

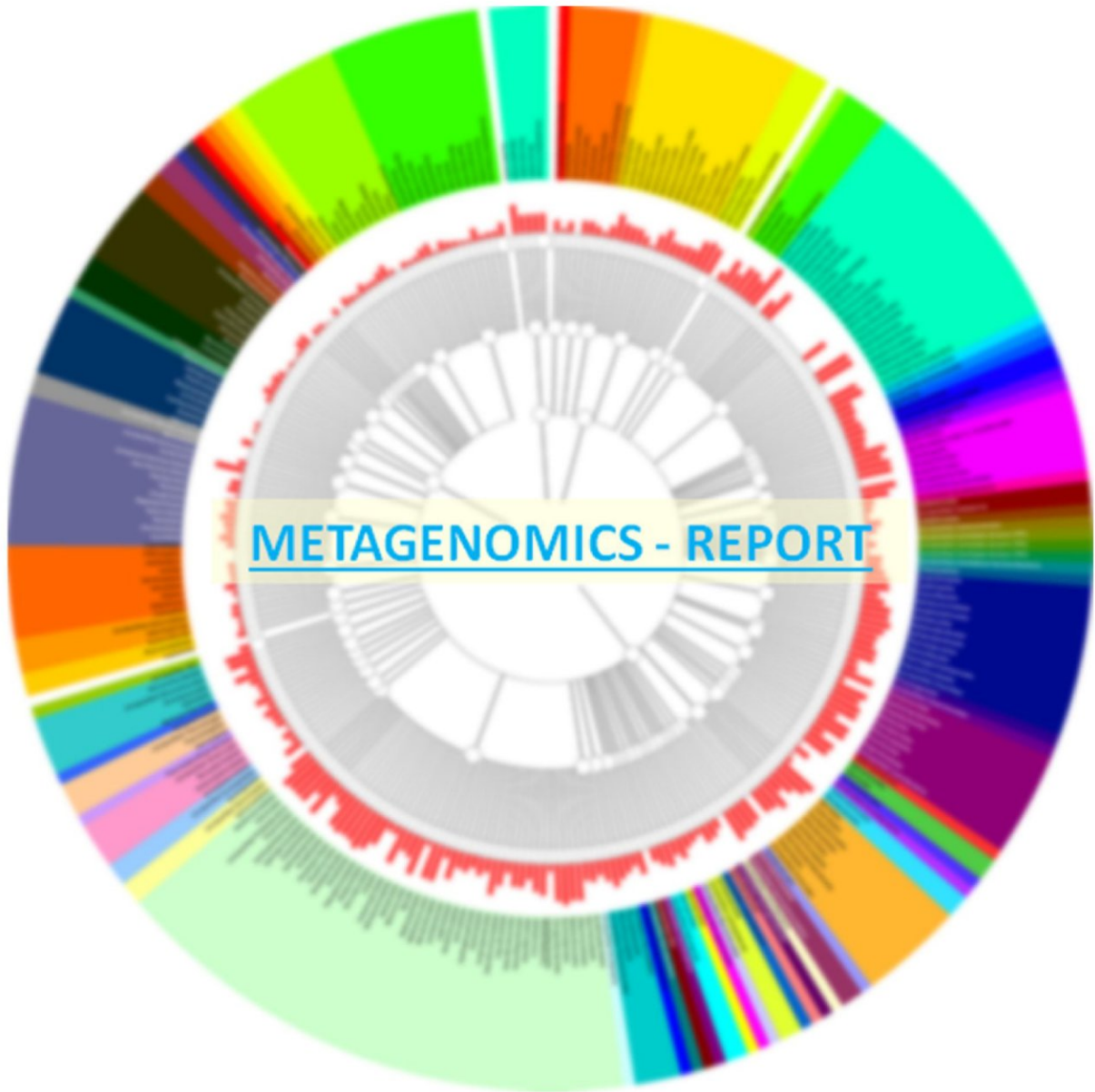


Biokart India Pvt Ltd

TIN: 29400662787

PAN: AAECB8426A

CIN: U74900KA2011PTC061787



Biokart India Pvt. Ltd.,

1<sup>st</sup> floor, No: 28 Income Tax Layout, Sadananda Nagar, NGEF East, Bangalore - 560038

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## METAGENOMICS \_ REPORT

### Client Details:

**Dr. Joseph  
Puducherry University**

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**Sample Name: M1, M2**

**Date of Report Generation: 02-04-2019**

**Sample Details: M1, M2**

### Services Availed:

- **DNA Extraction**
- **DNA QC**
- **PCR amplification with 16s Primers**
- **PCR Product Purification**
- **Sequencing using Nanopore Platform**
- **Data Analysis & Report Generation**



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## METAGENOMICS \_ REPORT

### Protocol:

#### DNA Extraction : (Optional)

DNA extraction is done using the suitable method for the sample type from commercially available kits such as QIAGEN, ZYMO RESEARCH, ThermoFisher

Dna extraction was done as per the manufacturer's recommendation.

#### PCR Amplification of 16S Gene:

Composition of TAQ Master MIX:

- 1) High-Fidelity DNA Polymerase
- 2) 0.5mM dNTPs
- 3) 3.2mM MgCl<sub>2</sub>
- 4) PCR Enzyme Buffer

#### Primer Details:

16F:- 5' AGAGTTTGATCMTGGCTCAG 3'

16R:- 5' TACGGYTACCTTGTTACGACTT 3'

#### Conditions Used:

40ng of Extracted DNA is used for amplification along with 10pM of each primer

Initial Denaturation: 95 degree C

25 Cycles of the following condition:

Denaturation @ 95 deg C for 15 sec, Annealing @ 60 deg C for 15 sec, Elongation @ 72 deg C for 2 mins

*Final Extension at 72 deg C for 10 mins and Hold at 4 deg C.*

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## METAGENOMICS \_ REPORT

### Overview of Sequencing Protocol:

- Nanopore sequencing was performed by using 1µg of DNA template.
- End repair/dA tailing Ligation of Barcode Adapter
- Barcoding PCR
- End repair/dA tailing
- Blunt end Adapter Ligation
- Purification using AMPure XP bead binding
- Priming and loading the SpotON flow cell

### Bioinformatics protocol:

EPI2ME 16S analysis workflow allows users to perform genus-level identification from single reads; with access to basecalled files for detailed investigations at the species and sub-species level. The phylogeny analysis of query sequence with the closely related sequence of blast results was performed followed by multiple sequence alignment.

The workflow is designed to BLAST basecalled sequence against the NCBI 16S bacterial database, which contains 16S sequences from different organisms. Each read is classified based on % coverage and identity.

The 16S workflow will be useful in identifying pathogens in a mixed sample or understanding the composition of a microbial community.



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## METAGENOMICS \_ REPORT

### **DNA QC :**

Extracted DNA from the samples were subjected to Nano Drop and GEL Check before being taken for PCR amplification:

The Nano Drop readings of 260/280 at an ~ value of 1.8 to 2 is used to determine the DNA's quality

### **PCR Ampliqon QC:**

The amplified 16s PCR Product is purified and subjected to GEL Check and Nanodrop QC

The Nano Drop readings of 260/280 at an ~ value of 1.8 to 2 is used to determine the DNA's quality



## METAGENOMICS REPORT

### Sample Details for 16s amplicon region

#### Untrimmed

##### Basic Statistics

Sample	Sequence Number	Mean GC	Sequence Range	Encoding
M1	24000	53	180-4428	Sanger / Illumina 1.9
M2	27868	53	294-4428	Sanger / Illumina 1.9

#### Trimmed

##### Basic Statistics

Sample	Sequence Number	Mean GC	Sequence Range	Encoding
M1.tr	15571	55	500-2480	Sanger / Illumina 1.9
M2.tr	18492	54	500-2615	Sanger / Illumina 1.9

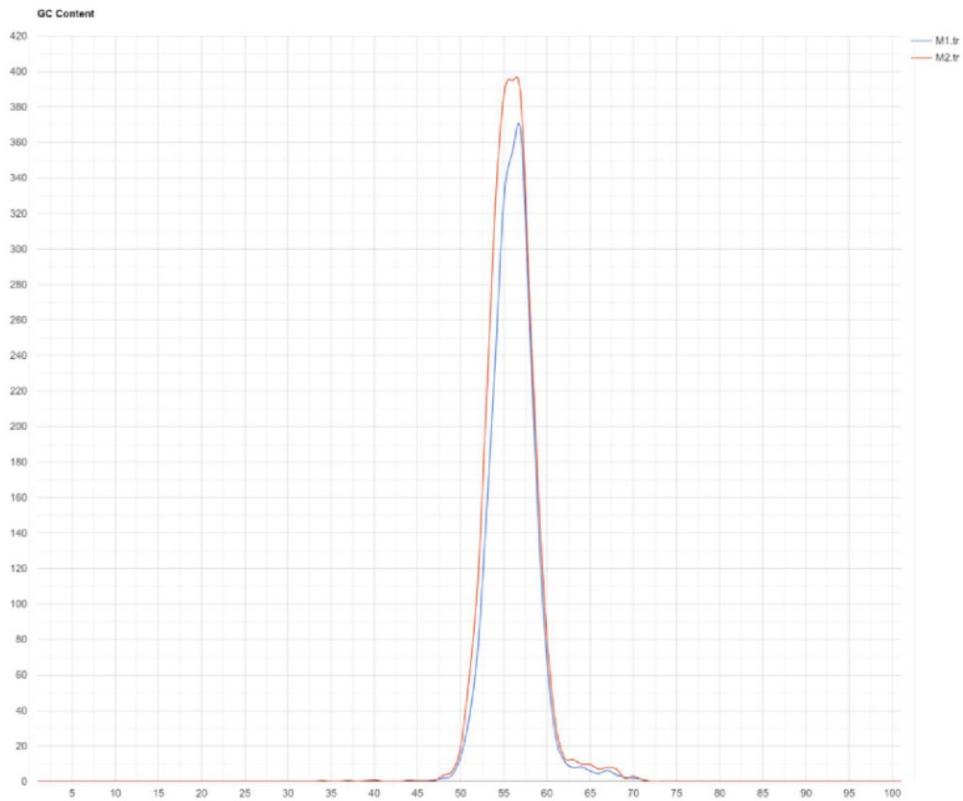


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## METAGENOMICS \_ REPORT

### METAGENOMICS REPORT

#### GC Content



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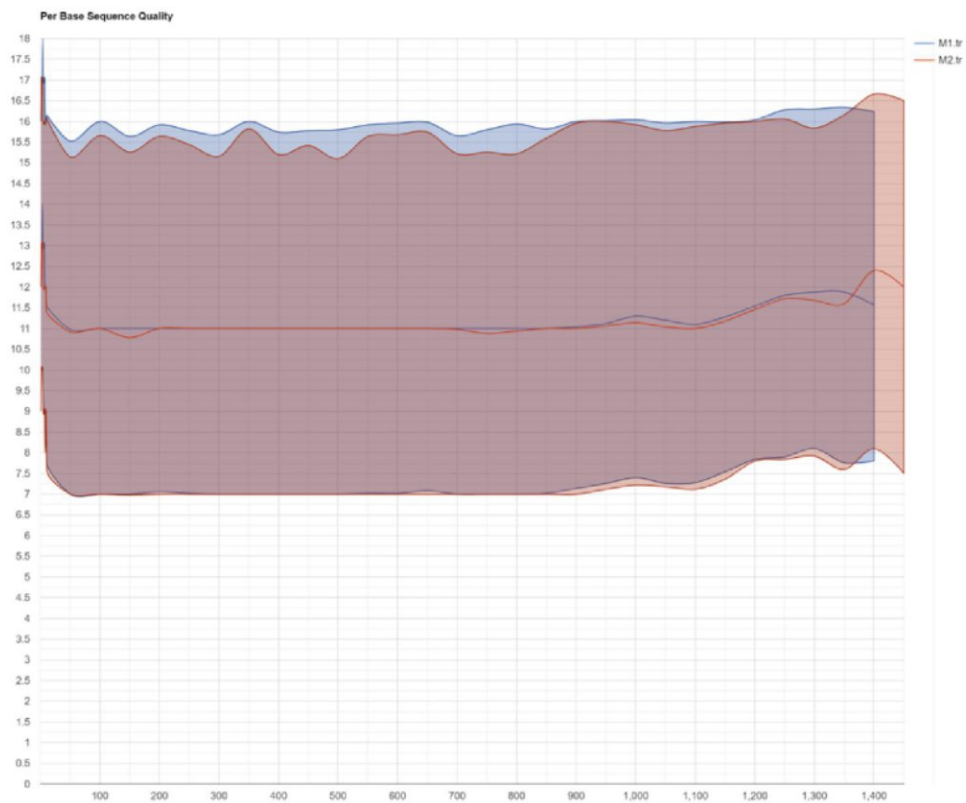


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## METAGENOMICS \_ REPORT

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### Per Base Sequence Quality



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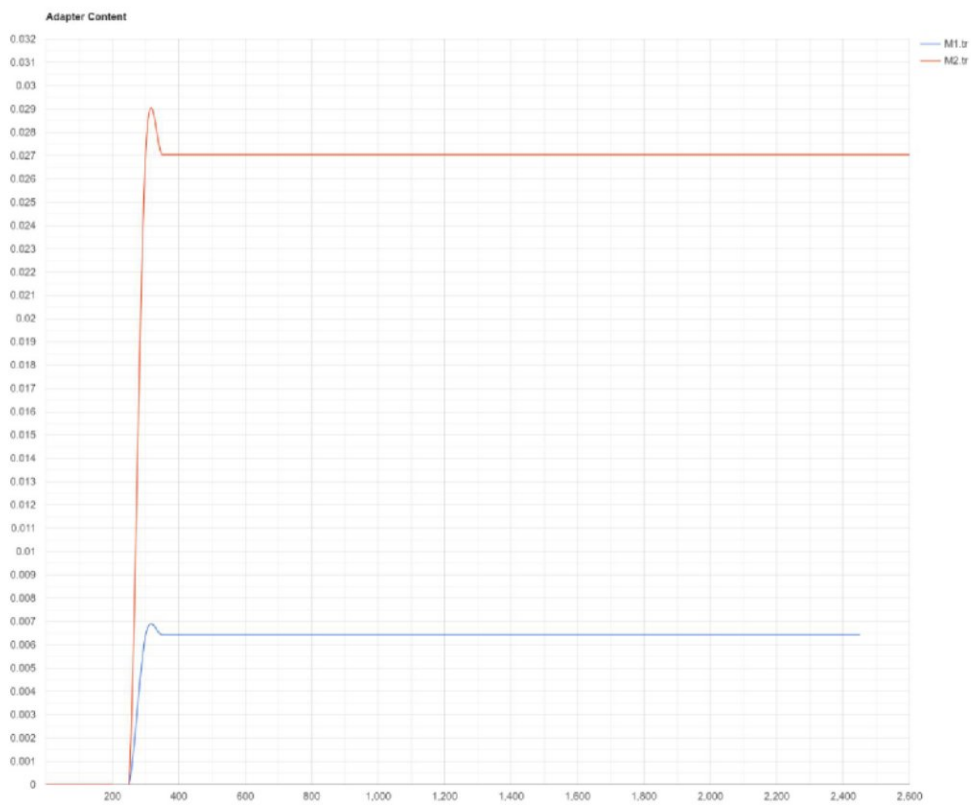


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# METAGENOMICS \_ REPORT

## METAGENOMICS REPORT

### Adapter Content

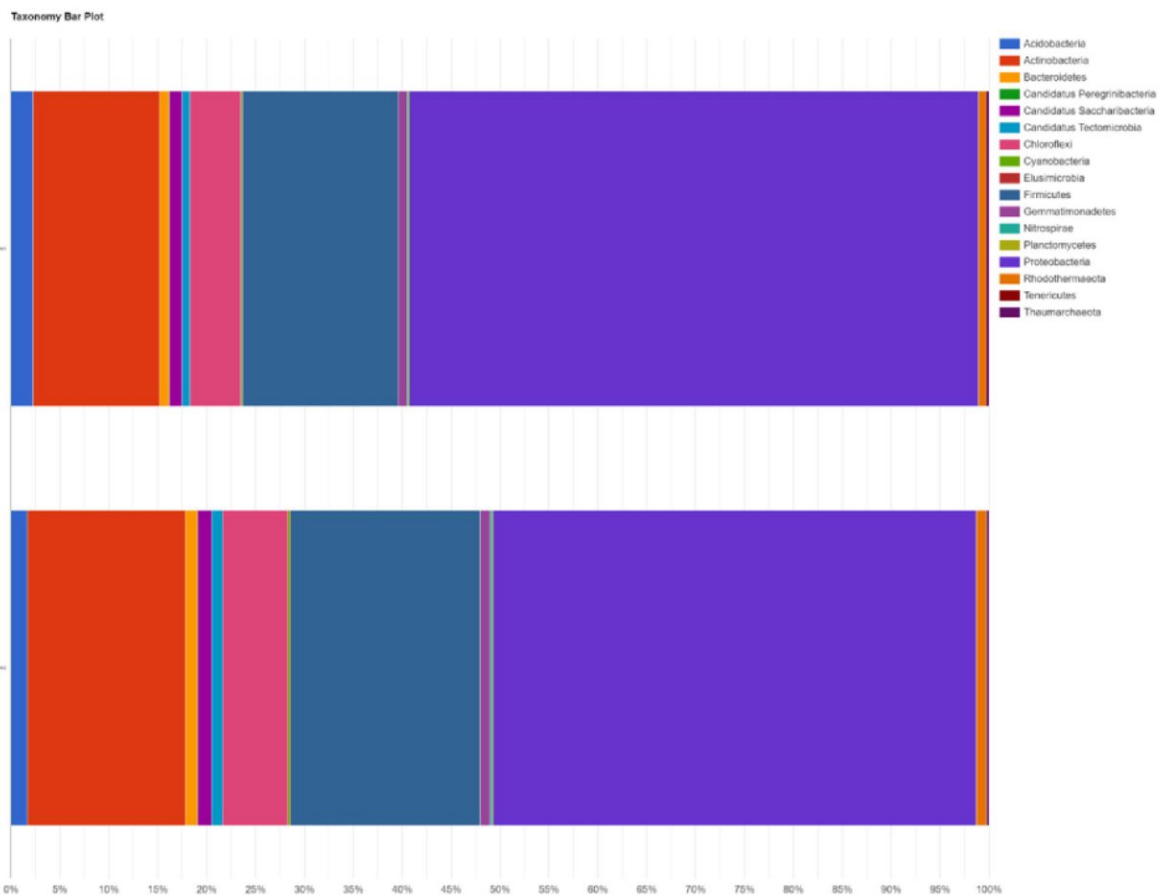


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## METAGENOMICS REPORT

### Phylum Level – Taxonomy Plot Analysis







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## METAGENOMICS \_ REPORT

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#### Alpha Diversity Analysis

Samples	Observed	Chao1	se.chao1	Shannon	Simpson	Fisher
M1	410	655	121.4292	3.97225	0.91301	77.18514
M2	403	403	0	4.02741	0.90917	72.71044

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#### Beta Diversity Analysis

Samples	M1	M2
M1	0	0.165
M2	0.165	0

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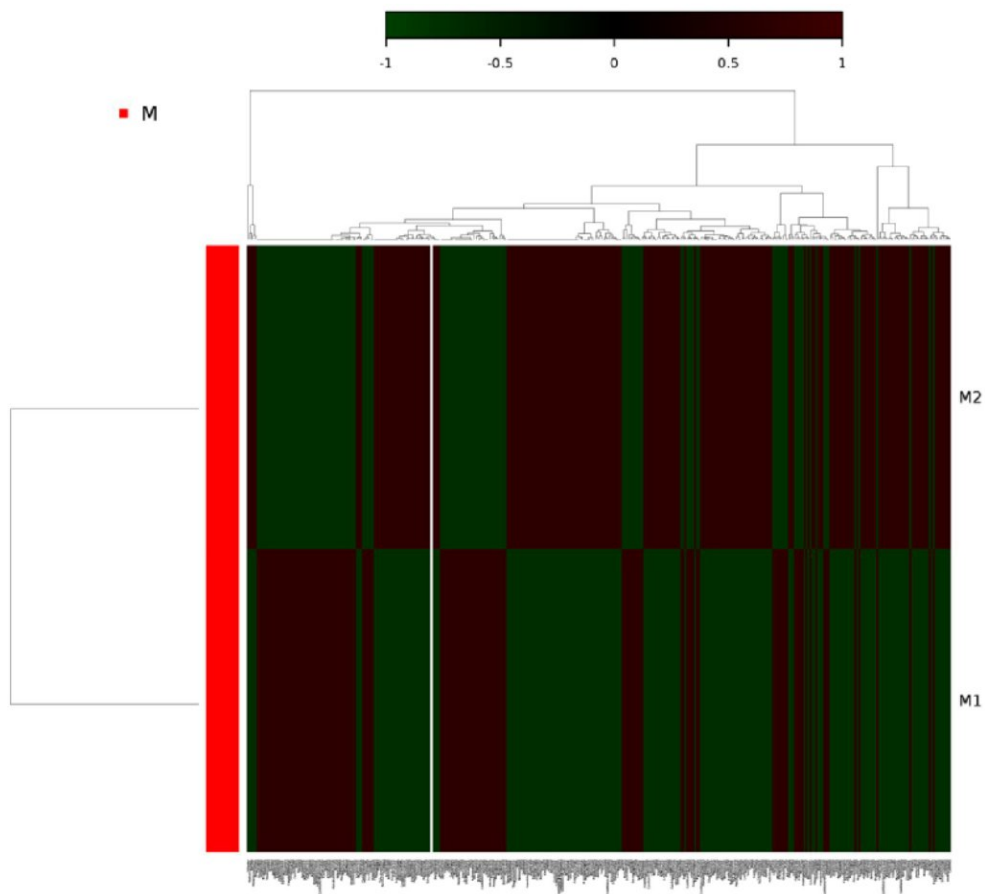
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## METAGENOMICS REPORT – Heat Map

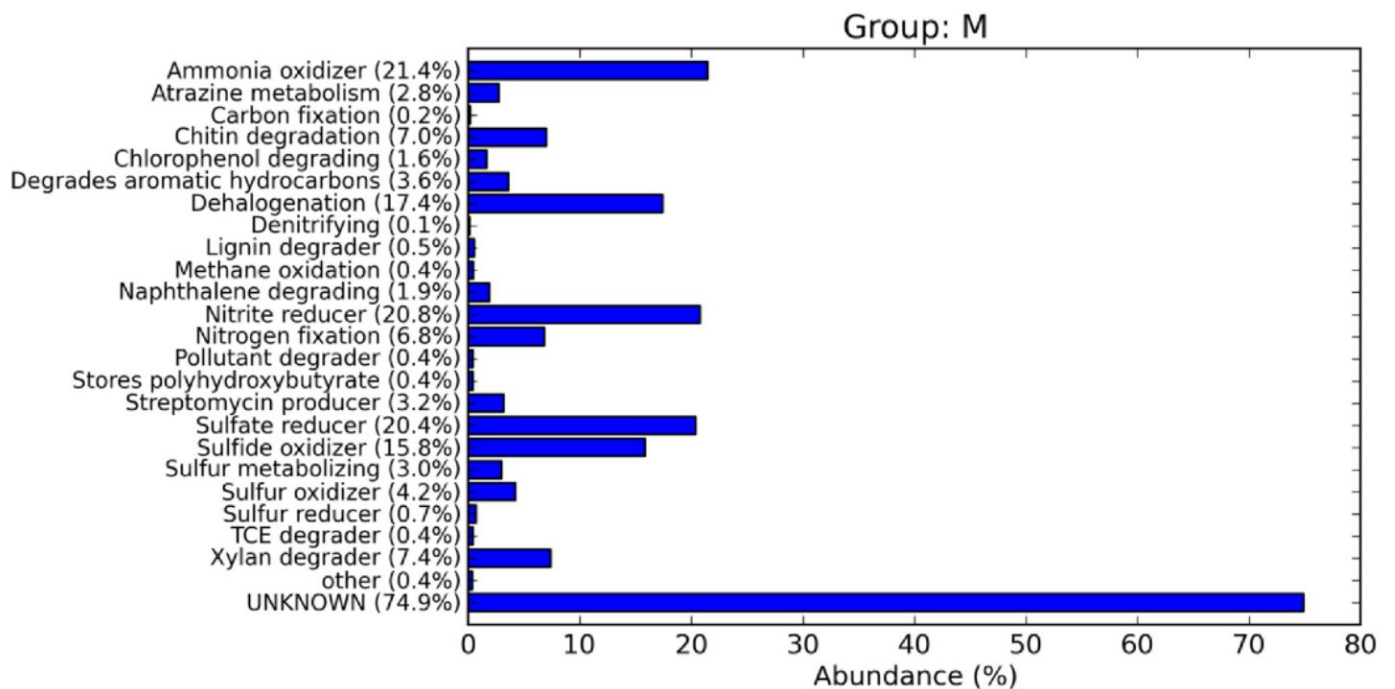
### Heat Map





## METAGENOMICS REPORT – Heat Map

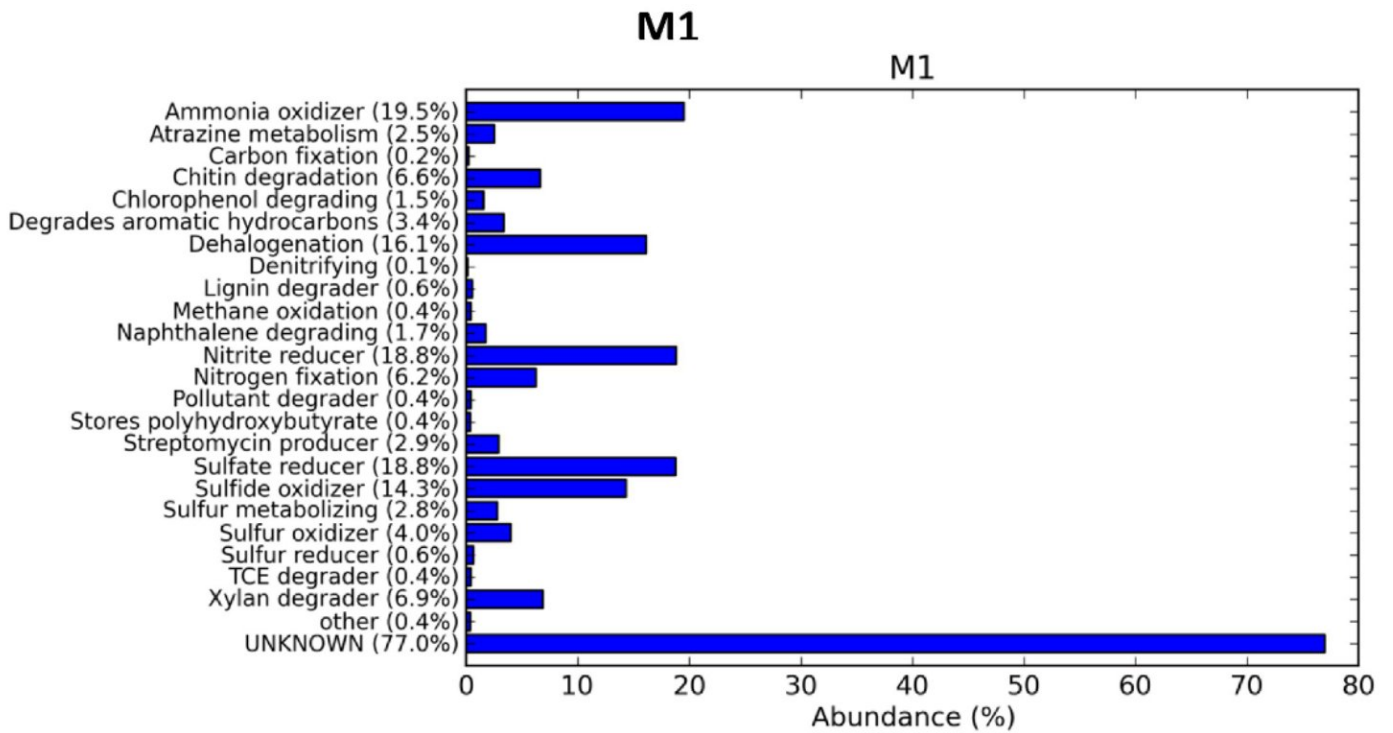
### Metabolic Pathways





**METAGENOMICS REPORT**

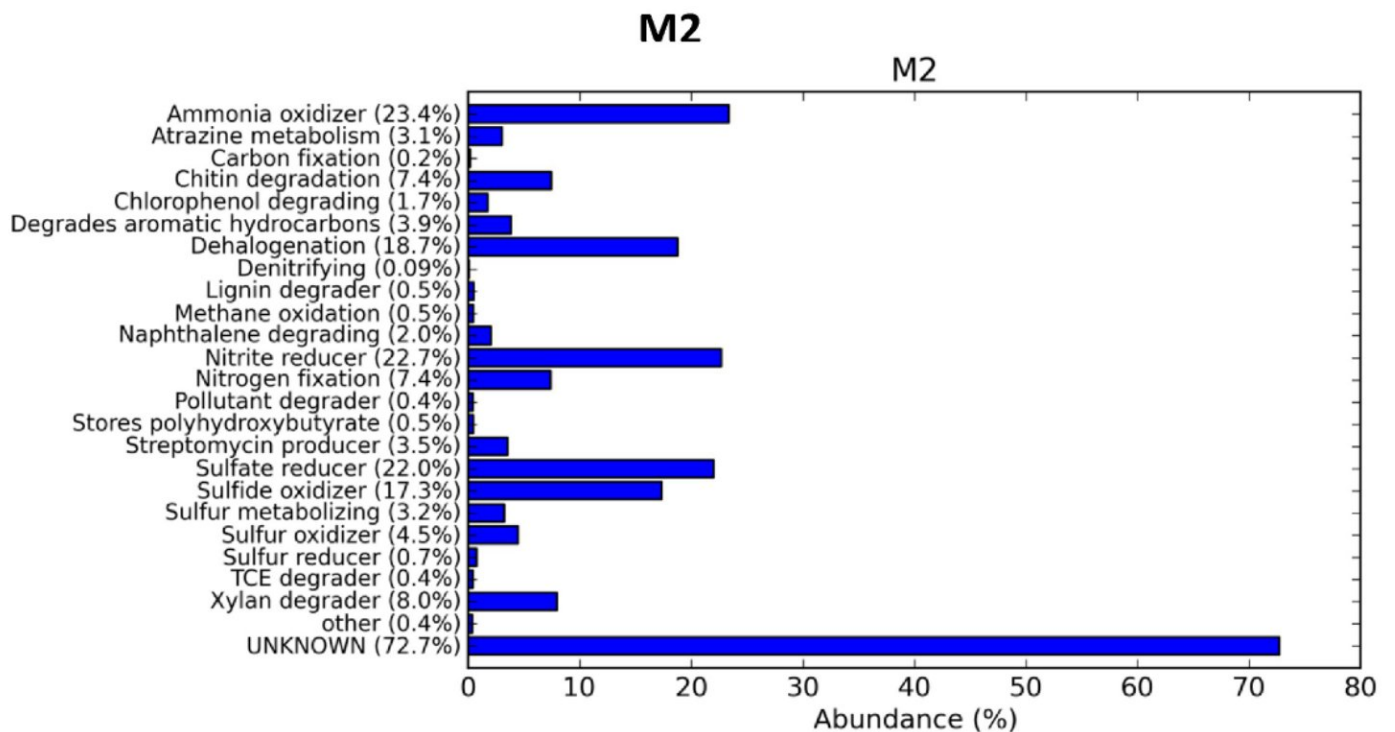
**Metabolic Pathways**





## METAGENOMICS REPORT

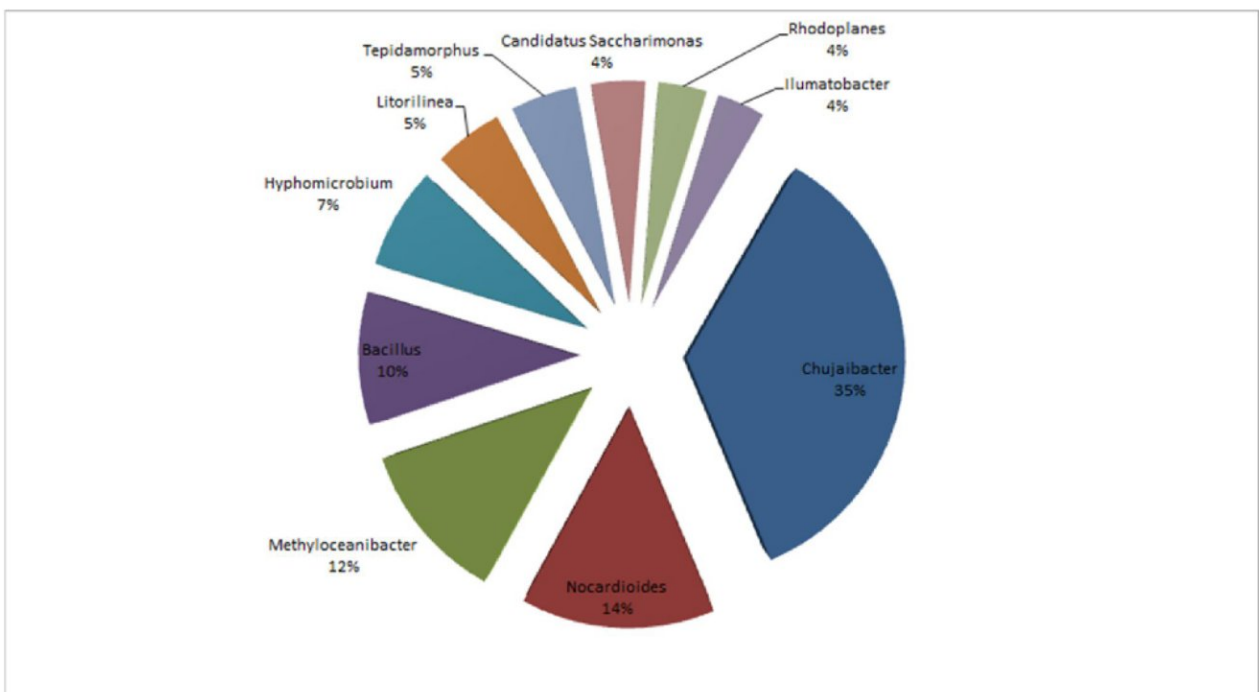
### Metabolic Pathways





**METAGENOMICS REPORT**

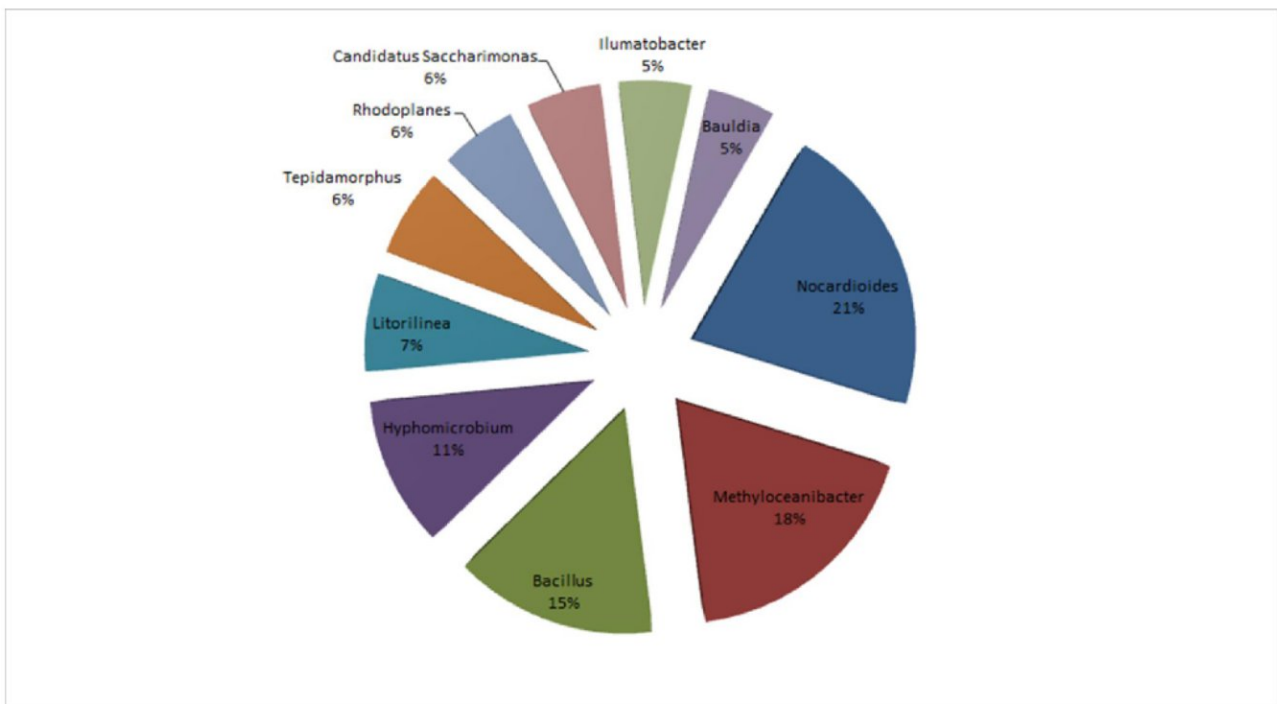
**Top 10 Enriched OTUs – M1**





**METAGENOMICS REPORT**

**Top 10 Enriched OTUs – M2**





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Comparative Analysis was performed using **GAIA 2.0**  
from **Sequentia Biotech SL**

END OF REPORT

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