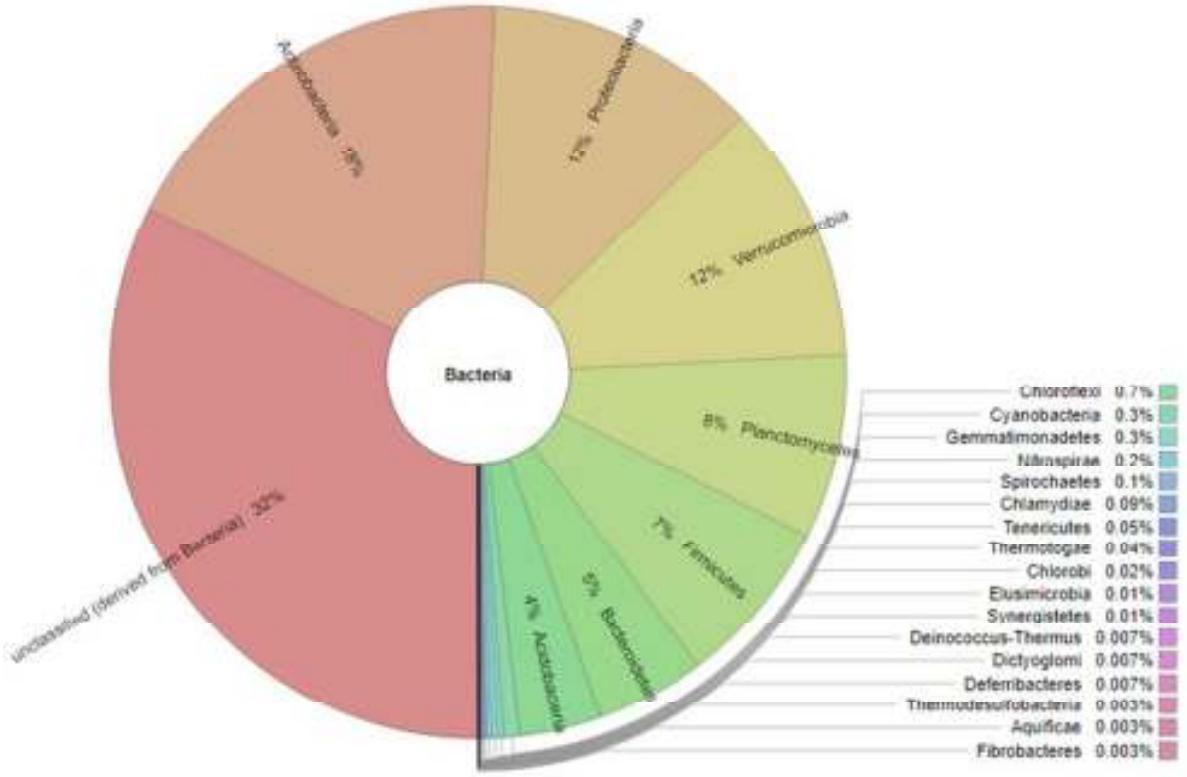


**Figure 1: MetagenomeAnalysis Showing the Percentage of Different Bacterial Phyla Present in the Soil from West of KnacheriTemple, Peripheral Area of Thattekad Bird Sanctuary**

16S amplicon of total soil DNA from west of Knacheri temple were analysed by MG-RAST. The percentage of different bacterial phyla received from the analysis are shown here.

**Table 4: Bacterial Phyla Present in the Soil from West of Knacheri Temple, Peripheral Area of Thattekad Bird Sanctuary**

| Sl No. | Bacterial phyla | No. of bacteria | Percentage |
|---|---|---|---|
| 1 | Actinobacteria | 5570 | 18 |
| 2 | Proteobacteria | 3633 | 12 |
| 3 | Verrucomicrobia | 3504 | 12 |
| 4 | Planctomycetes | 2484 | 8 |
| 5 | Firmicutes | 2255 | 7 |
| 6 | Bacteroidetes | 1448 | 5 |
| 7 | Acidobacteria | 1099 | 4 |
| 8 | Chloroflexi | 208 | 0.7 |
| 9 | Cyanobacteria | 85 | 0.3 |
| 10 | Gemmatimonadetes | 77 | 0.3 |
| 11 | Nitrospirae | 66 | 0.2 |
| 12 | Spirochaetes | 36 | 0.2 |
| 13 | Chlamydiae | 27 | 0.09 |
| 14 | Tenericutes | 16 | 0.05 |
| 15 | Thermotogae | 13 | 0.04 |
| 16 | Chlorobi | 7 | 0.02 |
| 17 | Elusimicrobia | 4 | 0.01 |

| | | | |
|---|---|---|---|
| 18 | Synergistetes | 3 | 0.01 |
| 19 | Deferribacteres | 2 | 0.007 |
| 20 | Deinococcus-Thermus | 2 | 0.007 |
| 21 | Dictyoglomi | 2 | 0.007 |
| 22 | Aquificae | 1 | 0.003 |
| 23 | Fibrobacteres | 1 | 0.003 |
| 24 | Thermodesulfobacteria | 1 | 0.003 |
| | Total bacteria | 30391 | 100 |
| | Total unclassified bacteria | 9847 | 32 |
| | Total Identified bacteria | 20544 | 64 |

16S amplicon of total soil DNA from west of Knacheri temple were analysed by MG-RAST. The number and percentage of different bacterial phyla received from the analysis are shown here.

The analysis can be done at many levels to study different phyla. Since in all our previous studies along with this work, the culturable bacteria belongs to predominantly the phylum Firmicutes (Remya KR, 2017; Neethumol S *et al.*, 2016; Remya SM *et al.*, 2016; Shelvin K *et al.*, 2015; Preethi KS, 2014), the detailed analysis focuses on the Firmicutes.

**Figure 2: MetagenomeAnalysis Showing the Percentage of Different Bacterial Genera amongFirmicutesPresent in the Soil from West of KnacheriTemple, Peripheral Area of Thattekad Bird Sanctuary**

16S amplicon of total soil DNA from west of Knacheri temple were analysed by MG-RAST. The percentage of different bacterial genera present in the class Firmicutes received from the analysis are shown here.

Phylum Firmicutes contains four classes Bacilli, Clostridia, Erysipelotrichi and Negativicutes; contains seven orders Bacillales, Clostridales, Erysipelotrichaales, Halanaerobiales, Lactobacillales, Selenomonadales and Thermoanaerobacterales; contains 31 Families with Bacillaceae (443), Clostridiaceae (183), Clostridiales Family XI (305), Paenibacillaceae (120), Peptococcaceae (569), being the abundant ones. This phylum contains 91 genus with *Tissierella* (101), *Bacillus* (324), *Clostridium* (176), *Desulfitobacterium* (410), *Desulfitomaculum* (118) and *Sporanaerobacter* (110) being the abundant ones (Fig. 2, Table 5).

The soil bacteria form this site belongs to nine genus within the family *Bacillaceae*. The most abundant one in this soil sample is genus *Bacillus*. The next abundant one being *Geobacillus*, followed by *Oceanobacillus*, *Lysinibacillus*, *Halobacillus*, *Anoxybacillus*, *Natronobacillus*, *Terribacillus* and *Virgibacillus*.

**Table 5: Metagenome Analysis Showing the Number of Firmicutes Present in the Soil from West of Knacheri Temple**

| Bacteria | Number |
|---|---|
| Identified Firmicutes | 3471 |
| Phyla | 1 |
| Class | 4 |
| Order | 7 |
| Family | 31 |
| Genus | 91 |
| Species | 268 * |

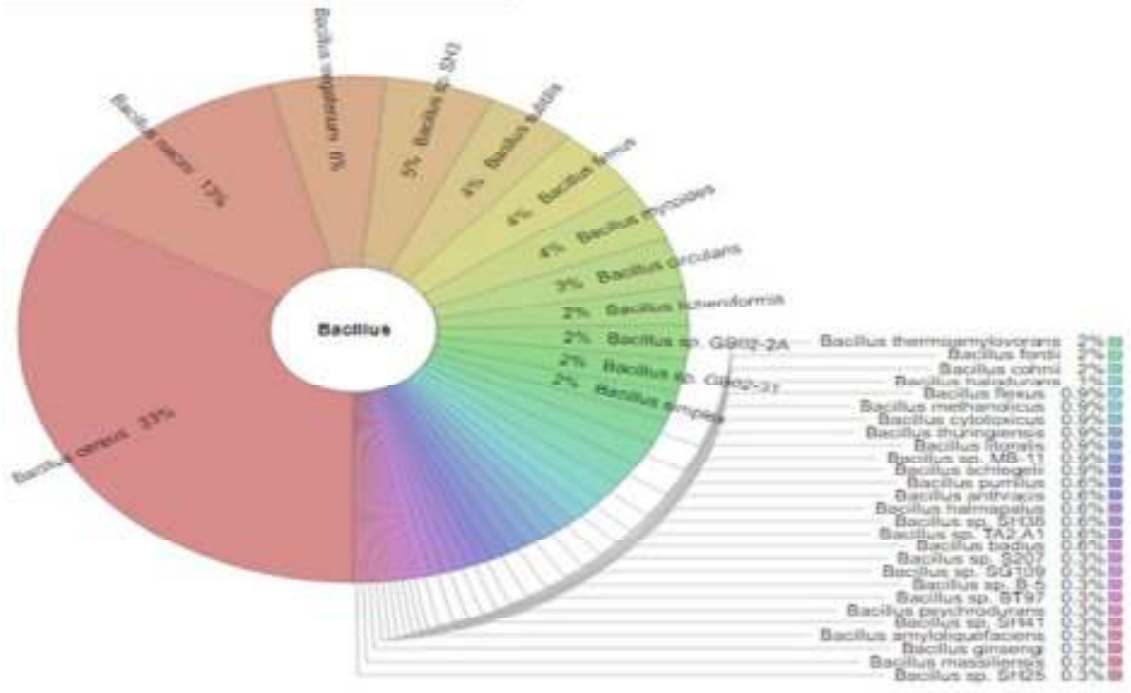


**Figure 3: The Species of Bacteria Present in the Genus Bacillusfrom the Soil from West of KnacheriTemple**

16S amplicon of total soil DNA from west of Knacheri temple were analysed by MG-RAST. The percentage of different bacterial species present in the Phyla *Bacillus* received from the analysis are shown here.

There are thirty nine species of Bacillus present in the soil (Fig. 3). The most abundant species in this soil sample is *Bacillus cereus*. The next abundant one in the sample is *B.niacini*, followed by *B. megaterium*, *Bacillus* sp. SH3, *B. subtilis*, *B. firmus*, *B. mycoides*, *B. circulans* etc. Few of these bacteria were identified in the previous studies through laboratory culture and microbiological techniques (Remya KR, 2017; Neethumol S *et al.*, 2016; Remya SM *et al.*, 2016; Shelvin K *et al.*, 2015; Preethi KS, 2014). These includes*Bacillus cereus*, *B. amyloliquifaciens*, *B. badius*, *B. subtilis*, *Lysinibacillus* sp., *Paenibacillus* sp.

## DISCUSSION

42 different bacteria were identified by laboratory culture and biochemical tests. In samples from all 3 sites, number of bacteria decreases with increase in depth. Sample from Pinavoorkudi showed the highest number of bacteria followed by KochuKnacheri and then West of Knacheri temple. In our study, top soil contained the highest number of bacteria compared to the other levels.The high numberand high diversity index value of *Bacillus* as compared to the other isolated genera in the study suggests that aerobic or facultative anaerobic, spore-forming bacteria are abundant in the forest soils, as is reported by Teixeira *et al.*, (2010) about Himalayan range which supports the patterns observed in temperate and tropical soils. Firmicutes have, however, been observed as being dominant among bacterial communities in forest soil of Kashmir, India (Ahmad *et al.*, 2009). Other members belongs to phyla representing Enterobacteriaceae and Proteobacteria. 8 species of *Staphylococcus*, 3 species of *Lysinibacillus* and *Enterobacter,* 2 species of *Solibacillus*, *Jeotgalicoccus*, *Pseudomonas* and *Pantoeadispersa*was

also seen.The other 3 of the species were identified as *Acinetobaterpittii, Eremococcussp.and Photorhabdussp.* Members of the phyla Proteobacteria and Firmicutes are the most abundant soil bacteria, as revealed by analysis of 16S rRNA gene (Janssen 2006, Bruce *et al.*, 2010, Lin *et al.*, 2010).

Metagenomic analysis showed the presence of 30391 organisms from the soil sample. Metagenomics helps us to provide the complete data of microbes in a particular area. However, most of the soil microorganisms need special handling in order to grow them in laboratory conditions andup to 99.9% remain uncultured (Hugenholtz and Pace, 1996; Lorenz and Schleper, 2002). The Raw data was analysed using MG-RAST and compiled by comparing it using data fromRDP database. 20544 bacteria were identified belonging to 24 phyla. The predominant phyla was Actinobacteria according to our study. Actinobacteria was reported to be the dominant one in soils (Jenkins *et al.*, 2009). We focused our studies on Firmicutesand mainly bacillus group to compare with the culturable ones, as in culturable bacteria Firmicutes were the predominant ones.3471 Firmicutes were identified. Further analysis is required to completely elucidate the results from the study. Although amplification bias still poses a non-negligible difficulty, single-cell genomic sequencing is expected to accelerate direct genome reconstruction from environmental samples (Eloe-Fadrosh *et al.*, 2016,Laske*et al.*, 2012, Rodrigue *et al.*, 2009), in which the combination of single cell genomic and metagenomic approaches may be a promising approach (Mende *et al.*, 2016). This study is the first metagenomic analyses of the soil bacterial diversity of this parts ofWestern Ghats taking the peripheral area of Thattekad bird sanctuary. This study has helped us to understand the identity of the un-culturable bacterial species present in the area. Only a small fraction of the total bacteria present in these sites were suitable to be cultured and identified by routine laboratory methods. These culturable bacteria were tested for their ability to synthesize different classes of enzymes which would have significant industrial and research applications. In addition, the metagenomic analysis has provided a huge amount of data and more insights into the actual bacterial diversity of the area which will be a stepping stone for further analysis and future prospects of utilizing microbiome for scientific benefits.

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## REFERENCES

Ahmad N., Johri S., Abdin M. and Qazi G., 2009. Molecular characterization of bacterial population in the forest soil of Kashmir, India. World J. Microbiol. Biotechnol., **25**: 107-113.

Brose U., Martinez N.D. and Williams R.J., 2003. Estimating species richness: Sensitivity to sample coverage and insensitivity to spatial patterns. Ecology, **84**: 2364-2377.

Bruce T., Martinez I.B., Neto O.M., Vicente A.C.P., Kruger R.H. and Thompson F.L., 2010. Bacterial community diversity in the Brazilian Atlantic forest soils. Microb. Ecol., **60**: 840-849.

Cowan D., Meyer Q., Stafford W., Muyanga S., Cameron R. and Wittwer P., 2005. Metagenomic gene discovery: past, present and future. Trends Biotechnol., **23**: 321-9.

Eloe-Fadrosh E.A., Paez-Espino D., Jarett J., Dunfield P.F., Hedlund B.P., Dekas A.N., Grasby S.E., Brady A. L., Dong H., Briggs B.R., Li W., Goudeau D., Malmstrom R., Pati A., Pett-Ridge J., Rubin E.M., Woyke T., Kyrpides N.C. and Ivanova N.N., 2016. Global metagenomic survey reveals a new bacterial candidate phylum in geothermal springs. Nat. Commun., **7**: 10476.

Godfray H.C.J., 2002. Challenges for taxonomy. Nature, **417**: 17-19.

Goldblatt P., Jonathan D.T., John C.M., van der Bank M., and Savolainen V., 2006. Phylogeny of Iridaceae Subfamily Crocoideae Based on a Combined Multigene Plastid DNA Analysis.Aliso: A Journal of Systematic and Evolutionary Botany, **22**: 399-411.

Hey J., 2001. Genes, categories and species: the evolutionary and cognitive causes of the species problem. Oxford University Press, Oxford.

Hugenholtz P. and Pace N.R., 1996. Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. Trends in Biotechnology, **14**: 190-197.

Janssen P.H., 2006. Identifying the Dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Appl. Environ. Microbiol., **72**: 1719-1728.

Jenkins S.N., Waite I.S., Blackburn A., Husbund R., Rushton S.P., Manning D.C. and O'Donnell A.G., 2009. Actinobacterial community dynamics in long term managed grasslands. Antonie van Leeuwenhoek, **95**:319–334.

Lasken R.S., 2012. Genomic sequencing of uncultured microorganisms from single cells. Nat. Rev. Microbiol., **10**: 631–640.

Lin Y.T., Huang Y.J., Tang S.L., Whitman W.B., Coleman D.C. and Chiu C.Y., 2010. Bacterial community diversity in undisturbed perhumidMontane forest soils in Taiwan. Microb. Ecol., **59**: 369-378.

Lorenz P. and Schleper C., 2002. Metagenome-a challenging source of enzyme discovery.Journal of Molecular Catalysis B: Enzymatic, **9-20**: 13-19.

McInerney J.O., Mullarkey M., Wernecke M.E. and Powell R., 2001. Bacteria and Archaea : Molecular techniques reveal astonishing diversity. Most, 8386: 3–10. https://doi.org/10.1080/14888386. 2002.

Mende D.R., Aylward F.O., Eppley J.M., Nielsen T.N. and DeLong E.F., 2016. Improved environmental genomes via integration of metagenomic and single-cell assemblies. Front. Microbiol., **7**: 143.

Neethumol S., Achamma T., Shelvin T.K. and Mohan S., 2016. Enzyme Production by Soil Bacteria Isolated From The Western Ghats, Proceedings of the 25$^{th}$Swadeshi Science Congress, pp: 370-375.

Preethi K.S., 2014. M Sc. Dissertation thesis submitted to Mahatma Gandhi University.

Rappe M.S. and Giovannoni S.J., 2003. The uncultured microbial majority.Annu Rev Microbiol., **57**: 369-94.

Remya K.R., 2017. M Sc. Dissertation thesis submitted to Mahatma Gandhi University.

Remya S.M. and Mohan S., 2016. Diversity of Soil Microbes in Amini Island of Lakshadweep, Proceedings of the 25$^{th}$Swadeshi Science Congress, pp: 382-386.

Rodrigue S., Malmstrom R.R., Berlin A.M., Birren B.W., Henn M.R. and Chisholm S.W., 2009. Whole genome amplification and de novo assembly of single bacterial cells. PLoS One, **4**:e6864.

Schloss P.D. and Handelsman J., 2004. Status of the microbial census. Microbiol Mol. Biol. Rev., **68**: 686–91.

Schloss P.D. and Handelsman J., 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol., **71**: 1501-1506.

Schloss P.D., Larget B.R. and Handelsman J., 2004. Integration of microbial ecology and statistics: at test to compare gene libraries. Appl. Environ. Microbiol., **70**: 5485-5492.

Shelvin T.K., Achamma T. and Mohan S., 2015. Soil microbial diversity of Mallana Forest in the Malayattoor division of Western Ghats, Perspectives and Paradigm Shifts, pp: 277-280.

Stanley J., 2002.Biodiversity of Microbial life. Wiley-Liss, New York, NY.

Teixeira L.C.R.S., Peixoto R.S., Cury J.C., Sul W.J., Pellizari V.H., Tiedje J. and Rosado A.S., 2010. Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. The ISME Journal, **4**: 989-1001.

Torsvik V.L., Sorheim R. and Goksoyr J., 1996. Total bacterial diversity in soil and sediment communities – a review. Journal of Industrial Microbiology, **17**: 170–178

Trevors J.T., 1998. Molecular evolution in bacteria: cell division. Revista de Microbiologia, **29**: 237-245.

Waksman S.A., 1927. Microbiological analysis of soil as an index of soil fertility: I. Mathematical interpretation of number of microorganisms in the soil. Soil Sci., **14**: 81.