

**METAGENOMIC ANALYSIS OF BACTERIAL DIVERSITY IN  
THE RHIZOSPHERE OF ARECANUT PALMS AFFECTED BY  
YELLOWING IN WAYANAD**

*by*

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**(2015-11-026)**

**THESIS**

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COLLEGE OF HORTICULTURE  
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KERALA, INDIA**

**2017**

## **DECLARATION**

I, hereby declare that this thesis entitled “**Metagenomic analysis of bacterial diversity in the rhizosphere of arecanut palms affected by yellowing in Wayanad**” is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place : Vellanikkara  
Date : 10-11-2017

  
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## CERTIFICATE

Certified that this thesis entitled “**Metagenomic analysis of bacterial diversity in the rhizosphere of arecanut palms affected by yellowing in Wayanad**” is a record of research work done independently by Mr. Mahesh Mohan, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Place : Vellanikkara

Date : 10-11-2017



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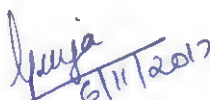
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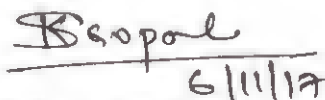
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## ABBREVIATIONS

CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxy ribonucleic acid
g	Grams
h	Hours
ha	Hectre
cm	Centimeter
m	Meter
Mg	Mega gram
kg	Kilo gram
kb	Kilo base
nm	Nano meter
min	Minutes
ml	Milliliter
mm	Millimeter
M	Molar
N	Normality
PCR	Polymerase Chain Reaction
pH	Hydrogen ion concentration
TAE	Tris acetate EDTA buffer
rpm	Revolutions per minute
μg	Micro gram
μl	Micro litre
μM	Micro molar
SDS	Sodium Dodecyl Sulfonate
cfu	Colony forming unit
OTU	Operational Taxonomic Unit
HCN	Hydrogen cyanide
CAS	Chrome azurol S

# INTRODUCTION

## 1. Introduction

Arecanut, a major masticatory crop widely used in India, has its major importance in cultural aspects and forms a major income source for numerous households in various parts of the nation. India leads in area and production of arecanut. The cultivation of arecanut palm is confined to the Western Ghats and the North-Eastern tracts, with the major share contributed by the states Karnataka, Kerala and Assam (Thomas *et al.* 2011). Along with a plethora of constrains like productivity stagnation, pests and diseases, yellowing of arecanut palms has also been emerged as a major threat to its cultivation. The manipulation of PGPR for the betterment of arecanut productivity has been considered as an effective strategy to overcome the loss imparted by yellowing (CPCRI, 2015). The arecanut crop faces nutritional insufficiencies and toxicity as the major cultivating tracts possess laterite soil, with low pH and increased Fe, Mn and Al contents, followed by rainfall resulting in the leaching of N and K (Bhat and Sujatha, 2004).

The disease considered to be first observed in 1949 has emerged to be one of the serious threat resulting in decreasing the production and destruction of arecanut palms over time. The etiological studies on the disease was conducted and the role of abiotic factors including nutritional imbalances and water stagnation were found to play pivotal role in the expression of yellowing in arecanut palms. A study conducted in the Sringeri taluk of Karnataka showed that yellowing of arecanut palms is on an uphill trend with a reduction of up to 67.8 per cent in yield (Shivakumara *et al.*, 2014). The exploitation of PGPR, including endophytic fungi and bacteria to improve the conditions of arecanut palms has been proposed in recent times (ICAR-CPCRI, 2015).

The rhizosphere, a narrow zone in the vicinity of plant roots directly influenced by the plants house a wide range of microorganisms, in which the beneficial ones are frequently studied and manipulated for crop improvement. It includes nitrogen-fixers, phosphate solubilizers, biocontrol agents and mycorrhizal fungi (Mendes *et al.*, 2013).

The PGPR community was then discovered to be diverse and more the diversity, more were the benefits imparted to the crop (Nihorimbere *et al.*, 2011). The selective pressure exerted by the root exudates in shaping the characteristic rhizosphere microbiome unique to the plant or cultivar in specific had been proved to be of immense potential (Bakker *et al.*, 2013).

The cultivation of bacterial cells using microbiological media was found to have a lot of limitations due to the plate count anomaly. It was later established that only 0.1 to 10 per cent of the bacterial cells from arable soils were culturable using the traditional agar plate methods while the major portion remained unrevealed (Torsvik *et al.*, 1998). The usage of DNA recovered directly from soil and further application of PCR technologies in enumerating the microbial population was found to be essential in identifying the unknown members of the population (Tiedje *et al.*, 1999). The term metagenomics was termed to this culture-independent technology of studying microbial composition in an environmental sample (Handelsman *et al.*, 1998).

The concept of manipulation of rhizosphere bacteria to improve plant health has provided ways to decrease the use of synthetic inputs and increase the manipulation of its characteristic microflora to provide better conditions for the plants. Thus, the application of sequencing technology in this aspect can provide us with immense probability of developing novel PGPR strains effective to a particular crop. A similar study to decipher the microbial communities residing in the rhizosphere zones of yellowing affected, apparently healthy and completely healthy rhizosphere arecanut palms may provide an insight on the difference in the taxonomic assemblage in the arecanut rhizosphere shaped as a result of the selective pressure exerted by the condition of the palm. The importance of phosphate solubilizers in the arecanut rhizosphere and its relation with arbuscular mycorrhizal fungi has been positively correlated in a study (Ambili *et al.*, 2012). A whole genome study has revealed the abundance of the family Enterobacteriaceae in the arecanut rhizosphere. The presence of IAA producing genes has been mentioned in the same study (Gupta *et al.*, 2014).

Realizing the importance of rhizosphere microbial diversity in the maintenance of plant health, this study entitled 'Metagenomic analysis of bacterial diversity in the rhizosphere of arecanut palms affected by yellowing in Wayanad' was undertaken. The yellowing of arecanut being a dreadful condition to the arecanut palms in many areas of Wayanad district, the study was conducted with an objective to do the metagenomic analysis of the rhizosphere bacterial diversity in the yellowing affected arecanut palms, so that the distinct bacterial taxa predominant in the healthy arecanut rhizosphere palms may be manipulated in the near future to mitigate yellowing.

# REVIEW OF LITERATURE



## 2. REVIEW OF LITERATURE

### 2.1 Arecanut

The arecanut palm, *Areca catechu* L., belongs to the family Palmaceae. Mainly cultivated for the production of betelnut, they are a significant masticatory crop in India and other Southeast Asian countries. In India, the cultivation of arecanut palms is restricted to a few states in the southern and eastern parts, with the major arecanut cultivating states being Karnataka, Kerala and Assam (Thomas *et al.* 2011).

Tracing the origin of arecanut palm could not be done due to the lack of fossil records of the genus *Areca*. It is considered to be a native of Cochin China, Malay Peninsula and the neighbouring islands and also considered to be indigenous to Indonesia, Sri Lanka, Southern China, Taiwan and Java. The highest species diversity with 24 species have been found in Philippines, Malaysia and Indonesia, suggesting the possible options of origins (Balasimha and Rajagopal, 2004).

#### 2.1.1 Uses

Arecanut is a major masticatory crop with both cultural and medicinal values. For masticatory purpose, the arecanut is usually chewed along with betel leaves, tobacco, lime and camphor or it is chewed alone. Apart from the medicinal importance of enhancing the gastric functions and anti-helminthic properties, arecanuts are often used in religious offerings. The usage of arecanut for chewing has been in practice from time immemorial and spread around the globe, especially in countries like Nepal, Sri Lanka, Burma, Thailand, Philippines, Africa, South China, Pakistan and Bangladesh (Balasimha and Rajagopal, 2004).

#### 2.1.2 Area and production

The leading countries in arecanut production are India, China, Burma, Bangladesh, Indonesia and Sri Lanka. A total area of 3.0 million hectares arecanut cultivation produced approximately 2.14 million tons arecanut in the year 1961. The

area and production was increased to 4.09 million hectares and 5.02 million tons respectively in the year 1990. The peak of arecanut production was recorded in the year 2012, with a total production of 13.4 million tons from an area of 9.92 million hectares, which then reduced to 11.03 million tons from 9.05 million hectares in the year 2014 (FAOSTAT, 2017).

India, being the major producer of arecanut, had a production of 1.20 million tons from a total area of 1.35 million hectares in the year 1961, which increased to 2.51 million tons from 2.09 million hectares in the year 1990. The highest production was recorded in the year 2012, with 6.81 million tons from an area of 4.64 million hectares. The production then declined to 6.22 million tons from an area of 4.52 million hectares in 2014 (FAOSTAT, 2017).

The area and production of arecanut cultivation has increased dramatically from the year 1961, as shown by the statistical report provided by the Food and Agriculture Organisation (FAO). Even though, an increase in the total area and production was observed, a profound increase in productivity was not observed. Among the states in India, Karnataka leads in area and production with an estimate of 2.35 million hectares and 4.36 million tons respectively. In the year 2015-16, followed by Kerala with a production of 1.32 million tons from an area of 0.99 million hectares.

### **2.1.3 Climatic conditions**

Arecanut palms prefer tropical climate with a temperature range of 14<sup>0</sup>C to 36<sup>0</sup>C. The susceptibility of the crop to extreme low and high temperatures make the cultivation confined to geographies below 1000 m below MSL elevation (Thomas *et al.* 2011). Even though a range of climatic conditions are found to be favourable for arecanut cultivation, extreme climatic conditions can have deleterious effect on arecanut yield (Bhat and Khader, 1982). The most critical factors for the growth of arecanut are altitude, relative humidity and rainfall. Among all the factors, relative

humidity, evaporation and rainfall was found to be the most important of all and reported to exert direct influence in arecanut yield (Thomas *et al.*, 2011).

The cultivation of arecanut palms are confined to the tropics, i.e. almost 28° North and South of the equator, with an altitude less than 1000m below MSL. Arecanut is also grown in high altitude area like Wayanad in Kerala and Coorg in Karnataka, but it was reported to produce inferior quality fruits in the high altitudes due to the lack of development of hardness in the endosperm (Pillai and Murthy, 1973). The crop grows well in high rainfall area and it is also grown in less rainfall area under irrigated conditions, especially in parts of Tamil Nadu, where the annual rainfall is about 750 mm. Extremity in the case of relative humidity was also observed to have detrimental effects on arecanut palms, as it directly influence water relation and indirectly affect plant growth (Bhat and Sujatha, 2004).

#### **2.1.4 Soil conditions**

The major share of arecanut growing area have red clay type gravelly laterite soil. It is also cultivated in other areas with clay loam soils (Thomas *et al.*, 2011). It was also reported that the soils with sticky clay, sandy texture, and alluvial, brackish and calcareous nature cannot support the growth of arecanut palms (Aiyer, 1966). A minimum soil depth of atleast 2 metres is required for the well establishment of the root system as the adventitious root system may penetrate upto 3 metres deep in deep soil conditions and 1.40 metres in the case of shallow soils (Bhat, 1978).

Arecanut cultivation is predominant in laterite soils of Western Ghats, Assam and West Bengal. The clay loams of Karnataka also support arecanut cultivation (Mohapatra and Bhat, 1982). The organic carbon was found to be high and available phosphorus in medium range in arecanut gardens with exceptions in Palode of Kerala and Vittal and Hirehalli of Karnataka, where both are low. The soil pH was reported to be acidic to neutral (Bhat and Sujatha, 2004). Due to the same reason, regular liming of the soil in arecanut gardens in suitable intervals were identified to be critical for

maintain the pH and increasing the nutrient uptake by the palms (Bellary and Patil, 2010). A slight increase in the soil acidity due to continuous manure application in arecanut garden was observed and reported by Mohapatra and Bhat in 1982. This continuous manuring is said to have accelerated the proliferation of soil microorganisms, especially in the laterite soil conditions (Bopaiah and Bhat, 1981).

### **2.1.5 Challenges faced in arecanut cultivation**

Arecanut cultivation is one of the most important sources of income for farming communities in India. Even though the cultivation of the crop is restricted to a few states, the consumption is nation-wide. As a result, 70 per cent area expansion in arecanut cultivation was observed in the past two decades, despite the fact that area expansion for arecanut cultivation is discouraged by the government to avoid clearing of forest area and paddy land conversion. Contradictory to the land expansion, the productivity has ceased to make a steady progress from a stagnant value of 1200 kg/ha due to multiple constraints like poor soil and crop management followed by cultivation in unsuitable lands (CPCRI, 2015).

Apart from the economic and agronomic hurdles faced in arecanut cultivation, the crop is attacked by a wide range of pests and diseases, which differs from one location to another (CPCRI, 2015). While the incidence of pests and diseases differs from one place to another, the incidence of koleroga was high in Sagar and Thirthahalli of Karnataka with 76 per cent loss in arecanut yield. Apart from koleroga, 7 per cent yield loss was contributed by yellowing and another 6 per cent by root grubs (Ramappa, 2013). The scenario deviated from the above mentioned case in the area of Sringeri taluk of Karnataka, where yellowing affected the crop and resulted in 67.76 per cent yield loss (Shivakumara *et al.*, 2014). It was also reported that yellowing of arecanut now poses a serious threat to the farmers, causing yield loss in large scale (Ramappa, 2013).

## **2.2 Yellowing of arecanut palms**

### **2.2.1 Origin and spread**

Yellowing has become the most deadly constraint that can affect arecanut palm. The disease was reported for the first time from the areas of Muvattupuzha, Meenachil and Chalakkudi in the year 1914. The disease was found to spread widely after a heavy flood in these areas. The disease has its vernacular name in Malayalam and it is called as “Kattuveezcha” (Nambiar, 1949). With the spread of yellowing, a series of studies, observations and investigations were conducted to understand the mode of spread and lethality of the disease. In a study conducted in Central Plantation Crops Research Institute, it was found out that the symptoms appeared in 80 per cent of the arecanut palms within 4 years of its first appearance in the arecanut garden (Rawther and Abraham, 1972). Later, studies were conducted on symptomatology and a method for indexing of yellowing was given by George *et al.* in 1980, in which parameters like yellowing, necrosis and crown size reduction were taken in account to measure the intensity of disease.

A survey conducted in the year 1960 illustrated the spread of yellowing in all parts of Kerala with the incidence at its peak in Quilon district with 90 per cent incidence (Chowdappa *et al.*, 2002). The spread of the disease was finally observed in all the districts of Kerala and rendered arecanut cultivation economically non-feasible due to high reduction in the yield (CPCRI, 2000). Low lying lands with high water table in the monsoon were the sites of severe disease incidence. The high water table was also seen to be the cause of root decay in the affected palms. The symptoms were initiated after three years of planting in the affected soil (Chandramohan and Nair, 1985).

### **2.3 Symptoms of yellowing**

The typical symptoms of yellowing is expressed soon after the onset of monsoon. Temperature at a maximum range of 30-32<sup>0</sup>C with cool wind currents were

found to be critical for the disease expression. A maximum intensity of disease expression was found in the month of August and less intensity before the arrival of South-West monsoon (Nayar, 1976).

### **2.3.1 Symptoms on leaves**

The symptoms were observed to be initiated with the yellowing of the tips of leaflets of a few leaves in the outermost whorls (Rawther, 1976). Drying up of leaf tips in the later stages, reduction in leaf size and finally a bunchy, shortened, pointy appearance of the leaves were also observed. The severity of yellowing at its maximum intensity also resulted in the falling off of the crown (Nayar and Selsikar, 1978).

### **2.3.2 Symptoms on roots**

The disease also had its impact on the root system. Reduction in the lateral root proliferation was observed in yellowing affected arecanut palms. Darkening of root tips with gradual rotting of the active absorbing regions of root tips were also detected (Rawther, 1976).

### **2.3.3. Symptoms on nuts**

The endosperm of the nuts often exhibited darkened colour with soft consistency, which made them unsuitable for its destined purpose. The darkening of the endosperm was sometimes observed to be independent of the yellowing symptoms. Apparently healthy palms in yellowing affected garden often produced normal nuts (Rawther, 1976). The disease also reduced the inflorescence production, which in turn led to 50 per cent loss in the yield in a time frame of three years (CPCRI, 2000).

## **2.4 Etiology of the disease**

The etiology of yellowing in arecanut was a matter of great debate. The primary hypothesis of fungi and bacteria being the causal organisms were rejected as the result of a series of studies undertaken.



#### 2.4.1 Fungi

The role of fungi in arecanut yellowing was first asserted by Khandinge *et al.* in 1957. Isolates of *Cercospora arecae*, *Exosporium arecae*, *Leptosphaeria* sp., *Diplodia* sp., *Phyllosticta* sp., *Dimerosporina* sp. and *Trametes corrupta* were recognized from the yellowing affected leaves (Menon, 1959). A number of non-pathogenic fungal isolates including *Trichoderma* sp., *Pestalotia* sp., *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Acremonium* sp. and *Colletotrichum* sp. were also isolated from the roots of yellowing affected arecanut palms (Chowdappa *et al.*, 2002). The advanced studies on inoculation of the isolates on palms yielded negative results (CPCRI, 2000).

#### 2.4.2 Bacteria

Inoculation studies using *Bacillus*, *Xanthomonas* and *Serratia* isolates from roots of yellowing affected arecanut roots displayed symptoms of discolouration and water soaked areas in arecanut seedlings and cowpea. The same isolates failed to prove its pathogenicity when it showed no symptoms in 18 months old arecanut seedlings (Chowdappa *et al.*, 2002). According to a study conducted by Bopaiah in 1990, a relatively high proportion of Gram positive bacteria (70-80 per cent) were present in the rhizosphere region of healthy arecanut palms, when compared to a reduced proportion of the Gram negative counterpart with 15-30 per cent in the same rhizosphere region.

#### 2.4.3 Virus

Presence of proteins and sub-units was detected in yellowing affected arecanut palms, which exhibited precipitation and antibody production with crude arecanut antigen (Menon, 1961). Subsequent transmission studies were undertaken on indicator plants that exhibited yellowing symptoms (Menon, 1963).

#### 2.4.4 Mites

The role of mites in arecanut yellowing was proposed by Khandige *et al.* in 1957, which was then proven to be distinct from the widely spread arecanut yellowing by Menon in the year 1960.

#### 2.4.5 Nematodes

The presence of *Meloidogyne javanica*, *Helicotylenchus* sp. and *Tylenchorhynchus* sp. from the rhizosphere of yellowing affected arecanut palms were detected in the samples collected from Palode, Kerala (Nair, 1964). Another study conducted by Weischer in 1967 showed the presence of almost seven genera of plant parasitic nematodes in the rhizosphere of yellowing affected and healthy arecanut palms, but no correlation with the disease was found out.

The role of *Radopholus similis* in the spread of yellowing was intensively studied and symptoms of orange discolouration of leaves, lesions and root rot were observed. Pathogenicity by this nematode was then proved and a reduction in growth and vigour was conformed (Koshy *et al.*, 1975). Establishing nematode as the causal agent of arecanut yellowing was not successful, however, the root rot symptom correlated with the yellowing of arecanut was considered to be associated with nematodes. The role of nematodes as an initiating agent of arecanut yellowing was suggested by Sundararaju and Koshy in 1986.

#### 2.4.6 Phytoplasma

The first report on the presence of phytoplasma as the causal agent of arecanut yellowing was given by Nayar in 1971, who was able to obtain colonies of phytoplasma from leaf bits of yellowing affected arecanut palms. The presence of phytoplasma in the sieve elements of yellowing affected arecanut palms was detected in an electron microscopic study (Selsikar and Wilson, 1981). The detection of phytoplasma in the sieve elements was followed by inoculation studies, which provided positive results



(CPCRI, 2000). The role of plant hopper, *Proutista moesta* as the vector of the disease was also found out (Ponnamma *et al.*, 1991).

A confirmation study conducted using phytoplasma specific universal primers to amplify specific genes from the total DNA isolated from the leaf samples of yellowing affected and healthy samples collected from various parts of Karnataka, failed to give amplicons of the specific region. The same primers were successful in amplifying R16F2/R2 regions in the case of samples collected from Hainan province of China, suggesting a different etiology for the arecanut yellowing in Indian sub-continent (Purushothama *et al.*, 2007). RFLP analysis of 16S rRNA gene and comparison with the phytoplasma from Hainan revealed 91 per cent sequence similarity. It was then identified as a 16SrXI-B phytoplasma and hence named it as Indian YLD phytoplasma (Ramaswamy *et al.*, 2013).

## **2.5 Physiological aspects associated with yellowing in arecanut**

The symptoms are highly pronounced during the months of August to October, which marks the onset of monsoon. The palms remain symptomless during December to May. The moisture level in the leaf samples from yellowing affected palms in the month of June was found to be 59.10 per cent. The moisture content in healthy leaf sample was found to be 70.84 per cent. This correlation was not applicable to root samples (Yadava *et al.*, 1972). Apart from the difference in the moisture percentage, the leaf sap acidity of yellowing affected palm was 3.29 while the pH in healthy palm was 4.63 (CPCRI, 2000).

Yellowing affected palms displayed low photosynthetic rate and increased carbon dioxide along with very low stomatal conductions (Chowdappa and Balasimha, 1992). An unbalanced chlorophyllase-chlorophyll system was identified to be associated with yellowing in arecanut. An increase in the chlorophyllase content resulted in the decrease of chlorophyll content was observed in yellowing affected leaves (Srinivasan, 1982). Disrupted translocation of metabolites followed by

accumulation of carbohydrates and starch was recorded to be higher in diseased palms than the healthy arecanut palms (Chowdappa *et al.*, 2002). In a study conducted to analyse the isozyme variation among arecanut cultivars, a significant difference in the production of peroxidase and polyphenol oxidase were observed in the cultivars that were reported to be tolerant to yellowing. Along with these observations, an increase in the total protein in yellowing tolerant cultivars were also reported, which was hypothesized to be inconspicuous with no significant importance (Swaminathan, 2002).

### **2.5.1 Mineral nutrition in yellowing of arecanut palms**

Deficiency of phosphorus in leaf tissues was reported by Mohapatra and Bhat in 1975. The low range of magnesium was considered to be a result of high CaO/MgO ratio. Application of MgSO<sub>4</sub> in the plant basin was recommended to increase the chlorophyll content. An increased concentration of acetic acid extractable aluminium was found to be higher in the yellowing affected leaves collected from Vittal. The soil samples from the same area was also found to possess increased exchangeable aluminium content (Mohapatra and Bhat., 1975). The variation in the concentration of Fe and Mn in the arecanut leaves were considered to be linked with the yellowing of leaves. The laterite soils with low pH due to the rainfall pattern, where arecanut cultivation is prevalent has increased aluminium content with less silicates. The release of Fe and Mn along with the presence of aluminium was considered to cause yellowing in arecanut palms (UAS, 1990).

An increased nutritional requirement, especially N, P, K and S were identified, followed by increased lime application from 100 g to 150 g per palm was recommended to increase the availability of Ca (Jacob *et al.*, 2014). Another study conducted by Gurumurthy and Ramaswamy in 2000 suggested the amelioration of yellowing by nutrient application. Deficiency in N, P and K in the leaf samples collected from Sringeri district of Karnataka were detected both in yellowing affected and apparently

healthy leaf samples, even though the soil nutrients were higher than the optimum (CPCRI, 2015). Deficiency in the major soil nutrients and the assimilation of micronutrients like Mn and Cu in the palm was considered to be the predisposing agents for yellowing in arecanut (CPCRI, 2013). Waterlogging and submergence of soil was identified as one of the pre-disposing agent of arecanut yellowing (Pal *et al.*, 1960). Presence of water table in the active root zone followed by the extreme decrease in soil pH after its temporary initial rise was considered to have a profound effect in causing yellowing (Mohapatra *et al.*, 1976).

## **2.6 Beneficial microorganisms in arecanut ecosystem**

After the estimation of PGPR population in arecanut ecosystem, 14 isolates of fungal endophytes and 32 isolates of bacterial endophytes were obtained from both yellowing affected and healthy arecanut leaves (CPCRI, 2015). The rhizosphere soil DNA analysed for PGPR strains in arecanut ecosystem revealed the presence of plant growth promoting rhizobacteria strains closely related to *Enterobacter cloacae* group. The PGPR traits identified from the whole genome sequence revealed through gene annotation revealed the presence of indolepyruvate decarboxylase coding gene, but very low in IAA production traits (Gupta *et al.*, 2014). As a part of enhancing the arecanut cultivation, development of superior isolates of PGPR strains, bio-control agents and endophytes are considered to be essential, especially when it comes to the management of yellowing in arecanut, as the curative measures are still not found to be completely effective (ICAR-CPCRI, 2015).

## **2.7 Soil as a habitat for microorganisms**

Soil, with its complex chemical components, is considered to be the most suitable, diverse and intricate habitat for living organisms. Soil is one of the most important source of microbial communities, due to its heterogeneity and complexity attributed by the interaction between minerals and organic compounds present in it. Due to the same reasons, soil has always been a platform for all the bio-geo chemical

cycles necessary for the sustenance of life (Mocali and Bendetti, 2010). The soil microbial community, being dependent on soil chemical and physical properties create this complexity. As a result, numerous microsites with diverse conditions are created even in a confined vicinity of a few square millimetres (Nunan *et al.* 2002).

Contradictory to the fact that microorganisms contribute only 0.5 per cent to soil mass, the fraction exerts a major impact in determining the soil properties and processes. The community dynamics of the living component is shaped because of these properties. The living component, especially, the microorganisms play a pivotal role in catalyzing the chemical reactions that change the soil. It happens, because of the high metabolic diversity present in the soil, even though it may not always be phenotypically expressed. Due to all the cumulative reasons we have seen, the soil gains its property of being a complex heterogeneous matrix that eventually shape the vegetation of an area, which is an unavoidable property to be considered for practicing agriculture (Tate, 1995).

Soil has always been known to conceal a hyperdiverse genetic diversity. Enumeration of microorganisms using conventional methods can only reveal about 0.1 per cent of the total diversity of microorganisms, while the rest 99.9 per cent of soil microflora remain unrevealed. The majority of the unrevealed microflora may even represent another phyla, as they are phylogenetically diverged from their culturable counterparts (Handelsman *et al.* 1998). Earlier, it was estimated that one gram soil housed almost  $10^7$  prokaryotic cells and it was later found that it was only a fraction of the real genetic pool present in the soil. A culture-independent method to estimate the diversity of bacterial DNA in soil done by Torsvik *et al.* in the year 1990a showed a total number of more than  $10^{10}$  bacterial cells per gram of soil (Torsvik *et al.* 1990b).

Apart from considering a huge mass of soil as the house of diverse microorganisms, it has also been observed that the spatial arrangement and distribution of bacterial communities may vary, as a result of nutrient transport, solute distribution

and other soil processes. Spatial arrangement studies on bacterial population suggested the affinity of bacteria towards the so called 'hotspots' or specific sites in the subsoil, rather than the topsoil (Nunan *et al.* 2002).

### **2.7.1 Soil microflora and plant health**

The soil microflora is considered to be a major factor that affects the plant health. The mechanism of the beneficial interaction may occur both directly and indirectly. The direct effects are further classified into positive and negative direct effects, in which, the positive direct effects mainly include the plant-microbe symbiotic associations, while the negative direct effects include the pathogenic interaction of microorganisms with the plant (Van Der Heijden *et al.* 2008). Among the positive direct interactions, the most common example being the symbiotic relationship between plants and diazotrophs, that assist the plants in acquiring one of the most crucial element that governs plant growth. Another classic example of the positive direct effect is the symbiotic association of mycorrhizal fungi with the roots of higher plants. The symbiotic association provides multiple benefits to the plant, including nitrogen, phosphorus, copper, iron and zinc nutrition in return of the carbon rewarded by the plant (Shtark *et al.* 2010).

Communication of soil microorganisms with plants are essential for their interaction, as the chemical signals produced by the microorganisms trigger the release of root exudates essential for their survival. The chemical properties of the exudates are unique to plant species and even subspecies (Uren, 2007). The root exudates are usually a mixture of compounds like amino acids, organic acids, pentoses and hexoses, pyrimidines and puridines, vitamins and enzymes. The chemical make, ratio and properties of the different compounds act as a major factor in shaping the rhizosphere microflora by providing selective advantages to certain species (Tate, 1995).



### 2.7.2 Root exudates

Root exudates remain as the major reason for the creation of a versatile platform for the interaction of plant roots with the microorganisms (Hirsch *et al.* 2003). The root exudates are generally composed of two groups of compounds. The high molecular weight compounds that include proteins and polysaccharides, which constitute the major portion of root exudates and contribute less to its diversity, while the low molecular weight compounds consisting of amino acids, organic acids, phenolics, and other secondary metabolites are responsible for the chemical diversity among root exudates of different plant species (Uren, 2007). Considering the effect of root exudates in shaping the rhizosphere microbiome, the process can be considered as the one governed by the plant that changes the soil parameters and rhizosphere microflora or vice versa. It varies from plant to plant and even among the accessions of the same species. T-RFLP analysis of the rhizosphere microflora of different accessions of *Arabidopsis thaliana* revealed that the quantitative and qualitative difference in the root exudates can shape difference in the rhizosphere microbiome pattern, even though a direct link or correlation among the variations could not be deciphered so easily (Micallef *et al.*, 2009). Another study on the culturable diversity of rhizosphere microflora of maize showed that the stages of plant development may have selective influence on specific groups of bacteria and fungi (Cavaglieri *et al.* 2009).

Apart from the root exudates, the disrupted root cells and mucilage secretions are also released into the rhizosphere region. Compounds like cellulose, lignin, pectin and other cell wall polymers are also found in higher concentration, which make the rhizosphere an active zone for cellulose and other organic matter decomposition. The decomposed substance may support other microbial communities by providing suitable carbon source. Among the bacterial population,  $\alpha$  and  $\beta$  classes of Proteobacteria were predominant apart from the other major groups like Firmicutes, Actinobacteria, Bacteroidetes, Planctomycetes, Verrucomicrobia and Acidobacteria (Turner, 2013).

The ecological interaction between plants and microbes can take many forms ranging from negative interactions like parasitism to neutralism to positive or beneficial interactions by plant growth promoting rhizobacteria (PGPR) (Bais, *et al.* 2006). PGPR are considered to perform multiple functions such as nutrient cycling, biological control of antagonistic microorganisms and seedling growth (Barea *et al.* 2005). Based on the functions, PGPR are classified into two groups. First group being those responsible for nutrient acquisition and plant growth stimulation. The second group consists of biological control agents that are antagonists of plant pathogens (Bashan and Holguin, 1998). These properties of PGPR are manipulated for crop production to increase the productivity, acquisition of nutrients and to induce stress tolerance, as they successfully colonize soils low in microbial biomass due to their selective advantage in thriving in peculiar conditions and occupying vacant spaces, which in turn exert a competition on the pathogen invasion (Chaparro *et al.*, 2012).

Increasing the plant productivity being one of the major contribution of PGPR, disease suppressiveness is also being provided by the same, which indirectly supports the crop productivity (Janvier *et al.* 2007). Production of antibiotics and other antifungal compounds induce the property of disease suppressiveness to the soil (Weller *et al.* 2002).

## **2.8 Disease suppressive soils**

Disease suppressive soils are characterized by the inherent capacity of the soils to repress pathogens up to a limit. The impact of the soil microbial community affects the proliferation of the pathogens. The saprophytic mode of nutrition followed by the pathogens are interfered by the soil microflora by exerting pressure by competing for space and nutrients. It results in achieving a character of general disease suppression (Berendsen *et al.* 2012). Another phenomenon called as specific disease suppression is observed when the microorganisms impart resistance against a specific disease. The

state of specific disease suppression can be attained by transferring 0.1 to 10 per cent of suppressive soil into disease prone soils (Gerbeva *et al.* 2004).

Disease suppressiveness is considered to be one of the factors contributing to soil health. It is considered to be derived as a result of soil biological diversity and their collective interactions. The property of less disease incidence even in the presence of a susceptible host and virulent pathogen along with a physical environment favourable for disease incidence is referred to as soil disease suppressiveness (Höper and Alabouvette, 1996).

### **2.8.1 General disease suppression**

Among the two types of disease suppressiveness, general suppression is considered to happen as a result of the collective interaction, antagonism and competition for nutrients and space. This phenomena is evident in natural soils (Workneh *et al.* 1993). The second type of suppression, known as, specific suppression occurs as a result of selective antagonism and parasitism imposed over specific pathogens by one or more antagonists (Hornby and Bateman, 1997). A popular example for specific suppression was given by Cook and Baker in 1983, who observed the decline in take-all disease caused by *Gaeumannomyces graminis*. The suppression is considered to be occurred as a result of mono-cropping.

The character of disease suppressiveness exhibited by healthy soils are considered to be imparted by the presence of functional units in the microbial population. The character may be determined by the communal composition, which plays an integral part in the organic matter decomposition followed by microbial succession (Van Bruggen and Semenov, 2000). As an exception, an example for individual organism contributing to disease suppressiveness was given by Raaijmakers and Weller in 1998. They observed the decline in take-all disease due to the presence of *Pseudomonas fluorescens* strains capable of producing phloroglucinol, which had a negative impact on the pathogen.



### 2.8.2 Specific disease suppression

Specific suppression, on the other hand is developed over general suppression and considered to be much more efficient. The mechanism involved in specific suppression is considered to be the presence of a specific microbial species or its related groups (Weller *et al.* 2002). It has also been experimented and proved that the property of specific suppression is transferable to fields and greenhouses (Weller, 2006).

Among the various examples given for specific disease suppression, the suppression of take-all disease is the most studied one due to its ubiquitous incidence in wheat monocropping area. The reason behind take-all disease suppression is considered to be the action of 2,4 diacetylphloroglucinol (2,4-DAPG) producing strains of fluorescent pseudomonads and parasitic activities of *Trichoderma* spp. (Simon and Sivasithamparam, 1989). A study conducted by Raaijmakers *et al.* in 1997 affirmed the presence of significant population of 2,4-DAPG producers in take-all suppressive soils, while the population was absent or less significant in disease conducive soils. FT-ARRA analysis and T-RFLP analysis of 16S rDNA amplicons followed by *in vitro* screening of isolates revealed the influence of 2,4-DAPG producing fluorescent pseudomonads in take-all disease suppression, even though other dominant genera, like *Chryseobacterium* and *Flavobacterium* were present (Gardner and Weller, 2001).

Another striking example for specific disease suppression is *Fusarium* wilt suppressiveness. A study on rhizosphere microflora of *Fusarium* wilt suppressive soils showed that intrageneric competition for carbon between pathogenic and non-pathogenic species of *Fusarium* played a substantial role in suppression of the pathogenic *Fusarium* sp. (Alabouvette, 1986). Another mechanism of suppression of *Fusarium* wilt was identified to be the competition for iron due to the presence of siderophore producing *Pseudomonas* spp. (Alabouvette, 1999).

## 2.9 Methods of soil microbial diversity analysis

It is evident that microbes play important roles in many activities in the ecosystem like nutrient cycling, plant nutrition and alteration in soil structure (Lakshmanan *et al.*, 2014). The soil microbial diversity is often considered as a function of all activities in the soil. The community is shaped by many anthropogenic activities, including agriculture (Kirt *et al.*, 2004).

The most conventional method used to analyse microbial diversity is the phenotypic characterization of isolated strains. This method limits the information, as only culturable microorganisms can be analysed using this method. It was observed that 99.5 to 99.9 per cent of soil bacteria observed under fluorescent microscope are unculturable under *in vitro* condition. It is therefore, an indisputable fact that only a minute portion of soil microflora can be cultured on artificial media (Torsvik *et al.*, 1998).

All the methods that are being practiced till this date were critically analysed to determine its efficiency in evaluating the community-level interactions of soil microflora, abundance of a species, shift in the microbial communities which represents the soil health (Hill *et al.*, 2000). The main objectives in the study of soil microflora has always been the enumeration of microorganisms in the soil and discovering the *in situ* functions. The methods used until now ranges from phenotypic characterization to metabolic fingerprinting to culture-independent methods (Hill *et al.*, 2000).

### 2.9.1 Dilution plating and culturing

The traditional culture-based analysis of soil microflora done using a wide variety of culture media is used mainly for the study of soil microbial composition. The method has been widely used for studying soil organic matter decomposition, soil quality and disease suppression (De Leij *et al.*, 1994). This method can be effectively used to evaluate the heterotrophic portion of the microbial population (Kirk *et al.*,

2004), while 99 percent of the population lies beyond the limit of being grown on a culture media (Rondon *et al.*, 1999).

### **2.9.2 Metabolic fingerprinting**

Assessment of bacterial population based on the utilization of sole the carbon source was developed as a standard method using 96-well microtitre plates by Garland and Mills in 1991. The microtitre plates comprised of 95 different carbon sources and one control (Hayward, CA, USA, [www.biolog.com](http://www.biolog.com)). The analysis was finished using tetrazolium salt, which produced a range of colour intensities based on carbon utilization (Becker and Stottmeister, 1998). Separate plates for Gram negative (GN), Gram positive (GP) and fungal specific plates (SFN2, SFP2) were developed (Classen *et al.*, 2003).

### **2.9.3 Fatty acid methyl ester (FAME) analysis**

A culture independent biochemical method, which was used to obtain information on microbial population dynamics based on the identification of fatty acids present in the soil was developed (Ibekwe and Kennedy, 1998). Fatty acids, being an integral part of microbial cells has its own specificity inn species level and can act as a signature compound that can represent the ingredients of a microbial population (Kelly *et al.*, 1999). The fatty acids that are extracted from soil directly is methylated, followed by analysis using gas chromatography. The FAME profiles thus obtained represents the microbial composition in a sample. A corresponding result to that obtained from metabolic fingerprinting using Biolog plates was obtained using phospholipid fatty acid (PLFA) analysis (Ibekwe and Kennedy, 1998).

The obscurities in FAME analysis include the misrepresentation of population due to the direct influence in the fatty acid composition in microbial cells due to factors like nutrition and temperature. In the case of fungal diversity analysis, almost 130 to 150 spores are required for the study and hence the minor fraction in the fungal population may get omitted (Graham *et al.*, 1995).

## **2.9.4 Molecular-based approaches**

### **2.9.4.1 Guanine plus cytosine (G+C) content**

The G+C content of microorganisms are different from one species to another and a difference of 3 to 5 per cent in guanine plus cytosine content is observed among closely related groups (Tiedje *et al.*, 1999). This variation in the G+C content among the taxa form the base of microbial diversity analysis (Nusslein and Tiedje, 1999). A quantitative analysis to decipher the rare members of the population, which is not influenced by PCR bias can be stated as an advantage of this method, while the requirement of large quantities of DNA (about 50 µg) for the analysis stand as a slight drawback of this method (Tiedje *et al.*, 1999).

### **2.9.4.2 Nuclei acid hybridization**

The rate of DNA hybridization after total DNA extraction, purification and denaturation if measured to find out the genetic complexity and diversity of microorganisms in the sample (Torsvik *et al.*, 1990a). The rate of hybridization is dependent on the sequence similarity, complexity and diversity as the hybridization rate decreases with the increase in complexity (Theron and Cloete, 2000).

The half time taken for DNA re-association and the extent of similarity of the DNA sequences analysed using hybridization kinetics was used to identify similarities between the populations (Griffiths *et al.*, 1998). Usage of fluorescent markers like fluorescein or rhodamine was used for the quantitative analysis and to find out the special distribution of microorganisms (Theron and Cloete, 2000). A modified method called as fluorescent *in situ* hybridization (FISH), using fluorescent labelled primers that are allowed to hybridize with the DNA was successfully practiced to study the special heterogeneity of microorganisms in environmental samples (Schramm *et al.*, 1996).

The usage of polymerase chain reaction (PCR) done by using the total DNA as template was done to eliminate the problem of not detecting the minor population (Van Elsas and Wolters, 1995). The amplified PCR product was then hybridized with the total DNA extracted from the environmental sample or with the oligonucleotide probes to find out the microbial population (Kirk *et al.*, 2004).

#### **2.9.4.3 PCR-based techniques**

The amplification of 16S rDNA region followed by the phylogenetic analysis has been commonly practiced to analyse the diversity of prokaryotes (Pace, 1996). For fungal population, analysis of 18S rDNA and internal transcribed spacer (ITS) regions have been extensively studied. The sequences of the amplicons are then compared with available database to identify the microbial communities (Ko *et al.*, 2011). Many modified methods like denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) have been used to study the microbial diversity. The amplified products are then separated on polyacrylamide gel using denaturants (formamide, urea or temperature) in increasing concentration gradient to analyse the changes in microbial population (Miller *et al.*, 1999).

### **2.11 Metagenomics - A culture independent approach**

#### **2.11.1 Requirement for a culture independent method**

The ground on which the study of soil microbial diversity has been constructed is that the microorganisms can be recovered and cultured in laboratory conditions (DeLong, 1998). It was also discovered that the microorganisms visualized under microscope by direct staining of natural samples yielded a number of cells two or three times greater than those obtained by culturing (Rondon *et al.*, 1999). The inability to culture the majority of microorganisms are argued to be due to the difference in the physiological state that prevents them from being cultured, even though they are considered to be phylogenetically similar to the culturable counterpart (McDougald *et al.*, 1998). A contradictory hypothesis states that the unculturable portion forms



phylogenetically divergent lineages, pointing out the presence of a huge unaccounted microbial wealth in the soil ecosystem (DeLong, 1998). The soil bacterial content was found to be highly heterogeneous in a DNA hybridization experiment, which revealed the presence of almost 4000 different bacterial genomes per gram of soil (Torsvik *et al.*, 1989).

## 2.12 Metagenomics

The idea of metagenomics was introduced as a result of the adaptation of BAC (Bacterial Artificial Chromosome) technology in bacterial genomics, which provided an additional advantage for gene expression studies, as the insert DNA was prokaryotic (Rondon *et al.*, 1999). The use of BAC library preparation thus introduced into the analysis of environmental samples for the estimation of microflora was not PCR-based. A combination of 16S rDNA sequences along with BAC studies has enabled the use of phylogenetics for the analysis of microbial communities from environmental samples (Rondon *et al.*, 1999).

The term 'metagenomics' was introduced to provide an insight on the unculturable microflora that remain unidentified and unexplored and the ones that has not yet been catalogued using 16S rDNA sequences (Handelsman, 2004). The possibility of analyzing the distribution of phylogenetic diversity (also termed as phylotypes) in natural ecosystem by isolating the total DNA from the environmental sample followed by rRNA gene sequencing was proposed by Pace in the year 1997. Metagenomic analysis involves the total DNA extraction from the environmental sample followed by gene expression studies and identification of phylogenetic makers after cloning it into a vector to prepare a cDNA library (Voget *et al.*, 2003). The sequences thus obtained may be utilized to study the ORFs by assigning it to the orthologous gene clusters obtained from databases like Kyoto Encyclopedia of Genes and Genomes (KEGG). Apart from all these possibilities, the sampling method and the molecular biology techniques involved in the metagenomic DNA extraction also play

pivotal roles in the analysis of microbial communities (Schloss and Handelsman, 2005).

### **2.12.1 Extraction of metagenomic DNA**

The objective of metagenomic DNA isolation should primarily be the extraction of DNA from a wide range of microorganisms to obtain the authentic representation of microbial population. Secondary objective should be then isolation of good quality DNA without being sheared. This can be achieved by avoiding harsh methods of DNA isolation. Tertiary objective is to obtain contaminant-free DNA, which should not hinder with the later steps like PCR, restriction and ligation (Schmeisser *et al.*, 2007). The susceptibility to the lysing procedures differ, as the soil microbial community is composed of archae, bacteria and protists (Kauffmann *et al.*, 2004). The physiologically dormant state in which some cells are present in the soil also influences the quantity of DNA obtained (Bertrand *et al.*, 2005).

The two major techniques in metagenomic DNA isolation are direct and indirect techniques. The direct extraction procedure involves the direct lysis of cells in the sample, while the indirect procedure involves the extraction of cells followed by the lysis to obtain the DNA. Direct method of DNA extraction was found to be more preferable, as it extracts more quantity of DNA than the indirect method (Schmeisser *et al.*, 2007). During the extraction of DNA, chemical or physical lysis procedures are followed either alone or in combination. The physical methods include the grinding of sample with liquid nitrogen, ultrasonication and thermal shock. For chemical lysis methods, detergents like sodium dodecyl sulfate (SDS) are used, as it dissolves hydrophobic substances that form the integral part of cytoplasmic membrane. Chelating agents like EDTA and various Tris buffers are also used (Robe *et al.*, 2003). The DNA thus extracted are purified to remove contaminants.



### 2.12.2 Purification of metagenomic DNA

As the soil a complex material, the metagenomic DNA is always associated with substances like humic acid and its removal is essential for further processes as it inhibits PCR and activities of restriction enzymes (Tebbe and Vahjen, 1993). Quinone, which is an oxidized form of humic acid and covalently bonds with DNA leading to its denaturation (Young *et al.*, 1993). Hence, the lysis for DNA extraction is usually followed by organic solvent extraction using phenol, chloroform and isopropanol (Steffan *et al.*, 1988). A purification technique using Q-Sepharose beads suspended in 10mM potassium phosphate buffer was done to obtain good quality DNA (Sharma *et al.*, 2007).

### 2.12.3 Next Generation Sequencing (NGS) and bioinformatics analysis

After the direct extraction and purification of metagenomic DNA, high-throughput sequencing (HTS) technologies are adopted to study the microbial diversity. For this purpose, Next Generation Sequencing technologies are utilized and considered to be much efficient (Shendure and Ji, 2008). NGS was proved to be comparatively reliable when it comes to the investigation of complex microbial communities, as it is lower in cost and higher throughput when compared to Sanger sequencing. The short reads generated from the illumine platform is a product of deep-coverage sequencing, but at the same time making the read based analysis difficult (Scholz *et al.*, 2012).

The primary objective of metagenomic analysis is to regenerate all the genomes present in an environmental sample. In order to obtain that, there are two methods followed: 1) Performing the taxonomic classification and functional assignments after the assembling of the contigs or 2) Reconstruction of the taxonomic and functional constituents of the metagenome (Scholz *et al.*, 2012). The metagenomic genome annotation for prokaryotic cells are usually done using online metagenome annotation services like IMG-M and MG-RAST (Meyer *et al.*, 2008). MG-RAST, being the most

commonly used genome annotation system, is an open source system used for comparative genomics (Overbeek *et al.*, 2005). The server provides access to an immense amount of data including metagenomes, metabolic and phylogenetic reconstructions to which the data in the hand can be compared (Meyer *et al.*, 2008).

### **2.13 Future trends in metagenomics**

Taking a step further from phylogenetic reconstruction and gene annotations, bioprospecting with the help of metagenomics is considered to be a possible area of utmost importance, as soil represents a valuable source of metabolite producing microorganisms (Daniel, 2005). The analysis of metabolic and functional ability of the microbial population in the soil can only be analyzed by manipulating the ribonucleic acid and hence the use of metatranscriptomics prove to be another technology to study the difference between expressed and non-expressed genes (Sorek and Cossart, 2010). Similar to the analysis of total DNA from an environmental sample, the analysis of total protein content extracted from the environmental sample was termed as 'metaproteomics' by Wilmes and Bond in 2006. The application of metagenomics has been commonly used in the field of medicine, health, food, industries and agriculture. Apart from genome studies, metatranscriptomics and metaproteomics has widened the horizons for the better understanding of the functional dynamics of soil microflora (Simon and Daniel, 2011).

# MATERIAL AND METHODS

### **3. MATERIAL AND METHODS**

The study entitled 'Metagenomic analysis of bacterial diversity in the rhizosphere of arecanut palms affected by yellowing in Wayanad' comprising laboratory experiments were carried out during the period of 2015-2017 at the department of Agricultural Microbiology, College of Horticulture, Vellanikkara. The materials used and the methods adopted in this study are presented below.

#### **3.1 MATERIAL**

##### **3.1.1 Chemicals, glassware and plasticware**

The chemicals utilized for the study were supplied by agencies Merck, SRL, Nice and HIMEDIA. The glass and plastic wares were obtained from Merck, Borosil, Tarson India Ltd. and Eppendorf respectively. The molecular biology reagents and buffers were purchased from GeNei and Sigma-Aldrich. The commercial kit for metagenomic DNA isolation, Nucleospin® soil, was obtained from Macherey-Nagel GmbH & Co. (Germany) supplied by Vision Scientific, Ernakulam.

##### **3.1.2 Equipment**

The equipment items available in the Department of Agricultural Microbiology were utilized and some facilities available in the Radio-Tracer Laboratory were also used for the study. The sterilization of microbiological media and glass wares were done using the autoclaves Equitron SLEFA and NatSteel horizontal autoclave. Incubation of cultures were carried out incubator cum shaker (GeNei OS-250) and centrifugation done using Eppendorf 5804R and SPINWIN MC-02 centrifuges. The microscopic examination of cells and photomicrography were done using a compound binocular microscope (Leica ICC50). To measure the pH of culture media, buffers and reagents, Eutech pH Tutor was used. For analyzing the total nitrogen from soil samples, KELPLUS VA DSL and DISTYL EMBA were used. The amplification of 16S rDNA was carried out using Eppendorf Mastercycler.

## **3.2 METHODOLOGY**

### **3.2.2 Collection of rhizosphere soil samples from Wayanad district**

Seven rhizosphere soil samples from each categories of yellowing affected, apparently healthy and completely healthy arecanut palms were collected from various parts of Wayanad district using HiDispo bags (Table 4). A trowel was used for collecting the rhizosphere soil samples. Locations were selected based on discussion with Officer of the Department of Agriculture, RARS, Ambalavayal and also farmers. The yellowing affected rhizosphere soil sample and its corresponding apparently healthy rhizosphere samples were collected from the same garden, while the completely healthy rhizosphere soil samples were collected from other regions where the yellowing of arecanut was not detected. Approximately 500 g rhizosphere soil was collected from each rhizosphere and the Global Positioning System (GPS) coordinates were also recorded. The soil samples were processed after thorough mixing and stored under refrigeration for further analysis.

### **3.2.3 Enumeration of rhizosphere microflora**

Enumeration of the culturable microorganisms from the arecanut rhizosphere soil samples was carried out by serial dilution and plating method using the appropriate dilution with the respective media (Sutton, 2011).

The enumeration of the culturable microflora was done using 10 different media for the differentiation of the different functional groups present in the soil samples. Nutrient agar and Luria Bertani agar were used for the enumeration of bacteria and potato dextrose agar and Martin's rose Bengal agar were used for the enumeration of fungi. For the enumeration of actinomycetes, Kenknight's agar and sodium caseinate agar was used. The enumeration of fluorescent pseudomonads, phosphate solubilizers and nitrogen fixers were done using King's B agar, Pikovskaya's agar and Jensen's agar and Ashby's agar respectively. The composition of the media are provided in

Appendix I. The dilutions were later standardized after conducting the experiments with the suitable media.

Ten gram of the soil sample was transferred under aseptic conditions, into 250 ml conical flask containing 90 ml sterilized distilled water and the contents were mixed by shaking for five minutes. One ml of the aliquot was taken and transferred to 9 ml water blank containing sterile distilled water. The suspension was then shaken for one minute for homogenization before further dilution. Dilutions up to  $10^{-6}$  were prepared for the isolation of microorganisms of specific groups. Dilutions were standardized for each media and its corresponding microorganism. Those dilutions which gave 20-200 colonies per plate was selected for further experiments (Table 1). One ml of the respective dilution was pipetted out and transferred aseptically into sterile Petri dishes. Twenty ml of molten and cooled agar media was poured into the Petri dishes. The plates were rotated clockwise and anti-clockwise manually for the uniform mixing of the aliquot with the agar media. The mixture was then allowed to solidify and incubated at room temperature in inverted position. The number of colonies in the respective agar media were observed, recorded and calculations were made to obtain the number of colony forming units per gram of soil (cfu/g) from each categories of soil samples. Based on the observations done from the dilution and plating, alterations in the dilutions were made to obtain optimum number of colony forming units.

#### **3.2.4 Purification and maintenance of isolates**

A total of 26 predominant bacterial isolates were purified and maintained as per standard procedures, as outlined below.

The bacterial colonies obtained were preserved in agar slants at a low temperature of  $4^{\circ}\text{C}$  in refrigerator and glycerol stock (broth culture containing 40% glycerol) at  $-80^{\circ}\text{C}$  in deep freezer. The fungal isolates obtained from the serial dilution experiment were also stored in PDA slants at  $4^{\circ}\text{C}$ .

**Table 1. Media used for serial dilution and plate count for the isolation of microorganisms**

<b>Microorganism</b>	<b>Media</b>
Bacteria	Nutrient agar
Bacteria	Luria Bertani agar
<i>Bacillus</i>	Nutrient agar
Fluorescent pseudomonads	King's B agar
Phosphate solubilisers	Pikovskaya agar
N-fixer	Jensen's agar
N-fixer	Ashby's agar
Actinomycetes	Kenknight's agar
Actinomycetes	Actinomycete isolation agar
Fungi	Potato dextrose agar
Fungi	Martin's Rose Bengal agar

### **3.3 Cultural characterization of purified isolates**

The cultural characters of the colonies on agar plates were observed. The size, shape, colour, optical properties and elevation of the colonies were recorded.

#### **3.3.1 Gram staining**

The purified isolates were studied for their morphological characteristics using the Gram staining method, differentiating them into Gram positive and Gram negative. A loopful of the pure culture was mixed with sterile water on a clean glass slide, to make a thin smear. A drop of the suspension was heat fixed to create a smear. The smear was heat-fixed before flooding with crystal violet (primary stain) for one minute and washed away. The smear was flooded with Gram's iodine for one minute, followed by decolourization using 95 per cent ethanol. The smear was then washed to remove



the decolourizer and flooded with safranin (counter stain) for one minute and then washed.

The smears were examined under compound, light microscope to visualize the shape and arrangement of cells. The cells were first observed at 40X magnification and then followed by 100X using oil immersion.

### **3.4 Biochemical characterization of isolates**

The isolate were subjected to biochemical tests in order to characterize the isolates based on their cellular metabolism (Cappuccino and Sherman, 1992).

#### **3.4.1 Carbohydrate fermentation test**

A carbohydrate fermentation medium containing ingredients of nutrient broth and carbohydrate source supplemented with a pH indicator, phenol red was prepared poured into boiling tubes. Durham tubes were inserted in an inverted position without any bubble formation and the tubes were autoclaved. The sterile fermentation medium was inoculated with the respective isolates with one un-inoculated tube kept as control and incubated for 24 to 48 hours at 28<sup>0</sup>C and observed for acid and gas production. The formation of yellow colour indicated acid production, while the presence of gas bubbles in the Durham tubes indicated gas production or the ability of the isolates to follow anaerobic fermentative pathways.

#### **3.4.2 IMViC test**

##### **3.4.2.1 Indole production test**

Trypticase soy broth was prepared in tubes and autoclaved. The isolates were inoculated in respective tubes with an un-inoculated tube as control and kept for incubation for a period of 24 to 48 hours at 28<sup>0</sup>C. After incubation, 10 drops of Kovac's reagent was added to all the tubes and agitated gently. The formation of cherry red layer above the broth indicated indole production.

### **3.4.2.2 Methyl red test**

The methyl red test was conducted to analyze the ability of the isolates to oxidize glucose and the subsequent production of acidic end products. Nutrient broth was prepared and the broth was distributed in tubes and sterilized. The bacterial isolates were inoculated in the respective tubes and an un-inoculated tube was kept as control. The inoculated tubes were incubated for 28 to 48 hours at a temperature of 28<sup>0</sup>C. About 5 drops of methyl red indicator was added and the change in colour was observed. The presence of red colour indicated the acid production.

### **3.4.2.3 Voges-Proskauer test**

The MR-VP medium was inoculated with the respective bacterial isolates in the same way as done for methyl red test. After inoculation and incubation for 24 to 48 hours at 37<sup>0</sup>C, 10 drops of Barritt's reagent A was added agitated gently. About 10 drops of Barritt's reagent B was added immediately and kept for 15 minutes with intermittent shaking at every 3 to 4 minutes. The tubes were observed for the presence of a pink complex that indicated the presence of acetylmethylcarbinol and hence a positive result.

### **3.4.2.4 Citrate utilization test**

Simmons' citrate agar slants were prepared and the isolates were streak inoculated into the sterile Simmons' citrate agar slants and one un-inoculated slant was kept as control. The slants were incubated at 37<sup>0</sup>C for 24 to 48 hours. The formation of deep Prussian blue colour in the slants indicated a positive result for citrate utilization, as the alkaline product sodium carbonate produced changes the colour of the indicator bromothymol blue from green to Prussian blue.

### 3.5 Chemical analysis of arecanut leaf samples

The index leaves of arecanut palms, which is the middle portion of the fourth leaf from the apex were collected (Bhat and Sujatha, 2013). The leaf samples were dried, powdered and sieved for the nutrient analysis. The methodologies followed are provided in Table 2.

**Table 2. Methodologies employed for the chemical analysis arecanut leaf samples**

Parameters	Method	Reference
Nitrogen	Micro-Kjeldahl method	Jackson, 1973
Phosphorus	Vanado-molybdo phosphoric yellow colour method	Jackson, 1973
Potassium	Diacid extract method using flame photometer	Jackson, 1973
Calcium, Magnesium	Diacid extract method using atomic absorption spectrophotometer	Jackson, 1973
Sulphur	Turbidimetric method	Hart, 1961
Iron, Manganese, Zinc, Copper	Diacid method using atomic absorption spectrophotometer	Lindsay and Norwell, 1978
Boron	Colorimetric method with Azomethine-H using diacid digest	Wolf, 1974

### 3.6 Physico-chemical analysis of the soil samples

The soil samples were analyzed for the nutrient concentrations, bulk density, pH and EC to discover the role of nutrient imbalance in the yellowing of arecanut palms (Table 3).

**Table 3. Methodologies employed for the physico-chemical and microbial biomass C analysis of rhizosphere soil samples**

Parameters	Method	Reference
Bulk density	Core sampler	Piper, 1966
Soil pH	Soil water suspension of 1:25 and read pH meter	Jackson, 1958
Electrical conductivity	Soil water suspension of 1:25 and read electrical conductivity meter	Jackson, 1958
Organic carbon	Walkley and Black method	Walkley and Black, 1934
Microbial biomass carbon	Fumigation-extraction method	Vance <i>et al.</i> , 1987
Total nitrogen	Micro-Kjeldahl method	Jackson, 1973
Available calcium and magnesium	Using atomic absorption spectrophotometer	Hesse, 1971
Available sulphur	Extraction using 0.15 per cent CaCl <sub>2</sub> turbidimetry method	Massoumi and Cornfield, 1963
Available phosphorus	Ascorbic acid reduced molybdophosphoric blue colour method	Watanabe and Olsen, 1965
Available potassium	Neutral normal ammonium acetate using photometry	Jackson, 1958
Available iron, manganese, zinc and copper	Extraction using 0.1 M HCl by atomic absorption spectrophotometer	Sims and Johnson, 1991
Available boron	Azomethine-H using spectrophotometer	Berger and Truog, 1939
Available aluminium	Aluminon colorimetric method	Jayman and Sivasubramaniam, 1974

### 3.7 *In vitro* screening for plant growth promoting (PGP) characters

The isolates obtained were screened for their ability for exhibiting PGP activities like indole acetic acid, HCN, siderophore and ammonia production and phosphate solubilization under *in vitro* conditions. The composition of the media used for the experiments are given in Appendix VI.

### 3.7.1 Screening for IAA production

The bacterial isolates were inoculated in sterile Luria-Bertani supplemented with tryptophan at the rate of 1mg/ml. The tubes were inoculated in the dark for 7 days. After incubation, the cultures were centrifuged at 3000 rpm for 30 minutes and the supernatant was collected. To the supernatant, 4 ml Salkowski reagent was added. The development of pink colour indicated the production of IAA (Ahmad *et al.*, 2008).

### 3.7.2 Estimation of IAA production

The quantification of the bacterial isolates for IAA production was carried out using the same procedure. The tubes that showed pink colouration were used for quantification by measuring the optical density at 530 nm using a spectrophotometer. The OD values were plotted on a standard graph to obtain the quantity of IAA produced by the isolates per unit volume of broth (Ahmad *et al.*, 2008).

### 3.7.3 Screening for phosphate solubilization activity

The bacterial isolates were screened for their ability to solubilize phosphate in Pikovskaya's agar. The cultures grown in Pikovskaya broth were spot inoculated on Pikovskaya's agar plates and incubated at 30<sup>0</sup>C for 7 days. The efficiency of phosphate solubilization was estimated in terms of per cent phosphate solubilization (Panhwar *et al.*, 2012).

$$\text{Solubilization efficiency (\%SE)} = \frac{\text{Solubilization diameter}}{\text{Colony diameter}} \times 100$$

### 3.7.4 Quantitative estimation of phosphate solubilization by bacterial isolates

Ten bacterial isolates that produced solubilization zones in the qualitative screening for phosphate solubilization were grown in Pikovskaya's broth for the purpose of quantification of P solubilized. Conical flasks containing 50 ml Pikovskaya's broth were inoculated with the respective isolates and incubated for 10

days at a temperature of 28<sup>0</sup>C. The quantity of soluble P in the broth after the incubation period was estimated by phospho-molybdic method and the comparison was done with an uninoculated broth used as control (Olsen, 1954). The protocol followed is mentioned below.

The inoculated Pikovskaya's broth was centrifuged at 10,000 rpm for 10 minutes to obtain the supernatant. Five ml of the supernatant was taken and made up to 8.6 ml using distilled water. To this solution, 1 ml of ammonium molybdate reagent was added followed by 0.4 ml ANSA reagent. The contents were thoroughly mixed and kept for 10 minutes for colour development. The absorbance was measured at 660 nm using spectrophotometer. The O.D values were plotted on a standard graph obtained by measuring the absorbance of solution containing known concentration of KH<sub>2</sub>PO<sub>4</sub>. The pH of the supernatant was also recorded after the incubation period to observe the production of organic acids.

### **3.7.5 Screening for HCN production**

The isolates were screened for their ability to produce hydrogen cyanide using the method introduced by Lorck (1948). Nutrient agar was supplemented with glycine at the rate of 4.4g/l. The bacterial isolates were streaked on the media and a Whatman No. 1 filter paper dipped in picric acid solution (2% sodium carbonate in 0.5% picric acid) was kept in the top portion of the Petri plates. The plates were sealed and incubated at 28<sup>0</sup>C for 4 days. The production of HCN was indicated by the formation of orange to red colour in the Whatman No.1 filter plate placed in the top of the Petri plates.

### **3.7.6 Screening for ammonia production**

Screening of the bacterial isolates was carried out by inoculating the isolates in 4 per cent peptone water and incubating at 28<sup>0</sup>C for 48 hours. After incubation, 0.5 ml of Nessler's reagent was added to the tubes. Formation of brown to yellow colour indicated the presence of ammonia (Cappuccino and Sherman, 1992).



### 3.7.7 Screening for siderophore production

The screening of bacterial isolates for siderophore production was done by Chrome Azurol Sulfonate (CAS) assay (John and Thankavel, 2015). The assay medium was prepared by dissolving 60.5 mg CAS in 50 ml distilled water, which was then mixed with Iron (III) solution (1mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 10mM HCl). The solution was then slowly added to 72.9 g hexadecyltrimethyl ammonium bromide (HDTMA) dissolved in 40 ml distilled water. The blue/green solution obtained was mixed with 100 ml nutrient agar, which was used for then screening. The bacterial isolates were spot-inoculated on the media and incubated at 30°C for 4-5 days. The presence of yellow to orange halo around the colony indicate the capability of producing siderophores by the isolates.

### 3.8 Metagenomic DNA extraction

The extraction of metagenomic DNA is done with an objective to construct metagenomic libraries of environmental samples. The three major constrains taken into consideration during the exrtraction of metagenomic DNA were: 1) Extraction of DNA from a wide range of microorganisms to obtain a representative of the microbial population 2) Shearing of DNA during the extraction procedure, as large molecular weight DNA is necessary for community analysis 3) Extraction of contaminant-free DNA for easy downstream processing (Schmeisser *et al.*, 2007). The extraction methods were also preferred to be not extremely harsh, as it may lyse recalcitrant structures like spores and Gram positive bacteria, but shear lyse-sensitive DNA (Robe *et al.*, 2003).

#### 3.8.1 Direct method of soil DNA extraction by soft lysis (Siddhapura *et al.*, 2010)

The soil sample (1g) was taken in a 30 ml centrifuge tube and suspended in 10 ml extraction buffer and kept for incubation at 37°C for 10-12 hours with continuous shaking at 150 rpm. The suspension was re-extracted using 1ml of extraction buffer, which was then centrifuged at 5000 rpm for 10 minutes to obtain the supernatant. The



supernatant was then mixed with 4ml lysis buffer and incubated at 65<sup>0</sup>C for 2 hours with vigorous shaking at an interval of 15 minutes. The sample was then centrifuged at 10,000 rpm for 10 minutes at 4<sup>0</sup>C and the upper aqueous layer was collected. A mixture of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the extracted aqueous layer and centrifuged at 10,000 rpm for 20 minutes at 4<sup>0</sup>C. The upper aqueous layer obtained was again extracted and mixed with chloroform: isoamyl alcohol (24:1) at 10,000 rpm for 10 minutes at 4<sup>0</sup>C. Subsequently, the preparation was treated with 1/10 volume of 7.5M potassium acetate and then precipitated using 2 volumes of chilled ethanol. The precipitate was obtained by centrifuging the suspension at 10,000 rpm for 10 minutes. It was air dried, and suspended in 50µl sterile distilled water.

### **3.8.2 Direct method- Short procedure (Siddhapura *et al.*, 2010)**

The soil sample (20 mg) was suspended in 400 µl extraction buffer and vortexed for 10-15 minutes. The suspension was then incubated for 1 hour at room temperature. After incubation, the supernatant was obtained by centrifuging it at 12,000 rpm for 5 minutes. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and centrifuged at 10,000 rpm for 20 minutes at 4<sup>0</sup>C and the supernatant was taken. The aqueous phase was separated and the DNA was precipitated by adding an equal volume of ice cold isopropanol followed by incubation for 15 minutes. The extract was then centrifuged at 13,000 rpm and the supernatant was discarded. The pellets obtained was washed by adding 600 µl ethanol followed by centrifugation at 10,000 rpm for 10 minutes. The pellets were air dried and suspended in 25 µl sterile distilled water.

### **3.8.3 Metagenomic DNA extraction using MACHEREY-NAGEL NucleoSpin® Soil**

The extraction of metagenomic DNA from the samples were done using the extraction buffers provided combined with mechanical sample lysis using ceramic beads. The lysis tubes were vortexed horizontally and re-extraction was also done to increase the DNA yield. The choice of lysis buffers were made based on the type of

sample and the extraction was followed by purification of DNA using NucleoSpin® Inhibitor Removal Column. The column was then washed using various buffers and the DNA was finally eluted using elution buffers to obtain 60 µl end product. The standard protocol provided in the user manual was followed to obtain the best result.

### 3.9 Agarose Gel Electrophoresis

The quality of the isolated metagenomic DNA was analyzed by agarose gel electrophoresis. The materials used for the preparation is given in Annexure IV.

The metagenomic DNA was run in 0.8 per cent agarose gel for the qualitative analysis. About 250 ml 1X TAE buffer was made from a stock solution of 50X TAE buffer (pH 8.0). The agarose gel was made by dissolving 0.8 g agarose in 100 ml 1X TAE buffer. The solution was heated for proper mixing and after cooling, ethidium bromide was added to it at a concentration of  $0.5 \mu\text{g ml}^{-1}$  prepared from a stock solution of  $10 \text{ mg ml}^{-1}$ . The agarose was then poured into a casting tray and a comb was placed with care after wiping both the casting tray and comb with alcohol. The agarose was allowed to solidify for 30.45 minutes. The comb was gently removed from the solidified gel to obtain wells and the gel was then placed in the buffer tank filled with 1X TAE buffer with the side with the well facing the cathode. About 5 µl of DNA was mixed with 2 µl gel loading dye and the mixture was carefully loaded into the respective wells using a micro-pipette. The molecular weight marker used was  $\lambda\text{DNA}/\text{EcoR1} + \text{Hind III}$  double digest (Sisco research laboratory; Biolit, Mumbai). The electrodes were connected to the power pack and a constant electric potential of 80 V was applied till the tracking dye reached almost 3 cm away from the end.

### 3.10 Gel documentation

The gel documentation was done using GeNei UVITEC Cambridge gel documentation system. The agarose gel was visualized in the presence of UV light to illuminate the DNA bands and images of the gel was captured using the software system attached to it and the band size was compared to the ladder.

### **3.11 Quantitative analysis of metagenomic DNA**

The quantity and purity of the DNA samples were analyzed using NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies., USA). The instrument was cleaned using distilled water and the blank value was taken using sterile distilled water in the case of DNA isolated using direct procedure and elution buffer in the case of DNA isolated using the commercial kit. The absorbance were measured at 260 nm and 280 nm. The 260/280 ratio and 260/230 ratio were calculated to understand the purity of the DNA samples. The quantity of DNA was also measured.

$$1 \text{ OD at } 260 \text{ nm} = 50 \mu\text{g DNA}/\mu\text{l}$$

Therefore,  $\text{OD}_{260} \times 50$  gives the quantity of DNA in  $\mu\text{g}/\mu\text{l}$

### **3.12 Metagenomic DNA sequencing**

Metagenomic DNA from nine samples (three from each category of yellowing affected, apparently healthy and completely healthy rhizosphere) were extracted and analyzed for the qualitative and quantitative standards recommended for sequencing. The samples were sequenced at a private scientific facility, AgriGenome Lab Pvt Ltd., Kochi.

#### **3.12.1 16S RNA gene amplicon library sequencing using Next Generation Illumina Miseq™**

The amplicon library was prepared by using specific primers to amplify the hypervariable regions V3 and V4 regions of 16S rRNA gene and the amplicons obtained were sequenced and subjected for the subsequent classification of microorganisms.

#### **3.12.2 16S rRNA gene library preparation**

The amplicon PCR of the soil metagenomic DNA was done using V3 and V4 primers after pooling and normalizing the metagenomic DNA to 5 ng/ $\mu\text{l}$ . The PCR

master mix was composed of 2 µl each 10 pmol/µl forward and reverse primers, 0.5 µl of 40 mM dNTP, 5 µl of 5X Phusion HF reaction buffer, 0.2 µl of 2 U/µl F-540 Special Phusion HS DNA polymerase, 5 ng input DNA and water to make up the total volumes to 25 µl. The PCR reactions were set at a preset programme of initial denaturation at 98°C for 30 seconds followed by denaturation in 30 cycles at 98°C for 10 seconds. The annealing temperature was set at 55°C for 30 seconds, primer extension at 72°C for 30 seconds and final extension at 72°C for 5 minutes followed by a hold at 4°C. The amplified product was quantified using fluorescence quantitative (Qubit 2.0®) fluorometer with the Qubit dsDNA HS assay kit (Invitrogen, USA).

### 3.12.3 PCR clean-up

The PCR clean-up to remove free primers and primer dimers were done using AMPure XP beads to obtain pure 16S rRNA gene V3 and V4 amplicon. The reagent used included 10 mM Tris pH 8.5 (52.5 µl per sample), AMPure XP beads (20 µl per sample), freshly prepared ethanol (EtOH) (80%) (400 µl per sample). Standard protocol was followed and the purified PCR product was stored at -20°C.

### 3.12.4 Index PCR

The index PCR is done with an objective to attach dual indices and Illumina sequencing adapters to the cleaned-up PCR products. The PCR master mix was composed of 2 µl each 10 pmol/µl forward and reverse primers, 1 µl of 40 mM dNTP, 10 µl of 5X Phusion HF reaction buffers, 0.4 µl of 2 U/ µl F-540 special Phusion HS DNA polymerase, 10 µl (minimum 5 ng) of PCR I amplicon and water to make up the total volume to 50 µl.

The PCR programme was set as follows: initial denaturation at 98°C for 30 seconds, denaturation at 98°C for 10 seconds repeated in 15 cycles, annealing at 55°C for 30 seconds, primer extension at 72°C for 30 seconds and final extension at 72°C for 5 minutes followed by 4°C hold.

### 3.12.5 PCR clean-up 2

The final product was cleaned-up using AMPure XP beads before the quantification. The reagents consisted of 10 mM Tris pH 8.5 (27.5 µl per sample), AMPure XP beads (56 µl per sample), freshly prepared 80 per cent ethanol (EtOH) (400 µl per sample). Standard protocol was followed and PCR product was stored at -20°C.

### 3.12.6 Library quantification, normalization and pooling

The library quantification was done using fluorometric quantification method using dsDNA binding dyes, as per Illumina recommendation. The concentrated final library was diluted using 10 mM Tris pH 8.5. Diluted DNA (5 µl) from each library was pooled with unique indices. A requirement of more than 100,000 reads per sample is considered to be sufficient for the estimation of the bacterial population of the provided sample.

### 3.12.7 Library denaturing and MiSeq sample loading

Denaturation of pooled libraries using NaOH, dilution using hybridization buffer and heat denaturation were done before MiSeq sequencing as a preparatory step before cluster generation and sequencing. The internal control of low-diversity libraries were attained using a minimum of 5% PhiX. The final step in the process was loading of the denatured library into the reagent cartridge of Illumina MiSeq™ sequencer for sequencing and the sequence were obtained in FastQ format, which was then further used for *in silico* analysis.

## 3.13 Analysis of NGS data

Quality control parameters like base quality, base composition, base distribution and GC distribution were applied on the total raw sequencing reads. The unwanted sequences were trimmed off from the original paired-end data and a consensus V3 and V4 region sequences were constructed with the help of Clustal

Omega program (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). To obtain the highest quality V3 and V4 region sequences, multiple filters like conserved region filter, spacer filter and mismatch filter were employed. Removal of singletons produced as a result of sequencing errors were removed to avoid errors in the estimation of operational taxonomic units (OTUs). The *de novo* program UCHIME implemented in the tool USEARCH was used to remove chimeras, which may be misinterpreted as novel species.

The clustering of sequences into operational taxonomic units based on sequence similarity was done using QIIME (Quantitative Insights into Microbial Ecology) software package. Identification of representative sequences from the OTUs followed by alignment against Greengenes, which offers a dataset of chimera-checked full-length 16S rRNA gene sequences were done using PyNAST program (DeSantis *et al.*, 2006).

The taxonomic classification was carried out employing MG RAST server, an open source system into which raw sequence data in fasta format was uploaded (Meyer *et al.*, 2008). The raw sequences were automatically processed and the taxonomic distribution data was generated by the MG RAST pipeline by comparing the sequences against the RDP database at 97 per cent identity and an e-value of 5. The taxonomic data was then represented using various graphs and charts and the plugin to krona was also used for the same purpose.

The manipulation of NCBI database to compare sequences using BLAST tool to obtain taxonomic information was done using the software MEGAN community edition v.6.8.13. MEGAN (MEtaGenome Analyzer) uses common ancestor algorithms to assign reads to provide graphical and statistical outputs (Huson *et al.*, 2007). The BIOM files which act as a representative of the metagenome table provides information on the number of reads and OTUs corresponding to each taxa ([www.biom-format.org](http://www.biom-format.org)), were used as the input for MEGAN. The BIOM file was inserted into MEGAN and



analysed to obtain phylogenetic trees and statistical data at various taxonomic levels, as the *in silico* tool uses the NCBI taxonomy to compare the taxonomical data to provide the graphical representation.

The Illumina sequence data was then submitted to Sequence Read Archive (SRA) of GenBank database and the accession number was obtained.

#### **Analysis of diversity between the samples using diversity indices.**

The diversity of the samples were compared with that of each other using the diversity indices *viz*, Shannon-Weaver index and Simpson index. The Shannon-Weaver index is considered to be an information statistic index using random sampling, assuming all the taxa are enlisted. The Shannon-Weaver index was calculated using the formula:

$$\text{Shannon-weaver index (H)} = - \sum_{i=1}^s P_i \ln p_i, \text{ where}$$

$P_i$  = Proportion of individuals in a particular taxa divided by the total number of individuals

$\ln$  = natural logarithm

$s$  = Number of taxa

The diversity was also compared using Simpson index, which is a dominance index as the priority given by this index is to the dominant taxa and the value is least affected by the presence of other taxa in low numbers. The Simpson index was calculated using the formula:

$$\text{Simpson index (D)} = 1 / \sum_{i=1}^s P_i^2, \text{ where}$$

$P_i^2$  = Square of the proportion of individuals in a particular taxa divided by the total number of individuals

$s$  = Number of taxa



# RESULTS

## 4. RESULTS

The results of the investigation entitled 'Metagenomic analysis of the bacterial diversity in the rhizosphere of arecanut palms affected by yellowing in Wayanad' carried out during the period of 2015-2017 at the Department of Agricultural Microbiology, College of Horticulture, are presented below.

### 4.1 Collection of rhizosphere soil and leaf samples

The rhizosphere soil samples from seven plantations containing yellowing affected, apparently healthy and completely healthy arecanut palms were collected from various parts of Wayanad district. The yellowing affected rhizosphere soil sample and its corresponding apparently healthy soil samples were collected from the same respective garden. Yellowing affected and apparently healthy rhizosphere soil samples were collected from the low lying areas like Meenangadi and Kakkavayal, which were mostly used for paddy cultivation. The completely healthy rhizosphere soil samples were collected from areas near Ambalavayal and Kolagappara (Plate 1). The corresponding index leaf from the palms were also collected. The coordinates and altitude of the locations of sample collection has been listed in Table 4.

**Table 4. Coordinates and altitudes of the area selected for sample collection**

Sample	Location	Latitude	Longitude	Elevation (ft)
YL-1, AH-1	Meenangadi	N 11.65687 <sup>0</sup>	E 076.15150 <sup>0</sup>	2439
YL-2, AH-2		N 11.65678 <sup>0</sup>	E 076.15096 <sup>0</sup>	2443
YL-3, AH-3		N 11.65538 <sup>0</sup>	E 076.15030 <sup>0</sup>	2440
YL-4, AH-4	Kakkavayal	N 11.64738 <sup>0</sup>	E 076.13892 <sup>0</sup>	2494
YL-5, AH-5		N 11.64691 <sup>0</sup>	E 076.13839 <sup>0</sup>	2516
YL-6, AH-6		N 11.64741 <sup>0</sup>	E 076.13796 <sup>0</sup>	2494
YL-7, AH-7		N 11.64790 <sup>0</sup>	E 076.13667 <sup>0</sup>	2462
CH-1	Kolagappara	N 11.64539 <sup>0</sup>	E 076.21572 <sup>0</sup>	2640
CH-2		N 11.64547 <sup>0</sup>	E 076.21534 <sup>0</sup>	2623
CH-3		N 11.64473 <sup>0</sup>	E 076.21590 <sup>0</sup>	2651
CH-4	Ambalavayal	N 11.64428 <sup>0</sup>	E 076.21619 <sup>0</sup>	2666
CH-5		N 11.63154 <sup>0</sup>	E 076.22269 <sup>0</sup>	2991
CH-6		N 11.62266 <sup>0</sup>	E 076.22204 <sup>0</sup>	2876
CH-7		N 11.62024 <sup>0</sup>	E 076.22100 <sup>0</sup>	2895



**Plate 1.a. Collection of rhizosphere samples.**



**Plate 1.a Identified locations**

**Plate 1. Collection of rhizosphere samples from the identified locations of Wayanad district.**

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**Symptoms on leaflets**



**Crown with bunchy appearance**

**Plate 2.a. Yellowing affected palms**



**Plate 2.b. Apparently healthy arecanut palms**



**Plate2.c. Completely healthy arecanut palms**

**Plate 2. Three categories of arecanut palms identified**

## **4.2 Physico-chemical properties of arecanut rhizosphere soil samples collected from Wayanad**

The rhizosphere soil samples collected from each category (yellowing affected, apparently healthy and completely healthy) were analysed for physico-chemical properties. The parameters measured included bulk density ( $\text{Mg m}^{-3}$ ), electrical conductivity ( $\text{dS m}^{-1}$ ), pH, organic carbon (%) and soil nutrient contents. The primary nutrients total nitrogen (%), available phosphorus ( $\text{kg ha}^{-1}$ ) and available potassium ( $\text{kg ha}^{-1}$ ) were analysed. The secondary nutrients analysed were available calcium ( $\text{mg/kg}$ ), available magnesium ( $\text{mg/kg}$ ) and available sulphur ( $\text{mg/kg}$ ). The micronutrients included copper ( $\text{mg/kg}$ ), iron ( $\text{mg/kg}$ ), zinc ( $\text{mg/kg}$ ), manganese ( $\text{mg/kg}$ ), boron ( $\text{mg/kg}$ ) and aluminium ( $\text{mg/kg}$ ) (Tables 5,6 and 7).

### **4.2.1 Bulk density**

Bulk density of all the soil samples was analysed and there was no significant difference in the bulk density between the categories of samples. Among the yellowing affected rhizosphere soil samples, the lowest value was observed in YL-6 with  $1.11 \text{ Mg m}^{-3}$  and the highest value in the samples YL-1 and YL-2 with a bulk density of  $1.33 \text{ Mg m}^{-3}$ . In the apparently healthy samples, the lowest bulk density was observed in AH-4 and AH-6 ( $1.05 \text{ Mg m}^{-3}$ ) and the highest in AH-2 ( $1.33 \text{ Mg m}^{-3}$ ). The completely healthy samples had the lowest bulk density of  $1.11 \text{ Mg m}^{-3}$  in the sample CH-6 and the highest in the samples CH-1, CH-2, CH-5 and CH-6 ( $1.33 \text{ Mg m}^{-3}$ ). The bulk density of all the soil samples has been provided in Table 5.

### **4.2.2 pH and electrical conductivity**

The pH and electrical conductivity showed variation among the samples. The soil samples from yellowing affected arecanut rhizosphere were found to be acidic in nature. The samples YL-1, YL-2 and YL-4 showed strong acidic nature with a pH of 5.4, 5.5 and 5.1 respectively and the samples YL-3, YL-5 and YL-7 were very strongly

acidic with a pH less than 5, while the sample YL-6 was moderately acidic with a pH 5.7.

The soil samples collected from the apparently healthy rhizosphere also showed acidic nature with the samples AH-1, AH-3 and AH-4 being strongly acidic with pH 5.1, 5.2 and 5.2 respectively, while the samples AH-2, AH-4, AH-5 and AH-7 showed very strongly acidic nature, with pH ranging from 4.8 to 5.0. The sample AH-6 was observed to be moderately acidic with a pH of 5.8. The soil samples collected from completely healthy arecanut rhizospheres were found to be comparatively less acidic than the yellowing affected and apparently healthy counterpart. The samples CH-1, CH-3, CH-4 and CH-6 were strongly acidic, while the samples CH-2 and CH-5 were found to be moderately acidic and slightly acidic with pH 5.6 and 6.1 respectively. The sample CH-7 was observed to be neutral with a pH of 7.3. A completely randomized design was used for the analysis of the samples in three categories and the treatment CH was found to exhibit significantly high pH at 5% level of significance, with the pH of the AH and YL samples on par with a CD (0.05) value 0.57.

The electrical conductivity of all the 21 samples were found to be normal with the lowest value of 0.03 dS m<sup>-1</sup> for the sample CH-2 and the highest value for the sample CH-6 with an electrical conductivity of 0.5 dS m<sup>-1</sup>.

#### **4.2.3 Organic carbon**

Among the rhizosphere soil samples collected from the yellowing affected rhizospheres, the sample YL-1 showed low organic carbon (0.68%), while the other samples showed medium level of organic carbon. The samples AH-1, AH-2 and AH-4 collected from the apparently healthy rhizospheres showed low organic carbon, while the samples AH-3, AH-5, AH-6 and AH-7 showed medium level of organic carbon. Among the completely healthy rhizosphere soil samples, CH-1 and CH-2 were found to have low organic carbon and samples CH-4 and CH-5 showed medium level of organic carbon. The samples CH-3 and CH-6 were high in organic carbon with a value

of 1.51 and 1.76 per cent respectively. No significant difference was observed between the yellowing affected, apparently healthy and completely healthy samples.

#### **4.2.4 Total nitrogen**

The total nitrogen was analysed from all the soil samples and the lowest value among the yellowing affected rhizosphere soil samples was observed in YL-7 with 0.028 per cent and the highest in YL-6 with 0.28 per cent. The lowest value of total nitrogen among the apparently healthy rhizosphere samples was found in AH-2 (0.056%) and highest in AH-7 (0.56%). The completely healthy rhizosphere soil samples showed highest total nitrogen in the sample CH-6 (0.448%) and lowest in the samples CH-2 and CH-7 with 0.056 per cent total nitrogen in both the samples. A significant difference in the total nitrogen content between the three categories of soil samples were not observed.

#### **4.2.5 Available phosphorus**

The available phosphorus was analysed from all the soil samples and a wide range of values were obtained from each category. In the yellowing affected rhizosphere soil samples, the lowest value was found to be 8.62 kg/ha in YL-3 and highest in YL-6 with 75.24 kg/ha. The apparently healthy rhizosphere soil samples showed the lowest of 7.47 kg/ha in AH-4 and highest in AH-5 with a value of 105.68 kg/ha. The completely healthy rhizosphere soil samples showed a less diversity in the values with the lowest being 13.21 kg/ha in CH-2 and the highest being 29.29 kg/ha in the sample CH-5. No significant difference in available phosphorus between the three soil categories were observed.

#### **4.2.6 Available potassium**

The highest value of potassium among the yellowing affected rhizosphere samples was recorded by YL-2 (621.60 kg/ha) and the lowest by YL-7 (41.44 kg/ha). Among the apparently healthy rhizosphere soil samples, the highest potassium content



was recorded in AH-6 (1025.92 kg/ha) and lowest in AH-2 (75.04 kg/ha). In completely healthy rhizosphere soil samples, the highest potassium content was detected in CH-6 (892.64 kg/ha) and lowest in 26.88 kg/ha). A significant difference in available potassium between the three soil categories was not established.

#### **4.2.7 Available calcium**

The available calcium was analysed from all samples and the highest value among the yellowing affected samples was recorded in YL-6 (1255 mg/kg) and the lowest in YL-3 (362.10 kg/ha). Among the apparently healthy rhizosphere soil samples, the highest calcium content was detected in AH-6 (885.40 mg/kg) and the lowest in AH-2 (262.60 mg/kg). The highest and lowest values in the completely healthy rhizosphere soil samples were observed in CH-7 (723.90 mg/kg) and CH-2 (116.30 mg/kg) respectively. There was no significant difference in available calcium between the three categories of soil samples.

#### **4.2.8 Available magnesium**

The highest available magnesium content in the yellowing affected rhizosphere soil samples was observed in YL-6 (165.70 mg/kg) and the lowest in YL-5 (65.00 mg/kg). Among the apparently healthy rhizosphere soil samples, the highest concentration was found in AH-4 (209.70 mg/kg) and lowest concentration in AH-2 (34.40 mg/kg). The highest and lowest values in completely healthy rhizosphere soil samples were recorded from CH-3 (160.40 mg/kg) and CH-2 (21.40 mg/kg) respectively. No significant difference in available magnesium among three soil categories was observed.

#### **4.2.9 Available sulphur**

Among the yellowing affected rhizosphere soil samples, the highest sulphur content was detected in YL-6 (7.06 mg/kg) and lowest in YL-3 (3.66 mg/kg). The highest sulphur content in apparently healthy rhizosphere soil samples was found in

AH-5 (9.41 mg/kg) and the lowest in AH-2 (3.92 mg/kg). The highest sulphur concentration in completely healthy rhizosphere soil samples were found in CH-6 and CH-7 with 10.98 mg/kg in both the samples and the lowest in CH-1 with 3.14 mg/kg sulphur. No significant difference in the available sulfur content between the three categories of soil was observed.

#### **4.2.10 Copper**

The yellowing affected rhizosphere soil sample showed the highest copper concentration in the sample YL-6 (11.35 mg/kg) and the lowest concentration in YL-5 (3.04 mg/kg). The highest and lowest concentration in apparently healthy rhizosphere soil samples were found in AH-7 (7.87 mg/kg) and AH-3 (2.17 mg/kg). Among the completely healthy rhizosphere soil samples, the highest and lowest values were observed in CH-6 (5.21 mg/kg) and CH-4 (0.51 mg/kg) respectively.

The copper content was found to be significantly high in yellowing affected samples (YL), which was on par with apparently healthy samples (AH), while the completely healthy samples (CH) were found to have significantly less copper content compared to the other two categories. The treatments were found to be significant at 5% level with a CD (0.05) value 3.247.

#### **4.2.11 Iron**

The highest concentration of iron among the yellowing affected rhizosphere soil samples was observed in YL-7 (198.90 mg/kg) and the lowest in YL-4 (59.81 mg/kg). The highest and lowest values in apparently healthy rhizosphere soil samples were observed in AH-6 (159.30 mg/kg) and AH-4 (27.34 mg/kg) respectively. Among the completely healthy rhizosphere soil samples, the highest and lowest concentrations of iron were found in CH-6 (118.80 mg/kg) and CH-3 (22.72 mg/kg). The iron concentrations in yellowing affected soil samples (YL) were found to be significantly high compared to the other two samples at 5% level with a CD (0.05) value 49.713.

The treatment YL was on par with AH, while CH was significantly less in iron concentration.

#### **4.2.12 Manganese**

The highest manganese concentration among the yellowing affected rhizosphere soil samples was found in YL-4 (50.22 mg/kg) and the lowest in YL-3 (8.01 mg/kg). Among the apparently healthy rhizosphere soil samples, the highest concentration was recorded in AH-6 (38.65 mg/kg) and the lowest in AH-2 (6.45 mg/kg). The highest and lowest concentrations among the completely healthy rhizosphere soil samples were observed in the samples CH-6 (85.97 mg/kg) and CH-2 (3.00 mg/kg). No significant difference in the manganese concentrations between the three soil categories was observed.

#### **4.2.13 Zinc**

Among the yellowing affected rhizosphere soil samples, the highest concentration of zinc was recorded in YL-6 (5.63 mg/kg) and the lowest in YL-1 (1.05 mg/kg). The highest zinc concentration among the apparently healthy rhizosphere soil samples was recorded in AH-6 (10.00 mg/kg) and the lowest in AH-2 (0.76 mg/kg). The highest and lowest concentrations in the completely healthy rhizosphere soil samples were recorded in CH-5 (3.03 mg/kg) and CH-2 (0.34 mg/kg). There was no significant difference in the zinc concentrations between the three categories of soil samples.

#### **4.2.14 Boron**

The highest boron concentration in yellowing affected rhizosphere soil samples was recorded in YL-3 (0.46 mg/kg) and the lowest in YL-1 (0.02 mg/kg). Among the apparently healthy rhizosphere soil samples, the highest boron concentration was found in AH-3 and AH-5 with 0.38 mg/kg in both samples. The highest and lowest concentrations of boron in completely healthy rhizosphere soil samples were recorded

in CH-6 (0.48 mg/kg) and CH-7 (0.24 mg/kg). No significant difference in the boron concentration was found between the three categories of soil samples.

#### **4.2.15 Aluminium**

The aluminium content in the rhizosphere soil samples were analysed and most of the samples with pH above 5.0 failed to show the concentration of available aluminium. Among the yellowing affected rhizosphere soil samples, the samples YL-3, YL-5 and YL-7 were observed to show 1.185, 0.585 and 0.453 cmol per 1 kg soil respectively. Among the apparently healthy rhizosphere soil samples, the highest value was recorded from AH-2 (1.217 cmol/kg) and lowest in AH-5 (0.173 cmol/kg) among the 4 samples that showed reading. The completely healthy rhizosphere soil samples had a comparatively less concentration of available aluminium recorded from the samples CH-2, CH-3 and CH-4 with 0.089, 0.068 and 0.637 cmol/kg respectively. Even though the difference in aluminium concentration was detected, statistical significance between three soil categories was not established.

**Table 5. Physico-chemical parameters of the yellowing affected and apparently healthy arecanut rhizosphere soil samples**

Samples	pH	EC	Bulk density (Mg/m <sup>3</sup> )	OC (%)	N (%)	P (kg/ha)	K (kg/ha)	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	B (mg/kg)	Al (cmol protons / kg)
YL-1	5.4	0.12	1.33	0.68	0.112	20.68	100.80	737.40	95.70	5.49	159.30	11.01	1.05	3.61	0.02	-
YL-2	5.5	0.13	1.33	0.95	0.056	45.95	621.60	458.00	89.80	6.54	148.30	16.44	1.71	3.99	0.36	-
YL-3	4.9	0.07	1.17	0.87	0.056	8.62	190.40	362.10	80.30	3.66	74.48	8.01	1.07	3.28	0.46	1.185
YL-4	5.1	0.18	1.17	1.45	0.14	17.81	498.40	833.50	124.70	5.49	59.81	50.22	2.68	7.60	0.30	-
YL-5	4.6	0.23	1.25	0.95	0.168	14.36	135.52	530.10	65.00	4.97	84.10	36.06	1.10	3.04	0.38	0.585
YL-6	5.7	0.23	1.11	1.10	0.28	75.24	364.00	1255.00	165.70	7.06	173.30	43.16	5.63	11.35	0.16	-
YL-7	4.7	0.14	1.25	0.93	0.028	12.64	41.44	393.10	81.20	4.97	198.90	8.28	1.33	11.32	0.42	0.453
<b>Apparently healthy rhizosphere soil samples</b>																
AH-1	5.1	0.07	1.25	0.69	0.112	14.36	211.68	370.60	57.10	5.23	74.07	12.77	1.16	3.30	0.22	0.803
AH-2	5.0	0.07	1.33	0.66	0.056	9.76	75.04	262.60	34.40	3.92	108.80	6.45	0.76	3.11	0.32	1.217
AH-3	5.2	0.23	1.11	1.10	0.112	10.34	95.20	503.80	135.40	4.18	98.89	10.59	1.49	2.17	0.38	-
AH-4	5.2	0.15	1.05	0.43	0.224	7.47	627.20	784.50	209.70	7.85	27.34	17.32	1.40	7.15	0.36	-
AH-5	4.8	0.18	1.11	1.45	0.224	105.68	274.40	754.60	121.90	9.41	70.63	35.56	3.85	7.67	0.38	0.173
AH-6	5.8	0.32	1.05	1.16	0.252	60.31	1025.92	885.40	150.10	8.11	159.30	38.65	10.00	8.64	0.30	-
AH-7	4.9	0.09	1.25	1.06	0.56	11.49	124.32	390.10	88.30	4.45	110.70	7.40	0.85	8.78	0.42	1.28

**Table 6. Physico-chemical parameters of the completely healthy arecanut rhizosphere soil samples**

Samples	pH	EC	Bulk density (Mg/m <sup>3</sup> )	OC (%)	N (%)	P (kg/ha)	K (kg/ha)	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	B (mg/kg)	Al (cmol protons/kg)
CH-1	5.5	0.05	1.33	0.56	0.168	15.51	81.76	116.50	54.50	3.14	65.62	9.74	2.87	1.01	0.28	-
CH-2	5.6	0.03	1.33	0.43	0.056	13.21	26.88	116.30	21.40	2.88	88.00	3.00	0.34	0.57	0.32	0.089
CH-3	5.4	0.12	1.25	1.51	0.112	18.38	285.60	573.00	160.40	6.28	22.72	45.14	2.73	0.66	0.44	0.068
CH-4	5.4	0.10	1.17	1.02	0.392	17.23	291.20	459.10	95.90	6.54	37.54	19.43	1.16	0.51	0.46	0.637
CH-5	6.1	0.14	1.33	1.00	0.224	29.29	212.80	435.00	75.40	5.23	28.00	20.53	3.03	1.03	0.44	-
CH-6	5.4	0.50	1.11	1.76	0.448	18.95	892.64	346.50	148.70	10.98	118.80	85.97	2.91	5.21	0.48	-
CH-7	7.3	0.13	1.33	1.00	0.056	22.97	257.60	723.90	77.70	10.98	66.01	75.18	1.74	1.70	0.24	-

**YL- Yellowing affected: AH- Apparently Healthy: CH- Completely Healthy**

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Table 7. Comparison of soil physico-chemical parameters among the three categories using CRD.

Samples	pH	EC	Bulk density (Mg/m <sup>3</sup> )	OC (%)	N (%)	P (kg/ha)	K (kg/ha)	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	B (mg/kg)	Al (mol protons/kg)
YL	5.13 <sup>b</sup>	0.16	1.23	0.99	0.12	27.9	278.88	652.74	100.34	5.45	128.31 <sup>a</sup>	24.74	2.08	6.31 <sup>a</sup>	0.3	0.84
AH	5.14 <sup>a</sup>	0.16	1.16	0.93	0.22	31.34	347.68	564.51	113.84	6.16	92.91 <sup>ab</sup>	18.39	2.79	5.83 <sup>a</sup>	0.34	0.89
CH	5.81 <sup>a</sup>	0.15	1.26	1.04	0.21	19.36	292.64	395.76	90.57	6.57	60.95 <sup>b</sup>	36.99	2.11	1.53 <sup>b</sup>	0.38	0.45
CD (0.05)	0.57	NS	NS	NS	NS	NS	NS	NS	NS	NS	49.713	NS	NS	3.247	NS	NS

YL- Yellowing affected; AH- Apparently Healthy; CH- Completely Healthy

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### **4.3 Nutrient status of arecanut leaf samples collected from Wayanad**

The index leaf of the arecanut palm, which is the middle portion of the fourth leaf from the apex was collected. Leaf samples from 7 palms of each categories *viz.*, yellowing affected, apparently healthy and completely healthy arecaut palms were collected. The leaves were then analysed for its nutrient contents after processing. The samples were analysed for nitrogen (%), phosphorus (%), potassium (%), calcium (%), magnesium (%), sulphur (%), copper (ppm), iron (ppm), manganese (ppm), zinc (ppm), boron (ppm) and aluminium (ppm) (Table 8 and 9).

#### **4.3.1 Nitrogen**

The nitrogen content in the leaf samples were analysed and the highest value among the yellowing affected samples was recorded in YL-6 (2.52%) and the lowest in YL-3 (1.59%). Among the apparently healthy leaf samples, the highest nitrogen content was observed in the sample AH-3 (2.56%) and the lowest in AH-5 (1.68%). The completely healthy leaf samples had the highest nitrogen content of 2.48% in CH-4 and lowest of 1.89% in CH-1.

#### **4.3.2 Phosphorus**

The highest phosphorus content among the yellowing affected leaf samples was from YL-7 (0.258%) and the lowest from YL-6 (0.156%). The apparently healthy leaf samples had the highest phosphorus content in AH-1 (0.298%) and the lowest in AH-6 (0.66%). Among the completely healthy leaf samples, the highest phosphorus concentration was recorded in CH-6 (0.386%) and the lowest in CH-4 (0.09%).

#### **4.3.3 Potassium**

The yellowing affected leaf samples were analysed for potassium content and the highest concentration among them was recorded in YL-7 (0.638%) and the lowest in YL-6 (0.511%). The highest potassium content among the apparently healthy leaf samples was recorded in AH-2 (0.69%) and the lowest in AH-6 (0.39%). Among the

completely healthy leaf samples, the highest concentration was recorded in CH-6 (0.861%) and the lowest in CH-3 (0.516%).

#### **4.3.4 Calcium**

The calcium content among the yellowing affected leaf samples showed its peak value in YL-5 (0.462%) and the lowest value in YL-3 (0.276%). The highest and lowest calcium concentration among the apparently healthy samples were observed in AH-1 (0.460%) and AH-6 (0.145%) respectively. The completely healthy leaf samples exhibited the highest concentration in CH-2 (0.489%) and the lowest in CH-3 (0.322%).

#### **4.3.5 Magnesium**

Among the yellowing affected leaf samples, the highest magnesium concentration was found in YL-4 (0.151%) and the lowest in YL-3 (0.045%). The apparently leaf samples showed its highest magnesium concentration in AH-5 (0.173%) and the lowest in AH-6 (0.056%). The highest concentration among the completely healthy leaf samples was recorded from CH-6 (0.220%) and the lowest from CH-3 (0.084%).

#### **4.3.6 Sulphur**

The sulphur concentration in the leaf samples were analysed and the highest concentration among the yellowing affected leaf samples was found in YL-1 (0.816%) and the lowest in YL-3 (0.433%). Among the apparently healthy leaf samples, the highest concentration was recorded in AH-5 (1.031%) and the lowest in AH-6 (0.259%). The completely healthy leaf samples had the highest sulphur concentration in CH-6 (1.622%) and the lowest in CH-1 (0.506%).

#### 4.3.7 Copper

The leaf samples were analysed for copper content and among the yellowing affected leaf samples, the highest concentration was found in YL-2 (61.9 mg/l) and the lowest in YL-5 (8.4 mg/l). Among the apparently healthy leaf samples, the highest concentration was recorded in AH-2 (64.8 mg/l) and the lowest in AH-6 (15.3mg/l). The completely healthy leaf samples had the highest concentration of copper in CH-6 (93.4 mg/l) and the lowest in CH-4 (9.4 mg/l).

#### 4.3.8 Iron

The highest iron concentration among the yellowing affected leaf samples was observed in YL-1 (874 mg/l) and the lowest in YL-6 (722 mg/l). The highest and lowest concentrations of iron among the apparently healthy leaf samples were recorded in AH-1 (852 mg/l) and AH-6 (550 mg/l) respectively. Among the completely healthy leaf samples, the highest iron concentration was found in CH-1 (713 mg/l) and the lowest in CH-4 (305 mg/l). The iron content in the treatment YL was found to be significantly higher, which was on par with AH at 5% level of significance, while the samples in CH category had comparatively less iron content.

#### 4.3.9 Manganese

The concentration of manganese was analysed from all the leaf samples and among the yellowing affected leaf samples, the highest concentration was detected in the sample YL-6 (87.8 mg/l) and the lowest in YL-5 (53.5 mg/l). Among the apparently healthy leaf samples, the highest manganese concentration was recorded in AH-5 (168.8 mg/l) and the lowest in AH-6 (22.1 g/l). The highest and lowest concentrations in completely healthy leaf samples were recorded in CH-6 (121.6 mg/l) and CH-3 (48.1mg/l) respectively. The concentration of manganese was found to be significantly high in the treatment AH, which was on par with YL. The treatment was found to be significantly high at 5% level of significance with a CD value 31.734.

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#### 4.3.10 Zinc

The highest concentration of zinc among the yellowing affected leaf samples was detected in YL-2 (33.3 mg/l) and the lowest in YL-3 (24.6 mg/l). Among the apparently healthy leaf samples, the highest concentration of zinc was recorded in AH-5 (33.9 mg/l) and the lowest in AH-6 (13.0 mg/l). The highest and lowest zinc content among the completely healthy leaf samples were recorded in the samples CH-6 (37.8 mg/l) and CH-3 (21.8 mg/l) respectively.

#### 4.3.11 Boron

The boron content among the yellowing affected leaf samples were found to be highest in YL-6 (35.238 mg/l) and lowest in YL-2 (3.809 mg/l). Among the apparently healthy leaf samples, the highest boron concentration was recorded in AH-3 (23.809 mg/l) and the lowest in AH-1 (4.762 mg/l). The highest and lowest values in completely healthy leaf samples were recorded in CH-4 (19.99 mg/l) and CH-3 (3.809 mg/l) respectively.

**Table 8. Comparison of leaf nutrient concentrations of the three sample categories using CRD**

Samples	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
YL	2.19	0.196	0.577	0.367	0.115	0.697	793.429 <sup>a</sup>	73.6	27.886	46.15	14.286
AH	2.273	0.251	0.601	0.386	0.126	0.759	727.857 <sup>ab</sup>	101.143	25.914	48.317	15.476
CH	2.264	0.241	0.659	0.453	0.142	0.931	640.143 <sup>b</sup>	85.414	29.557	58.617	14.828
CD (0.05)	NS	NS	NS	NS	NS	NS	119.253	NS	NS	NS	NS

YL- Yellowing affected; AH- Apparently Healthy; CH- Completely Healthy

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**Table 9. Nutrient content analysed from the arecanut leaf samples**

Samples	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
<b>Yellowing affected leaf samples</b>											
YL 1	2.15	0.233	0.608	0.333	0.119	0.816	874	74.8	29	47.7	4.762
YL 2	2.03	0.233	0.617	0.369	0.138	0.794	806	70	33.3	61.9	3.809
YL 3	1.59	0.163	0.522	0.276	0.045	0.433	788	67.8	24.6	49.4	22.857
YL 4	2.2	0.159	0.597	0.403	0.151	0.618	753	101	27.9	45.8	19.047
YL 5	2.42	0.172	0.545	0.462	0.109	0.692	791	53.5	24.9	8.4	15.238
YL 6	2.52	0.156	0.511	0.311	0.119	0.804	722	87.8	26.8	30.5	35.238
YL 7	2.42	0.258	0.638	0.417	0.121	0.719	820	60.3	28.7	41.6	9.524
<b>Apparently healthy leaf samples</b>											
AH 1	2.06	0.298	0.64	0.460	0.136	0.856	852	102	27.7	40.8	4.762
AH 2	2.53	0.297	0.69	0.346	0.136	0.899	796	103	30.3	64.8	9.524
AH 3	2.56	0.229	0.591	0.454	0.119	0.642	759	78.8	29.5	28.1	23.809
AH 4	2.08	0.258	0.665	0.333	0.115	0.858	729	149.1	25.5	56.4	11.428
AH 5	1.68	0.218	0.593	0.519	0.173	1.031	717	168.8	33.9	48.2	13.333
AH 6	2.52	0.066	0.39	0.145	0.054	0.259	550	22.1	13.0	15.3	9.524
AH 7	2.48	0.208	0.637	0.448	0.146	0.767	692	84.2	21.5	51.6	13.333
<b>Completely healthy leaf samples</b>											
CH 1	1.89	0.175	0.573	0.342	0.108	0.506	713	67	31.1	39.2	14.285
CH 2	2.14	0.229	0.639	0.489	0.149	0.798	726	90.7	26.3	57.7	15.238
CH 3	2.11	0.118	0.516	0.322	0.084	0.541	669	48.1	21.8	20.5	3.809
CH 4	2.48	0.09	0.63	0.513	0.130	0.850	305	67.5	28.4	9.8	19.999
CH 5	2.38	0.304	0.714	0.488	0.157	1.154	656	113	29.6	78.4	17.143
CH 6	2.25	0.386	0.861	0.545	0.220	1.622	709	121.6	37.8	93.4	5.714
CH 7	2.6	0.234	0.681	0.469	0.148	1.043	703	90	31.9	62.5	7.619

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## **4.4 Biological properties of the soil**

### **4.4.1 Microbial biomass carbon**

The microbial biomass carbon in all the samples were estimated and among the yellowing affected rhizosphere soil samples, the highest value of microbial biomass carbon was observed in the sample YL-4 ( $675.2 \mu\text{g g}^{-1}$ ) and the lowest in YL-6 ( $153.6 \mu\text{g g}^{-1}$ ). Among the apparently healthy rhizosphere soil samples, the highest microbial organic content was observed in the sample AH-4 ( $576.8 \mu\text{g g}^{-1}$ ) and the lowest in AH5 ( $132.31 \mu\text{g g}^{-1}$ ). The completely healthy rhizosphere soil samples had the highest microbial biomass carbon in the sample CH3 ( $912.1 \mu\text{g g}^{-1}$ ) and the lowest in the sample CH5 ( $190.68 \mu\text{g g}^{-1}$ ). The microbial biomass carbon values for all the samples has been enlisted in Table 10, 11 and 12.

### **4.4.2 Enumeration of culturable microbial population from the soil samples**

The population of bacteria, fungi, actinomycetes, phosphate solubilizers, nitrogen fixers, fluorescent pseudomonads, *Trichoderma* and *Bacillus* were estimated using an array of media and suitable dilutions (Plate 3). The population of the above mentioned microorganisms from all the 21 rhizosphere soil samples are illustrated in Tables 10, 11 and 12.

### **4.4.3 Bacteria**

The bacterial population in all the samples were enumerated and the results were found to be not significantly different. Among the yellowing affected rhizosphere soil samples, the highest population of bacteria was found in the sample YL-6 with  $54.1 \times 10^5 \text{ cfu g}^{-1}$  and the lowest bacterial population in YL-1 ( $6.6 \times 10^5 \text{ cfu g}^{-1}$ ). The apparently healthy rhizosphere soil samples were also analysed for the bacterial population and the highest population was recorded in the sample AH-7 with  $6.2 \times 10^6 \text{ cfu g}^{-1}$  soil sample and the lowest in the sample AH-6 with  $12.8 \times 10^5 \text{ cfu g}^{-1}$  soil sample. Among the completely healthy rhizosphere soil samples, the highest bacterial



population was estimated from the sample CH-3 with  $9.25 \times 10^6$  cfu  $g^{-1}$  soil sample and the lowest in the sample CH-1 with  $16.2 \times 10^5$  cfu  $g^{-1}$  soil sample.

#### 4.4.4 Fungi

There was no significant difference among the three categories of samples with respect to fungal population. The yellowing affected rhizosphere soil samples were analysed for the fungal population and the highest population was recorded from the sample YL-7 with  $34.4 \times 10^2$  cfu  $g^{-1}$  soil sample and the lowest from the YL-1 with  $6.6 \times 10^2$  cfu  $g^{-1}$  soil. Among the apparently healthy rhizosphere soil samples, the highest fungal population was recorded from AH-4 ( $41.2 \times 10^2$  cfu  $g^{-1}$ ) and the lowest from AH-1 ( $10.5 \times 10^2$  cfu  $g^{-1}$ ). The highest fungal population among the completely healthy rhizosphere soil samples was recorded from YL-7 ( $42.2 \times 10^2$  cfu  $g^{-1}$ ) and the lowest from CH-2 ( $9.5 \times 10^2$  cfu  $g^{-1}$ ).

#### 4.4.5 Actinomycetes

Among the yellowing affected rhizosphere soil samples, the highest population was recorded from the sample YL-7 with  $42.1 \times 10^2$  cfu  $g^{-1}$  soil sample and no colonies were obtained from the samples YL-1 and YL-2 due to high fungal growth on the plates. Among the apparently healthy rhizosphere soil samples, the highest population was recorded from AH-7 ( $36.3 \times 10^2$  cfu  $g^{-1}$  soil sample) and no colonies were found in AH-1 and AH-2 due to high fungal growth. The actinomycete population in the completely healthy rhizosphere soil samples was found to be highest in CH-7 ( $41.1 \times 10^2$  cfu  $g^{-1}$ ) and no colonies were obtained from the samples CH-1 and CH-2.

#### 4.4.6 Phosphate solubilizers

The enumeration of phosphate solubilizers bacteria and fungi was carried out. The yellowing affected rhizosphere soil samples had the highest population of phosphate solubilizers in the sample YL-5 ( $10.5 \times 10^3$  cfu  $g^{-1}$  soil sample) and the lowest in YL-3 ( $2.2 \times 10^3$  cfu  $g^{-1}$  soil sample). Among the apparently healthy



rhizosphere soil samples, the highest population was observed in AH-2 ( $13.7 \times 10^3$  cfu  $g^{-1}$  soil sample) and the lowest population in the sample AH-3 with  $1 \times 10^3$  cfu  $g^{-1}$  soil sample. The completely healthy rhizosphere soil samples showed the highest population of phosphate solubilizers in the sample CH-5 ( $52 \times 10^3$  cfu  $g^{-1}$  soil) and the lowest in CH-3 ( $2.3 \times 10^3$  cfu  $g^{-1}$  soil sample).

#### **4.4.7 Fluorescent pseudomonads**

Among the yellowing affected rhizosphere soil samples, the maximum population of fluorescent pseudomonads was recorded in the sample in YL-4 ( $6.2 \times 10^3$  cfu  $g^{-1}$  soil sample) and the samples YL-2 and YL-6 were not found to show the presence of fluorescent pseudomonads. A highest population of  $8.3 \times 10^3$  cfu  $g^{-1}$  soil sample was obtained from the sample AH-2 from the apparently healthy rhizosphere soil samples and no colonies were observed from the samples AH-1, AH-4 and AH-6. From the completely healthy rhizosphere soil samples, the highest population of fluorescent pseudomonads was recorded in CH-7 ( $16.5 \times 10^3$  cfu  $g^{-1}$  soil sample) and no colonies were obtained from the samples CH-1, CH-2 and CH-5.

#### **4.4.8 Nitrogen Fixers**

The population of nitrogen fixers were enumerated and the highest population among the yellowing affected rhizosphere soil samples was recorded in YL-2 ( $12 \times 10^3$  cfu  $g^{-1}$  soil) and no population was observed in YL-5 and YL-6. Among the apparently healthy rhizosphere soil samples, the highest population was recorded in AH-1 ( $21 \times 10^3$  cfu  $g^{-1}$  soil), but the samples AH-5 and AH-6 failed to show the presence of nitrogen fixers. The maximum population of nitrogen fixers was recorded in the sample CH-1 ( $10.8 \times 10^3$  cfu  $g^{-1}$  soil sample) and it was found to be absent in the samples CH-4, CH-5 and CH-6.

#### 4.4.9 *Trichoderma*

The population of *Trichoderma* among the yellowing affected rhizosphere soil samples was enumerated and the highest population was observed in YL-3 ( $6.75 \times 10^2$  cfu g<sup>-1</sup> soil sample) and the sample YL-6 failed to show colonies of *Trichoderma*. Among the apparently healthy rhizosphere soil samples, the population was highest in AH-2 ( $5.75 \times 10^2$  cfu g<sup>-1</sup> soil sample) and the lowest in AH-6 ( $0.1 \times 10^2$  cfu g<sup>-1</sup> soil sample). The completely healthy rhizosphere soil samples had the highest population of *Trichoderma* was recorded from CH-1 ( $20 \times 10^2$  cfu g<sup>-1</sup> soil sample) and the lowest in the samples CH-4 and CH-6 ( $0.1 \times 10^2$  cfu g<sup>-1</sup> soil sample).

#### 4.4.10 *Bacillus*

The population of *Bacillus* sp. was analysed by serial dilution and plating of the soil suspension heated at 80°C for 10 minutes. The yellowing affected rhizosphere soil samples were analysed for *Bacillus* sp. and the highest population was recorded from YL-3 ( $4.5 \times 10^4$  cfu g<sup>-1</sup> soil sample) and lowest in YL-6 ( $0.8 \times 10^4$  cfu g<sup>-1</sup> soil sample). Among the apparently healthy rhizosphere soil samples, the highest population was recorded in AH-2 ( $9.5 \times 10^4$  cfu g<sup>-1</sup> soil sample) and the lowest in AH-7 ( $2.15 \times 10^4$  cfu g<sup>-1</sup> soil sample). The completely healthy soil samples had their highest *Bacillus* population in the sample CH-3 ( $23.6 \times 10^4$  cfu g<sup>-1</sup> soil sample) and the lowest in CH-5 ( $1.85 \times 10^4$  cfu g<sup>-1</sup> soil sample). The completely healthy rhizosphere soil samples were found to harbor significantly higher population of *Bacillus* sp. than the yellowing affected rhizosphere soil samples.

**Table 10. Microbial biomass carbon and the culturable microbial diversity in yellowing affected and apparently healthy rhizosphere soil samples**

Samples	Microbial biomass carbon ( $\mu\text{g g}^{-1}$ )	Microbial population in cfu/g soil									
		Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^3$	P-Solubilizers $\times 10^3$	N-fixers $\times 10^3$	Fluorescent pseudomonads $\times 10^3$	<i>Trichoderma</i> $\times 10^2$	<i>Bacillus</i> $\times 10^4$		
YL-1	350.4	6.6	6.6	0	9.3	7	1.5	1.5	1.4		
AH-1	295.93	29.4	10.5	0	10	21	0	3.75	6.7		
YL-2	265.18	8.8	21.5	0	5.2	12	0	0.5	4		
AH-2	534.5	20.8	16.3	0	13.7	16.5	8.3	5.75	9.5		
YL-3	565.34	40.2	18.7	5.7	2.2	3.4	5.2	6.75	4.5		
AH-3	309.3	46.5	21	4.5	1	2.2	5.6	4.25	11		
YL-4	675.2	24	14.7	14.5	0	1.2	6.2	4.75	3.4		
AH-4	576.8	33	41.2	17.2	0	0.4	0	1.4	3.2		
YL-5	233.15	19.1	24.6	6.3	0	0	1	1.75	1.4		
AH-5	132.31	20.3	25.3	4.3	0	0	1	5.15	3.15		
YL-6	153.6	54.1	25.8	8.4	5.6	0	0	0	0.8		
AH-6	167.32	12.8	28.2	9.3	2.6	0	0	0.1	2.75		
YL-7	233.12	43.4	34.4	42.1	4.2	2	5.5	0.2	1.15		
AH-7	256.4	62	34.4	36.3	4.3	4.8	2	1.3	2.15		

**Table 11. Culturable microbial diversity in the completely healthy rhizosphere soil samples**

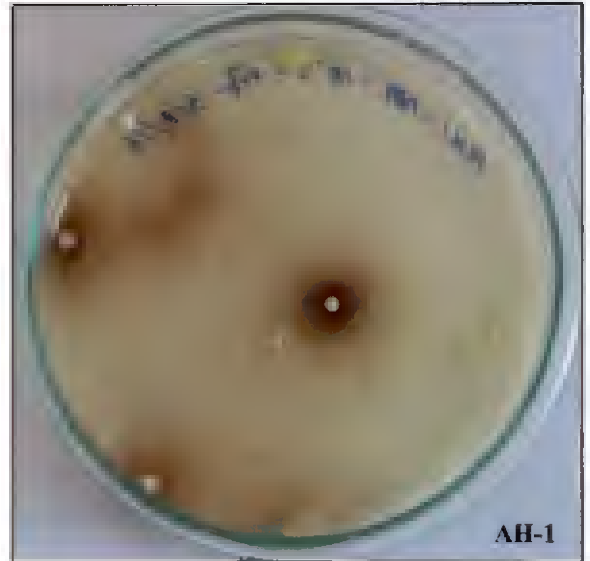
Samples	Microbial biomass carbon ( $\mu\text{g g}^{-1}$ )	Microbial population in cfu/g soil									
		Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^3$	P-Solubilizers $\times 10^3$	N-fixers $\times 10^3$	Fluorescent pseudomonads $\times 10^3$	<i>Trichoderma</i> $\times 10^2$	<i>Bacillus</i> $\times 10^4$		
CH-1	734.1	16.2	19.3	0	15	10.8	0	20	19.4		
CH-2	825.8	19.6	9.5	0	15	8.8	0	1.25	23.6		
CH-3	912.1	92.5	24.2	5.3	2.3	2.8	3.2	4.5	12.5		
CH-4	554.67	48.2	39.2	13.6	0	0	4.8	0.1	6.60		
CH-5	190.68	19.8	31.2	8.8	0	0	0	2.4	1.85		
CH-6	286.69	41.8	38.6	12.4	6.3	0	10.5	0.1	4.15		
CH-7	200.62	42	42.2	41.3	6.4	1.3	16.5	2.4	6.85		

**Table 12. Comparison of microbial biomass carbon and microbial population using CRD**

Samples	Microbial biomass carbon ( $\mu\text{g g}^{-1}$ )	Microbial population in cfu/g soil							
		Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^3$	P-Solubilizers $\times 10^3$	N-fixers $\times 10^3$	Fluorescent pseudomonads $\times 10^3$	<i>Trichoderma</i> $\times 10^2$	<i>Bacillus</i> $\times 10^4$
YL	353.71 (2.49)	2.8 (6.3)	2.1 (3.3)	1.1 (2.2)	3.8 (2.6)	3.6 (2.5)	2.7 (2.5)	0.76 (1.6)	2.4 <sup>b</sup> (4.3)
AH	324.65 (2.46)	3.2 (6.4)	2.5 (3.4)	1.02 (2.1)	4.5 (2.6)	6.0 (2.5)	2.4 (1.9)	3.1 (2.3)	5.5 <sup>b</sup> (4.6)
CH	529.24 (2.65)	4.0 (6.5)	2.9 (3.4)	1.1 (2.2)	6.4 (2.8)	3.4 (2.07)	5.0 (2.2)	4.4 (2.1)	10.7 <sup>a</sup> (4.9)
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	0.366

YL- Yellowing affected: AH- Apparently Healthy: CH- Completely Healthy

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**Plate 3.a. Culturable bacterial diversity enumerated using serial dilution and plating**



**Plate 3.b. Fungal diversity on Martin's Rose Bengal agar**



**Plate 3.c. Culturable diversity of actinomycetes obtained using dilution**



**Plate 3.d. *Bacillus* colonies on nutrient agar**

**Plate 3. Diversity of culturable microflora in the rhizosphere of arecanut palms in Wayanad**





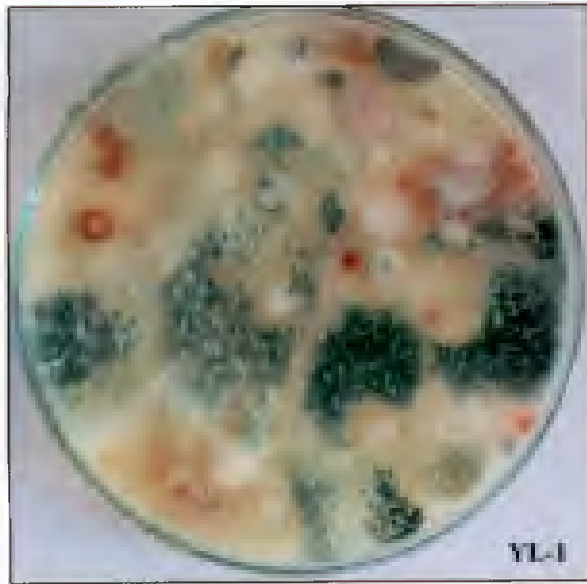


Plate 4. Increased *Trichoderma* population in AH samples

#### **4.5 Purification and maintenance of isolates**

A total of 27 predominant bacterial isolates comprising of 6 *Bacillus* isolates, 8 nitrogen fixers, 5 fluorescent pseudomonads, 2 phosphate solubilizers and 6 antagonistic bacterial isolates were purified and maintained as per the standard procedures (Plate 5).

#### **4.6 *In vitro* screening for Plant Growth Promoting (PGP) activities**

The bacterial isolates were screened for IAA, HCN, siderophore and ammonia production. The qualitative estimation for IAA, siderophore, HCN and ammonia production has been given in Table 13. The isolates were also screened of its capacity for solubilizing phosphorus and the solubilization index was estimated. The solubilization efficiency and the quantity of phosphate solubilized has been provided in Table 13. A quantitative estimation of IAA production was done and provided in the table 13. About 7 bacterial isolates were found to show IAA production and only 1 isolate exhibited siderophore production. Among the bacterial isolates, 2 isolates were able to produce HCN and 15 isolates showed ammonia production, which was ranked based on the intensity of colour produced during the screening. About 10 bacterial isolates displayed the capacity of phosphate solubilization due to the production of organic acids (Plate 6).

##### **4.6.1 Screening for phosphate solubilization**

All the 27 bacterial isolates were screened for the ability of phosphate solubilization using Pikovskaya's agar. Among them, 10 isolates were capable of phosphate solubilization and the amount of P solubilized was quantified. The maximum solubilization efficiency based on the size of solubilization zone was observed in the bacterial isolate CH3FP2.



**Nitrogen fixer (AH2NF)**



**Fluorescent pseudomonad (CH3FP2)**



***Bacillus* spp. isolate (CH3BAC)**

**Plate 5. Predominant bacterial isolates**

**Table 13. Screening for Plant Growth Promoting (PGP) activities of the predominant bacterial isolates**

Isolate	N-Fixation	Phosphate solubilization	Volatile metabolite		Non-volatile metabolite	
			HCN production	Ammonia production	IAA production	Siderophore production
CH2BC	-	=	-	+	+	=
AH2BC	=	=	=	=	=	=
CH7BC	-	-	-	-	-	-
AH2BC1	-	-	-	++	+	-
AH2BC2	=	=	=	+	=	=
AH2BC3	-	-	-	-	-	-
CH6NF	=	+	-	-	-	-
YL7NF	+	=	=	+	+	=
AH7NF	+	+	-	+	-	-
AH2NF	+	=	=	+	=	=
CH1NF	=	-	-	+	-	-
AH5NF	+	-	-	+	-	-
AH1NF	+	=	=	+++	=	=
CH4NF	+	-	-	-	-	-
CH3FP	-	=	-	-	-	-
YL4FP	=	+	=	++	+	=
AH4FP	-	+	+	+++	+	=
CH3FP1	-	+	-	-	+	-
CH3FP2	=	=	=	=	=	=
YL4PS	-	+	-	-	-	+
AH4PS	=	+	=	+++	=	=
YL3BAC1	-	+	+	-	+	-
YL3BAC2	-	+	-	+	-	-
YL3BAC3	=	+	=	+	=	=
CH3BAC	-	-	-	-	-	-
YL4BAC	-	-	-	+	-	-
YL3BAC4	=	=	=	=	=	=

BC- Predominant bacterial isolate: NF- N-fixer: FP- Fluorescent pseudomonad:

PS- P-solubilizer: BAC- *Bacillus*

#### 4.6.2 Quantitative estimation of phosphate solubilization

The quantitative estimation of phosphate solubilization was one using spectrophotometry and among the selected bacterial isolates, the highest production was recorded in the isolate AH4FP with 13.1  $\mu\text{g ml}^{-1}$  of available P in the Pikovskaya's broth. The lowest production was recorded in the isolate YL4PS with 2.7  $\mu\text{g ml}^{-1}$  of

available P. The solubilization efficiency and quantitative estimate of phosphate solubilization has been given Table 14.

**Table 14. Solubilization efficiency and quantity of phosphate solubilized by the selected bacterial isolates**

Isolate name	Solubilization efficiency in Pikovskaya's agar in 7 days (%)	Quantity of P solubilized in Pikovskaya's broth in 15 days ( $\mu\text{g ml}^{-1}$ )
AH2BC3	68	2.9
YL7NF	64	4.2
CH3FP	41	3.6
YL4FP	60	6.8
AH4FP	33	13.1
CH3FP2	70	4.1
YL4PS	55	2.7
AH4PS	48	3.2
YL3BAC1	47	8.5
YL3BAC2	55	2.9

BC- Predominant bacterial isolate: NF- N-fixer: FP- Fluorescent pseudomonad:

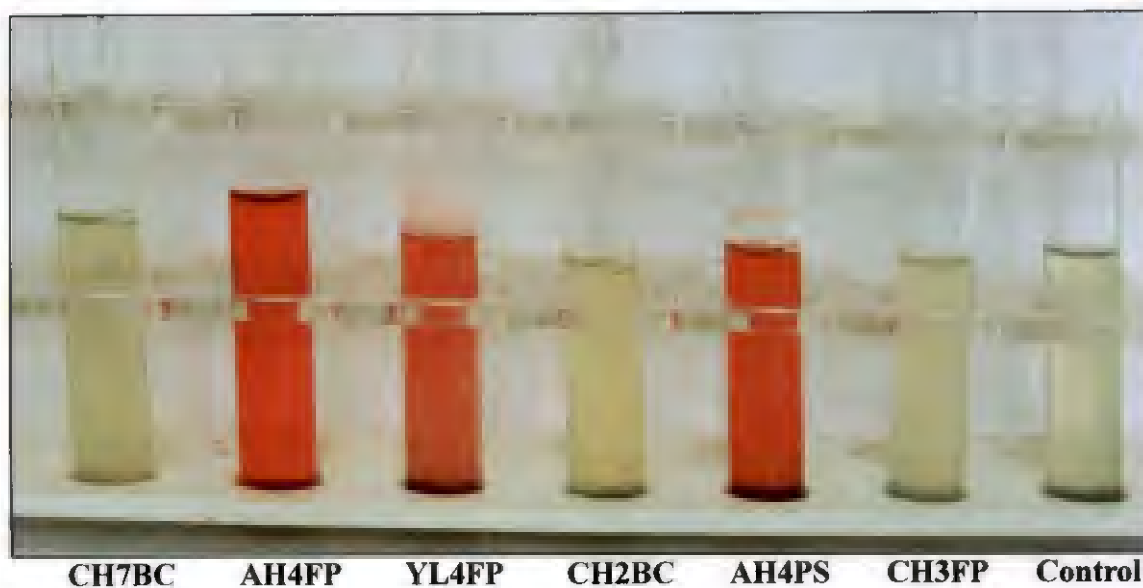
PS- P-solubilizer: BAC- *Bacillus*

#### 4.6.3 Screening for and quantitative estimation of indole acetic acid (IAA) production

Among the 25 predominant bacterial isolates, 7 were selected for IAA production potential based on the colour development during the screening procedure. The intensity of the pink colour was taken as the parameter and further quantification was done. The quantitative estimation of the 7 selected bacterial isolates for IAA production was done and the highest production was detected in the isolate CH2BC



**Plate 6.a Phosphate solubilization by bacterial isolates on Pikovskaya's agar**



**Plate 6.b. Screening of bacterial isolates for NH<sub>3</sub> production**



**Plate 6.c. Sideropore production by the isolate YL4PS on CAS agar**



AH4FP

YL4FP

**Plate 6.d. HCN production by the isolate AH4FP**



**Plate 6.e. IAA production by bacterial isolates in nutrient broth enriched with tryptophan**

**Plate 6. PGP characterization of the bacterial isolates**



with 26.3  $\mu\text{g ml}^{-1}$  IAA and the lowest in the isolate YL4FP with 11.2  $\mu\text{g ml}^{-1}$  IAA (Table 15).

**Table 15. IAA production by selected bacterial isolates**

Isolate	Quantity of IAA produced ( $\mu\text{g ml}^{-1}$ )
AH4FP	17.25
CH2BC	26.3
YL4FP	11.2
AH2BC1	12.3
YL4PS	10.8
YL3BAC1	13.2
YL7NF	17.7

BC- Predominant bacterial isolate: NF- N-fixer: FP- Fluorescent pseudomonad:

PS- P-solubilizer: BAC- *Bacillus*

#### 4.7 Quality and quantity of metagenomic DNA

The quality of the metagenomic DNA isolated from 9 soil samples (YL-2, AH-2, CH-2, YL-5, AH-5, CH-5, YL-7, AH-7 and CH-7) using MN Nucleospin soil DNA isolation kit was analysed using agarose gel electrophoresis. Electrophoresis on 0.8 per cent agarose gel revealed a single intact band corresponding to the 21226 bp band in the marker. The quantitative analysis of the metagenomic DNA was done by spectrophotometry using NanoDrop. The concentration of DNA and the ratio of absorbance between 260/280 nm and 260/230 nm were estimated and provided in Table 16. The samples YL-5 and CH-2 failed to give amplicons in the 16S rDNA region and the amplicons obtained from the other seven samples were used for the downstream processes.

**Table 16. Qualitative and quantitative parameters of the isolated metagenomic DNA**

Sample Name	A 260/280	A 260/230	Quantity (ng/ $\mu$ l)	A 230
YL-2	1.52	0.70	36.2	1.028
YL-5	1.45	0.63	41.7	1.319
YL-7	1.60	0.50	13.3	0.529
AH-2	1.11	0.75	27.1	0.719
AH-5	1.91	0.56	42.8	1.520
AH-7	1.66	0.68	13.8	0.409
CH-2	1.30	0.67	25.8	0.593
CH-5	1.73	0.90	26.3	0.581
CH-7	1.55	0.97	54.3	1.121

YL- Yellowing affected; AH- Apparently Healthy; CH- Completely Healthy

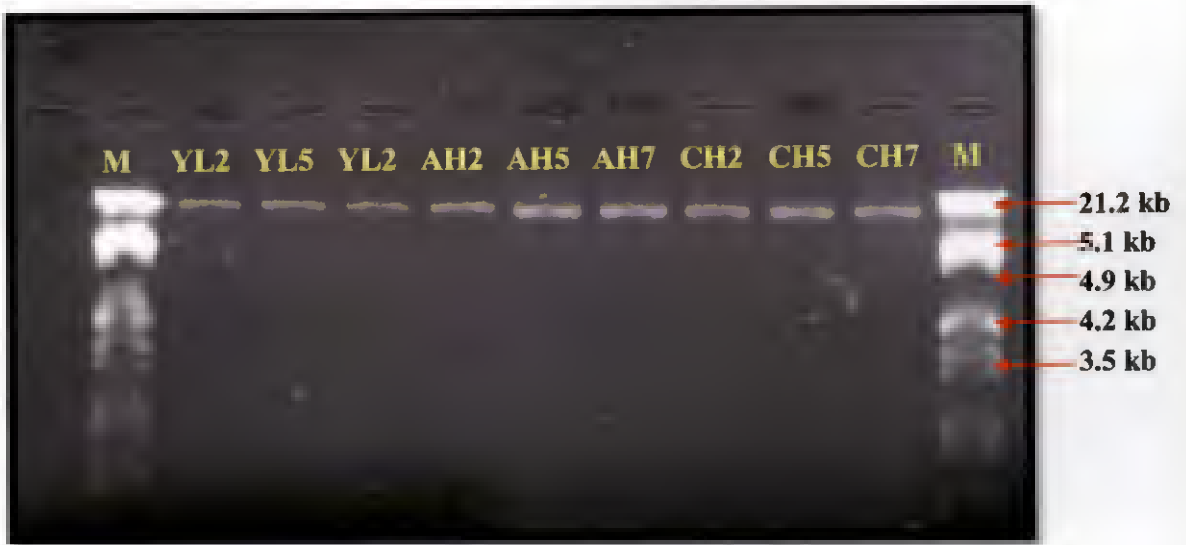
#### 4.8 Quality checking of Fastq sequences

Sequences were analysed for quality parameters like base quality score distributions, average base content and GC distribution for all the samples (YL-2, YL-7, AH-2, AH-5, AH-7, CH-5 and CH-7).

#### 4.9 Base quality score distribution

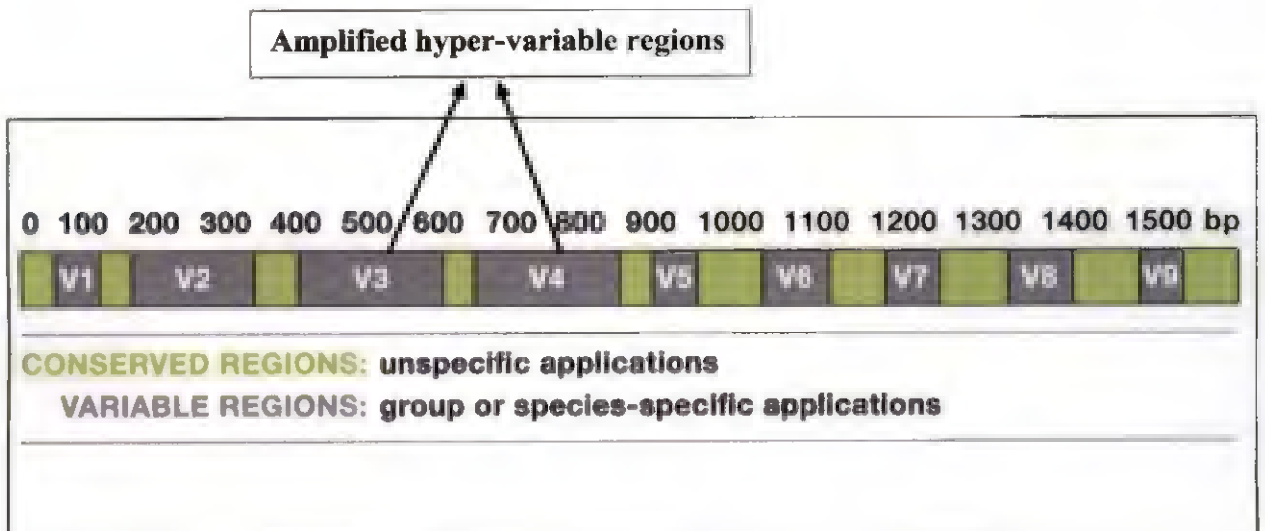
The base quality distribution for all the sequences were observed to be greater than 80 per cent with a Phred score above 30. The Phred score represents the base quality and a value above 30 indicates an error probability around 0.001. The diagrammatic representation shows the sequencing cycle in the X-axis and the percentage of total reads in the Y-axis.

1911



**Plate 7. Metagenomic DNA on 0.8 % agarose gel**

**M: Marker ( $\lambda$ *EcoR*I + *Hind*III double digest)**



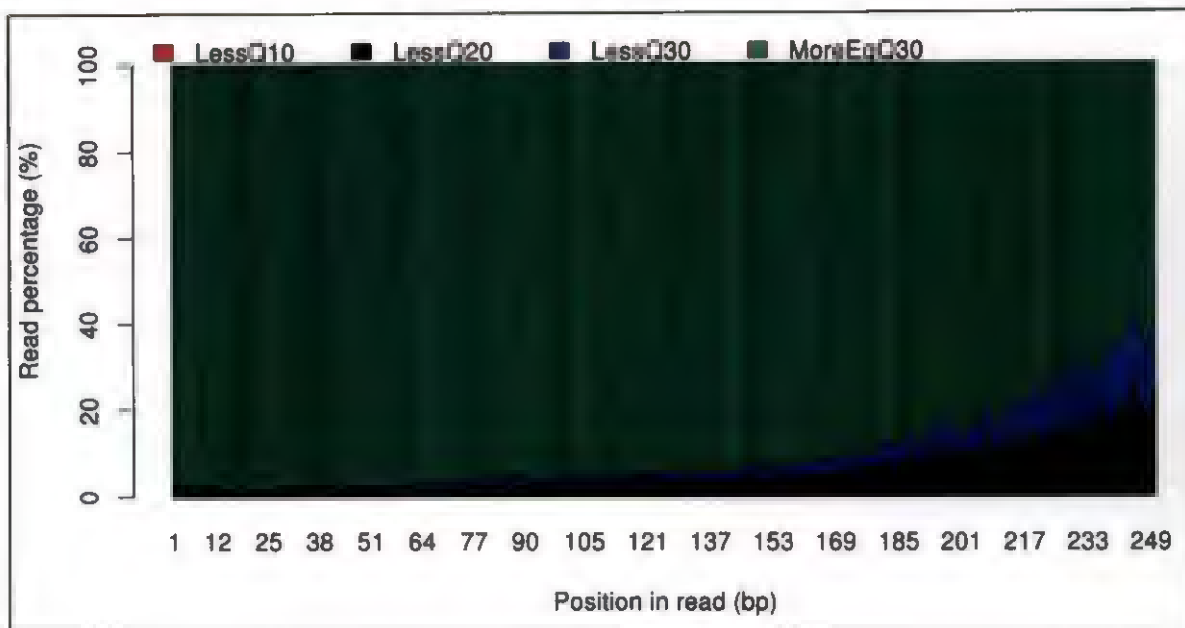
**Plate 8. V3 and V4 regions of 16S rRNA gene**

**Table 17. Raw read summary: Read quantity and quality**

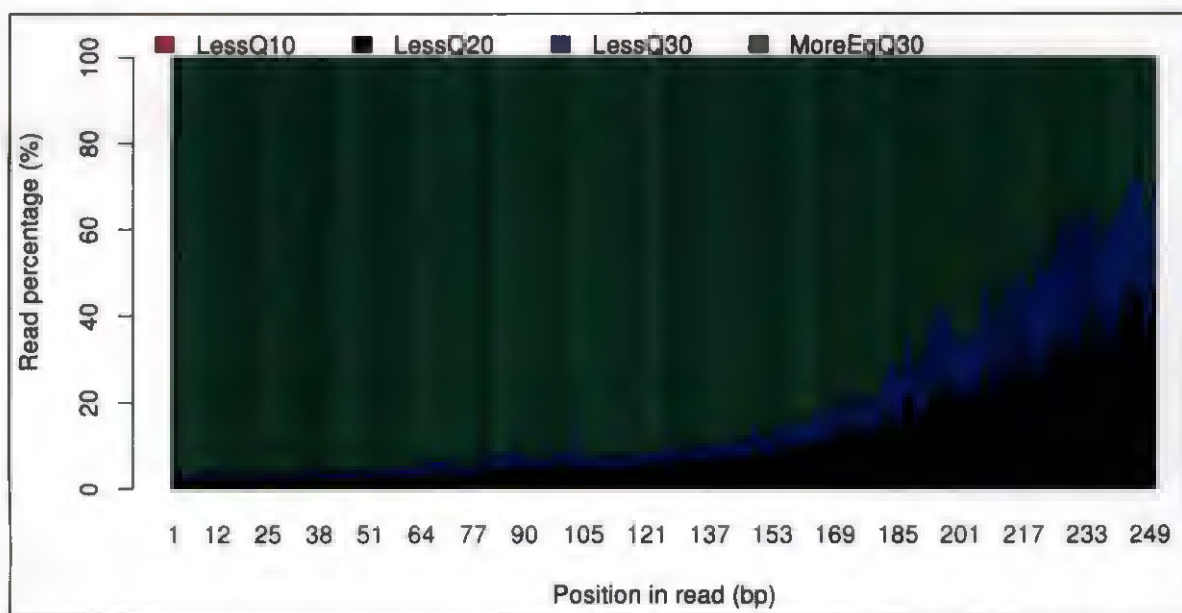
Sample Name	Read orientation	Mean read quality (Phred score)	Number of reads	% GC	% Q < 10	% Q 10-20	% Q 20-30	% Q > 30	Number of bases (MB)	Mean read length (bp)
YL-2	R1	36.33	558634	54.99	0.07	5.8	3.97	90.16	139.66	250.0
	R2	33.79	558634	55.5	0.02	11.72	8.1	80.16	139.66	250.0
YL-7	R1	36.68	887278	55.59	0.05	5.34	3.7	90.9	221.82	250.0
	R2	33.13	887278	55.92	0.3	13.62	8.9	77.18	221.82	250.0
AH-2	R1	36.84	848955	54.93	0.05	4.9	3.47	91.58	212.24	250.0
	R2	34.2	848955	55.06	0.3	10.81	7.25	81.64	212.24	250.0
AH-5	R1	36.68	773725	56.45	0.05	5.41	3.7	90.84	193.43	250.0
	R2	34.07	773725	56.61	0.3	11.28	7.4	81.02	193.43	250.0
AH-7	R1	36.72	930829	55.34	0.05	5.27	3.62	91.05	232.71	250.0
	R2	33.92	930829	55.6	0.3	11.55	7.67	80.47	232.71	250.0
CH-5	R1	36.68	860521	54.63	0.05	5.4	3.68	90.87	215.13	250.0
	R2	34.06	860521	54.88	0.3	11.19	7.42	81.09	215.13	250.0
CH-7	R1	36.03	573716	56.93	0.07	6.77	4.26	88.9	143.43	250.0
	R2	33.62	573716	57.18	0.02	12.43	8.25	79.3	143.43	250.0

#### 4.10 Base composition

The base composition of each sample was analysed and assembled in Table 18. The graphical representation of the base composition indicated the bases in its respective assigned colours with the sequencing cycle in X-axis and nucleotide percentage in the Y-axis.



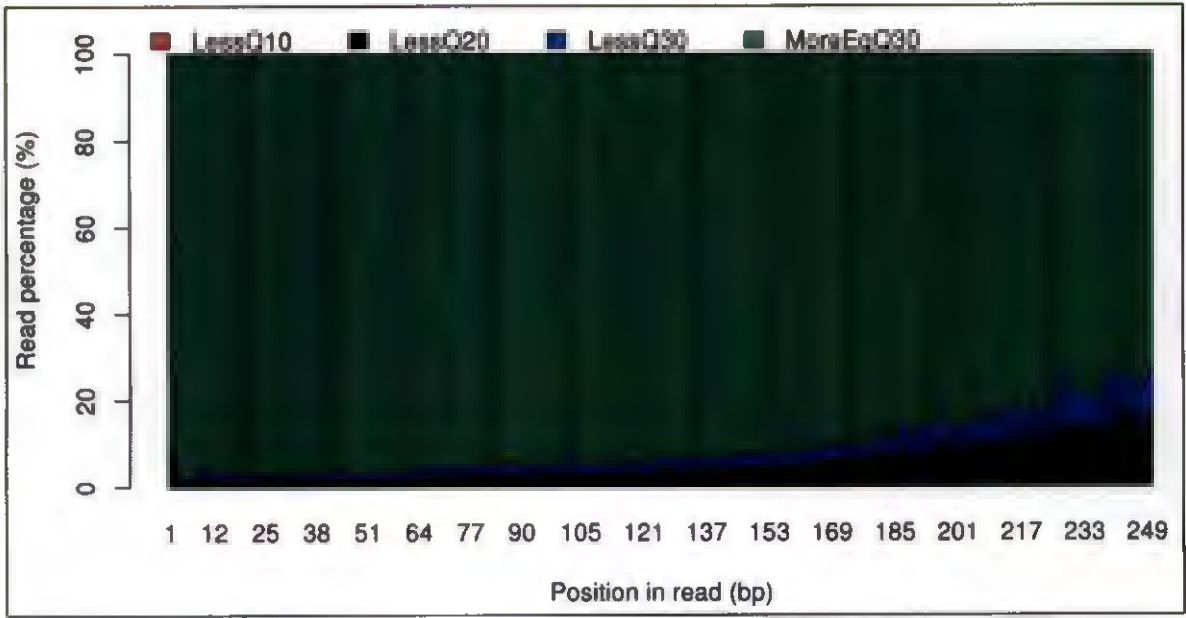
**Fig. a. Read 1**



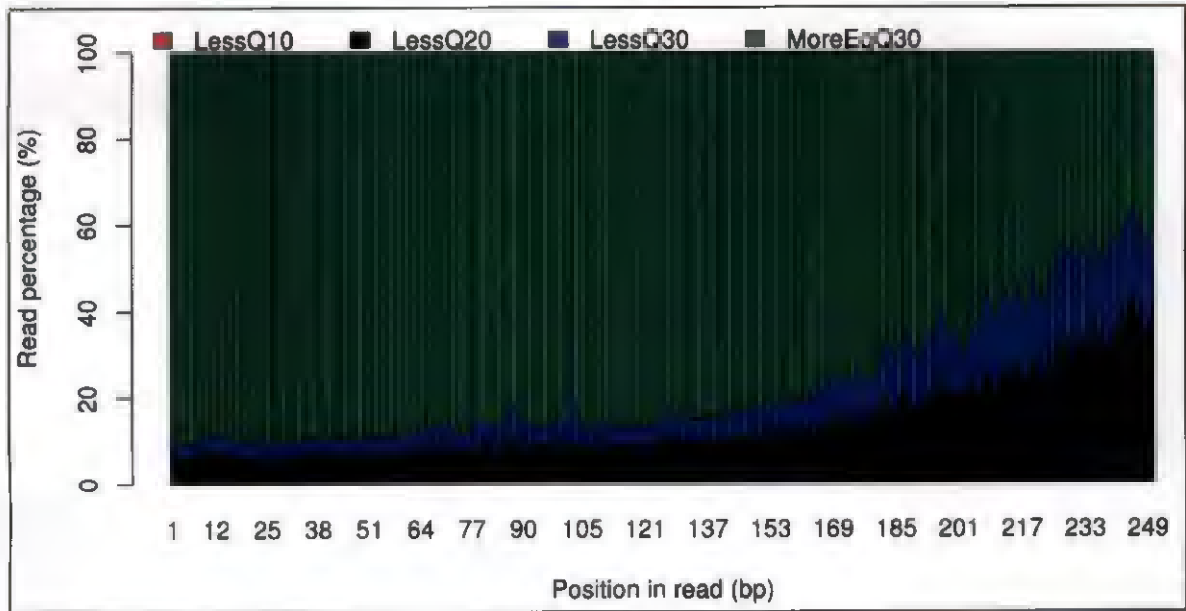
**Fig. b. Read 2**

**Plate 9.a. Base quality distribution of YL-2**

24

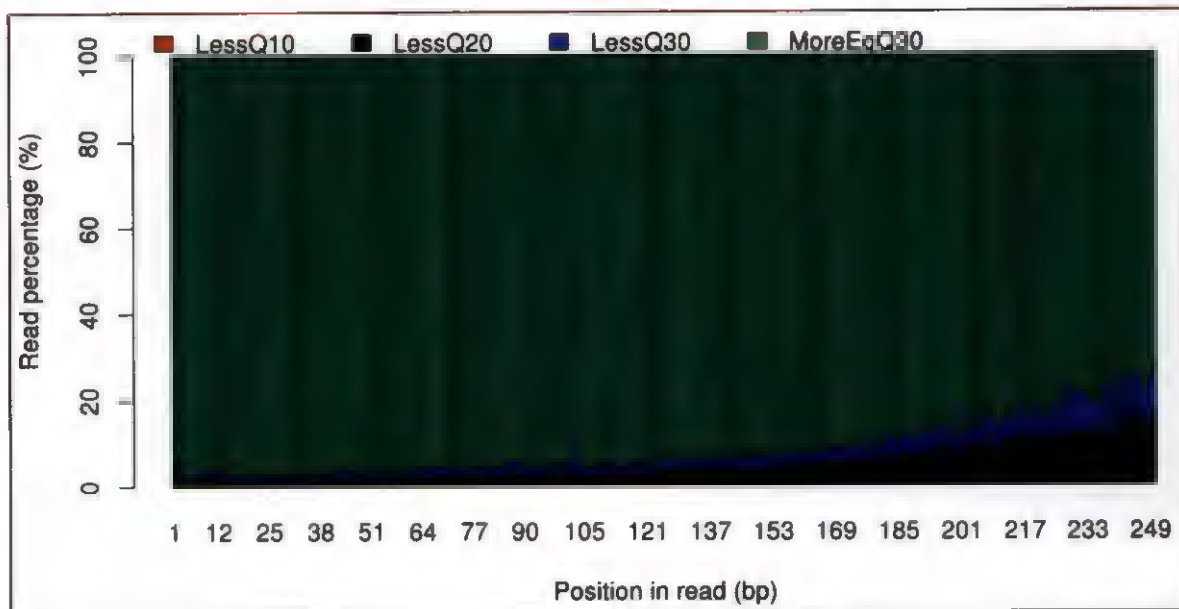


**Fig. a. Read1**

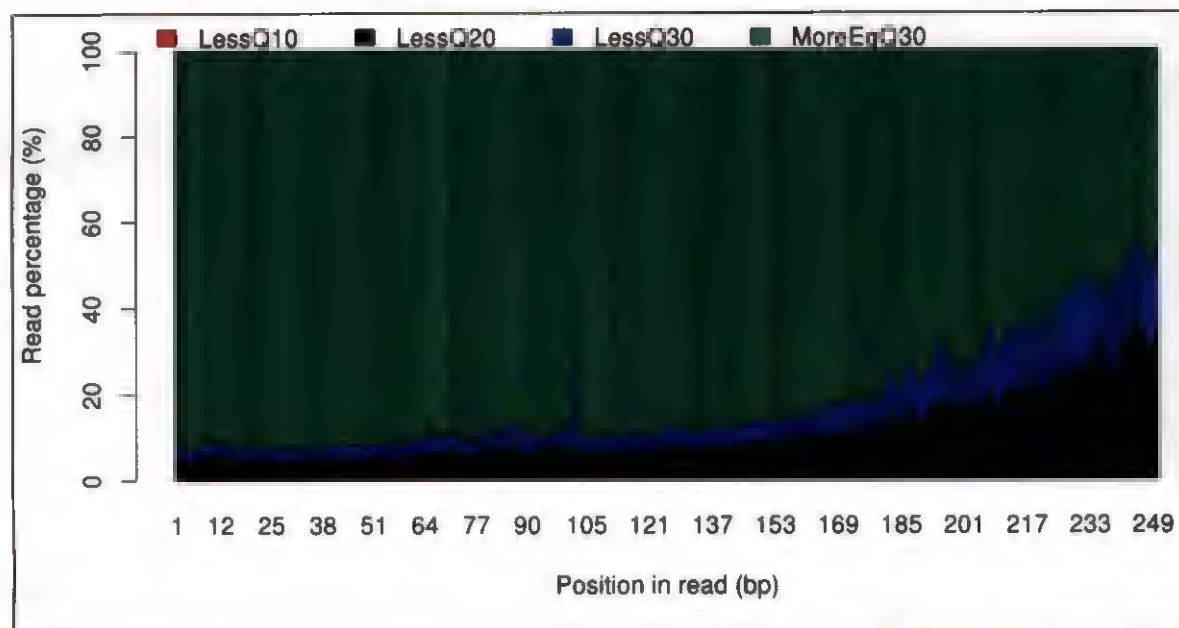


**Fig. b. Read2**

**Plate 9.b. Base quality distribution of YL-7**



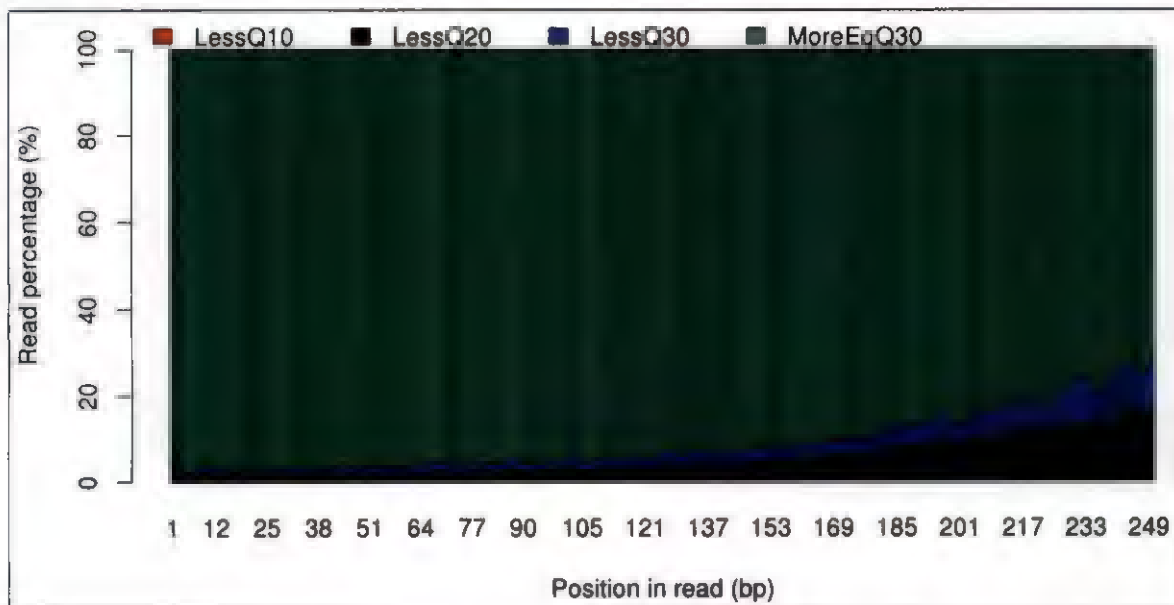
**Fig. a. Read 1**



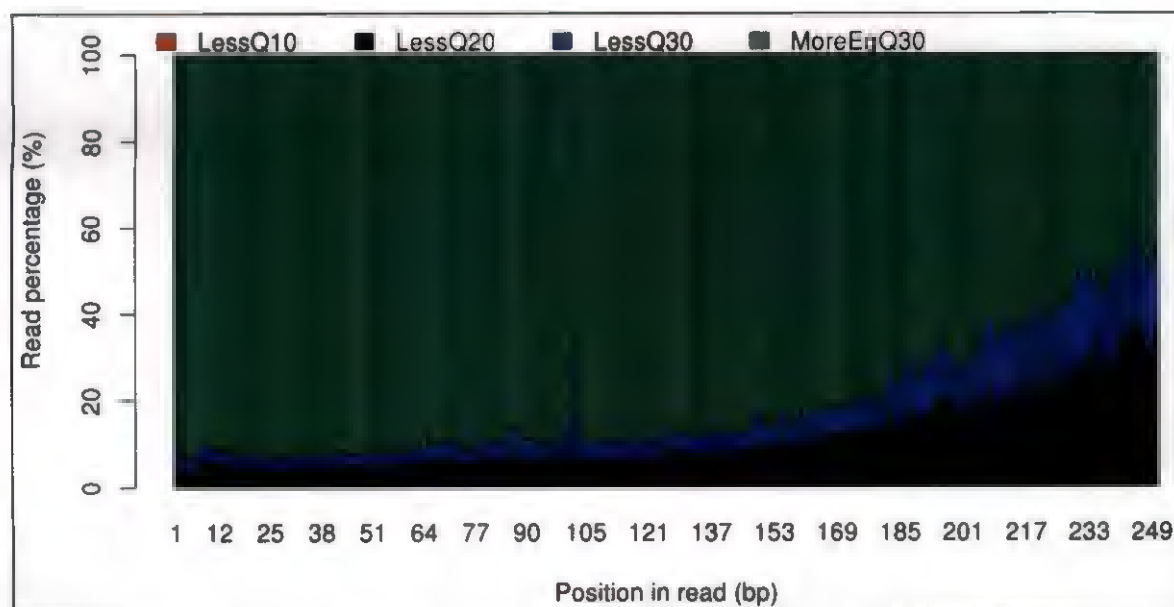
**Fig. b. Read 2**

**Plate 9.c. Base quality distribution AH-2**



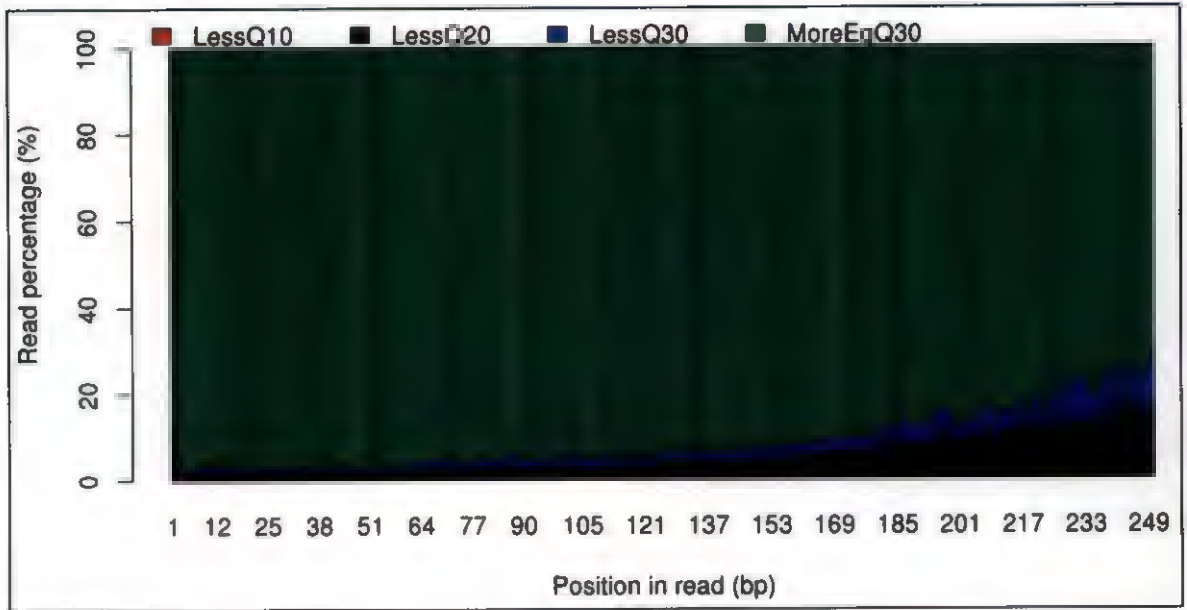


**Fig. a. Read 1**

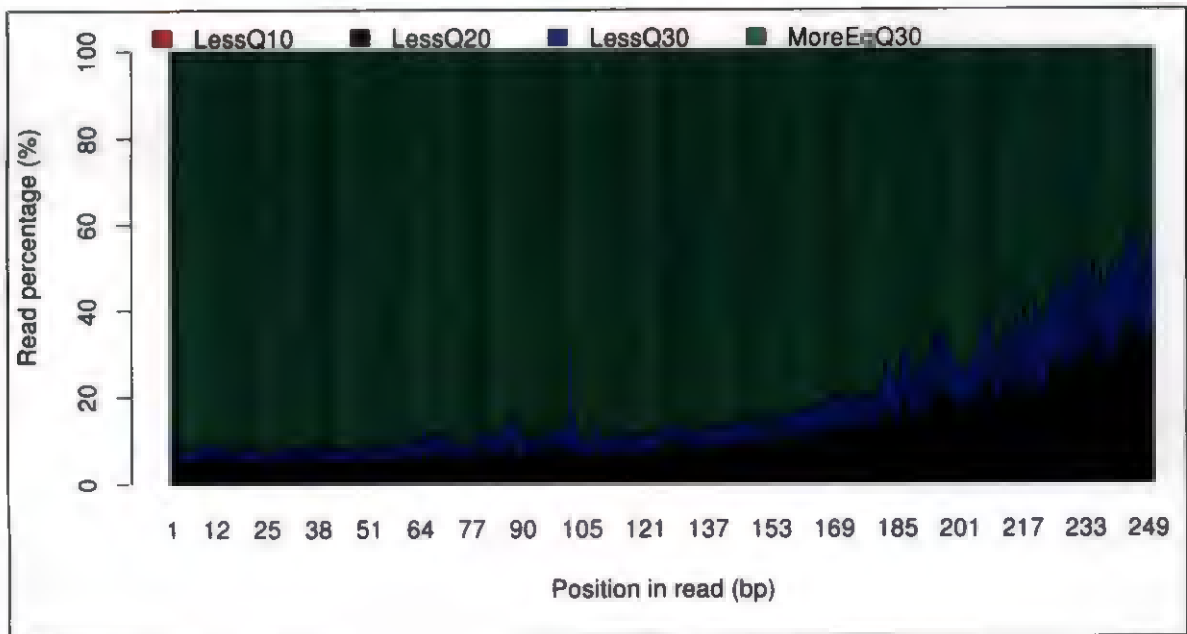


**Fig. b. Read 2**

**Plate 9.d. Base quality distribution of AH-5**



**Fig. a. Read 1**



**Fig. b. Read 2**

**Plate 9. e. Base quality distribution of AH-7**

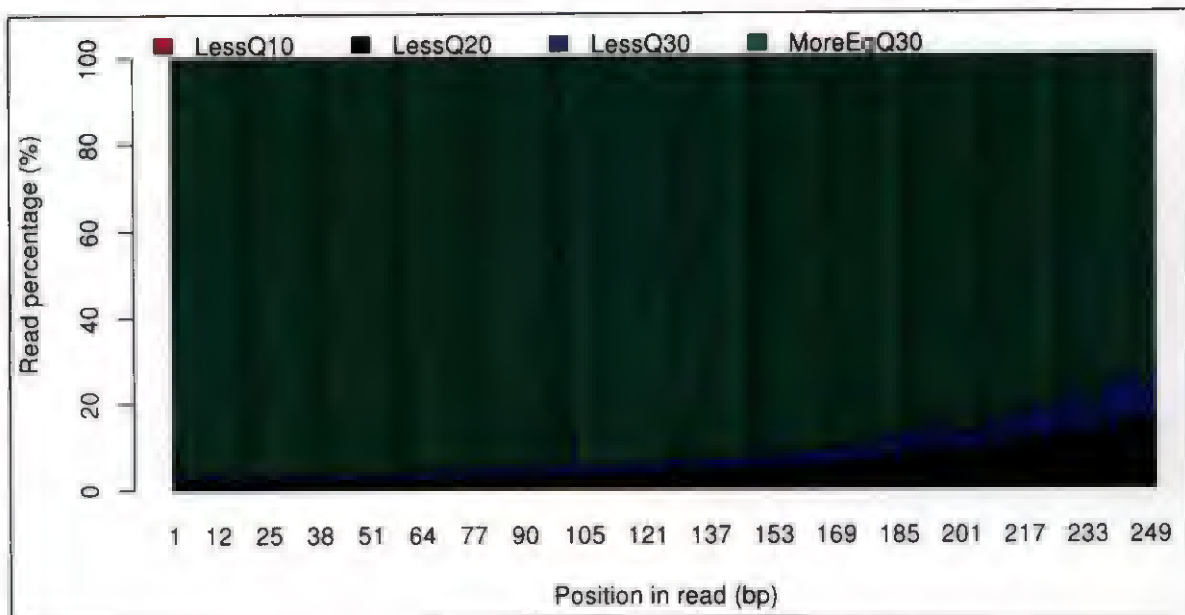


Fig. a. Read 1

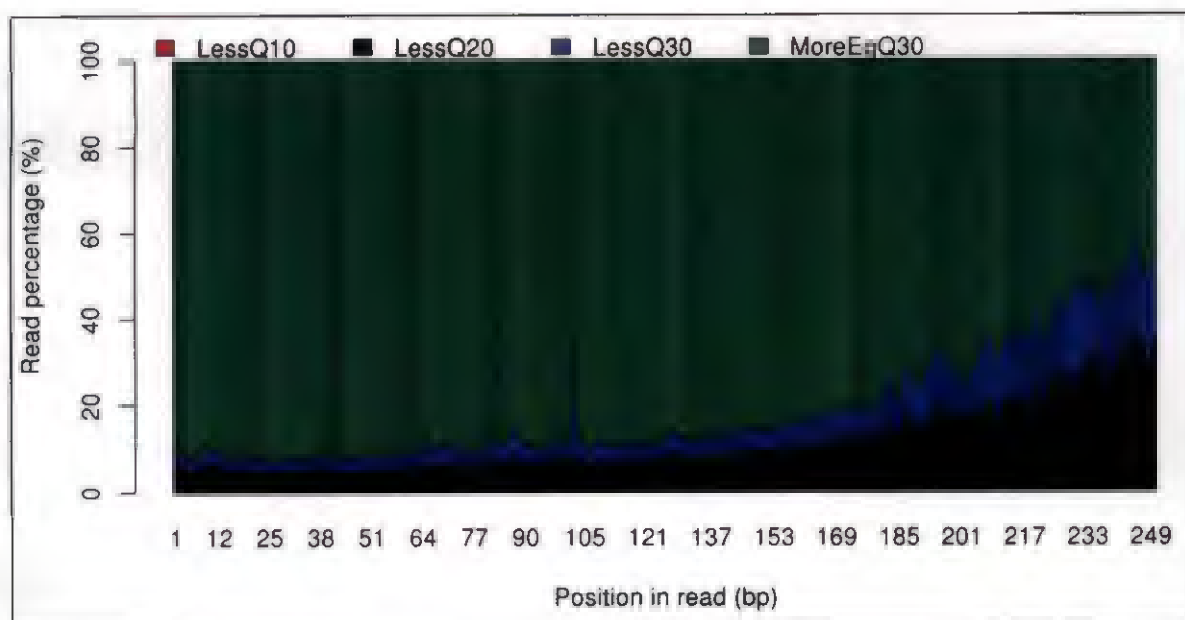
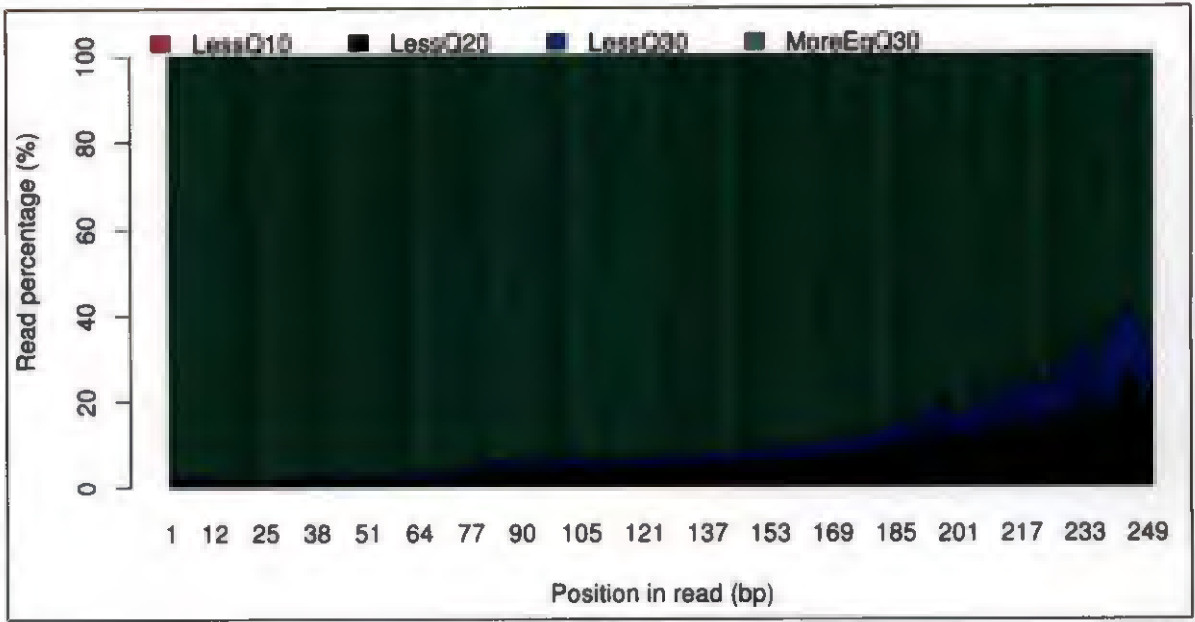


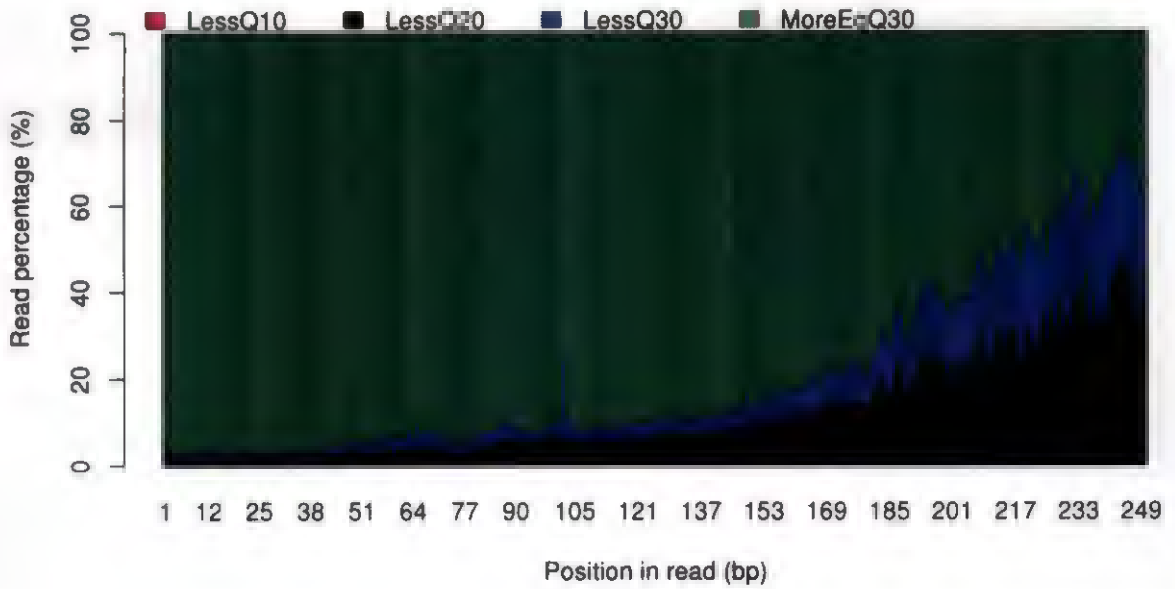
Fig.b. Read 2

Plate 9.f. Base quality distribution of CH-5

109



**Fig.a. Read 1**



**Fig.b. Read 2**

**Plate 9. g. Base quality distribution of CH-7**

**Plate 9. Base quality distribution of all samples**

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**Table 18. Base composition of the 16S rDNA amplicons of the isolated metagenomic DNA samples**

Sample	Base composition (%)			
	A	C	G	T
YL-2	22.47	28.10	27.15	22.25
YL-7	22.21	28.44	27.32	21.85
AH-2	22.46	27.93	27.07	22.36
AH-5	21.87	28.66	27.87	21.43
AH-7	22.25	28.12	27.35	22.10
CH-5	22.63	27.87	26.89	22.44
CH-7	21.71	28.99	28.07	21.19

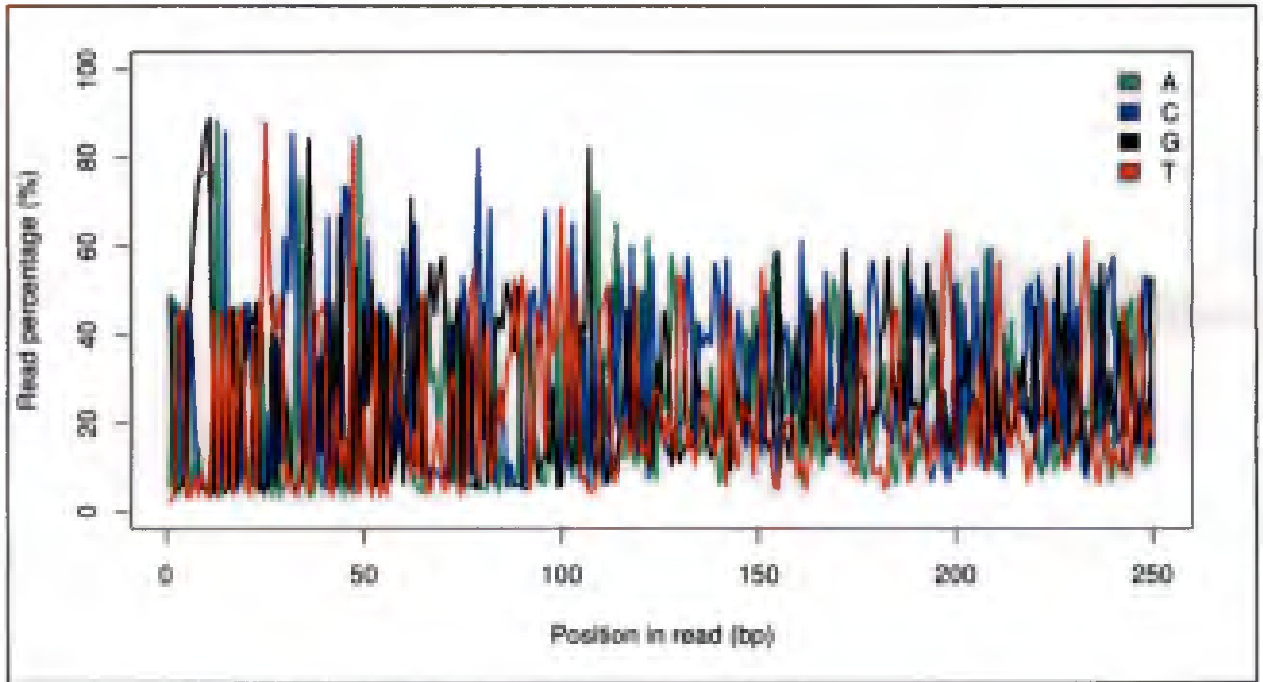
#### 4.11 GC distribution

The average GC composition of all the reads of samples were analysed (Table 17). The graphical representation of the average GC content with X-axis showing the average GC content and Y-axis showing percentage of sequences indicates that all the reads of samples has an average GC content in the range of 30-60 per cent.

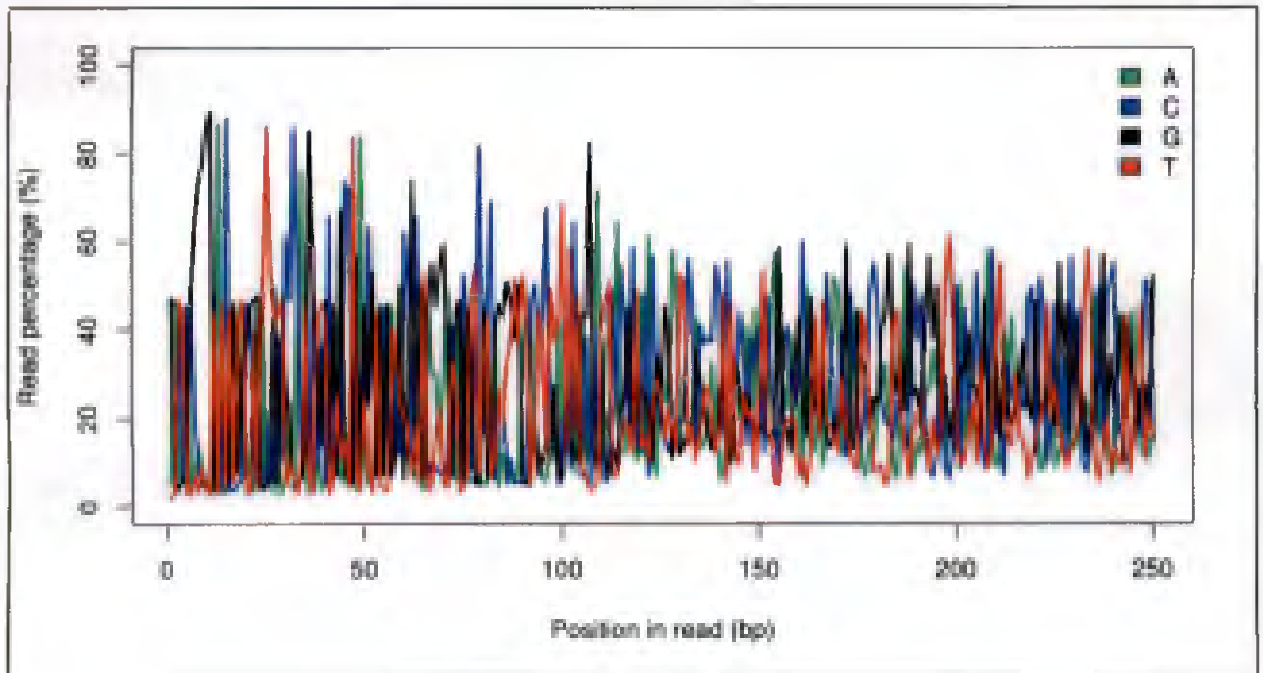
#### 4.12 Identification of V3 and V4 regions of the paired end sequences

The consensus reads were obtained after separating them from the paired-end sequences followed by trimming of unwanted sequences and finally the construction of the V3 and V4 regions using FLASH program. An average contig length of 350-450 bp with 0 mismatch was obtained for all the samples. The total number of reads obtained are given in Table 19.

1619

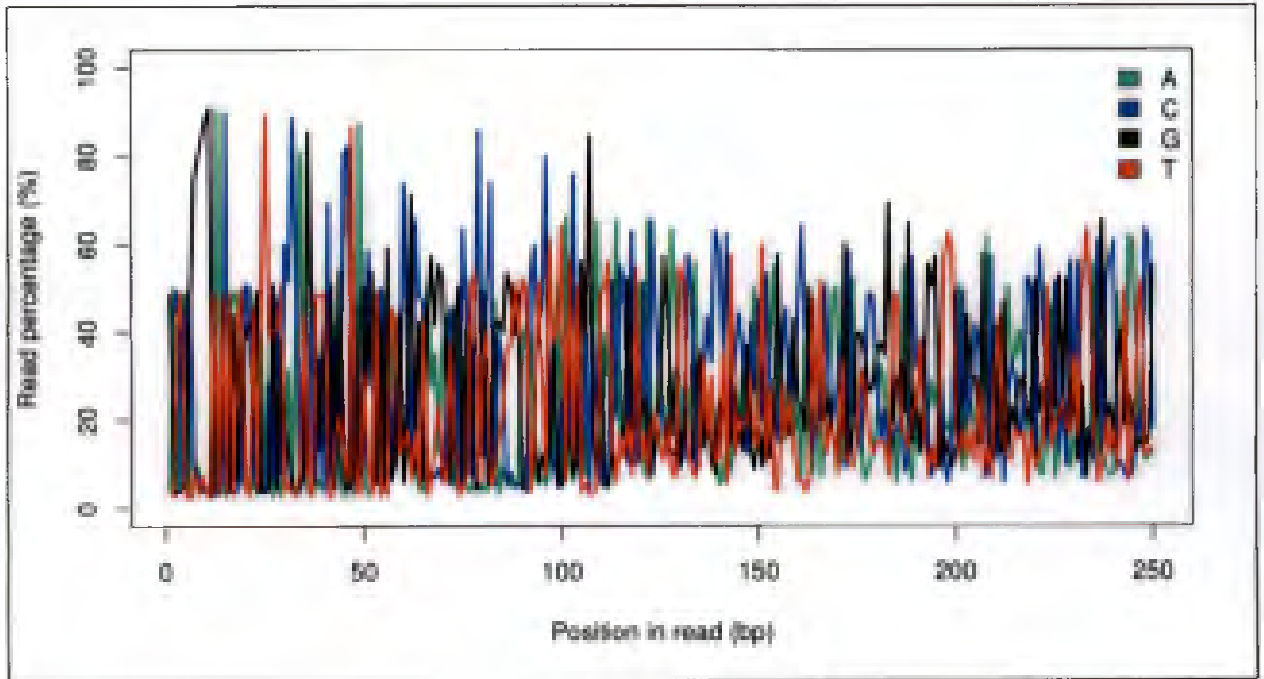


**Fig. a. Read 1**

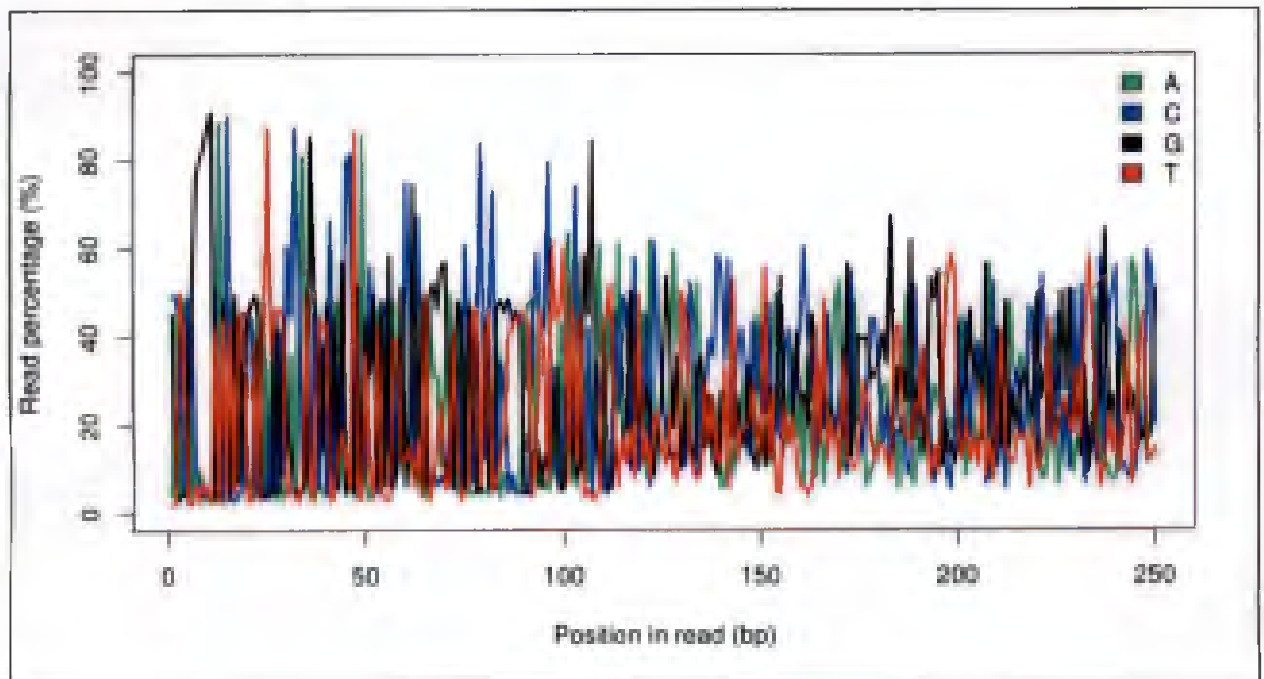


**Fig. b. Read 2**

**Plate 10.a. Base composition of YL-2**



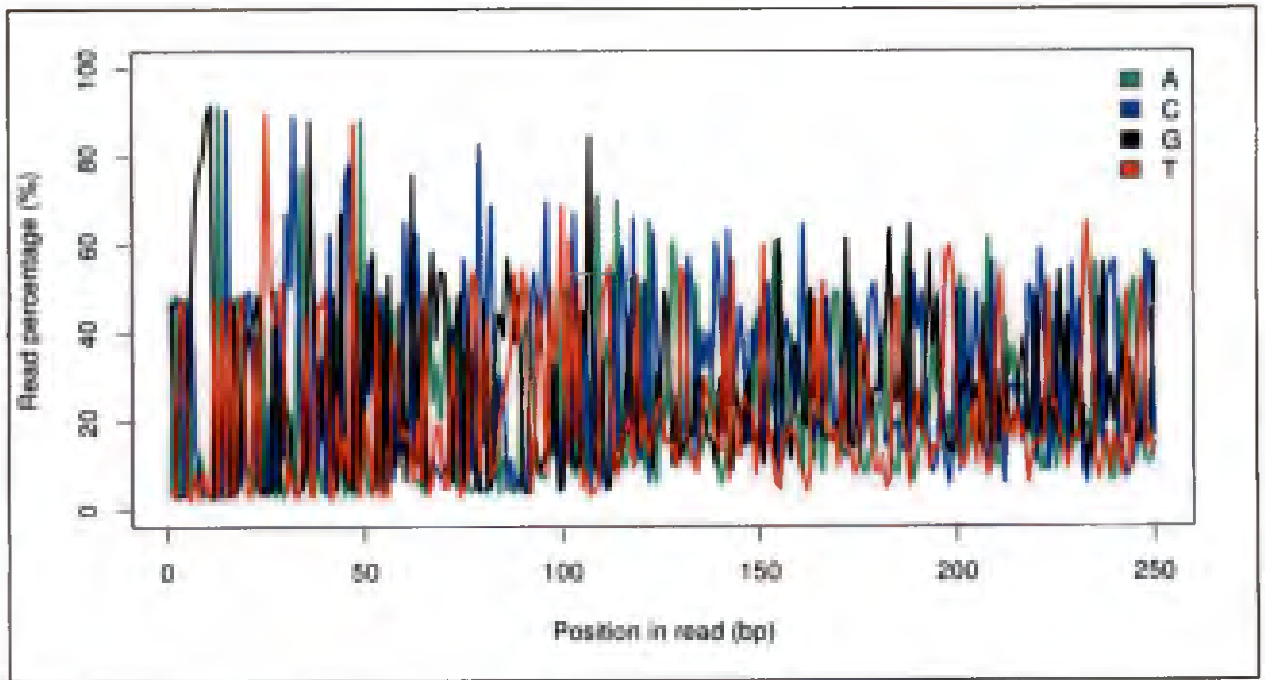
**Fig. a. Read 1**



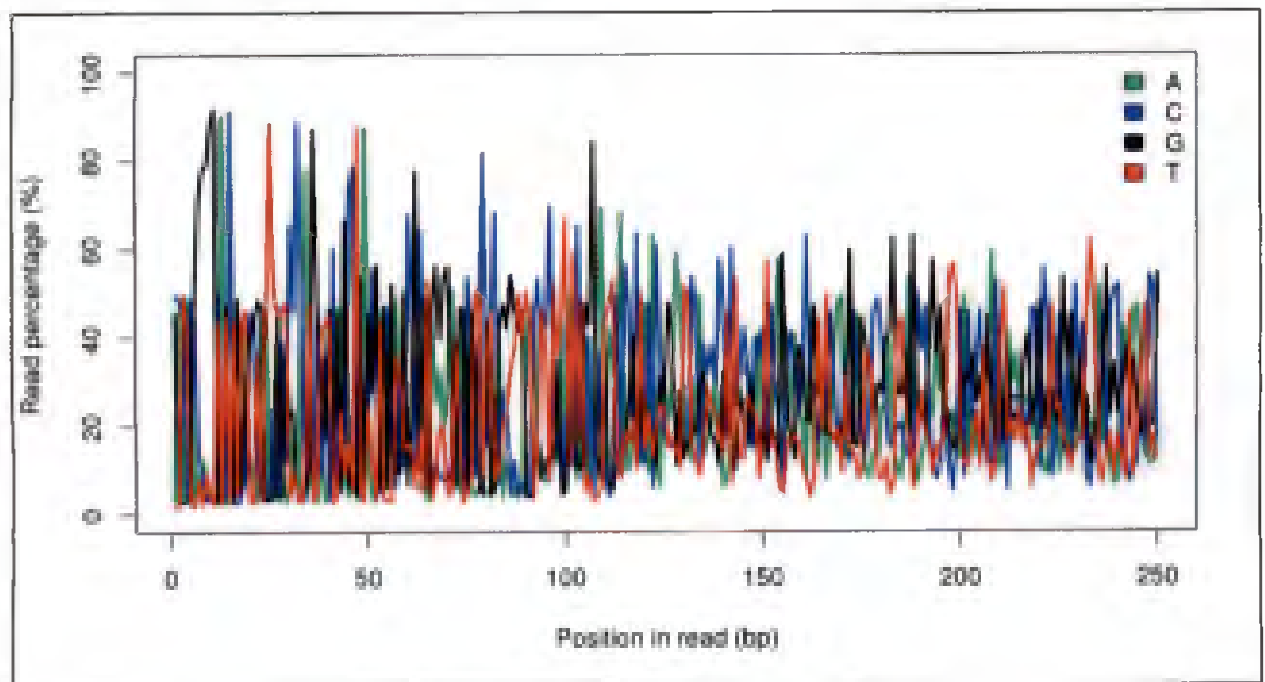
**Fig.b. Read 2**

**Plate 10.b. Base composition of YL-7**



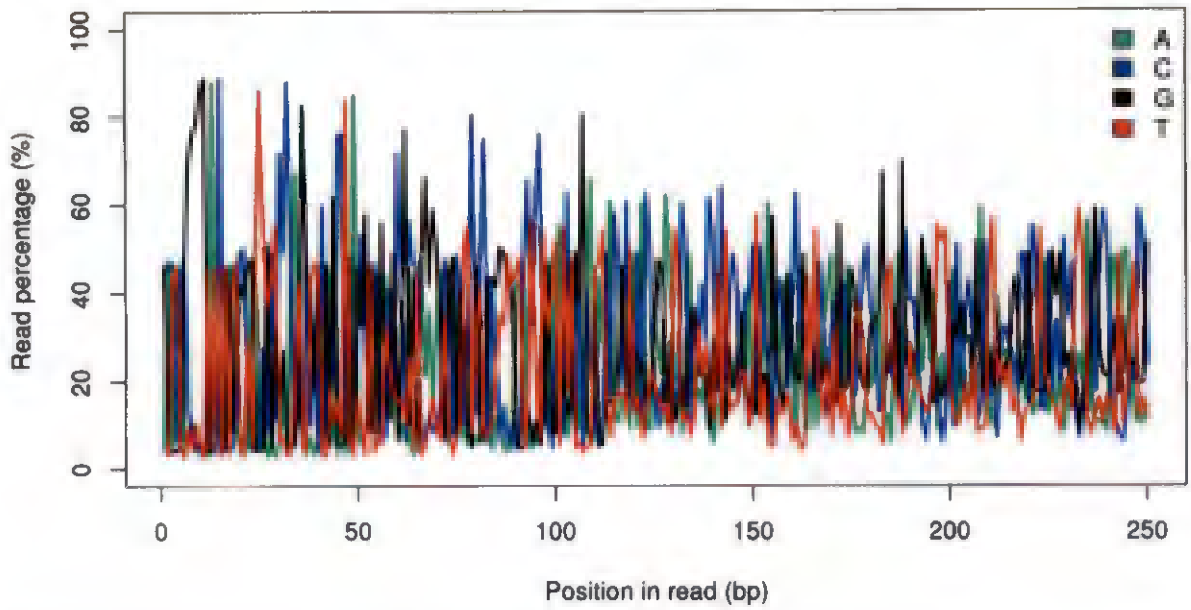


**Fig. a. Read 1**

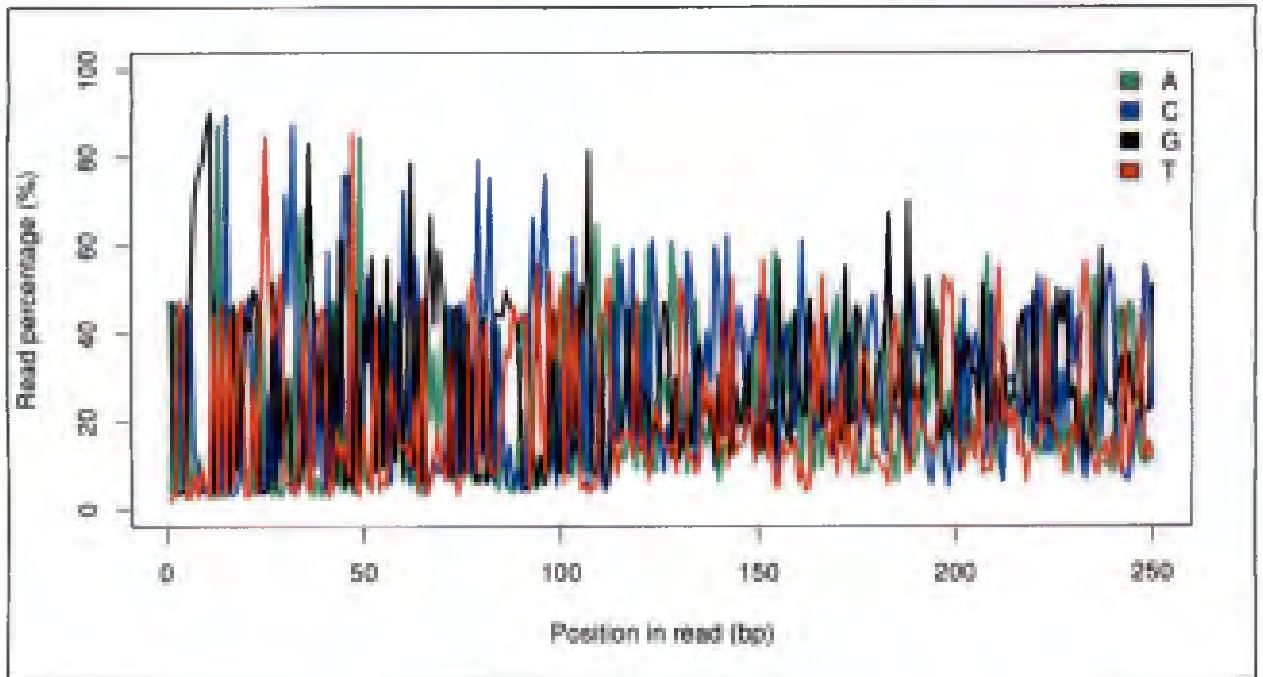


**Fig. b. Read 2**

**Plate 10. c. Base composition of AH-2**

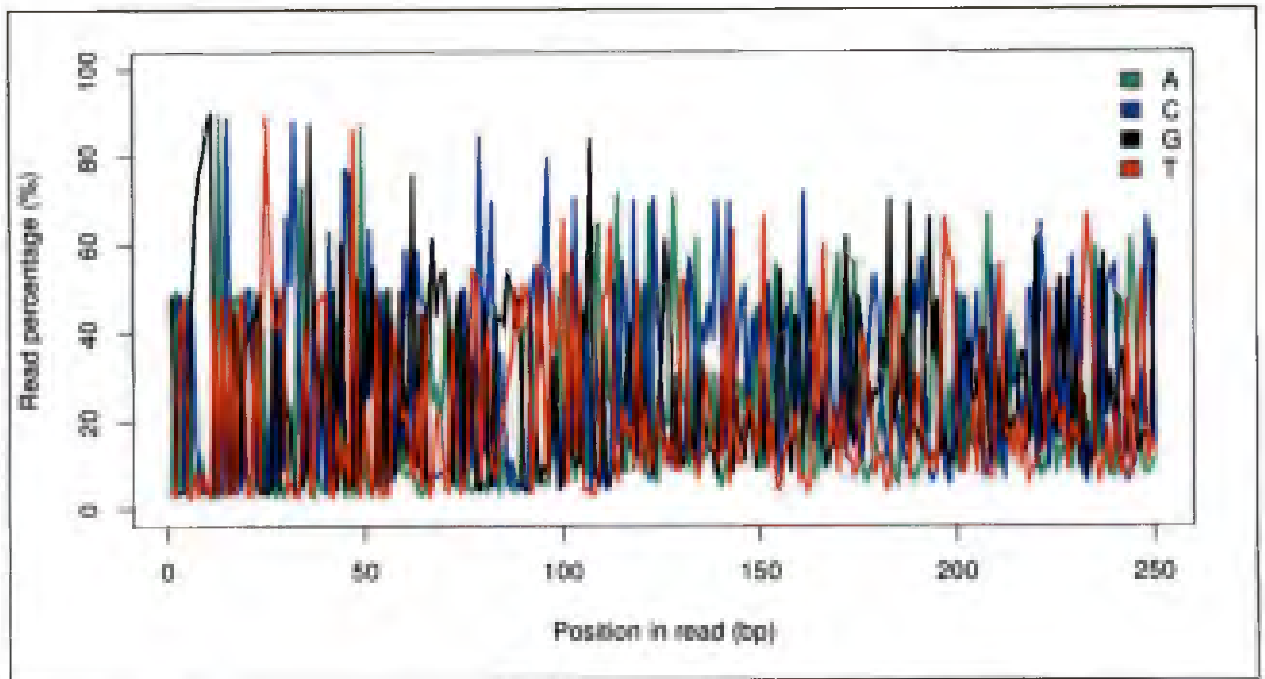


**Fig. a. Read 1**

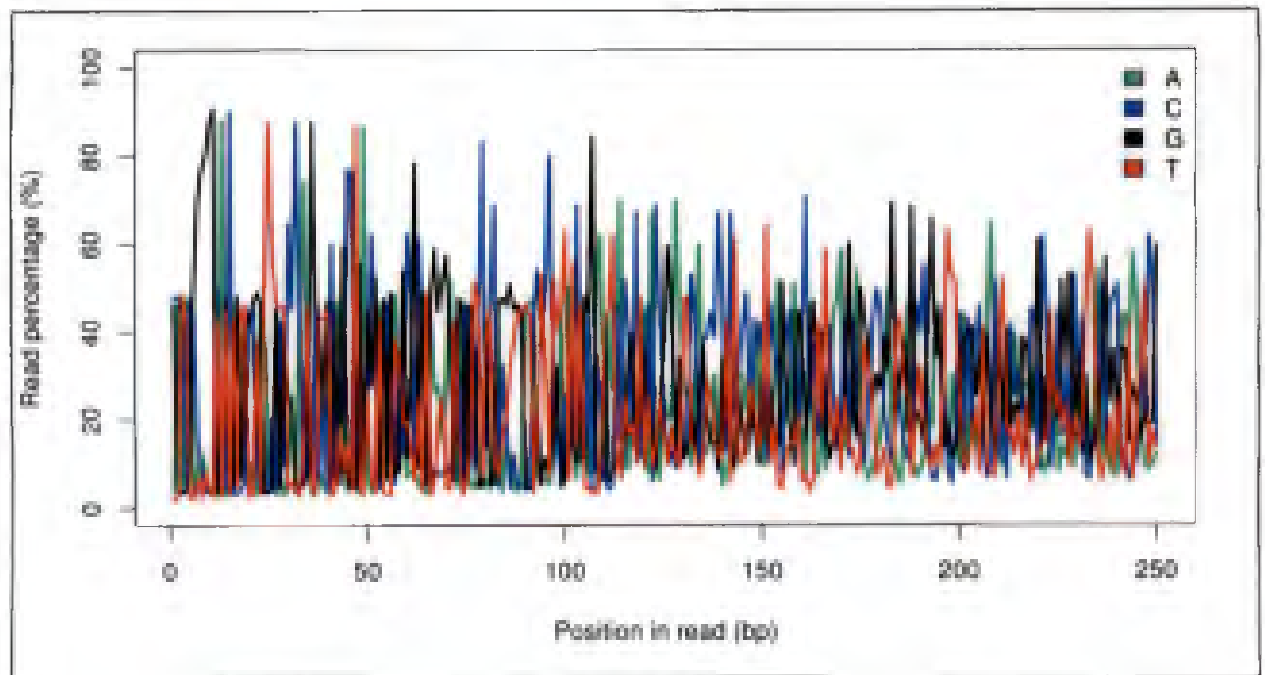


**Fig. b. Read 2**

**Plate 10. d. Base composition of AH-5**

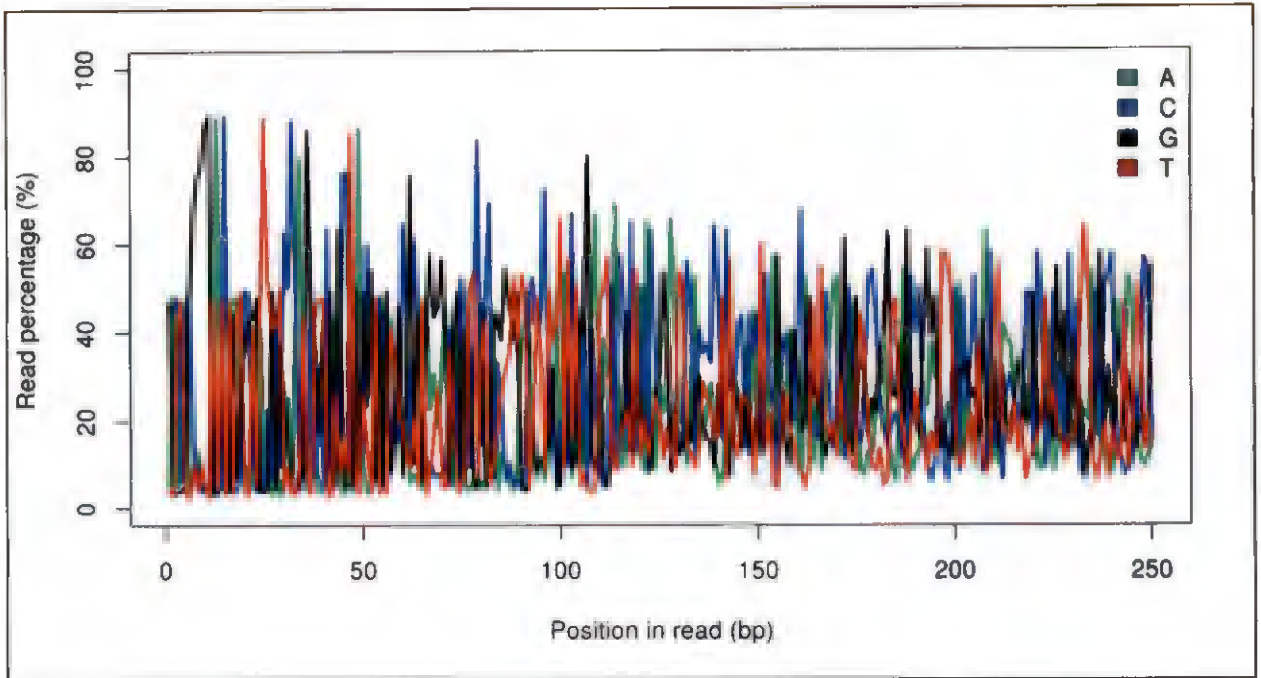


**Fig. a. Read 1**

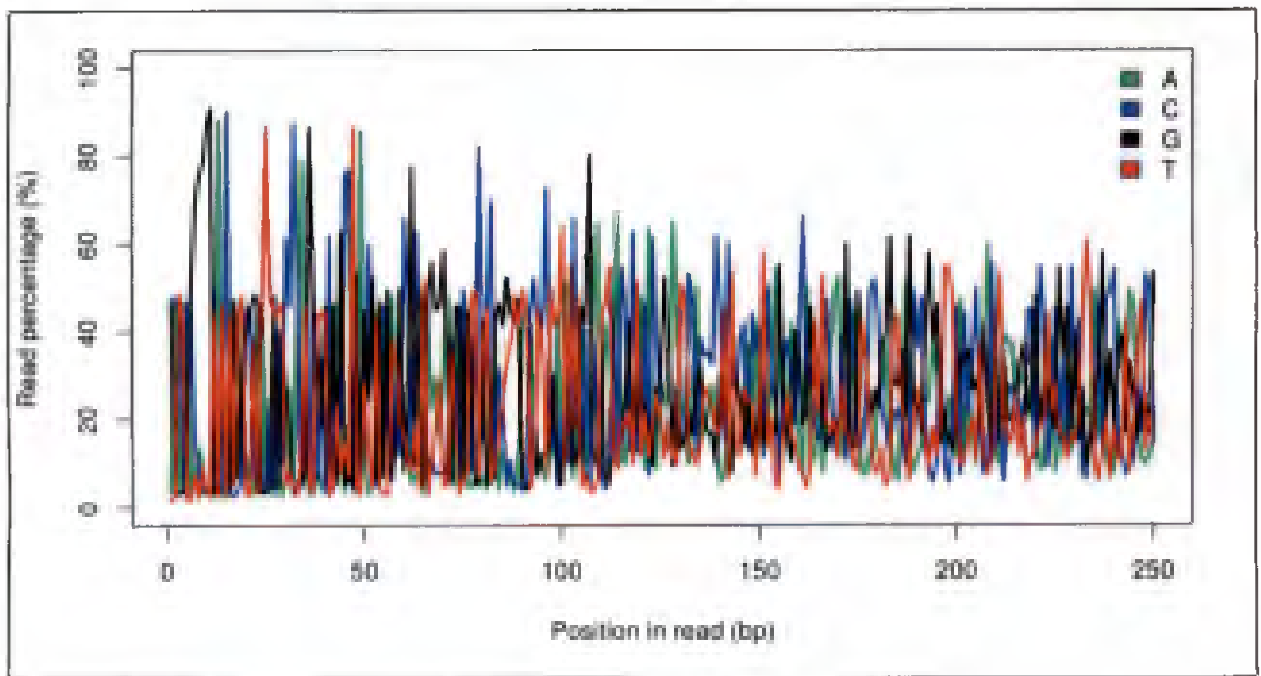


**Fig. b. Read 2**

**Plate 10. e. Base composition of AH-7**

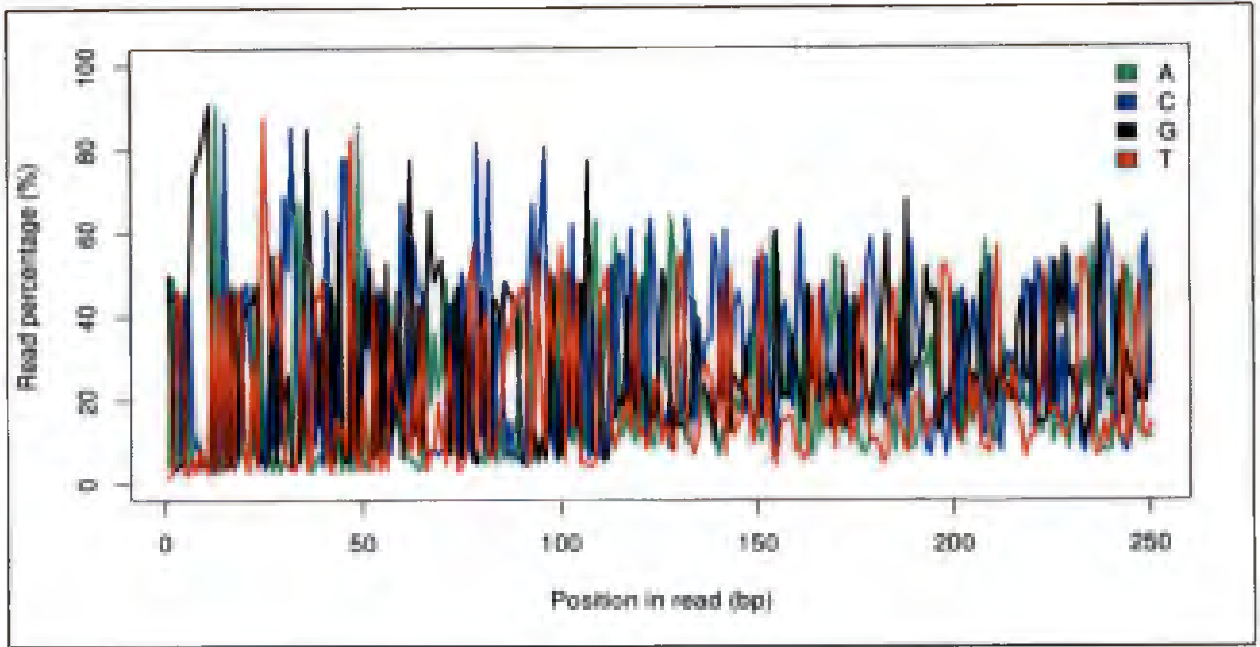


**Fig. a. Read 1**

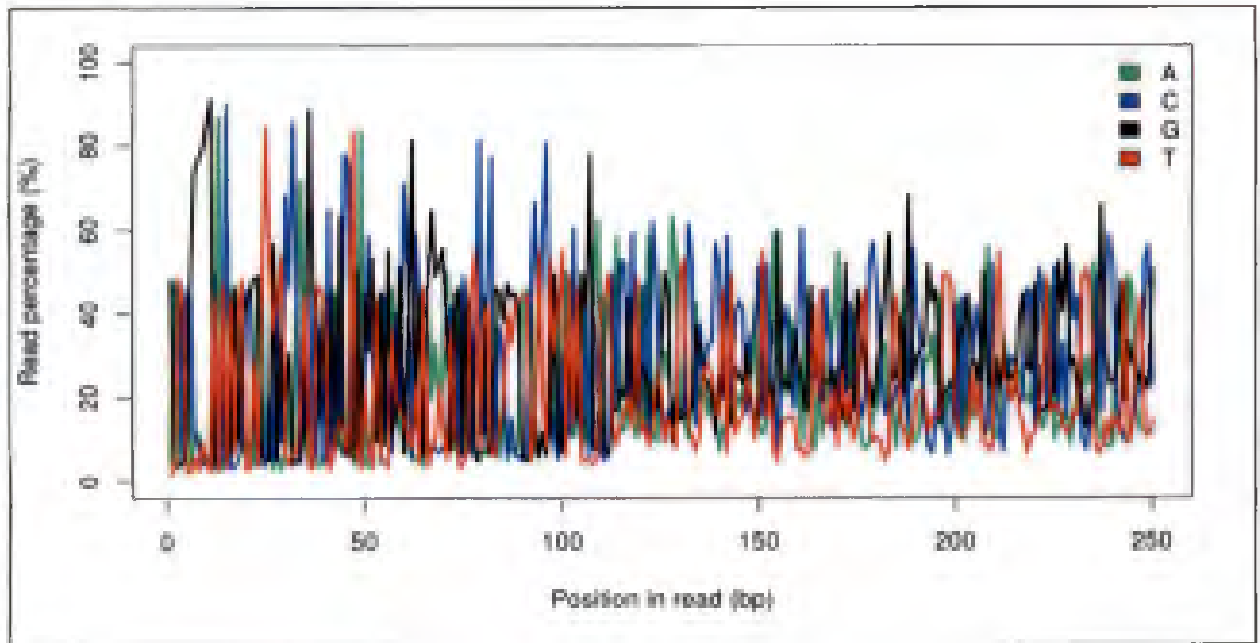


**Fig. b. Read 2**

**Plate 10. f. Base composition of CH-5**



**Fig. a. Read 1**



**Fig. b. Read 1**

**Plate 10. g. Base composition of CH-7**

**Plate 10. Base composition of all samples**

**Table 19. Total reads passed through each filter**

Sample Name	Total Reads	Passed Conserved Region Filter	Passed Mismatch Filter
YL-2	558634	558634	557866
YL-7	887278	887278	0
AH-2	848955	848955	0
AH-5	773725	773725	0
AH-7	930829	930829	0
CH-5	860521	860521	0
CH-7	573716	573716	572658

#### 4.13 Chimera filter

The chimeric sequences were removed from the consensus reads to obtain the pre-processed reads using the tool UCHIME implemented in the tool USEARCH. The number of chimeric sequences and the pre-processed reads are provided in the table. The reads thus obtained were analysed for the taxonomical classification.

**Table 20. Pre-processed reads obtained after chimera filter**

Sample Name	Consensus Reads	Chimeric Sequences	Pre-processed Reads
YL-2	557866	24255	533611
YL-7	885364	64369	820995
AH-2	847510	42355	805155
AH-5	771892	22443	749449
AH-7	929040	39313	889727
CH-5	858723	37768	820955
CH-7	572658	11561	561097

#### 4.14 Taxonomical classification and relative abundance of the OTUs

The Operational Taxonomic Units (OTUs) were obtained by the pooling and clustering of the pre-processed reads based on the sequence similarity using Uclust program. The total number of OTUs obtained after the removal of singletons are provided in Table 20.

**Table 21. Total OTUs obtained after singleton removal**

Sample Name	Total Reads	Total OTUs Picked	Total Singleton OTUs	Total OTUs After Singleton Removal
YL-2	533611	261308	223572	37736
YL-7	820995	302299	256284	46015
AH-2	805155	417341	360650	56691
AH-5	749449	441387	387956	53431
AH-7	889727	384539	327676	56863
CH-5	820955	455005	398939	56066
CH-7	561097	304936	264760	40176

Identification of the OTUs was done using the QIIME program and PyNAST program was used to align the representative OTUs against the Greengenes core set, followed by the alignment against the chimeric datasets. The taxonomical classification was done by comparing datasets against the SILVA database using RDP classifier. The taxonomical classification was done and the top ten taxa (phylum, class, order, family, genus and species) were represented graphically using this method.

#### **4.15 Bacterial diversity analysed using QIIME program**

The analysis of bacterial diversity from the sequences obtained after the filtering process was done using three tools namely, QIIME, PyNAST and RDP classifier. Bacterial diversity analysed using the QIIME program yielded a wide array of bacterial taxa indicating the population dynamics of the various categories of samples. The apparently healthy rhizosphere soil samples had a similar population make, as the majority of the bacterial population was composed of phylum Proteobacteria. In the sample AH-2, the phylum Proteobacteria contributed to almost 29 per cent of the bacterial population followed by Actinobacteria (10.7 %) and the third largest phylum being Firmicutes (10 %). The sample AH-5 was composed of a population with Proteobacteria being the majority, but in a lesser proportion of almost 20 per cent compared to the other apparently healthy rhizosphere soil samples, followed by 19 per cent of Actinobacteria which was closely followed by Acidobacteria with 18 per cent of the total population. In the sample AH-7, the larger



proportion of the bacterial assemblage was occupied by Proteobacteria (40.7 %), followed by Acidobacteria at 14.2 per cent and thirdly by Actinobacteria (12.11 %). The samples also yielded novel bacterial phyla like Candidate division BRC1, OD1, OP11, TM7 and WS3 at lesser proportions. The unknown categories of bacterial phyla were present in a proportion of 11.8, 13.5 and 10.8 per cent in the samples AH-2, AH-5 and AH-7 respectively.

The sample CH-5 was analysed for its bacterial diversity and the predominant phyla was found to be Proteobacteria (42 %) followed by Actinobacteria (9.7 %) and Acidobacteria (9.05 %). The sample YL-7 was found to be majorly composed of Proteobacteria (38 %), followed by Actinobacteria (21.3%) while using the QIIME program to compare against the Greengenes core set of sequences. The major phylum in the sample YL-2 was observed to be Proteobacteria being 42 per cent of the total OTUs, followed by 10 per cent of the total OTUs occupied by phylum Bacteroidetes. The major phylum in the sample CH7 was also observed to be Proteobacteria (31.5%), followed by Acidobacteria (27.5%).

#### **4.16 Bacterial diversity analysed using MEGAN V6.8.13**

A BIOM file provided was provided as a supplementary concise information on the bacterial diversity expressed in terms of the total and assigned number of reads. The BIOM file was used as the input file for MEGAN V.6.8.13 to obtain bacterial taxonomic composition of the given sample using NCBI taxonomy. A phylogenetic tree for each sample was generated and comparative analysis between the yellowing affected and apparently healthy rhizosphere soil samples was carried out. Graphical representations for all the analyses were closely studied for the community variations among them. A comparison between the samples YL-7 and AH-7 provided an insight into the increased proportion of Actinobacteria in YL-7 when compared to AH-7 and an increased population of Proteobacteria in AH-7. The same pattern was observed

during the comparison of the sample YL-7 with CH-5, even though both the samples were collected from different location.

#### **4.17 Bacterial diversity analysed using MG-RAST pipeline**

The fasta sequences were uploaded into the MG-RAST pipeline and subjected to taxonomical analysis. The graphical representations using krona was obtained for easy comparison and tab separated files in csv format were exported for the calculation of population indices. The bacterial diversity at phylum and genus level was studied and an increased population of Proteobacteria in the sample CH-5 was observed. A considerably increased proportion of Actinobacteria in the sample YL-7 was also observed and the percentage composition of the specific phylum was observed to be comparatively less in other samples.

The phylum level bacterial and archaeal diversity was estimated to find out the dominant phyla in each sample. Among the apparently healthy rhizosphere soil samples, Actinobacteria was found to be dominant in AH-2 and AH-5, while the dominant phylum in AH-7 was observed to be Acidobacteria. The completely healthy rhizosphere soil samples (CH-5 and CH-7) had a dominant population of Proteobacteria. In sample YL-2, the dominant phylum was found Bacteroidetes and in the sample YL-7, phylum Actinobacteria was the dominant one.

The diversity of archaeobacteria at phylum level was studied and in all the seven samples studied, the phylum Thaumarchaeota was the dominant phylum in the domain Archaea, while the status of Euryarchaeota and Crenarchaeota varied among the samples. The sample YL-2 consisted of bacterial population from 22 phyla, while the corresponding apparently healthy sample AH-2 had 25 bacterial phyla. The yellowing affected rhizosphere soil sample YL-7 was observed to house 21 bacterial genera, while AH-7 had 24 bacterial phyla. The number of bacterial phyla in the samples AH-5, CH-5 and CH-7 was observed to be 24, 22 and 23 respectively. The phylum level bacterial and archaeal distribution has been enlisted in Table 22.

**Table 22. Phylum-level bacterial and archaeal diversity rhizosphere samples**

Domain	Phylum	No. of reads assigned						
		YL-2	AH-2	YL-7	AH-7	AH-5	CH-5	CH-7
Bacteria	Actinobacteria	55394	92594	409823	75716	91689	66485	55034
	Firmicutes	42979	84380	42068	44889	53651	64180	18172
	Bacteroidetes	88619	75301	45190	40323	6074	81690	4625
	Proteobacteria	40796	67218	113063	61372	54223	132502	65237
	Acidobacteria	16839	40194	12819	108535	56331	28663	5834
	Verrucomicrobia	4507	19217	7077	9405	26821	12468	5858
	Gemmatimonadetes	2678	12051	564	0	2715	1884	2986
	Chloroflexi	2976	4035	1372	9172	2724	1194	2849
	Planctomycetes	1953	3351	1941	1861	2309	2717	5290
	Spirochaetes	617	1554	1378	1820	697	1595	2377
	Cyanobacteria	211	370	440	1385	146	410	197
	Chlamydiae	252	330	434	226	63	431	39
	Nitrospirae	186	327	2266	1187	3191	4035	501
	Synergistetes	16	66	47	100	16	13	9
	Deinococcus-Thermus	221	63	215	30	236	793	179
	Aquificae	33	52	8	29	127	5	27
	Thermotogae	26	43	29	155	66	72	31
	Tenericutes	9	27	5	29	3	17	9
	Chlorobi	0	26	60	23	2	77	64
	Elusimicrobia	1	8	0	4	4	0	0
	Deferribacteres	0	1	0	1	6	1	3
	Dictyoglomi	1	1	1	4	4	0	3
	Fibrobacteres	0	1	0	1	0	4	14
Fusobacteria	1	1	4	7	1	0	0	
Thermodesulfobacteria	2	1	0	9	26	1	36	
Unclassified	151434	167933	138678	265653	165159	169080	142930	
Archaea	Thaumarchaeota	20	937	122	405	1545	464	1717
	Euryarchaeota	4	32	114	43	1	20	8
	Crenarchaeota	0	5	1	53	107	9	4
	Unclassified	28	10	0	52	1	5	0

#### 4.18 Diversity of Archaeobacteria in the rhizosphere soil samples

A total of 7 genera was observed in the sample AH2, while only 4 genera were present in the corresponding yellowing affected rhizosphere soil sample. The sample YL-7 housed 7 genera of archaeobacteria, while the corresponding apparently healthy sample AH-7 consisted of 6 archaeal genera, but an increased number of individuals were found in the sample AH-7. The samples AH-5, CH-5 and CH-7 had a total number 5, 8 and 7 archaeal genera respectively. The genus-level archaeal diversity is provided in Table 23.

**Table 23. Genus-level diversity of domain Archaeobacteria in the samples**

<b>Genus-level archaeal assemblage in the sample YL-2</b>				
<b>Phylum</b>	<b>Class</b>	<b>Order</b>	<b>Family</b>	<b>Genus</b>
Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	<i>Haloterrigena</i> (2)
	Methanobacteria	Methanobacteriales	Methanobacteriaceae	<i>Methanobacterium</i> (1)
	Methanomicrobia	Methanocellales	Methanocellaceae	<i>Methanocella</i> (1)
Thaumarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrososphaera</i> (20)
Unclassified	Unclassified	Unclassified	Unclassified	Unclassified (28)
<b>Genus-level archaeal assemblage in the sample AH-2</b>				
Crenarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrosocaldus</i> (5)
Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	<i>Halopiger</i> (1)
	Methanobacteria	Methanobacteriales	Methanobacteriaceae	<i>Methanobacterium</i> (2)
	Methanomicrobia	Methanocellales	Methanocellaceae	<i>Methanocella</i> (5)
		Methanosarcinales	Methanosaeataceae	<i>Methanosaeta</i> (4)
Methanosarcinales	Methanosarcinaceae	<i>Methanosarcina</i> (20)		
Thaumarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrososphaera</i> (937)
Unclassified	Unclassified	Unclassified	Unclassified	Unclassified (10)
<b>Genus-level archaeal assemblage in the sample YL-7</b>				
Crenarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrosocaldus</i> (1)
Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	<i>Halopiger</i> (24), <i>Natrinema</i> (1), <i>Natronobacterium</i> (1)
	Methanobacteria	Methanobacteriales	Methanobacteriaceae	<i>Methanobacterium</i> (1)
	Methanomicrobia	Methanosarcinales	Methanosaeataceae	<i>Methanosaeta</i> (87)

Thaumarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrososphaera</i> (122)
<b>Genus-level archaeal assemblage in the sample AH-7</b>				
Crenarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrosocaldus</i> (53)
Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	<i>Methanobacterium</i> (3)
	Methanomicrobia	Methanocellales	Methanocellaceae	<i>Methanocella</i> (14)
		Methanomicrobiales	Methanocorpusculaceae	<i>Methanocorpusculum</i> (1)
		Methanosarcinales	Methanosaetaceae	<i>Methanosaeta</i> (24)
	Unclassified	Unclassified	Unclassified	Unclassified (1)
Thaumarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrososphaera</i> (405)
Unclassified	Unclassified	Unclassified	Unclassified	Unclassified (52)
<b>Genus-level archaeal assemblage in the sample AH-5</b>				
Crenarchaeota	Thermoprotei	Fervidicoccales	Fervidicoccaceae	<i>Fervidicoccus</i> (1)
		Thermoproteales	Thermoproteaceae	<i>Pyrobaculum</i> (1)
	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrosocaldus</i> (105)
Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae	<i>Methanosaeta</i> (1)
Thaumarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrososphaera</i> (1545)
Unclassified	Unclassified	Unclassified	Unclassified	Unclassified (1)
<b>Genus-level archaeal assemblage in the sample CH-5</b>				
Crenarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrosocaldus</i> (9)
Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	<i>Halopiger</i> (1), <i>Natrinema</i> (1)
	Methanobacteria	Methanobacteriales	Methanobacteriaceae	<i>Methanobacterium</i> (3), <i>Methanobrevibacter</i> (1)
			Unclassified	<i>Methanolinea</i> (1)
		Methanosarcinales	Methanosaetaceae	<i>Methanosaeta</i> (2)
	Unclassified	Unclassified	Unclassified	Unclassified (11)
Thaumarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrososphaera</i> (464)
Unclassified	Unclassified	Unclassified	Unclassified	Unclassified (5)
<b>Genus-level archaeal assemblage in the sample CH-7</b>				
Crenarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrosocaldus</i> (4)
Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	<i>Haloferax</i> (2)
	Methanobacteria	Methanobacteriales	Methanobacteriaceae	<i>Methanobrevibacter</i> (1), <i>Methanothermobacter</i> (1)
			Methanosarcinales	Methanosaetaceae
		Methanosarcinaceae	<i>Methanosarcina</i> (3)	
Thaumarchaeota	Unclassified	Unclassified	Unclassified	<i>Candidatus Nitrososphaera</i> (1717)

\*Figures given in the parenthesis indicate number of assigned reads



#### 4.19 Bacterial diversity in the sample YL-2

The most abundant phylum in the sample YL-2 was observed to be Bacteroidetes, occupying 22 per cent of the total bacterial population, followed by Actinobacteria (14%). The third abundant phylum was Proteobacteria and Firmicutes each occupying 10 per cent of the bacterial population. In the phylum Bacteroidetes, genus *Terrimonas* was found to be the dominant one being 40 per cent of the phylum followed by *Chitinophaga* (28%). The genus *Athrobacter* being the dominant genera in phylum Actinobacteria occupied 41 per cent of the phylum with *Thermoleophilum* (10%) being the second abundant genera.

The phylum Proteobacteria was mainly composed of the class Betaproteobacteria being 49 per cent of the phylum, followed by 22 per cent of Deltaproteobacteria and thirdly by Alphaproteobacteria (17%). The phylum Proteobacteria was found to harbor the most number of unclassified bacterial population in all the three dominant classes of the phylum. The most abundant known genus of the phylum Proteobacteria was observed to be *Massilia* (11%) followed by *Burholderia* (4%). The phylum Firmicutes was majorly composed of the genus *Bacillus* (59%) followed by *Clostridium* (8%) and *Paenibacillus* (4%). The genus level bacterial diversity of the 10 most abundant phyla has been provided in Table 24.

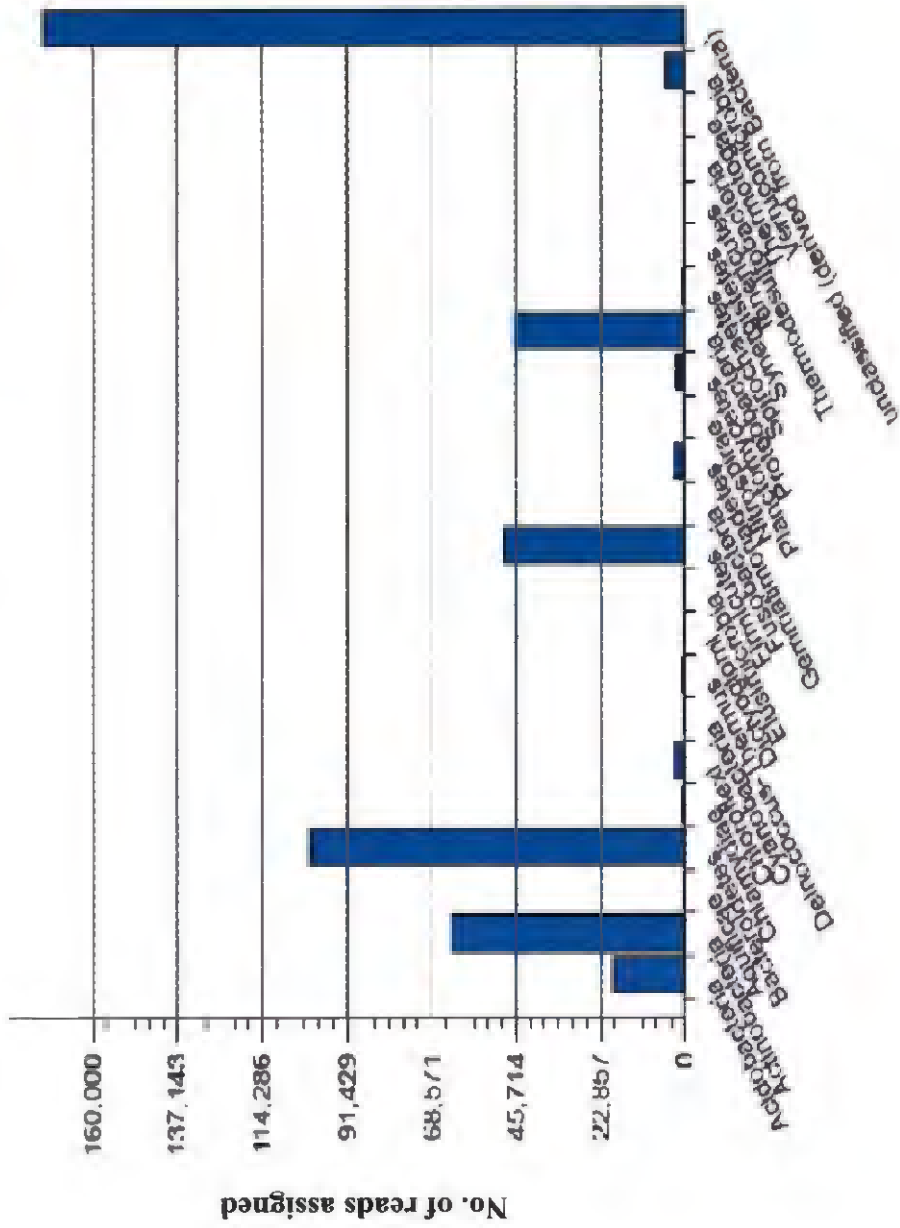


Plate 11. Phylum-level bacterial diversity in the sample YL-2 obtained using MG-RAST pipeline



**Table 24. Genus-level taxonomic assemblage of bacterial diversity from 10 predominant phyla in the sample YL-2**

Phylum	Class	Order	Family	Genus	
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	<i>Bacteroides</i> (163)	
			Porphyromonadaceae	<i>Butyrivimonas</i> (8), <i>Odoribacter</i> (1), <i>Parabacteroides</i> (45), <i>Porphyromonas</i> (12)	
			Prevotellaceae	<i>Prevotella</i> (34)	
	Cytophagia	Cytophagales	Rikenellaceae	<i>Alistipes</i> (328), <i>Rikenella</i> (322)	
			Cyclobacteriaceae	<i>Cyclobacterium</i> (63)	
			Cytophagaceae	<i>Cytophaga</i> (978), <i>Dyadobacter</i> (1), <i>Flectobacillus</i> (2), <i>Flexibacter</i> (6960), <i>Hymenobacter</i> (553), <i>Marinoscillum</i> (2), <i>Microscilla</i> (21), <i>Spirosoma</i> (57)	
			Flammeovirgaceae	<i>Flammeovirga</i> (7), <i>Flexithrix</i> (87), <i>Persicobacter</i> (6)	
	Flavobacteria	Flavobacteriales	Flavobacteriaceae	<i>Aquimarina</i> (1), <i>Arenibacter</i> (49), <i>Bergeyella</i> (1), <i>Chryseobacterium</i> (4), <i>Coenonia</i> (1), <i>Dokdonia</i> (1), <i>Elizabethkingia</i> (941), <i>Empedobacter</i> (55), <i>Flavobacterium</i> (980), <i>Gramella</i> (1024), <i>Leeuwenhoekella</i> (4), <i>Myroides</i> (6), <i>Ornithobacterium</i> (3), <i>Riemerella</i> (52), <i>Robignitalea</i> (43), <i>Salegentibacter</i> (1), <i>Tenacibaculum</i> (48), <i>Waytarsiella</i> (2), <i>Zurongwangia</i> (10)	
				Rhodothermaceae	<i>Rhodothermus</i> (9), <i>Salinibacter</i> (12)
				Saprospiraceae	<i>Saprospira</i> (8)
Sphingobacteriaceae				<i>Pedobacter</i> (1188), <i>Sphingobacterium</i> (3356), Unclassified (7321)	
Unclassified				<i>Chitinophaga</i> (24954), <i>Terrimonas</i> (35852)	
Unclassified				<i>Candidatus Amoebophilus</i> (2462), <i>Candidatus Cardinium</i> (3), <i>Proloxibacter</i> (318), Unclassified (260)	
Unclassified				<i>Acidimicrobium</i> (398), <i>Acidithiomicrobium</i> (29)	
Actinobacteria	Actinobacteria	Actinobacteriales	Acidimicrobiaceae	<i>Acidothermus</i> (136)	
			Actinotherrmaceae	<i>Actinobaculum</i> (7), <i>Actinomycetes</i> (6), <i>Arcanobacterium</i> (10), <i>Mobiluncus</i> (1)	
			Actinomycetaceae	<i>Actinokineospora</i> (25), <i>Actinosynnema</i> (1), <i>Lechevalieria</i> (13), <i>Lentzea</i> (10), <i>Saccharothrix</i> (3)	
			Actinosynnemataceae		
			Actinosynnemataceae		

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Beutenbergiaceae	<i>Beutenbergia</i> (53)
Brevibacteriaceae	<i>Brevibacterium</i> (416)
Catenulisporaceae	<i>Catenulispora</i> (17)
Cellulomonadaceae	<i>Actinotalea</i> (4), <i>Cellulomonas</i> (37)
Corynebacteriaceae	<i>Corynebacterium</i> (1167)
Dermabacteraceae	<i>Brachybacterium</i> (26)
Dermacoccaceae	<i>Dermacoccus</i> (7), <i>Kytococcus</i> (11)
Dermatophilaceae	<i>Dermatophilus</i> (21)
Dietziaceae	<i>Dietzia</i> (14)
Frankiaceae	<i>Frankia</i> (549)
Geodermatophilaceae	<i>Geodermatophilus</i> (72)
Glycomycetaceae	<i>Stackebrandtia</i> (25)
Gordoniaceae	<i>Gordonia</i> (255), <i>Skermania</i> (1)
Intrasporangiaceae	<i>Intrasporangium</i> (290), <i>Janibacter</i> (1067), <i>Serinicoccus</i> (15), <i>Terrabacter</i> (856), <i>Terracoccus</i> (6), <i>Tetrasphaera</i> (133)
Kineosporiaceae	<i>Kineococcus</i> (10)
Microbacteriaceae	<i>Agrococcus</i> (27), <i>Agromyces</i> (36), <i>Candidatus Aquiluna</i> (1), <i>Clavibacter</i> (8), <i>Cryobacterium</i> (46), <i>Curtobacterium</i> (5), <i>Glaciibacter</i> (2), <i>Leifsonia</i> (57), <i>Leucobacter</i> (1), <i>Microbacterium</i> (367), <i>Mycteocola</i> (17), <i>Pseudoclavibacter</i> (3), <i>Rathayibacter</i> (28), <i>Subtercola</i> (1)
Micrococcaceae	<i>Arthrobacter</i> (22550), <i>Kocuria</i> (31), <i>Micrococcus</i> (109), <i>Nesterenkonia</i> (9), <i>Renibacterium</i> (161), <i>Rothia</i> (192)
Micromonosporaceae	<i>Actinoplanes</i> (60), <i>Catenuloplanes</i> (280), <i>Dactylosporangium</i> (151), <i>Micromonospora</i> (1406), <i>Salinispora</i> (42), <i>Verrucosipora</i> (22)
Mycobacteriaceae	<i>Mucobacterium</i> (1579)
Nakamurellaceae	<i>Nakamurella</i> (56)
Nocardiaceae	<i>Nocardia</i> (46), <i>Rhodococcus</i> (1326), <i>Smaragdicooccus</i> (29)

			Nocardioideae	<i>Aeromicrobium</i> (18), <i>Kribbella</i> (664), <i>Nocardioidea</i> (227), <i>Pimelobacter</i> (64)
			Nocardioaceae	<i>Nocardopsis</i> (175), <i>Streptomonospora</i> (1), <i>Thermobifida</i> (3)
			Promicromonosporaceae	<i>Cellulosimicrobium</i> (40), <i>Promicromonospora</i> (31)
			Propionibacteriaceae	<i>Microbacterium</i> (26), <i>Propionibacterium</i> (247), Unclassified (220)
			Pseudonocardaceae	<i>Amicolatopsis</i> (142), <i>Kutzneria</i> (11), <i>Pseudonocardia</i> (310), <i>Saccharomonospora</i> (2), <i>Saccharopolyspora</i> (910), <i>Streptoalloeichus</i> (6), <i>Thermobispora</i> (30)
			Rarobacteraceae	<i>Rarobacter</i> (22)
			Sanguibacteraceae	<i>Sanguibacter</i> (7)
			Segniliparaceae	<i>Segniliparus</i> (13)
			Streptomycetaceae	<i>Kitasatospora</i> (14), <i>Streptacidiphilus</i> (2), <i>Streptomyces</i> (1116)
			Streptosporangiaceae	<i>Microbispora</i> (62), <i>Microtetraspora</i> (9), <i>Nonomuraea</i> (29), <i>Planomonospora</i> (10), <i>Streptosporangium</i> (365)
			Thermomonosporaceae	<i>Atinocallomurus</i> (326), <i>Actinocorallia</i> (44), <i>Actinomadura</i> (705), <i>Spirillospora</i> (3), <i>Thermomonospora</i> (565), Unclassified (230)
			Tsakamurellaceae	<i>Tsakamurella</i> (9)
			Williamsiaceae	<i>Williamsia</i> (1866)
			Unclassified	<i>Tropheryma</i> (5), Unclassified (4)
		Bifidobacteriales	Bifidobacteriaceae	<i>Alloscardovia</i> (1), <i>Bifidobacterium</i> (2672), <i>Gardereila</i> (8)
		Coriobacteriales	Coriobacteriaceae	<i>Atopobium</i> (1856), <i>Collinsella</i> (80), <i>Cryptobacterium</i> (9), <i>Eggerthella</i> (2), <i>Enterorhabdus</i> (6), <i>Gordonibacter</i> (176), <i>Slackia</i> (2)
		Rubrobacterales	Rubrobacteraceae	<i>Rubrobacter</i> (341)
		Thermoleophilales	Thermoleophilaceae	<i>Thermoleophilum</i> (5366)
		Unclassified	Unclassified	Unclassified (3)
		Bacillales	Alicyclobacillaceae	<i>Alicyclobacillus</i> (649), Unclassified (1)
Firmicutes		Bacilli		

	Bacillaceae	<i>Amphibacillus</i> (15), <i>Anaerobacillus</i> (11), <i>Anoxybacillus</i> (894), <i>Bacillus</i> (25495), <i>Geobacillus</i> (174), <i>Halobacillus</i> (143), <i>Lysinibacillus</i> (735), <i>Marinococcus</i> (1), <i>Oceanobacillus</i> (617), <i>Terribacillus</i> (7), <i>Virgibacillus</i> (240)
	Listeriaceae	<i>Listeria</i> (6)
	Paenibacillaceae	<i>Aneurinibacillus</i> (55), <i>Brevibacillus</i> (445), <i>Paenibacillus</i> (1614), <i>Thermobacillus</i> (5)
	Pasteuriaceae	<i>Pasteuria</i> (8)
	Planococcaceae	<i>Jeotgalibacillus</i> (2), <i>Kurthia</i> (680), <i>Planococcus</i> (4), <i>Planomicrobium</i> (121), <i>Sporosarcina</i> (377), <i>Ureibacillus</i> (49), <i>Viridibacillus</i> (108)
	Sporolactobacillaceae	<i>Sporolactobacillus</i> (19)
	Staphylococcaceae	<i>Macroccocus</i> (1), <i>Staphylococcus</i> (77)
	Thermoactinomycetaceae	<i>Laceyella</i> (133), <i>Mechercharimyces</i> (3), <i>Seinonella</i> (41), <i>Thermoactinomycetes</i> (186)
	Unclassified	<i>Alkalilactibacillus</i> (1), <i>Bhargavaea</i> (2), <i>Exiguobacterium</i> (64), <i>Gemella</i> (14), <i>Pultulanibacillus</i> (9), Unclassified (1)
	Aerococcaceae	<i>Abiotrophia</i> (7), <i>Aerococcus</i> (2)
	Carnobacteriaceae	<i>Atopostipes</i> (2), <i>Granulicatella</i> (9), <i>Trichococcus</i> (15)
	Enterococcaceae	<i>Enterococcus</i> (10), <i>Tetragenococcus</i> (7)
	Lactobacillaceae	<i>Lactobacillus</i> (200)
	Leuconostocaceae	<i>Weissella</i> (1)
	Streptococcaceae	<i>Lactococcus</i> (1), <i>Streptococcus</i> (4)
	Clostridiaceae	<i>Alkaliphilus</i> (219), <i>Butyricoccus</i> (14), <i>Caloramator</i> (15), <i>Clostridium</i> (3436), <i>Oxobacter</i> (15), <i>Tepidimicrobium</i> (4)
	Clostridiales Family XI. Incertae	<i>SedisAnaerococcus</i> (18), <i>SedisHelcococcus</i> (1), <i>SedisPeptinophilus</i> (1461), <i>SedisSedimentibacter</i> (1), <i>SedisSoehngenia</i> (2), <i>SedisSporanaerobacter</i> (726), <i>SedisTissierella</i> (466)
	Clostridiales Family XII. Incertae	<i>SedisFusibacter</i> (1)
	Lactobacillales	
	Clostridia	
	Clostridiales	

			Clostridiales Family XIV. Incertae	<i>SedisAnaerobranca</i> (6)
			Clostridiales Family XVII. Incertae	<i>SedisSulfobacillus</i> (13), <i>SedisThermaerobacter</i> (3)
			Clostridiales Family XVIII. Incertae	<i>SedisSymbiobacterium</i> (39)
			Eubacteriaceae	<i>Acetobacterium</i> (9), <i>Eubacterium</i> (30)
			Heliobacteriaceae	<i>Heliobacillus</i> (23), <i>Heliobacterium</i> (232), <i>Heliophilum</i> (23), <i>Heliorestis</i> (4)
			Lachnospiraceae	<i>Anerostipes</i> (2), <i>Butyrivibrio</i> (4), <i>Cellulosilyticum</i> (8), <i>Hespellia</i> (2), <i>Lachnospira</i> (10), <i>Robinsoniella</i> (16), <i>Roseburia</i> (9), Unclassified (58)
			Peptococcaceae	<i>Candidatus Desulfurodis</i> (2), <i>Dehalobacter</i> (22), <i>Desulfitobacterium</i> (118), <i>Desulfonispota</i> (152), <i>Desulfosporosimus</i> (228), <i>Desulfotomaculum</i> (684), <i>Pelotomaculum</i> (4), <i>Thermincola</i> (4), Unclassified (32)
			Peptostreptococcaceae	<i>Peptostreptococcus</i> (41)
			Ruminococcaceae	<i>Eithanoligenens</i> (14), <i>Faecalibacterium</i> (24), <i>Ruminococcus</i> (501), Unclassified (29)
			Syntrophomonadaceae	<i>Syntrophomonas</i> (29)
			Unclassified	<i>Epulopiscium</i> (30), Unclassified (139)
			Halanaerobiaceae	<i>Halarsenatibacter</i> (2)
			Natranaerobiaceae	<i>Natranaerobius</i> (80)
			Thermonanerobacteraceae	<i>Ammonifex</i> (1), <i>Caldanaerobacter</i> (11), <i>Caldanaerobium</i> (93), <i>Carboxydotherrmus</i> (1), <i>Moorella</i> (15), <i>Thermacetogenium</i> (2), <i>Thermoanaerobacter</i> (9)
			Thermoanaerobacterales Family III. Incertae	<i>SedisCaldicellulosiruptor</i> (142)
			Thermodesulfobiaceae	<i>Thermodesulfobium</i> (65)
			Unclassified	Unclassified (8)
			Unclassified	<i>Desulfitibacter</i> (14)
			Erysipelotrichaceae	<i>Erysipelotricha</i> (1), Unclassified (4)
			Acidaminococcaceae	<i>Acidaminococcus</i> (6), <i>Phascolarctobacterium</i> (6)
			Unclassified	
			Erysipelotrichales	
			Selenomonadales	
			Erysipelotrichi	
			Negativicutes	

Proteobacteria	Alphaproteobacteria		Veillonellaceae	<i>Dialister</i> (4), <i>Megasphaera</i> (43), <i>Mitsuokella</i> (4), <i>Pectinatus</i> (3), <i>Selenomonas</i> (119), <i>Sporomusa</i> (121), <i>Veillonella</i> (38)
		Caulobacterales	Caulobacteraceae	<i>Asticcacaulis</i> (45), <i>Brevundimonas</i> (33), <i>Caulobacter</i> (7), <i>Phenylbacterium</i> (92)
		Rhizobiales	Beijerinckiaceae	<i>Beijerinckia</i> (2), <i>Methyllocapsa</i> (2)
			Bradyrhizobiaceae	<i>Afpia</i> (7), <i>Balneimonas</i> (19), <i>Bosea</i> (190), <i>Bradyrhizobium</i> (617), <i>Nitrobacter</i> (33), <i>Rhodoblastus</i> (2), <i>Rhodopseudomonas</i> (43), Unclassified (22)
			Brucellaceae	<i>Mycoplana</i> (2), <i>Ochrobactrum</i> (6)
			Hyphomicrobiaceae	<i>Blastochloris</i> (26), <i>Hyphomicrobium</i> (148), <i>Rhodomicrobium</i> (12), <i>Rhodoplanes</i> (40)
			Methylobacteriaceae	<i>Methylobacterium</i> (290)
			Methylocystaceae	<i>Methylocystis</i> (27), <i>Methylosinus</i> (2), Unclassified (205)
			Phyllobacteriaceae	<i>Aminobacter</i> (10), <i>Chelativorans</i> (2), <i>Mesorhizobium</i> (200), <i>Phyllobacterium</i> (9)
			Rhizobiaceae	<i>Agrobacterium</i> (5), <i>Candidatus Liberibacter</i> (3), <i>Rhizobium</i> (115), <i>Sinorhizobium</i> (1)
			Rhodobiaceae	<i>Afpella</i> (1), <i>Rhodobium</i> (1)
			Xanthobacteraceae	<i>Xanthobacter</i> (1)
			Unclassified	Unclassified (52)
		Rhodobacteriales	Hyphomonadaceae	<i>Hirschia</i> (1), <i>Maricaulis</i> (1)
			Rhodobacteraceae	<i>Pannonibacter</i> (15), <i>Paracoccus</i> (1), <i>Rhodobacter</i> (1), <i>Rhodovulum</i> (2), Unclassified (2)
		Rhodospirillales	Acetobacteraceae	<i>Acidiphilum</i> (1), <i>Gluconacetobacter</i> (8), <i>Gluconobacter</i> (2), <i>Granulibacter</i> (73), <i>Kozakia</i> (1), <i>Neosassa</i> (1), <i>Rubritepida</i> (4)
			Rhodospirillaceae	<i>Azospirillum</i> (24), <i>Magnetospirillum</i> (1), <i>Rhodospirillum</i> (1), <i>Rhodovibrio</i> (1), <i>Telmatospirillum</i> (1), Unclassified (73)
		Rickettsiales	Anaplasmataceae	<i>Anaplasma</i> (1), <i>Neorickettsia</i> (1), <i>Wolbachia</i> (1)
			Rickettsiaceae	<i>Orientia</i> (1), Unclassified (2)
			Unclassified	<i>Candidatus Odysseella</i> (3)



	Sphingomonadales	Erythrobacteraceae Sphingomonadaceae	<i>Erythrobacter</i> (2), <i>Erythromicrobium</i> (3) <i>Blastomonas</i> (1), <i>Novosphingobium</i> (90), <i>Sandarakanorhabdus</i> (2), <i>Sphingobium</i> (54), <i>Sphingomonas</i> (125), <i>Sphingopyxis</i> (3), <i>Sphingosinicella</i> (1)
Betaproteobacteria	Unclassified	Unclassified	<i>Geminicoccus</i> (1), Unclassified (4132)
	Burkholderiales	Alcaligenaceae	<i>Achromobacter</i> (14), <i>Alcaligenes</i> (5), <i>Azohydromonas</i> (11), <i>Bordetella</i> (6), <i>Oligella</i> (1), <i>Pelstega</i> (1), Unclassified (3)
		Burkholderiaceae	<i>Burkholderia</i> (1490), <i>Candidatus Glomeribacter</i> (5), <i>Cupriavidus</i> (212), <i>Pandoraea</i> (13), <i>Paucimonas</i> (133), <i>Polynucleobacter</i> (22), <i>Ralstonia</i> (314)
		Comomonadaceae	<i>Acidovorax</i> (105), <i>Alicyclophilus</i> (1), <i>Brachymonas</i> (1), <i>Comamonas</i> (35), <i>Delftia</i> (4), <i>Pelomonas</i> (5), <i>Variovorax</i> (348), <i>Verminephrobacter</i> (36), <i>Xenophilus</i> (144), Unclassified (408)
		Oxalobacteraceae	<i>Collimonas</i> (68), <i>Duganella</i> (38), <i>Herbaspirillum</i> (353), <i>Herminiimonas</i> (27), <i>Janthinobacterium</i> (113), <i>Massilia</i> (4489), <i>Oxalicibacterium</i> (544), <i>Oxalobacter</i> (19), <i>Telluria</i> (332)
		Unclassified	<i>Leptothrix</i> (4), <i>Mitsuaria</i> (5), <i>Rubrivivax</i> (3), <i>Sphaerotilus</i> (9), <i>Thiobacter</i> (2), <i>Thiomonas</i> (2), Unclassified (548)
		Hydrogenophilales	<i>Thiobacillus</i> (1)
		Methylophilales	<i>Methylobacillus</i> (4)
		Neisseriales	<i>Aquaspirillum</i> (3), <i>Aquitalea</i> (5), <i>Chromobacterium</i> (14), <i>Laribacter</i> (1), <i>Neisseria</i> (5), <i>Stenoxobacter</i> (1)
		Nitrosomonadales	Nitrosomonadaceae
Deltaproteobacteria		Unclassified	Unclassified (6)
		Rhodocyclales	<i>Azoarcus</i> (2), <i>Dechloromonas</i> (3), <i>Sterolibacterium</i> (5), Unclassified (514)
		Unclassified	<i>Candidatus Trembalaya</i> (3), <i>Kinetoplastibacterium</i> (3), Unclassified (9336)
		Bdellovibrionales	<i>Bacteriovorax</i> (61), <i>Pereidibacter</i> (5)



			<i>Bdellovibrio</i> (30)
		Bdellovibrionaceae	<i>Desulfaiibacillum</i> (1), <i>Desulfobacter</i> (1), <i>Desulfobacterium</i> (3), <i>Desulfobotulus</i> (10), <i>Desulfocella</i> (90), <i>Desulfococcus</i> (4), <i>Desulfofaba</i> (1), <i>Desulfofrigus</i> (8), <i>Desulfonema</i> (115), Unclassified (2)
		Desulfobacteriales	<i>Desulfobulbus</i> (2), <i>Desulforhopalus</i> (5), Unclassified (5)
		Desulfovibrionales	<i>Desulfohalobium</i> (3), <i>Desulfonatronovibrio</i> (49), <i>Desulfothermus</i> (1), <i>Desulfomicrobium</i> (5)
		Desulfurellales	<i>Desulfonatronum</i> (10)
		Desulfuromonadales	<i>Desulfovibrio</i> (447), <i>Lawsonia</i> (1), Unclassified (2)
			<i>Desulfurella</i> (1)
			<i>Desulfuromonas</i> (26)
		Geobacteraceae	<i>Geothalibacter</i> (602), <i>Geobacter</i> (349)
		Pelobacteraceae	<i>Malonomonas</i> (2), <i>Pelobacter</i> (43)
		Cystobacteraceae	<i>Cystobacter</i> (51), <i>Melittangium</i> (7), <i>Stigmatella</i> (2)
		Haliangiaceae	<i>Haliangium</i> (4)
		Myxococcaceae	<i>Anaeromyxobacter</i> (31), <i>Corallocooccus</i> (46), <i>Myxococcus</i> (52)
		Nannocystaceae	<i>Nannocystis</i> (6)
		Polyangiaceae	<i>Chondromyces</i> (3), <i>Sorangium</i> (21)
		Syntrophaceae	<i>Desulfobacca</i> (1)
		Syntrophobacteraceae	<i>Syntrophobacter</i> (2), <i>Thermodesulforhabdus</i> (1)
		Unclassified	<i>Spirobacillus</i> (1), Unclassified (6973)
		Campylobacteriales	<i>Arcobacter</i> (18), <i>Campylobacter</i> (399), <i>Sulfurospirillum</i> (1)
		Helicobacteraceae	<i>Helicobacter</i> (5)
		Nautiliaceae	<i>Caminibacter</i> (11), <i>Nautilia</i> (7)
		Unclassified	<i>Nitratiraptor</i> (1), Unclassified (56)
		Acidithiobacillales	<i>Acidithiobacillus</i> (21)
		Aeromonadales	<i>Aeromonas</i> (4)
		Succinivibrionaceae	<i>Succinimonas</i> (1)
		Alteromonadaceae	<i>Alteromonas</i> (1), <i>Microbulbifer</i> (1)
		Epsilonproteobacteria	
		Nautiliales	
		Unclassified	
		Acidithiobacillales	
		Aeromonadales	
		Succinivibrionales	
		Alteromonadales	

		Idiomarina (1)	
		Shewanella (42)	
Chromatiales		Lamprocytis (1), Nitrosococcus (2), Thiocapsa (7), Thiorhodovibrio (4)	
		Ectothiorhodospiraceae	Ectothiorhodospira (55), Thiohalospira (5), Unclassified (7)
		Halothiobacillaceae	Halothiobacillus (2)
Enterobacteriales		Enterobacteriaceae	Buchnera (1), Buttiauxella (1), Candidatus Hamiltonella (7), Citrobacter (6), Cronobacter (3), Enterobacter (27), Escherichia (7), Klebsiella (14), Morganella (1), Pantoea (12), Pectobacterium (8), Photorhabdus (7), Plesiomonas (1), Raoultella (6), Salmonella (1), Serratia (27), Shigella (1), Unclassified (601)
		Coxiellaceae	Coxiella (22), Rickettsiella (5)
Legionellales		Legionellaceae	Fluoribacter (1), Legionella (25)
		Methylococcales	Methylocaldum (3), Methylococcus (2), Methylohalobius (2), Methylothermus (2)
		Oceanospirillales	Alcanivorax (2)
		Halomonadaceae	Halomonas (164), Candidatus Portiera (1), Zymobacter (1)
		Unclassified	Unclassified (1)
Pasteurellales		Pasteurellaceae	Aggregatibacter (1), Gallibacterium (1), Haemophilus (1)
		Moraxellaceae	Acinetobacter (7), Moraxella (4)
Pseudomonadales		Pseudomonadaceae	Azotobacter (2), Pseudomonas (149)
		Salinisphaerales	Salinisphaera (1)
		Thiotrichales	Francisella (1)
		Piscirickettsiaceae	Cycloclasticus (2)
		Thiotrichaceae	Beggiatoa (8), Thiothrix (2)
		Sinobacteraceae	Sinobacter (1)
Xanthomonadales		Xanthomonadaceae	Dyella (5), Lysobacter (45), Pseudoxanthomonas (1), Rhodanobacter (2), Stenotrophomonas (2), Wohlfahrtiimonas (3), Xanthomonas (15), Xylella (1)

		Unclassified	Unclassified	<i>Candidatus Carsonella</i> (2), <i>Dechloromarinus</i> (1), <i>Methylhalomonas</i> (42), <i>Methylostratum</i> (3), Unclassified (1294)
Acidobacteria	Unclassified	Unclassified	Unclassified	Unclassified (1364)
	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Acidobacterium</i> (4358), <i>Terriglobus</i> (91)
Verrucomicrobia	Solibacteres	Solibacterales	Solibacteraceae	<i>Candidatus Solibacter</i> (3224)
	Unclassified	Unclassified	Unclassified	<i>Candidatus Koribacter</i> (9166)
	Opitutae	Pumiceococcales	Pumiceococcaceae	<i>Coralimargarita</i> (1)
		Unclassified	Opitutaceae	<i>Opitutus</i> (24), Unclassified (4)
	Spartobacteria	Unclassified	Unclassified	<i>Chthoniobacter</i> (3079)
	Unclassified	Methylacidiphilales	Methylacidiphilaceae	<i>Methylacidiphilum</i> (5)
	Verrucomicrobiae	Verrucomicrobiales	Unclassified (2)	
		Verrucomicrobia	Verrucomicrobia	Unclassified (593)
			subdivision 3	
			Verrucomicrobiaceae	<i>Akkermansia</i> (7), <i>Prostheco bacter</i> (615), <i>Rubritalea</i> (76), <i>Verrucomicrobium</i> (4), Unclassified (76)
Chloroflexi	Unclassified	Unclassified	Unclassified	Unclassified (21)
	Chloroflexi	Chloroflexales	Chloroflexaceae	<i>Chloroflexus</i> (470), <i>Roseiflexus</i> (17)
			Oscillochloridaceae	<i>Oscillochloris</i> (88)
		Herpetosiphonales	Herpetosiphonaceae	<i>Herpetosiphon</i> (389)
	Dehalococcoidetes	Unclassified	Unclassified	<i>Dehalococcoides</i> (13)
	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	<i>Ktedonobacter</i> (1374)
		Unclassified	Unclassified	Unclassified (613)
	Thermomicrobia	Sphaerobacterales	Sphaerobacteraceae	<i>Sphaerobacter</i> (4)
		Thermomicrobiales	Thermomicrobiaceae	<i>Thermomicrobium</i> (8)
	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	<i>Gemmatimonas</i> (2678)
	Planctomycetes	Planctomycetales	Planctomycetaceae	<i>Blastopirellula</i> (166), <i>Isosphaera</i> (1347), <i>Pirellula</i> (183), <i>Planctomyces</i> (151), <i>Rhodopirellula</i> (8), Unclassified (93)
			Unclassified	<i>Candidatus Kuenenia</i> (5)
Spirochaetes	Spirochaetes	Spirochaetales	Brachyspiraceae	<i>Brachyspira</i> (217)
			Leptospiraceae	<i>Leptospira</i> (318)
			Spirochaetaceae	<i>Spirochaeta</i> (82)

#### 4.20 Bacterial diversity in the sample AH-2

The most dominant phylum in the sample AH-2 was found to be Actinobacteria (16.24%), followed by Firmicutes (14.8%) and thirdly by Bacteroidetes (13.27%). About 45 per cent of the Actinobacterial population was found to be occupied by genus *Arthrobacter*, followed by 7 per cent of *Thermoleophilum* and 3 per cent of *Atopobium*. A considerable portion of Firmicutes was composed of the genus *Bacillus* (58%) and 10 per cent of population in the phylum was composed of the genus *Clostridium*, while the genus *Paenibacillus* occupied only 2 per cent of the population. The phylum Bacteroidetes was found to be majorly composed of genus *Terrimonas*, with the second most abundant genera being *Chitinophaga*.

Among the different classes in the phylum Proteobacteria, class Betaproteobacteria was found to be the dominant one. The most abundant genus in phylum Proteobacteria was observed to be *Ralstonia* (12%), followed by *Burkholderia* (10%). The proportion of unclassified bacteria was found to be very high in the phylum Proteobacteria. The genus-level taxonomic assemblage of the bacterial diversity from the sample AH2 has been provided in Table 25.

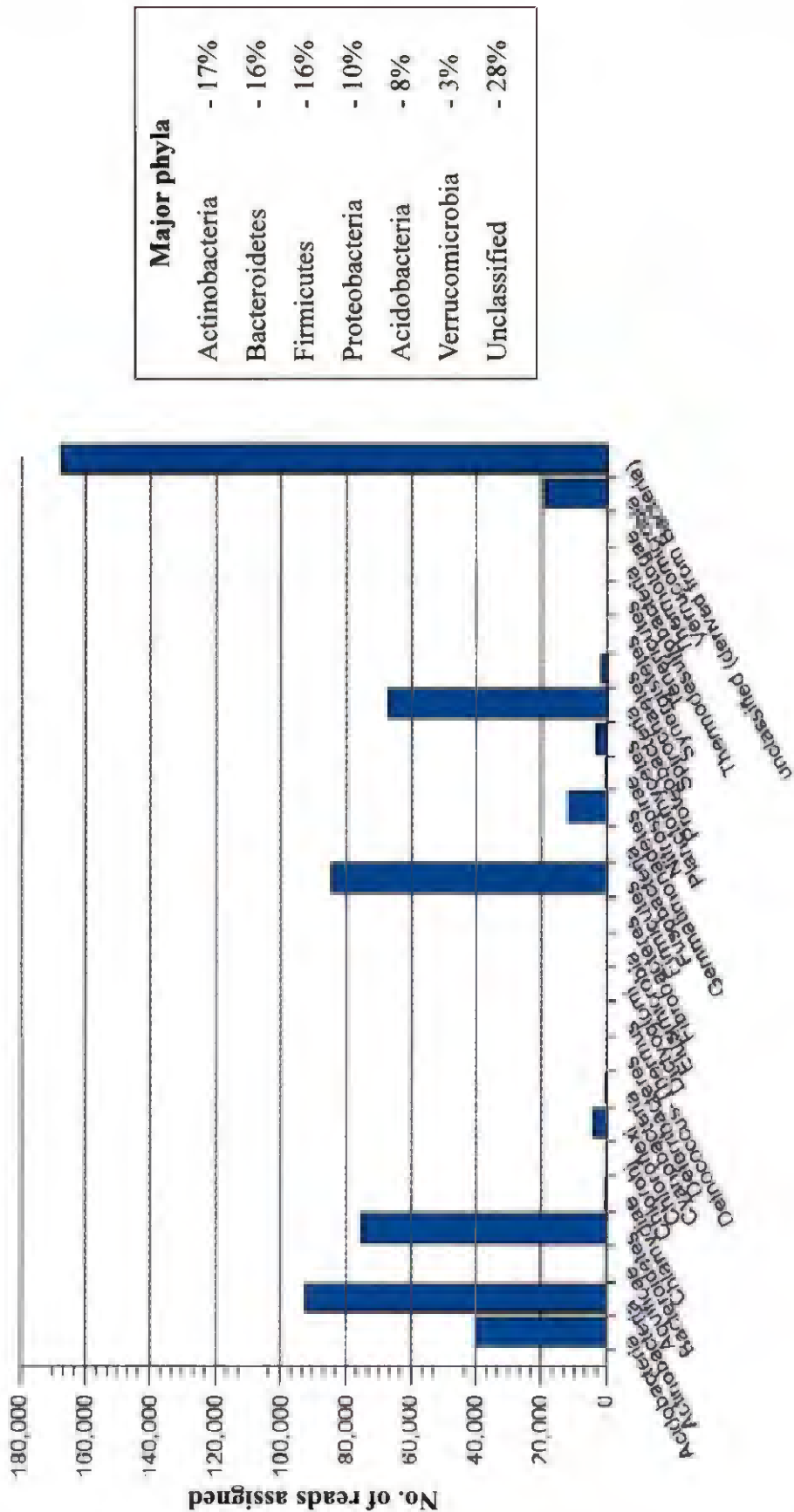


Plate 13. Phylum-level bacterial diversity in the sample AH-2 obtained using MG-RAST pipeline

**Table 25. Genus-level taxonomic assemblage of bacterial diversity from 10 predominant phyla in the sample AH-2**

Phylum	Class	Order	Family	Genus
Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	<i>Acidimicrobium</i> (253), <i>Acidithiomicrobium</i> (101)
			Acidothermaceae	<i>Acidothermus</i> (248)
			Actinomycetaceae	<i>Actinobaculum</i> (4), <i>Actinomyces</i> (19), <i>Arcanobacterium</i> (14), <i>Mobiluncus</i> (21)
			Actinosynnemataceae	<i>Actinokineospora</i> (65), <i>Actinosynnema</i> (3), <i>Lechevalieria</i> (11), <i>Lentzea</i> (40), <i>Saccharothrix</i> (4)
			Beutenbergiaceae	<i>Beutenbergia</i> (344)
			Brevibacteriaceae	<i>Brevibacterium</i> (613)
			Catenulisporaceae	<i>Catenulispora</i> (17)
			Cellulomonadaceae	<i>Cellulomonas</i> (453)
			Corynebacteriaceae	<i>Corynebacterium</i> (3090)
			Dermabacteraceae	<i>Brachybacterium</i> (77)
			Dermacoccaceae	<i>Dermacoccus</i> (18), <i>Kytococcus</i> (32)
			Dermatophilaceae	<i>Dermatophilus</i> (50)
			Dietziaceae	<i>Dietzia</i> (29)
			Frankiaceae	<i>Frankia</i> (1238)
			Geodermatophilaceae	<i>Geodermatophilus</i> (119)
			Glycomycetaceae	<i>Glycomyces</i> (7), <i>Stackebrandtia</i> (12)
			Gordonaceae	<i>Gordonia</i> (661)
			Intrasporangiaceae	<i>Intrasporangium</i> (370), <i>Janibacter</i> (559), <i>Serinicoccus</i> (65), <i>Terrabacter</i> (3259), <i>Terracoccus</i> (11), <i>Tetrasphaera</i> (338)
			Kineosporiaceae	<i>Kineococcus</i> (58)
			Microbacteriaceae	<i>Agreia</i> (6), <i>Agrococcus</i> (16), <i>Agromyces</i> (199), <i>Candidatus Aquiluna</i> (3), <i>Candidatus rhodoluna</i> (4), <i>Clavibacter</i> (78), <i>Cryobacterium</i> (135), <i>Curtobacterium</i> (3),



		<p><i>Frigoribacterium</i> (5), <i>Glaciibacter</i> (20),  <i>Leifsonia</i> (348), <i>Leucobacter</i> (4),  <i>Microbacterium</i> (560), <i>Mycetocola</i> (41),  <i>Okibacterium</i> (6), <i>Plantibacter</i> (1),  <i>Pseudolavibacter</i> (3), <i>Rathayibacter</i> (17),  <i>Salinibacterium</i> (25), <i>Subtercola</i> (33)</p>
Micrococcaceae		<p><i>Arthrobacter</i> (41661), <i>Kocuria</i> (39),  <i>Micrococcus</i> (146), <i>Nesterenkonia</i> (21),  <i>Renibacterium</i> (432), <i>Rothia</i> (355)</p>
Micromonosporaceae		<p><i>Actinoplanes</i> (208), <i>Catenuloplanes</i> (203),  <i>Couchioplanes</i> (7), <i>Dactylosporangium</i>  (178), <i>Micromonospora</i> (1595),  <i>Polymorphospora</i> (11), <i>Salinispora</i> (125),  <i>Verrucosipora</i> (143)</p>
Mycobacteriaceae		<i>Mycobacterium</i> (2910)
Nakamurellaceae		<i>Nakamurella</i> (268)
Nocardiaceae		<p><i>Nocardia</i> (160), <i>Rhodococcus</i> (1370),  <i>Smaragdicooccus</i> (70)</p>
Nocardiodaceae		<p><i>Aeromicrobium</i> (56), <i>Kribbella</i> (602),  <i>Nocardioides</i> (3457), <i>Pimelobacter</i> (204),  <i>Nocardiopsis</i> (74), <i>Streptomonospora</i> (2),  <i>Thermobifidia</i> (18)</p>
Promicromonosporaceae		<p><i>Cellulosimicrobium</i> (122),  <i>Promicromonospora</i> (52),  <i>Xylanimicrobium</i> (14), <i>Xylanimonas</i> (11)</p>
Propionibacteriaceae		<p><i>Microlunatus</i> (23), <i>Propionibacterium</i> (21),  Unclassified (232)</p>
Pseudonocardiaceae		<p><i>Amycolatopsis</i> (508), <i>Pseudonocardia</i>  (1203), <i>Saccharomonospora</i> (14),  <i>Saccharopolyspora</i> (1283),  <i>Streptoalloteichus</i> (7), <i>Thermobispora</i> (70)</p>
Rarobacteraceae		<i>Rarobacter</i> (46)
Sanguibacteraceae		<i>Sanguibacter</i> (19)
Streptomycetaceae		<p><i>Kitasatospora</i> (56), <i>Streptacidiphilus</i> (5),  <i>Streptomyces</i> (2983)</p>



			Streptosporangiaceae	<i>Microspora</i> (94), <i>Microtetraspora</i> (18), <i>Nonomurea</i> (128), <i>Planomonospora</i> (13), <i>Streptosporangium</i> (420)
			Thermomonosporaceae	<i>Actinoallomurus</i> (886), <i>Actinocorallia</i> (86), <i>Actinomadura</i> (1416), <i>Spirillospora</i> (17), <i>Thermomonospora</i> (1067), Unclassified (393)
			Tsukamurellaceae	<i>Tsukamurella</i> (39)
			Williamsiaceae	<i>Williamsia</i> (2545)
			Unclassified	<i>Tomitella</i> (5), <i>Tropheryma</i> (5), Unclassified (1)
		Bifidobacteriales	Bifidobacteriaceae	<i>Aeriscardovia</i> (1), <i>Bifidobacterium</i> (245), <i>Gerdnerella</i> (30), <i>Metascardovia</i> (1)
		Coriobacteriales	Coriobacteriaceae	<i>Atopobium</i> (3151), <i>Collinsella</i> (61), <i>Eggerthella</i> (10), <i>Enterorhabdus</i> (6), <i>Gordonibacter</i> (175), <i>Slackia</i> (8)
		Rubrobacterales	Rubrobacteraceae	<i>Rubrobacter</i> (607)
		Thermoleophilales	Thermoleophilaceae	<i>Thermoleophilum</i> (6462)
		Bacillales	Alicyclobacillaceae	<i>Alicyclobacillus</i> (636)
			Bacillaceae	<i>Amphibacillus</i> (25), <i>Anaerobacillus</i> (31), <i>Anoxybacillus</i> (3286), <i>Bacillus</i> (48593), <i>Geobacillus</i> (298), <i>Halobacillus</i> (122), <i>Lysinibacillus</i> (2110), <i>Marinococcus</i> (6), <i>Oceanobacillus</i> (3285), <i>Teribacillus</i> (6), <i>Virgibacillus</i> (114)
			Listeriaceae	<i>Brochothrix</i> (2), <i>Listeria</i> (6)
			Paenibacillaceae	<i>Aneurinibacillus</i> (74), <i>Brevibacillus</i> (2722), <i>Cohnella</i> (1), <i>Paenibacillus</i> (2073), <i>Thermobacillus</i> (6)
			Pasteuriaceae	<i>Pasteuria</i> (17)
			Planococcaceae	<i>Jeotgalibacillus</i> (34), <i>Kurthia</i> (317), <i>Planococcus</i> (14) <i>Planomicrobium</i> (177), <i>Sporosarcina</i> (61), <i>Ureibacillus</i> (105), <i>Viridibacillus</i> (157)
			Sporolactobacillaceae	<i>Sporolactobacillus</i> (2)
Firmicutes	Bacilli			

		Staphylococcaceae	<i>Macroccoccus</i> (3), <i>Staphylococcus</i> (57)
		Thermoactinomycetaceae	<i>Laceyella</i> (81), <i>Mechercharimyces</i> (5), <i>Seinonella</i> (98) <i>Thermoactinomycetes</i> (249), <i>Thermoflavimicrobium</i> (1)
		Unclassified	<i>Alkalilactibacillus</i> (3), <i>Exiguobacterium</i> (264), <i>Gemella</i> (74), <i>Pullulanibacillus</i> (18)
	Lactobacillales	Aerococcaceae	<i>Abiotrophia</i> (7), <i>Aerococcus</i> (1)
		Carnobacteriaceae	<i>Alkalibacterium</i> (2), <i>Atopostipes</i> (1), <i>Granulicatella</i> (7), <i>Trichococcus</i> (152)
		Enterococcaceae	<i>Enterococcus</i> (24), <i>Tetragenococcus</i> (25), <i>Vagococcus</i> (1)
		Lactobacillaceae	<i>Lactobacillus</i> (207)
		Leuconostocaceae	<i>Oenococcus</i> (1), <i>Weisella</i> (2)
		Streptococcaceae	<i>Lactococcus</i> (8), <i>Streptococcus</i> (12)
	Clostridia	Clostridiaceae	<i>Alkaliphilus</i> (671), <i>Butyricoccus</i> (6), <i>Caloramator</i> (51), <i>Clostridium</i> (8815), <i>Oxobacter</i> (7), <i>Tepidimicrobium</i> (3)
		Family XI. Incertae	<i>SedisAnaerococcus</i> (8), <i>SedisHelcococcus</i> (2), <i>SedisPeptoniphilus</i> (792), <i>SedisSedimentibacter</i> (2), <i>SedisSoehngenia</i> (4), <i>SedisSporanaerobacter</i> (933), <i>SedisTissierella</i> (1468)
		Family XIV. Incertae	<i>SedisAnaerobranca</i> (7)
		Family XVII. Incertae	<i>SedisSulfobacillus</i> (14), <i>SedisThermaerobacter</i> (7)
		Eubacteriaceae	<i>Acetobacterium</i> (17), <i>Eubacterium</i> (40)
		Heliobacteriaceae	<i>Heliobacillus</i> (49), <i>Heliobacterium</i> (536), <i>Heliophilum</i> (11), <i>Heliorexis</i> (4)
		Lachnospiraceae	<i>Anaerostipes</i> (12), <i>Butyrivibrio</i> (3), <i>Cellulostypticum</i> (17), <i>Hespellia</i> (16), <i>Lachnospira</i> (31), <i>Pseudobutyrvibrio</i> (1), <i>Robinsoniella</i> (35), <i>Roseburia</i> (5), Unclassified (35)
		Peptococcaceae	<i>Candidatus Desulforubis</i> (12), <i>Dehalobacter</i> (30), <i>Desulfotobacterium</i> (193),

			<i>Desulfonispota</i> (463), <i>Desulfosporosinus</i> (83), <i>Desulfotomaculum</i> (1266), <i>Pelotomaculum</i> (6), <i>Peptococcus</i> (1), <i>Thermincola</i> (25), Unclassified (76) <i>Peptostreptococcus</i> (59) <i>Ethanoligenens</i> (56), <i>Faecalibacterium</i> (30), <i>Ruminococcus</i> (819), Unclassified (87) <i>Syntrophomonas</i> (13) <i>Blautia</i> (3), <i>Epulopiscium</i> (56), Unclassified (153) <i>Haloetherothrix</i> (1) <i>Caldanaerobacter</i> (52), <i>Caldanaerobius</i> (377), <i>Carboxydotherrmus</i> (1), <i>Moorella</i> (23), <i>Thermacetogenium</i> (2), <i>Thermoanaerobacter</i> (18) <i>SedisCaldicellulosiruptor</i> (134) <i>Coprothermobacter</i> (2), <i>Thermodesulfobium</i> (21) Unclassified (11) <i>Desulfifibacter</i> (13) Unclassified (7) <i>Acidaminococcus</i> (24), <i>Phascolarctobacterium</i> (1) <i>Dialister</i> (9), <i>Megamonas</i> (2), <i>Megasphaera</i> (49), <i>Mitsuokella</i> (1), <i>Pectinatus</i> (5), <i>Selenomonas</i> (552), <i>Sporomusa</i> (231), <i>Veillonella</i> (193) Unclassified (35) <i>Bacteroides</i> (661) <i>Marinilabilia</i> (11) <i>Odoribacter</i> (1), <i>Parabacteroides</i> (711), <i>Porphyromonas</i> (24), <i>Tannerella</i> (1) <i>Prevotella</i> (124) <i>Alistipes</i> (1366), <i>Rikenella</i> (384)
			<i>Peptostreptococcaceae</i> <i>Ruminococcaceae</i> <i>Syntrophomonadaceae</i> Unclassified <i>Halanaerobiaceae</i> Thermoanaerobacteraceae Family III. Incertae Thermodesulfobiaceae Unclassified Unclassified Erysipelotrichaceae Acidaminococcaceae Veillonellaceae Unclassified Bacteroidaceae Marinilabiaceae Porphyromonadaceae Prevotellaceae Rikenellaceae
			Halanaerobiales Thermoanaerobacterales Unclassified Erysipelotrichales Selenomonadales Unclassified Bacteroidales Unclassified Bacteroidia Unclassified Bacteroidetes

	Cytophagia	Cytophagales	Cyclobacteriaceae	Cyclobacterium (449)
			Cytophagaceae	Cytophaga (1608), <i>Dyadoacter</i> (6), <i>Flectobacillus</i> (28), <i>Flexibacter</i> (168), <i>Hymenobacter</i> (3200), <i>Marinoscillum</i> (14), <i>Microscilla</i> (3)
			Flammeovirgaceae	<i>Flammeovirga</i> (35), <i>Flexithrix</i> (465), <i>Persicobacter</i> (34)
	Flavobacteria	Flavobacteriales	Flavobacteriaceae	<i>Aquimarina</i> (70), <i>Arenibacter</i> (58), <i>Bergeyella</i> (5), <i>Candidatus Hemobacterium</i> (1), <i>Cappocytophaga</i> (1), <i>Chryseobacterium</i> (24), <i>Coenonia</i> (1), <i>Dokdonia</i> (3), <i>Elizabethkingia</i> (424), <i>Empedobacter</i> (24), <i>Flavobacterium</i> (1032), <i>Gaetbulibacter</i> (1), <i>Gramella</i> (4812), <i>Leeuwenhoekella</i> (11), <i>Myroides</i> (7), <i>Riemerella</i> (64), <i>Robiginitalea</i> (209), <i>Salegentibacter</i> (1), <i>Tenacibaculum</i> (148), <i>Zunongwangia</i> (38)
			Unclassified	<i>Candidatus Sulcia</i> (1)
	Sphingobacteria	Sphingobacteriales	Rhodothermaceae	<i>Rhodothermus</i> (22), <i>Salinibacter</i> (17)
			Saprosiraceae	<i>Saprosira</i> (5)
			Sphingobacteriaceae	<i>Pedobacter</i> (5578), <i>Sphingobacterium</i> (4609), Unclassified (12299)
			Unclassified	<i>Chitiphaga</i> (13464), <i>Terrimonas</i> (20723)
			Unclassified	<i>Candidatus Amoebophilus</i> (1829), <i>Prolixibacter</i> (355), Unclassified (172)
	Unclassified	Unclassified	Caulobacteraceae	<i>Phenylbacterium</i> (337)
	Alphaproteobacteria	Caulobacterales	Aurantimonadaceae	<i>Mariella</i> (1)
		Rhizobiales	Bartonellaceae	<i>Bartonella</i> (5)
			Beijerinckiaceae	<i>Beijerinckia</i> (7), <i>Chelatococcus</i> (1), <i>Methylocapsa</i> (11), <i>Methylocella</i> (2)
			Bradyrhizobiaceae	<i>Afipia</i> (21), <i>Balheimonas</i> (4), <i>Blastobacter</i> (2), <i>Bosea</i> (51), <i>Bradyrhizobium</i> (2023), <i>Nitrobacter</i> (135), <i>Rhodoblastus</i> (1), <i>Rhodopseudomonas</i> (164), Unclassified (31)
Proteobacteria			Brucellaceae	<i>Ochrobactrum</i> (13), <i>Pseudochrobactrum</i> (1)

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			Spingomonadaceae	<i>Blastomonas</i> (1), <i>Novosphingobium</i> (40), <i>Sandarakinorhabdus</i> (3), <i>Sphingobium</i> (275), <i>Sphingomonas</i> (420), <i>Sphingopyxis</i> (29), <i>Sphingocinicella</i> (4)
	Unclassified	Unclassified	Unclassified	Unclassified (5039)
Betaproteobacteria	Burkholderiales	Alcaligenaceae	Alcaligenaceae	<i>Alcaligenes</i> (9), <i>Azohydromonas</i> (16), <i>Bordetella</i> (6), <i>Oligella</i> (2), Unclassified (6)
		Burkholderiaceae	Burkholderiaceae	<i>Burkholderia</i> (6973), <i>Candidatus</i> <i>Glomeribacter</i> (6), <i>Cupriavidus</i> (537), <i>Pandoraea</i> (60), <i>Paucimonas</i> (358), <i>Polynucleobacter</i> (16), <i>Ralstonia</i> (8086)
		Comamonadaceae	Comamonadaceae	<i>Acidovorax</i> (206), <i>Alicyclophilus</i> (3), <i>Brachimonas</i> (7), <i>Caldimonas</i> (1), <i>Comamonas</i> (126), <i>Delftia</i> (3), <i>Diaphorobacter</i> (2), <i>Hydrogenophaga</i> (3), <i>Pelomonas</i> (11), <i>Polaromonas</i> (1), <i>Variovorax</i> (290), <i>Verminephrobacter</i> (156), <i>Xenophilus</i> (170), Unclassified (331)
		Oxalobacteraceae	Oxalobacteraceae	<i>Collimonas</i> (166), <i>Duganella</i> (33), <i>Herbaspirillum</i> (462), <i>Herminiimonas</i> (67), <i>Janthinobacterium</i> (68), <i>Massilia</i> (5874), <i>Oxalicibacterium</i> (587), <i>Oxalobacter</i> (17), <i>Telluria</i> (68)
		Unclassified	Unclassified	<i>Aquicola</i> (1), <i>Leptothrix</i> (5), <i>Mitsuaria</i> (6), <i>Rubrivivax</i> (6), <i>Sphaerotilus</i> (57), <i>Thiobacter</i> (7), <i>Thiomonas</i> (16), Unclassified (2196)
	Gallionellales	Gallionellaceae	Gallionellaceae	<i>Sideroxydans</i> (11)
	Hydrogenophilales	Hydrogenophilaceae	Hydrogenophilaceae	<i>Thiobacillus</i> (7)
	Methylophilales	Methylophilaceae	Methylophilaceae	<i>Methylobacillus</i> (1)
	Neisseriales	Neisseriaceae	Neisseriaceae	<i>Aquitalea</i> (3), <i>Chromobacterium</i> (24), <i>Laribacter</i> (12), <i>Neisseria</i> (12), <i>Vogesella</i> (1)
	Nitrosomonadales	Nitrosomonadaceae	Nitrosomonadaceae	<i>Nitrosomonas</i> (19), <i>Nitrospira</i> (50), <i>Nitrosovibrio</i> (8), Unclassified (864)
		Unclassified	Unclassified	Unclassified (8)



	Rhodocyclales	Rhodocyclaceae	<i>Aromatoleum</i> (1), <i>Azoarcus</i> (15), <i>Azonexus</i> (1), <i>Azospira</i> (1), <i>Azovibrio</i> (7), <i>Dechloromonas</i> (5), <i>Georgfuchsia</i> (9), <i>Sterolibacterium</i> (10), <i>Zooglea</i> (1), Unclassified (552)
	Unclassified	Unclassified	<i>Candidatus Tremblaya</i> (1), <i>Kinetoplastibacterium</i> (4), Unclassified (5817)
Deltaproteobacteria	Bdellovibrionales	Bacteriovoraceae	<i>Bacteriovorax</i> (40), <i>Peredibacter</i> (8)
	Desulfobacterales	Bdellovibrionaceae	<i>Bdellovibrio</i> (351)
		Desulfobacteraceae	<i>Desulfatibacillum</i> (3), <i>Desulfatiferula</i> (1), <i>Desulfobacterium</i> (15), <i>Desulfobotulus</i> (30), <i>Desulfocella</i> (4), <i>Desulfococcus</i> (13), <i>Desulfofrigus</i> (16), <i>Desulfonema</i> (136), <i>Desulforegula</i> (1), Unclassified (2)
		Desulfobulbaceae	<i>Desulfobulbus</i> (4), <i>Desuforhopatus</i> (3), <i>Desulfurivibrio</i> (3), Unclassified (33)
	Desulfovibrionales	Desulfohalobiaceae	<i>Desulfohalobium</i> (5), <i>Desulfonatronovibrio</i> (101), <i>Desulfonauticus</i> (2), <i>Desulfothermus</i> (1), <i>Desulfomicrobium</i> (24), <i>Desulfonatronum</i> (10), <i>Desulfovibrio</i> (671), <i>Lawsonia</i> (1)
		Unclassified	Unclassified (3)
	Desulfurellales	Desulfurellaceae	<i>Desulfurella</i> (4)
	Desulfuromonadales	Desulfuromonadaceae	<i>Desulfuromonas</i> (1)
		Geobacteraceae	<i>Geokalibacter</i> (121), <i>Geobacter</i> (1328), <i>Geopsychrobacter</i> (1), Unclassified (10)
		Pelobacteraceae	<i>Malomonas</i> (7), <i>Pelobacter</i> (22)
	Myxococcales	Cystobacteraceae	<i>Cystobacter</i> (125), <i>Melittangium</i> (20), <i>Stigmatella</i> (12)
		Haliangiaceae	<i>Haliangium</i> (5)
		Myxococcaceae	<i>Anaeromyxobacter</i> (57), <i>Corallocooccus</i> (124), <i>Myxococcus</i> (153)
		Nannocystaceae	<i>Plesiocystis</i> (1)
		Polyangiaceae	<i>Chondromyces</i> (12), <i>Sorangium</i> (27)



	Syntrophobacteriales	Syntrophobacteraceae	<i>Desulforhabdus</i> (1), <i>Desulfovirga</i> (29), <i>Syntrophobacter</i> (2), <i>Thermodesulforhabdus</i> (4)		
Epsilonproteobacteria	Campylobacteriales	Unclassified	Unclassified (6920)		
		Campylobacteraceae	<i>Arcobacter</i> (5), <i>Campylobacter</i> (264), Unclassified (8)		
Gammaproteobacteria	Nautiliales	Helicobacteraceae	<i>Helicobacter</i> (5)		
		Nautiliaceae	<i>Caminibacter</i> (37), <i>Lebetimonas</i> (1), <i>Nautilia</i> (6)		
	Unclassified	Unclassified (140)			
	Acidithiobacillales	Acidithiobacillaceae	<i>Nitratiruptor</i> (12), Unclassified (140)		
	Aeromonadales	Aeromonadaceae	<i>Acidithiobacillus</i> (3)		
		Succinivibrionaceae	<i>Aeromonas</i> (4), <i>Oceanimonas</i> (1)		
	Alteromonadales	Pseudoalteromonadaceae	<i>Ruminobacter</i> (1), <i>Succinimonas</i> (3)		
		Shewanellaceae	<i>Pseudoalteromonas</i> (1)		
	Chromatiales	Chromatiaceae	Chromatiaceae	<i>Shewanella</i> (40)	
			Ectothiorhodospiraceae	<i>Halochromatium</i> (1), <i>Nitrosococcus</i> (4), <i>Thiocapsa</i> (36), <i>Thiorhodovibrio</i> (10)	
Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae	<i>Alkalispirillum</i> (5), <i>Ectothiorhodospira</i> (22), <i>Halorhodospira</i> (1), <i>Nitrosococcus</i> (1), <i>Thioalkalivibrio</i> (4), <i>Thiohalospira</i> (2), <i>Thiorhodospira</i> (1), Unclassified (1)		
		Halotheiobacillaceae	<i>Halotheiobacillus</i> (7)		
			<i>Buchnera</i> (2), <i>Butiauxella</i> (3), <i>Candidatus</i> <i>Curculioniphilus</i> (1), <i>Candidatus</i> <i>Hamiltonella</i> (16), <i>Citrobacter</i> (17), <i>Cronobacter</i> (2), <i>Enterobacter</i> (13), <i>Escherichia</i> (7), <i>Klebsiella</i> (26), <i>Pantoea</i> (20), <i>Photorhabdus</i> (5), <i>Plesiomonas</i> (1), <i>Proteus</i> (1), <i>Salmonella</i> (1), <i>Serratia</i> (26) <i>Trabulsiella</i> (1), <i>Wigglesworthia</i> (1), Unclassified (479)		
			Legionellales	Coxiellaceae	<i>Coxiella</i> (17), <i>Rickettsiella</i> (2)
			Methylococcales	Legionellaceae	<i>Fluoribacter</i> (1), <i>Legionella</i> (24)
				Methylococcaceae	<i>Methylobacter</i> (1), <i>Methylocaldum</i> (19), <i>Methylococcus</i> (2), <i>Methylohalobius</i> (5),

				<i>Methylomicrobium</i> (1), <i>Methylosarcina</i> (1), Unclassified (1)
	Oceanospirillales		Alcanivoraceae	<i>Alcanivorax</i> (3)
			Halomonadaceae	<i>Halomonas</i> (198)
			Oceanospirillaceae	<i>Marinomonas</i> (1)
			Unclassified	Unclassified (1)
	Pasteurellales		Pasteurellaceae	<i>Aggregatibacter</i> (1), <i>Gallibacterium</i> (2), <i>Haemophilus</i> (4), <i>Mannheimia</i> (2)
	Pseudomonadales		Moraxellaceae	<i>Acinetobacter</i> (18), <i>Moraxella</i> (3)
			Pseudomonadaceae	<i>Azotobacter</i> (4), <i>Pseudomonas</i> (132)
	Thiotrichales		Francisellaceae	<i>Francisella</i> (201)
			Piscirickettsiaceae	<i>Cycloasticus</i> (1)
			Thiotrichaceae	<i>Beggiatoa</i> (9), <i>Thiothrix</i> (2)
	Xanthomonadales		Sinobacteraceae	<i>Sinobacter</i> (4)
			Xanthomonadaceae	<i>Dyella</i> (774), <i>Ignatzschineria</i> (1), <i>Lysobacter</i> (76), <i>Pseudoxanthomonas</i> (3), <i>Rhodanobacter</i> (1), <i>Stenotrophomonas</i> (8), <i>Wohlfahrtiimonas</i> (25), <i>Xanthomonas</i> (21), <i>Xylella</i> (3)
	Unclassified		Unclassified	<i>Candidatus Carsonella</i> (4), <i>Methylohalomonas</i> (34), <i>Methylostratum</i> (2), <i>Solimonas</i> (2), Unclassified (3891)
Acidobacteria	Unclassified	Unclassified	Unclassified	Unclassified (3053)
	Acidobacteriales		Acidobacteraceae	<i>Acidobacterium</i> (13607), <i>Terriglobus</i> (301)
	Soilbacteres		Solibacteraceae	<i>Candidatus Solibacter</i> (9635)
	Unclassified		Unclassified	<i>Candidatus Koribacter</i> (16651)
Verrucomicrobia	Opitutae		Puniceococcaceae	<i>Coraliomargarita</i> (1)
			Opitutaceae	<i>Opitutus</i> (300), Unclassified (21)
	Spartobacteria		Unclassified	<i>Chthoniobacter</i> (14403)
	Unclassified		Methylacidiphilales	<i>Methylacidiphilum</i> (74)
	Verrucomicrobia		Verrucomicrobiales	<i>Akkermansia</i> (13), <i>Prostheco bacter</i> (2086), <i>Rubritalea</i> (5), <i>Verrucomicrobium</i> (45), Unclassified (141)

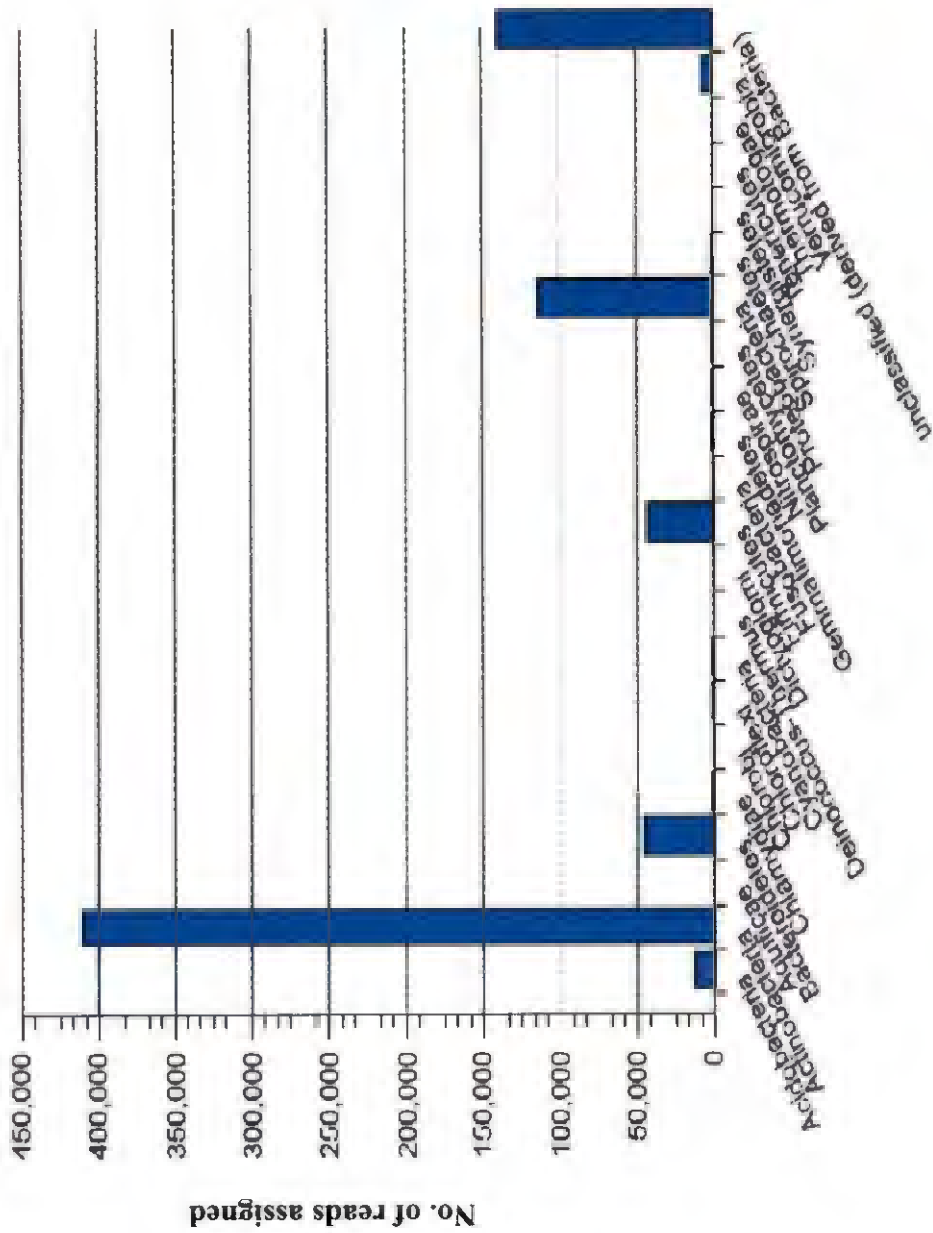
				Verrucomicrobia subdivision 3	Unclassified (2010)
Gemmatimonadetes	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified (114)
Chloroflexi	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	<i>Gemmatimonas</i> (12051)	
	Chloroflexi	Chloroflexales	Chloroflexaceae	<i>Chloroflexus</i> (134), <i>Roseiflexus</i> (55)	
			Oscillochloridaceae	<i>Oscillochloris</i> (203)	
		Herpetosiphonales	Herpetosiphonaceae	<i>Herpetosiphon</i> (666)	
Ktedonobacteria	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	<i>Ktedonobacter</i> (1795)	
		Unclassified	Unclassified	Unclassified (1143)	
Thermomicrobia	Thermomicrobia	Sphaerobacterales	Sphaerobacteraceae	<i>Sphaerobacter</i> (6)	
		Thermomicrobiales	Thermomicrobiaceae	<i>Thermomicrobium</i> (25)	
Dehalococcoidetes	Dehalococcoidetes	Unclassified	Unclassified	<i>Dehalococcoides</i> (8)	
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	<i>Blastopirellula</i> (167), <i>Isosphaera</i> (2681), <i>Pirellula</i> (196), <i>Planctomyces</i> (159), <i>Rhodopirellula</i> (1), Unclassified (143)	
			Unclassified	<i>Candidatus Kuenenia</i> (4)	
			Brachyspiraceae	<i>Brachyspira</i> (173)	
Spirochaetes	Spirochaetes	Spirochaetales	Leptospiraceae	<i>Leptospira</i> (1318)	
			Spirochaetaceae	<i>Borrelia</i> (1), <i>Spirochaeta</i> (59), <i>Treponema</i> (3)	

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#### 4.21 Bacterial diversity in the sample YL-7

The sample YL-7 was analysed for the phylum level bacterial diversity and the most abundant bacterial phylum was found to be Actinobacteria forming 52 per cent of the bacterial population followed by 14 per cent of Proteobacteria and thirdly by Bacteroidetes (6%). Phylum Actinobacteria was found to be exceptionally high in the population of genus *Williamsia* forming 59 per cent of the phylum Actinobacteria followed by 10 per cent of *Brevibacterium* and *Gordonia*. The second largest phylum being Proteobacteria, was largely composed of the class Alphaproteobacteria and the most abundant genera in the class was observed to be *Methylobacterium* occupying 33 per cent of the population of phylum Proteobacteria.

The phylum Bacteroidetes possessed 16 per cent of *Terrimonas* and 14 per cent of *Prevotella*, followed by 11 per cent of *Flavobacterium*. Other genera like *Chitinophaga*, *Sphingobacterium* and *Pedobacter* were found to be present in notable proportion. The phylum Firmicutes was dominated by the genus *Bacillus*, occupying 49 per cent of the population in the phylum, followed by 8 per cent of *Clostridium*. Other genera like *Paenibacillus*, *Brevibacillus*, *Desulfotomaculum* and *Ruminococcus* was found to be present in notable numbers. The genus-level bacterial diversity in the 10 most abundant phyla has been provided in Table 26.



Major phyla	
Actinobacteria	- 52%
Proteobacteria	- 14%
Bacteroidetes	- 6%
Firmicutes	- 5%
Acidobacteria	- 2%
Verrucomicrobia	- 0.9%
Unclassified	- 16%

Plate 12. Phylum-level bacterial diversity in the sample YL-7 obtained using MG-RAST pipeline

Table 26. Genus-level taxonomic assemblage of bacterial diversity from 10 predominant phyla in the sample YL-7

Phylum	Class	Order	Family	Genus		
Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	<i>Acidimicrobium</i> (82), <i>Acidithiomicrobium</i> (18)		
			Actinomycetales	<i>Acidothermus</i> (214)		
					Actinomycetaceae	<i>Actinobaculum</i> (68), <i>Actinomyces</i> (67), <i>Arcanobacterium</i> (3), <i>Mobiluncus</i> (1)
					Actinosynnemataceae	<i>Actinokineospora</i> (1), <i>Lechevalieria</i> (59), <i>Lentzea</i> (3)
					Beutenbergiaceae	<i>Beutenbergia</i> (121),
					Brevibacteriaceae	<i>Brevibacterium</i> (41567)
					Catenulisporaceae	<i>Catenulispora</i> (21)
					Cellulomonadaceae	<i>Actinotalea</i> (24), <i>Cellulomonas</i> (539)
					Corynebacteriaceae	<i>Corynebacterium</i> (4806)
					Dermabacteraceae	<i>Brachybacterium</i> (336)
					Dermacoccaceae	<i>Dermacoccus</i> (403), <i>Kytococcus</i> (121)
					Dermatophilaceae	<i>Dermatophilus</i> (673)
					Dietziaceae	<i>Dietzia</i> (5648)
					Frankiaceae	<i>Frankia</i> (369)
					Geodermatophilaceae	<i>Geodermatophilus</i> (68)
					Gordoniaceae	<i>Gordonia</i> (41863), <i>Stermania</i> (21)
					Intrasporangiaceae	<i>Intrasporangium</i> (70), <i>Janibacter</i> (191), <i>Serinicoccus</i> (19), <i>Terrabacter</i> (710), <i>Tetrasphaera</i> (57)
					Kineosporiaceae	<i>Kineococcus</i> (9)
					Microbacteriaceae	<i>Agreia</i> (5), <i>Agrococcus</i> (86), <i>Agromyces</i> (775), <i>Candidatus Aquiluna</i> (5), <i>Candidatus Rhodoluna</i> (1), <i>Clavibacter</i> (254), <i>Cryobacterium</i> (249), <i>Curtobacterium</i> (245), <i>Frigoribacterium</i> (5), <i>Glaciibacter</i> (214), <i>Leifsonia</i> (697), <i>Leucobacter</i> (105), <i>Microbacterium</i> (31198), <i>Mycetocola</i> (1064), <i>Okibacterium</i> (31), <i>Plantibacter</i> (3), <i>Pseudoclavibacter</i>

		(46), <i>Rathayibacter</i> (26), <i>Salinibacterium</i> (155), <i>Subtercola</i> (235), Unclassified (1)
Micrococcaceae		<i>Arthrobacter</i> (6093), <i>Kocuria</i> (36), <i>Micrococcus</i> (576), <i>Nesterenkonia</i> (19), <i>Renibacterium</i> (33), <i>Rothia</i> (767)
Micromonosporaceae		<i>Actinoplanes</i> (42), <i>Catenuloplanes</i> (2), <i>Dactylosporangium</i> (126), <i>Micromonospora</i> (382), <i>Salinispora</i> (181), <i>Verrucosipora</i> (41)
Mycobacteriaceae		<i>Mycobacterium</i> (2191)
Nakamurellaceae		<i>Nakamurella</i> (150)
Nocardiaceae		<i>Nocardia</i> (1203), <i>Rhodococcus</i> (8891)
Nocardioidaceae		<i>Aeromicrobium</i> (59), <i>Kribbella</i> (507), <i>Nocardioides</i> (1554)
Nocardiopsaceae		<i>Nocardiopsis</i> (159), <i>Streptomonospora</i> (2), <i>Thermobifodia</i> (26)
Promicromonosporaceae		<i>Cellulosimicrobium</i> (277), <i>Promicromonospora</i> (9)
Propionibacteriaceae		<i>Microtholunatus</i> (109), <i>Propionibacterium</i> (199), Unclassified (142)
Pseudonocardiaceae		<i>Amylocolatopsis</i> (176), <i>Prauserella</i> (61), <i>Pseudonocardia</i> (1415), <i>Saccharomonospora</i> (30), <i>Thermobispora</i> (4), <i>Thermocrispum</i> (1)
Rarobacteraceae		<i>Rarobacter</i> (6)
Segniliparaceae		<i>Segniliparus</i> (10)
Streptomycetaceae		<i>Kitasatospora</i> (87), <i>Streptacidiphilus</i> (1), <i>Streptomyces</i> (1044)
Streptosporangiaceae		<i>Microbispora</i> (6), <i>Microtetraspora</i> (1), <i>Nonomuraea</i> (34), <i>Planomonospora</i> (2), <i>Streptosporangium</i> (58), <i>Thermopolyspora</i> (1)
Thermomonosporaceae		<i>Actinoallomurus</i> (172), <i>Actinocoralia</i> (67), <i>Actinomadura</i> (277),



Proteobacteria	Alphaproteobacteria			<i>Thermomonospora</i> (478), Unclassified (124)
				Tsukamurellaceae
				<i>Tsukamurella</i> (32)
				Unclassified
				Williamsiaceae
				<i>Williamsia</i> (241198)
				Bifidobacteriales
				Bifidobacteriaceae
				<i>Bifidobacterium</i> (502), <i>Gardnerella</i> (3), <i>Metascardovia</i> (1)
				Coriobacteriales
				Coriobacteriaceae
				<i>Atopobium</i> (1824), <i>Collinsella</i> (11), <i>Cryptobacterium</i> (1), <i>Eggerthella</i> (21), <i>Enterorhabdus</i> (1), <i>Gordonibacter</i> (230), <i>Slackia</i> (33)
				Rubrobacterales
				Rubrobacteraceae
				<i>Rubrobacter</i> (376)
				Thermoleophilales
				Thermoleophilaceae
<i>Thermoleophilum</i> (3297)				
Unclassified				
Unclassified (19)				
Caulobacteriales				
Caulobacteraceae				
<i>Asticcacaulis</i> (32), <i>Brevundimonas</i> (54), <i>Caulobacter</i> (28), <i>Phenyllobacterium</i> (209)				
Kordiimonadales				
Unclassified				
<i>Kordiimonas</i> (1)				
Rhizobiales				
Bartonellaceae				
<i>Bartonella</i> (19)				
Beijerinckiaceae				
<i>Chelatococcus</i> (1), <i>Methylocapsa</i> (3), <i>Methylocella</i> (1)				
Bradyrhizobiales				
Bradyrhizobiaceae				
<i>Afpia</i> (639), <i>Balneimonas</i> (6), <i>Bosea</i> (157), <i>Bradyrhizobium</i> (1342), <i>Nitrobacter</i> (22), <i>Rhodopseudomonas</i> (80), Unclassified (14)				
Brucellaceae				
<i>Mycoplana</i> (1), <i>Ochrobactrum</i> (2)				
Hyphomicrobiaceae				
<i>Blastochloris</i> (72), <i>Hyphomicrobium</i> (66), <i>Rhodomicrobium</i> (2)				
Methylobacteriaceae				
<i>Methylobacterium</i> (37603)				
Methylocystaceae				
<i>Methylocystis</i> (5), <i>Methylophila</i> (2), <i>Methylosinus</i> (1), Unclassified (92)				
Phyllobacteriaceae				
<i>Aminobacter</i> (6), <i>Chelativorans</i> (6), <i>Mesorhizobium</i> (345), <i>Phyllobacterium</i> (37)				
Rhizobiaceae				
<i>Agrobacterium</i> (1), <i>Ensifer</i> (21), <i>Rhizobium</i> (447), <i>Sinorhizobium</i> (20)				

		Rhodobiaceae	<i>Aifella</i> (3), <i>Rhodobium</i> (5)
		Unclassified	Unclassified (207)
Rhodobacterales		Rhodobacteraceae	<i>Jannaschia</i> (3), <i>Nereida</i> (1), <i>Pannonibacter</i> (4), <i>Paracoccus</i> (4), <i>Pseudoruegeria</i> (62), <i>Rhodobaca</i> (1), <i>Rhodobacter</i> (18), <i>Rhodothalassium</i> (1), <i>Rhodovulum</i> (2), <i>Roseobacter</i> (3), <i>Ruegeria</i> (1), Unclassified (8)
		Unclassified	Unclassified (4)
Rhodospirillales		Acetobacteraceae	<i>Acetobacter</i> (2), <i>Acidiphillum</i> (10), <i>Asaia</i> (1), <i>Gluconacetobacter</i> (17), <i>Gluconobacter</i> (4), <i>Granulibacter</i> (56), <i>Kozakia</i> (4), <i>Roseococcus</i> (1), <i>Rubritepida</i> (2), <i>Swaminathana</i> (4)
		Rhodospirillaceae	<i>Azospirillum</i> (47), <i>Magnetospirillum</i> (30), <i>Rhodocista</i> (1), <i>Rhodospirillum</i> (5), <i>Rhodovibrio</i> (2), <i>Roseospira</i> (1), <i>Tisterella</i> (3), Unclassified (135)
Rickettsiales		Anaplasmataceae	<i>Anaplasma</i> (3), <i>Ehrlichia</i> (1), <i>Neorickettsia</i> (1), <i>Wolbachia</i> (1)
		Holosporaceae	<i>Holospora</i> (1)
		Rickettsiaceae	<i>Orientia</i> (15)
		Rickettsiaceae	<i>Candidatus Midichloria</i> (1)
Sphingomonadales		Erythrobacteraceae	<i>Erythrobacter</i> (3), <i>Erythromicrobium</i> (1), <i>Porphyrobacter</i> (4)
		Sphingomonadaceae	<i>Blastomonas</i> (3), <i>Novosphingobium</i> (50), <i>Sandarakanorhabdus</i> (1), <i>Sphingobium</i> (126), <i>Sphingopyxis</i> (197), <i>Sphingosinicella</i> (2), <i>Zymomonas</i> (5), Unclassified (5)
		Unclassified	<i>Micavibrio</i> (1), Unclassified (6822)
Betaproteobacteria		Alcaligenaceae	<i>Achromobacter</i> (19), <i>Alcaligenes</i> (21), <i>Azohydromonas</i> (1), <i>Bordetella</i> (6), <i>Pigmentiphaga</i> (1)

		Burkholderiaceae	<i>Burkholderia</i> (3171), <i>Candidatus Glomeribacter</i> (2), <i>Cupriavidus</i> (346), <i>Pandoraea</i> (7), <i>Paucimonas</i> (2), <i>Polynucleobacter</i> (4), <i>Ralstonia</i> (71), Unclassified (12)
		Comamonadaceae	<i>Acidovorax</i> (99), <i>Brachymonas</i> (9), <i>Comamonas</i> (24), <i>Pelomonas</i> (35), <i>Simplicispira</i> (1), <i>Variovorax</i> (410), <i>Yerminephrobacter</i> (32), <i>Xenophilus</i> (32), Unclassified (224)
		Oxalobacteraceae	<i>Collimonas</i> (102), <i>Duganella</i> (7), <i>Herbaspirillum</i> (258), <i>Herminiimonas</i> (84), <i>Janihinobacterium</i> (33), <i>Massilia</i> (58), <i>Oxalicibacterium</i> (33), <i>Oxalobacter</i> (6), <i>Telluria</i> (20)
		Unclassified	<i>Aquicola</i> (11), <i>Leptothrix</i> (20), <i>Mitsuaria</i> (1), <i>Roseateles</i> (11), <i>Rubrivivax</i> (7), <i>Sphaerotilus</i> (15), <i>Thiomonas</i> (5), Unclassified (808)
	Hydrogenophilales	Hydrogenophilaceae	<i>Thiobacillus</i> (1)
	Methylophilales	Methylophilaceae	<i>Methylophilus</i> (11), <i>Methylotenera</i> (2)
	Neisseriales	Neisseriaceae	<i>Aquitalea</i> (1), <i>Chromobacterium</i> (3), <i>Laribacter</i> (1), <i>Neisseria</i> (5), <i>Stenoxybacter</i> (1), <i>Vitreoscilla</i> (1), <i>Vogosella</i> (2)
	Nitrosomonadales	Nitrosomonadaceae	<i>Nitrosomonas</i> (3), <i>Nitrosospira</i> (1684), <i>Nitrosovibrio</i> (332), Unclassified(272)
		Unclassified	Unclassified (111)
	Rhodocyclales	Rhodocyclaceae	<i>Dechloromonas</i> (1), <i>Georgfuchsia</i> (3), <i>Sterolibacterium</i> (13), <i>Thauera</i> (1), Unclassified (204)
	Unclassified	Unclassified	Unclassified (7603)
	Bdellovibrionales	Bacteriovoracaceae	<i>Bacteriovorax</i> (12)
		Bdellovibrionaceae	<i>Bdellovibrio</i> (96)
	Deltaproteobacteria		

	Desulfobacterales	Desulfobacteraceae	<i>Desulfatibacillum</i> (1), <i>Desulfobotulus</i> (13), <i>Desulfocella</i> (4), <i>Desulfococcus</i> (2), <i>Desulfofaba</i> (1), <i>Desulfonema</i> (618), <i>Desulforegula</i> (1), <i>Desulfosarcina</i> (5)
	Desulfovibrionales	Desulfobulbaceae	<i>Desulfobulbus</i> (15), <i>Desulfovibrio</i> (1)
		Desulfohalobiaceae	<i>Desulfohalobium</i> (1), <i>Desulfonatronovibrio</i> (39), <i>Desulfonauticus</i> (61)
		Desulfomicrobiaceae	<i>Desulfomicrobium</i> (2)
		Desulfonatronumaceae	<i>Desulfonatronum</i> (15)
		Desulfovibrionaceae	<i>Desulfovibrio</i> (624)
		Unclassified	Unclassified (1)
	Desulfuromonadales	Geobacteraceae	<i>Geoalkalibacter</i> (223), <i>Geobacter</i> (872)
		Pelobacteraceae	<i>Malomonas</i> (3), <i>Pelobacter</i> (3)
	Myxococcales	Cystobacteraceae	<i>Cystobacter</i> (62), <i>Melittangium</i> (9), <i>Stigmatella</i> (7)
		Myxococcaceae	<i>Anaeromyxobacter</i> (19), <i>Coralloccoccus</i> (42), <i>Myxococcus</i> (101)
		Nannocystaceae	<i>Plesiocystis</i> (1)
		Polyangiaceae	<i>Chondromyces</i> (1), <i>Sorangium</i> (9)
	Syntrophobacterales	Syntrophobacteraceae	<i>Desulforhabdus</i> (1), <i>Desulfovirga</i> (1)
	Unclassified	Unclassified	<i>Spirobacillus</i> (1), Unclassified (3477)
	Campylobacterales	Campylobacteraceae	<i>Arcobacter</i> (128), <i>Campylobacter</i> (36)
		Helicobacteraceae	<i>Helicobacter</i> (6)
	Nautiliales	Nautiliaceae	<i>Caminibacter</i> (4)
	Unclassified	Unclassified	<i>Nitratiraptor</i> (3), Unclassified (31)
	Acidithiobacillales	Acidithiobacillaceae	<i>Acidithiobacillus</i> (2)
	Aeromonadales	Aeromonadaceae	<i>Aeromonas</i> (64), <i>Oceanimonas</i> (1)
		Succinivibrionaceae	<i>Succinimonas</i> (1)
	Alteromonadales	Alteromonadaceae	<i>Marinobacterium</i> (1), Unclassified (1)
		Colwelliaceae	<i>Colwellia</i> (1)
		Idiomarinaceae	<i>Idiomarina</i> (1)
		Pseudoalteromonadaceae	<i>Pseudoalteromonas</i> (2)
		Shewanellaceae	<i>Shewanella</i> (1677)
Epsilonproteobacteria			
Gammaproteobacteria			

Chromatiales	Chromatiaceae	<i>Marichromatium</i> (2), <i>Nitrosococcus</i> (2), <i>Rheinheimera</i> (10), <i>Thermochromatium</i> (1), <i>Thiorhodovibrio</i> (1)
	Ectothiorhodospiraceae	<i>Alkalispirillum</i> (1), <i>Ectothiorhodosinus</i> (1), <i>Ectothiorhodospira</i> (1), <i>Nitrococcus</i> (1), <i>Thioalkalispira</i> (1), <i>Thioalkalivibrio</i> (1), <i>Thiorhodospira</i> (1), Unclassified (4)
	Halothiobacillaceae	<i>Halothiobacillus</i> (21)
Enterobacteriales	Enterobacteriaceae	<i>Buchnera</i> (1), <i>Buttiauxella</i> (4), <i>Candidatus</i> <i>Hamiltoniella</i> (3), <i>Citrobacter</i> (88), <i>Cronobacter</i> (77), <i>Edwardsiella</i> (7), <i>Enterobacter</i> (112), <i>Erwinia</i> (44), <i>Escherichia</i> (190), <i>Klebsiella</i> (326), <i>Kluyvera</i> (6), <i>Morganella</i> (23), <i>Pantoea</i> (45), <i>Pectobacterium</i> (3), <i>Photorhabdus</i> (6), <i>Rahnella</i> (12), <i>Salmonella</i> (43), <i>Serratia</i> (731), <i>Shigella</i> (1), <i>Xenorhabdus</i> (1), <i>Yersinia</i> (5), Unclassified (291)
Legionellales	Coxiellaceae	<i>Coxiella</i> (2), <i>Rickettsiella</i> (2)
	Legionellaceae	<i>Legionella</i> (8)
Methylococcales	Methylococcaceae	<i>Methylocaldum</i> (2), <i>Methylococcus</i> (1), <i>Methylohalobius</i> (1), <i>Methylomicrobium</i> (8), <i>Methylothermus</i> (3)
Oceanospirillales	Alcanivoracaceae	<i>Alcanivorax</i> (8)
	Halomonadaceae	<i>Candidatus Portiera</i> (1), <i>Chromohalobacter</i> (1), <i>Halomonas</i> (3350), <i>Zymobacter</i> (1)
	Oceanospirillaceae	<i>Marinomonas</i> (8)
	Oleiphilaceae	<i>Oleiphilus</i> (1)
Pasteurellales	Pasteurellaceae	<i>Actinobacillus</i> (3), <i>Aggregatibacter</i> (64), <i>Gallibacterium</i> (1), <i>Haemophilus</i> (317), <i>Pasteurella</i> (3), Unclassified (2)
Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i> (613), Unclassified (1)
	Pseudomonadaceae	<i>Azomonas</i> (2), <i>Azotobacter</i> (46), <i>Pseudomonas</i> (5450)

		Thiotrichales	Thiotrichaceae	<i>Beggiotoa</i> (35)
		Xanthomonadales	Xanthomonadaceae	<i>Aquimonas</i> (27), <i>Dyella</i> (89), <i>Ignatzschinia</i> (1), <i>Lysobacter</i> (8), <i>Rhodanobacter</i> (9771), <i>Stenotrophomonas</i> (22), <i>Wohlfahrtiimonas</i> (3), <i>Xanthomonas</i> (77), <i>Xyella</i> (5)
		Unclassified	Unclassified	<i>Methylothalomonas</i> (32), <i>Methylonatronum</i> (4), <i>Solimonas</i> (2), <i>Thiohalophilus</i> (1), Unclassified (7532)
	Unclassified	Unclassified	Unclassified	Unclassified (2098)
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	<i>Bacteroides</i> (711)
			Porphyromonadaceae	<i>Butyrivimonas</i> (15), <i>Odoribacter</i> (13), <i>Parabacteroides</i> (122), <i>Porphyromonas</i> (1854)
			Prevotellaceae	<i>Prevotella</i> (6191)
			Rikenellaceae	<i>Alistipes</i> (398), <i>Rikenella</i> (81)
	Cytophagia	Cytophagales	Cyclobacteriaceae	<i>Cyclobacterium</i> (80)
			Cytophagaceae	<i>Cytophaga</i> (1200), <i>Dyadobacter</i> (1883), <i>Flexibacter</i> (619), <i>Hymenobacter</i> (370), <i>Spirosoma</i> (202), <i>Sporocytophaga</i> (2)
	Flavobacteria	Flavobacteriales	Flammeovirgaceae	<i>Flammeovirga</i> (16), <i>Flexithrix</i> (85)
			Flavobacteriaceae	<i>Aquimarina</i> (3), <i>Arenibacter</i> (17), <i>Capnocytophaga</i> (13), <i>Cellulophaga</i> (8), <i>Chryseobacterium</i> (11), <i>Dokdonia</i> (5), <i>Elizabethkingia</i> (142), <i>Flavobacterium</i> (4868), <i>Gramella</i> (112), <i>Leeuwenhoekella</i> (3), <i>Mariniflexile</i> (1), <i>Myroides</i> (5), <i>Ornithobacterium</i> (1), <i>Riemerella</i> (155), <i>Robiginitalea</i> (47), <i>Tenacibaculum</i> (11), <i>Wautersiella</i> (1), <i>Zumongwangia</i> (62), Unclassified (3)
		Unclassified	Unclassified	Unclassified (57)
Sphingobacteria	Sphingobacteriales	Sphingobacteriales	Rhodothermaceae	<i>Rhodothermus</i> (5), <i>Salinibacter</i> (2)
			Saprospiraceae	<i>Saprospira</i> (16)

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Firmicutes	Unclassified	Unclassified	Spingobacteriaceae	<i>Pedobacter</i> (3600), <i>Sphingobacterium</i> (3900), Unclassified (5683)
			Unclassified	<i>Chitinophaga</i> (3721), <i>Terrimonas</i> (7046)
Bacilli	Unclassified	Bacillales	Alicyclobacillaceae	<i>Candidatus Amoebophilus</i> (1391), <i>Candidatus Cardinium</i> (27), <i>Proloxibacter</i> (3), Unclassified (429)
			Bacillaceae	<i>Alicyclobacillus</i> (328) <i>Amphibacillus</i> (28), <i>Anaerobacillus</i> (29), <i>Anoxybacillus</i> (499), <i>Bacillus</i> (20433), <i>Geobacillus</i> (172), <i>Halobacillus</i> (28), <i>Lysinibacillus</i> (804), <i>Oceanobacillus</i> (157), <i>Terribacillus</i> (2), <i>Virgibacillus</i> (61)
			Listeriaceae	<i>Brochothrix</i> (3), <i>Listeria</i> (1)
			Paenibacillaceae	<i>Aneurinibacillus</i> (32), <i>Brevibacillus</i> (1344), <i>Cohnella</i> (2), <i>Paenibacillus</i> (1568), <i>Thermobacillus</i> (5)
			Pasteuriaceae	<i>Pasteuria</i> (7)
			Planococcaceae	<i>Kurthia</i> (95), <i>Planococcus</i> (7), <i>Planomicrobium</i> (15), <i>Sporosarcina</i> (69), <i>Ureibacillus</i> (74), <i>Viridibacillus</i> (17)
			Sporolactobacillaceae	<i>Sporolactobacillus</i> (18)
			Staphylococcaceae	<i>Staphylococcus</i> (1049)
			Thermoactinomycetaceae	<i>Laceyella</i> (166), <i>Seimonella</i> (25), <i>Thermoactinomyces</i> (487), <i>Thermoflavomicrobium</i> (3)
			Unclassified	<i>Exiguobacterium</i> (31), <i>Gemella</i> (46), <i>Pullulanibacillus</i> (5)
			Aerococcaceae	<i>Abiotrophia</i> (94)
			Carnobacteriaceae	<i>Alkalibacterium</i> (4), <i>Atopostipes</i> (1), <i>Carnobacterium</i> (27), <i>Granulicatella</i> (66), <i>Trichococcus</i> (5)
			Enterococcaceae	<i>Bavariicoccus</i> (1), <i>Enterococcus</i> (298), <i>Tetragenococcus</i> (1)
			Lactobacillaceae	<i>Lactobacillus</i> (816), <i>Pediococcus</i> (1)
			Leuconostocaceae	<i>Leuconostoc</i> (15), <i>Weissella</i> (59)



	Clostridia	Clostridiales	<p>Streptococcaceae  <i>Lactococcus</i> (3), <i>Streptococcus</i> (218)</p> <p>Clostridiaceae  <i>Alkaliphilus</i> (97), <i>Butyricoccus</i> (99),  <i>Caloramator</i> (15), <i>Candidatus Arthromitus</i> (1), <i>Clostridium</i> (3171),  <i>Oxobacter</i> (3)</p> <p>Clostridiales Family XI.  Incertae  <i>SedisAnaerococcus</i> (2), <i>SedisFinegoldia</i> (27), <i>SedisHelcococcus</i> (6),  <i>SedisPeptoniphilus</i> (255),  <i>SedisSporanaerobacter</i> (373),  <i>SedisTissierella</i> (586)</p> <p>Clostridiales Family XII.  Incertae  <i>SedisFusibacter</i> (5)</p> <p>Clostridiales Family XIV.  Incertae  <i>SedisAnaerobranca</i> (5)</p> <p>Clostridiales Family XVII. Incertae  <i>SedisSulfobacillus</i> (27),  <i>SedisThermaerobacter</i> (5)</p> <p>Clostridiales Family XVIII. Incertae  <i>SedisSymbiobacterium</i> (16)</p> <p>Eubacteriaceae  <i>Acetobacterium</i> (24), <i>Eubacterium</i> (275)</p> <p>Heliobacteriaceae  <i>Heliobacillus</i> (7), <i>Heliobacterium</i> (31),  <i>Heliophilum</i> (8)</p> <p>Lachnospiraceae  <i>Anaerostipes</i> (159), <i>Butyrivibrio</i> (148),  <i>Cellulosilyticum</i> (38), <i>Hespellia</i> (5),  <i>Lachnospira</i> (2), <i>Robinsoniella</i> (1),  <i>Roseburia</i> (224), Unclassified (21)</p> <p>Peptococcaceae  <i>Candidatus Desulforudis</i> (2),  <i>Dehalobacter</i> (9), <i>Desulfotobacterium</i> (71), <i>Desulfonispota</i> (99),  <i>Desulfosporosinus</i> (13),  <i>Desulfotomaculum</i> (1063), <i>Pelotomaculum</i> (1), Unclassified (1)</p> <p>Peptostreptococcaceae  <i>Peptostreptococcus</i> (65)</p> <p>Ruminococcaceae  <i>Acetivibrio</i> (94), <i>Ethanoligenens</i> (21),  <i>Faecalibacterium</i> (790), <i>Ruminococcus</i> (2434), Unclassified (10)</p>
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			Syntrophomonadaceae Unclassified	<i>Syntrophomonas</i> (9) <i>Blautia</i> (57), <i>Epulopiscium</i> (2), Unclassified (255)
		Halanaerobiales	Halanaerobiaceae	<i>Halofermotherix</i> (1)
		Natranaerobiales	Natranaerobiaceae	<i>Natranaerobius</i> (2)
		Thermoanaerobacteriales	Thermoanaerobacteraceae	<i>Caldanaerobacter</i> (1), <i>Caldanaerobius</i> (176), <i>Carboxydotherrmus</i> (1), <i>Thermacetogenium</i> (2), <i>Thermoanaerobacter</i> (44)
			Thermoanaerobacteriales Family III. Incertae	<i>SedisCaldicellulosiruptor</i> (192)
			Thermodesulfobiaceae	<i>Thermodesulfobium</i> (1)
			Unclassified	Unclassified (10)
		Unclassified	Unclassified	<i>Desulfotribacter</i> (1)
	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	<i>Erysipelotrich</i> (1), Unclassified (62)
	Negativicutes	Selenomonadales	Acidaminococcaceae	<i>Acidaminococcus</i> (2), <i>Phascocartobacterium</i> (45)
			Veillonellaceae	<i>Dialister</i> (151), <i>Megasphaera</i> (652), <i>Pectinatus</i> (76), <i>Selenomonas</i> (129), <i>Sporomusa</i> (20), <i>Veillonella</i> (637)
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Acidobacterium</i> (5459), <i>Terriglobus</i> (327)
	Solibacteres	Solibacterales	Solibacteraceae	<i>Candidatus Solibacter</i> (7033)
Verrucomicrobia	Opitutae	Unclassified	Opitutaceae	<i>Opitutus</i> (709), Unclassified (35)
	Sporolactobacteria	Unclassified	Unclassified	<i>Chthoniobacter</i> (3116)
	Unclassified	Methylacidiphilales	Methylacidiphilaceae	<i>Methylacidiphilum</i> (70)
	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	<i>Akkermansia</i> (196), <i>Prostheco bacter</i> (621), <i>Rubritalea</i> (37), <i>Verrucomicrobium</i> (1), Unclassified (56)
			Verrucomicrobia subdivision 3	Unclassified (2187)
			Unclassified	Unclassified (2)
Nitrospirae	Unclassified	Unclassified	Unclassified	Unclassified (47)
	Nitrospira	Nitrospirales	Nitrospiraceae	<i>Leptospirillum</i> (44), <i>Nitrospira</i> (2222)

Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	<i>Blastopirellula</i> (93), <i>Isosphaera</i> (630), <i>Pirellula</i> (615), <i>Planctomyces</i> (425), <i>Rhodopirellula</i> (6), Unclassified (172)
Spirochaetes	Spirochaetes	Spirochaetales	Brachyspiraceae	<i>Brachyspira</i> (283)
			Leptospiraceae	<i>Leptonema</i> (1), <i>Leptospira</i> (1057)
			Spirochaetaceae	<i>Spirochaeta</i> (35), <i>Treponema</i> (2)

#### 4.22 Bacterial diversity in the sample AH-7

An abundance of the phylum Acidobacteria was recorded from the sample AH-7 with the phylum being less diverse. The phylum Actinobacteria was the second most abundant phylum in the sample followed by Proteobacteria. The phylum Acidobacteria was majorly composed of the genus *Candidatus Koribacter* (59%) as seen in the previous sample. The abundant genus in the phylum Actinobacteria was observed to be *Thermoleophilum* (19%) followed by *Arthrobacter* (14%) and then by *Williamsia* (10%). The class Betaproteobacteria dominated the phylum Proteobacteria, with 15 per cent of the population being *Burkholderia*. Among the phylum Firmicutes, the Bacillus population occupied 37 per cent of the phylum. The population of other important genera was also recorded for comparative purposes. The genus-level bacterial diversity has been provided in Table 27.

The dominant phylum in the sample AH-7, being Acidobacteria was observed to be less diverse with three genera *Acidobacterium*, *Candidatus Solibacter* and *Candidatus Koribacter* constituting the entire Acidobacterial population with 18, 23 and 59 per cent of the phylum respectively. The phylum Actinobacteria was found to have 19 per cent of *Thermoleophilum* followed by 14 per cent *Arthrobacter* and 10 per cent of *Williamsia* being notably abundant genera. Among the different classes in the phylum Proteobacteria, the class Betaproteobacteria was found to be abundant followed by Alphaproteobacteria.

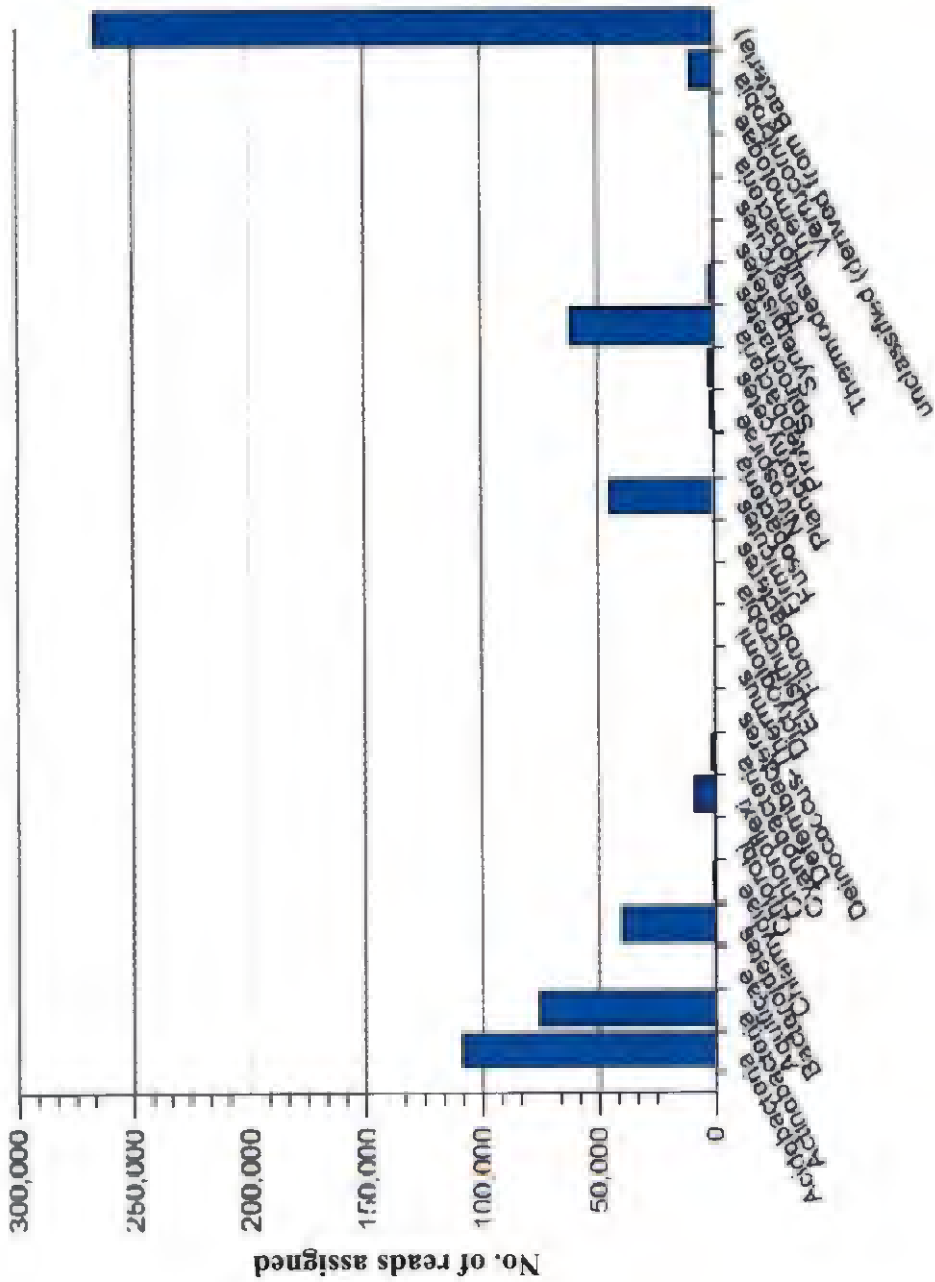


Plate 15. Phylum-level bacterial diversity in the sample AH-7 obtained using MG-RAST pipeline

**Table 27. Genus-level taxonomic assemblage of bacterial diversity from 10 predominant phyla in the sample AH-7**

Phylum	Class	Order	Family	Genus
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Acidobacterium</i> (20042)
	Solibacteres	Solibacterales	Solibacteraceae	<i>Candidatus Solibacter</i> (24637)
	Unclassified	Unclassified	Unclassified	<i>Candidatus Koribacter</i> (63856)
Actinobacteria	Actinobacteria	Actinomicroniales	Acidimicrobiaceae	<i>Acidimicrobium</i> (246), <i>Acidithiomicrobium</i> (149)
			Acidothermaceae	<i>Acidothermus</i> (240)
			Acidomycetaceae	<i>Actinobaculum</i> (12), <i>Actinomyces</i> (8), <i>Arcanobacterium</i> (5)
			Actinosynnemataceae	<i>Actinokineospora</i> (17), <i>Actinosynnema</i> (3), <i>Lechevalieria</i> (17), <i>Lentzea</i> (67), <i>Saccharothrix</i> (5)
			Beutenbergiaceae	<i>Beutenbergia</i> (543)
			Brevibacteriaceae	<i>Brevibacterium</i> (1566)
			Catenulisporaceae	<i>Catenulispora</i> (159)
			Cellulomonadaceae	<i>Actinotalea</i> (7), <i>Cellulomonas</i> (956), <i>Oerskovia</i> (1)
			Corynebacteriaceae	<i>Corynebacterium</i> (3339)
			Dermabacteraceae	<i>Brachybacterium</i> (77)
			Dermacoccaceae	<i>Dermacoccus</i> (12), <i>Kytococcus</i> (10)
			Dermatophilaceae	<i>Dermatophilus</i> (69)
			Dietziaceae	<i>Dietzia</i> (30)
			Frankiaceae	<i>Frankia</i> (2740)
			Geodermatophilaceae	<i>Geodermatophilus</i> (182)
			Glycomycetaceae	<i>Glycomyces</i> (1), <i>Stacchebrandtia</i> (3)
			Gordoniaceae	<i>Gordonia</i> (901), <i>Sklermania</i> (2)
			Intrasporangiaceae	<i>Intrasporangium</i> (95), <i>Janibacter</i> (829), <i>Serinicoccus</i> (21), <i>Terrabacter</i> (649), <i>Tetrasphaera</i> (332)
			Kineosporiaceae	<i>Kineococcus</i> (6)
			Microbacteriaceae	<i>Agreia</i> (1), <i>Agrococcus</i> (58), <i>Agromyces</i> (51), <i>Candidatus Aquiluna</i> (2),

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		<p><i>Clavibacter</i> (53), <i>Cryobacterium</i> (47), <i>Curtobacterium</i> (55), <i>Glaciibacter</i> (9), <i>Leifsonia</i> (364), <i>Leucobacter</i> (4), <i>Microbacterium</i> (1212), <i>Mycetocola</i> (28), <i>Okibacterium</i> (4), <i>Pseudoclavibacter</i> (5), <i>Rathayibacter</i> (33), <i>Salinibacterium</i> (2), <i>Subiercola</i> (1)</p> <p><i>Arthrobacter</i> (10236), <i>Kocuria</i> (72), <i>Micrococcus</i> (71), <i>Nesterenkonia</i> (13), <i>Renibacterium</i> (37), <i>Rothia</i> (535)</p> <p><i>Actinoplanes</i> (254), <i>Catenuloplanes</i> (111), <i>Couchioplanes</i> (1), <i>Dactylosporangium</i> (181), <i>Micromonospora</i> (1244), <i>Salinispora</i> (64), <i>Verrucosipora</i> (92)</p> <p><i>Mycobacterium</i> (1958)</p> <p><i>Nakamurella</i> (24)</p> <p><i>Nokardia</i> (243), <i>Rhodococcus</i> (1319), <i>Smaragdicooccus</i> (139)</p> <p><i>Aeromicrobium</i> (14), <i>Kribbella</i> (454), <i>Nocardioides</i> (2461), <i>Pimelobacter</i> (112), <i>Nocardopsis</i> (361), <i>Streptomonospora</i> (2), <i>Thermobifida</i> (3)</p> <p><i>Cellulosimicrobium</i> (312), <i>Isoptricola</i> (9), <i>Promicromonospora</i> (50), <i>Xylanimicrobium</i> (13), <i>Xylanimonas</i> (1)</p> <p><i>Microtunatus</i> (13), <i>Propionibacterium</i> (49), Unclassified (302)</p> <p><i>Amycolatopsis</i> (474), <i>Kibdelosporangium</i> (4), <i>Kutzneria</i> (82), <i>Prauserella</i> (21), <i>Pseudonocardia</i> (951), <i>Saccharomonospora</i> (11), <i>Saccharopolyspora</i> (2978), <i>Sireptocalloteichus</i> (9), <i>Thermobispora</i> (18)</p>
	Micrococaceae	
	Micromonosporaceae	
	Mycobacteriaceae	
	Nakamurellaceae	
	Nocardiaceae	
	Nocardioideae	
	Promicromonosporaceae	
	Propionibacteriaceae	
	Pseudonocardiaceae	



			Rarobacteraceae	<i>Rarobacter</i> (64)
			Sanguibacteraceae	<i>Sanguibacter</i> (13)
			Segniliparaceae	<i>Segniliparus</i> (1)
			Streptomycetaceae	<i>Kitasatospora</i> (135), <i>Streptacidiphilus</i> (920), <i>Streptomyces</i> (1964)
			Streptosporangiaceae	<i>Microbispora</i> (54), <i>Microtetrastroma</i> (8), <i>Nonomuraea</i> (56), <i>Planobispora</i> (1), <i>Streptosporangium</i> (902)
			Thermomonosporaceae	<i>Actinoallomurus</i> (761), <i>Actinocorallia</i> (107), <i>Actinomadura</i> (1849), <i>Spirillospora</i> (2), <i>Thermomonospora</i> (765), Unclassified (406)
			Tsakamurellaceae	<i>Tsakamurella</i> (16)
			Unclassified	<i>Tropheryma</i> (11), Unclassified (3)
			Williamsiaceae	<i>Williamsia</i> (7243)
		Bifidobacteriales	Bifidobacteriaceae	<i>Aeriscardovia</i> (8), <i>Bifidobacterium</i> (150), <i>Gardnerella</i> (17), <i>Metascardovia</i> (2)
		Coriobacteriales	Coriobacteriaceae	<i>Atopobium</i> (3946), <i>Collinsella</i> (138), <i>Eggerthella</i> (26), <i>Enterorhabdus</i> (8), <i>Gordonibacter</i> (253), <i>Slackia</i> (12)
		Rubrobacterales	Rubrobacteraceae	<i>Rubrobacter</i> (715)
		Thermoleophilales	Thermoleophilaceae	<i>Thermoleophilum</i> (14601)
		Unclassified	Unclassified	Unclassified (42)
		Caulobacteriales	Caulobacteraceae	<i>Asticcacaulis</i> (18), <i>Brevundimonas</i> (30), <i>Caulobacter</i> (12), <i>Phenyllobacterium</i> (1016)
		Rhizobiales	Bartonellaceae	<i>Bartonella</i> (3)
			Beijerinckiaceae	<i>Beijerinckia</i> (1)
			Bradyrhizobiaceae	<i>Afipia</i> (32), <i>Balneimonas</i> (3), <i>Blastobacter</i> (3), <i>Bosea</i> (4), <i>Bradyrhizobium</i> (3152), <i>Nitrobacter</i> (183), <i>Rhodopseudomonas</i> (111), Unclassified (20)
Proteobacteria	Alphaproteobacteria		Brucellaceae	<i>Ochrobactrum</i> (5)

		Hyphomicrobiaceae	<i>Blastochloris</i> (128), <i>Hyphomicrobium</i> (124), <i>Rhodomicrobium</i> (60)
		Methylobacteriaceae	<i>Methylobacterium</i> (1179)
		Methylocystaceae	<i>Methylocystis</i> (61), <i>Methylosinus</i> (10), Unclassified (281)
		Phyllobacteriaceae	<i>Aminobacter</i> (2), <i>Chelativorans</i> (2), <i>Mesorhizobium</i> (1034)
		Rhizobiaceae	<i>Agrobacterium</i> (1), <i>Candidatus Liberibacter</i> (2), <i>Rhizobium</i> (256), <i>Sinorhizobium</i> (27)
		Rhodobiaceae	<i>Aifella</i> (1)
		Unclassified	Unclassified (109)
		Xanthobacteraceae	<i>Ancylobacter</i> (1), <i>Azorhizobium</i> (5), <i>Xanthobacter</i> (6)
		Hyphomonadaceae	<i>Hirschia</i> (1)
		Rhodobacteraceae	<i>Jannaschia</i> (1), <i>Pannonibacter</i> (16), <i>Paracoccus</i> (1), <i>Pelagibaca</i> (1), <i>Phaeobacter</i> (2), <i>Rhodobacter</i> (1), <i>Rhodotalassium</i> (1), <i>Rhodovulvum</i> (2), <i>Roseobacter</i> (2), <i>Sulfitobacter</i> (2), Unclassified (11)
		Unclassified	Unclassified (8)
		Rhodospirillaceae	<i>Azospirillum</i> (63), <i>Magnetospirillum</i> (1), <i>Nisaea</i> (1), <i>Rhodovibrio</i> (1), <i>Roseospira</i> (1), <i>Telmatospirillum</i> (8), Unclassified (537)
		Anaplasmataceae	<i>Anaplasma</i> (1), <i>Ehrlichia</i> (5), <i>Neorickettsia</i> (1), <i>Wolbachia</i> (1)
		Rickettsiaceae	<i>Orientia</i> (15), <i>Rickettsia</i> (6)
		Unclassified	<i>Caedibacter</i> (5), <i>Candidatus Midichloria</i> (1), <i>Candidatus Odysseella</i> (6)
		Erythrobacteraceae	<i>Erythrobacter</i> (191), <i>Erythromicrobium</i> (1)
		Sphingomonadaceae	<i>Blastomonas</i> (17), <i>Novosphingobium</i> (10), <i>Sandarakinorhabdus</i> (15),
		Rhodobacterales	
		Rhodospirillales	
		Rickettsiales	
		Sphingomonadales	

Betaproteobacteria	Unclassified	Unclassified	<i>Sphingobium</i> (356), <i>Sphingomonas</i> (432), <i>Sphingopyxis</i> (100), <i>Sphingosinicella</i> (19), <i>Zymomonas</i> (6)
	Burkholderiales	Alcaligenaceae	Unclassified (7522) <i>Achromobacter</i> (5), <i>Alcaligenes</i> (9), <i>Azohydromonas</i> (13), <i>Bordetella</i> (3), <i>Oligella</i> (2), <i>Pigmentiphaga</i> (1), <i>Taylorella</i> (1), Unclassified (1)
		Burkholderiaceae	<i>Burkholderia</i> (9152), <i>Candidatus Glomeribacter</i> (8), <i>Cupriavidus</i> (707), <i>Pandoraea</i> (27), <i>Paucimonas</i> (2), <i>Polynucleobacter</i> (2), <i>Ralstonia</i> (507)
		Commonadaceae	<i>Acidovorax</i> (290), <i>Alicyclophilus</i> (25), <i>Brachymonas</i> (8), <i>Caldimonas</i> (2), <i>Comamonas</i> (324), <i>Hydrogenophaga</i> (1), <i>Pelomonas</i> (11), <i>Pseudoacidovorax</i> (2), <i>Schlegelella</i> (7), <i>Simplicispira</i> (1), <i>Variovirax</i> (818), <i>Verminephrobacter</i> (596), <i>Xenophilus</i> (40), Unclassified (269)
		Oxalobacteraceae	<i>Collimonas</i> (38), <i>Duganella</i> (23), <i>Herbaspirillum</i> (39), <i>Herminimonas</i> (5), <i>Janthinobacterium</i> (61), <i>Massilia</i> (800), <i>Oxalobacterium</i> (37), <i>Oxalobacter</i> (2), <i>Telluria</i> (83)
		Unclassified	<i>Aquabacterium</i> (1), <i>Aquicola</i> (1), <i>Leptothrix</i> (9), <i>Mitsuaria</i> (2), <i>Roseateles</i> (4), <i>Rubrivivax</i> (5), <i>Sphaerotilus</i> (72), <i>Thiobacter</i> (2), <i>Thiomonas</i> (4), Unclassified (914)
	Gallionellales	Gallionellaceae	<i>Sideroxydans</i> (7)
	Hydrogenophilales	Hydrogenophilaceae	<i>Thiobacillus</i> (1)
	Methylophilales	Methylophilaceae	<i>Methylobacillus</i> (3), <i>Methylophilus</i> (1), <i>Methylotenera</i> (2)

	Neisseriales	Neisseriaceae	<i>Aquaspirillum</i> (2), <i>Chromobacterium</i> (5), <i>Eikenella</i> (1), <i>Laribacter</i> (4), <i>Neisseria</i> (14), <i>Stenoxobacter</i> (1), <i>Yogesella</i> (1)
	Nitrosomonadales	Nitrosomonadaceae	<i>Nitrosomonas</i> (12), <i>Nitrosospira</i> (563), <i>Nitrosovibrio</i> (49), Unclassified (720)
		Spirillaceae	<i>Spirillum</i> (1)
		Unclassified	Unclassified (43)
	Rhodocyclales	Rhodocyclaceae	<i>Aromatoleum</i> (2), <i>Azoarcus</i> (6), <i>Azonexus</i> (1), <i>Azospira</i> (1), <i>Methyloversatilis</i> (1), <i>Sterolibacterium</i> (3), <i>Thauera</i> (1), <i>Zooglea</i> (1), Unclassified (177)
	Unclassified	Unclassified	<i>Candidatus Tremblaya</i> (5), Unclassified (7900)
Deltaproteobacteria	Bdellovibrionales	Bacteriovoraceae	<i>Bacteriovorax</i> (23)
		Bdellovibrionaceae	<i>Bdellovibrio</i> (121)
	Desulfobacterales	Desulfobacteraceae	<i>Desulfatibacillus</i> (14), <i>Desulfobacter</i> (1), <i>Desulfobacterium</i> (3), <i>Desulfobotulus</i> (45), <i>Desulfocella</i> (134), <i>Desulfococcus</i> (7), <i>Desulfonema</i> (259), <i>Desulforegula</i> (3), Unclassified (2)
		Desulfobulbaceae	<i>Desulfobulbus</i> (5), Unclassified (2)
		Desulfohalobiaceae	<i>Desulfonatronovibrio</i> (11)
		Desulfomicrobiaceae	<i>Desulfomicrobium</i> (2)
		Desulfonatronumaceae	<i>Desulfonatronum</i> (2)
		Desulfovibrionaceae	<i>Desulfovibrio</i> (1679)
		Unclassified	Unclassified (1)
	Desulfuromonadales	Geobacteraceae	<i>Geoalkalibacter</i> (240), <i>Geobacter</i> (777)
		Pelobacteraceae	<i>Maonomonas</i> (17), <i>Pelobacter</i> (10)
	Myxococcales	Cystobacteraceae	<i>Cystobacter</i> (222), <i>Melittangium</i> (45), <i>Stigmatella</i> (4)
		Haliangiaceae	<i>Haliangium</i> (5)
		Myxococcaceae	<i>Anaeromyxobacter</i> (108), <i>Corallocooccus</i> (246), <i>Myxococcus</i> (125)

			Nannocystaceae	<i>Nannocystis</i> (13)
			Polyangiaceae	<i>Chondromyces</i> (20), <i>Sorangium</i> (41)
	Syntrophobacterales		Syntrophobacteraceae	<i>Desulfovirga</i> (1), <i>Syntrophobacter</i> (1)
	Unclassified		Unclassified	<i>Spirobacillus</i> (1), Unclassified (7734)
Epsilonproteobacteria	Campylobacterales		Campylobacteraceae	<i>Arcobacter</i> (1), <i>Campylobacter</i> (65), <i>Sulfurospirillum</i> (1), Unclassified (3)
			Helicobacteraceae	<i>Helicobacter</i> (6)
	Nautiliales		Nautiliaceae	<i>Caminibacter</i> (13), <i>Lebetimonas</i> (1)
	Unclassified		Unclassified	<i>Nitratifactor</i> (1), <i>Nitratiruptor</i> (2), Unclassified (126)
Gammaproteobacteria	Acidithiobacillales		Acidithiobacillaceae	<i>Acidithiobacillus</i> (1)
	Aeromonadales		Aeromonadaceae	<i>Aeromonas</i> (2), <i>Oceanimonas</i> (1)
			Succinivibrionaceae	<i>Succinimonas</i> (5)
	Alteromonadales		Colwelliaceae	<i>Colwellia</i> (1)
			Shewanellaceae	<i>Shewanella</i> (167)
	Chromatiales		Chromatiaceae	<i>Halochromatium</i> (1), <i>Marichromatium</i> (2), <i>Nitrosococcus</i> (3), <i>Thiobaca</i> (1), <i>Thiocapsa</i> (4), <i>Thiorhodococcus</i> (1), <i>Thiorhodovibrio</i> (3), Unclassified (1)
			Ectothiorhodospiraceae	<i>Alkalispirillum</i> (2), <i>Ectothiorhodosinus</i> (1), <i>Ectothiorhodospira</i> (397), <i>Halorhodospira</i> (3), <i>Thioalkalivibrio</i> (48), <i>Thiohalospira</i> (6), <i>Thiorhodospira</i> (2)
			Halothiobacillaceae	<i>Halothiobacillus</i> (4)
	Enterobacteriales		Enterobacteriaceae	<i>Buttiauxella</i> (5), <i>Candidatus Hamiltonella</i> (6), <i>Citrobacter</i> (28), <i>Cronobacter</i> (4), <i>Enterobacter</i> (1), <i>Erwinia</i> (6), <i>Escherichia</i> (9), <i>Klebsiella</i> (8), <i>Morganella</i> (1), <i>Pantoea</i> (5), <i>Photorhabdus</i> (5), <i>Proteus</i> (1), <i>Serratia</i> (29), Unclassified (105)
	Legionellales		Coxiellaceae	<i>Coxiella</i> (15), <i>Rickettsiella</i> (9)
			Legionellaceae	<i>Legionella</i> (47)

	Methylococcales	Methylococcaceae	<i>Methylobacter</i> (1), <i>Methylocaldum</i> (68), <i>Methylococcus</i> (7), <i>Methylolobolus</i> (10), <i>Methylomicrobium</i> (1), <i>Methylotarcina</i> (1), <i>Methylotermus</i> (2)
	Oceanospirillales	Alcanivoraceae	<i>Alcanivorax</i> (5)
		Halomonadaceae	<i>Halomonas</i> (219)
		Oceanospirillaceae	<i>Marinomonas</i> (3), <i>Oceanospirillum</i> (2)
		Oleiphilaceae	<i>Oleiphilus</i> (1)
	Pasteurellales	Pasteurellaceae	<i>Aggregatibacter</i> (1), <i>Haemophilus</i> (1)
	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i> (30), <i>Moraxella</i> (6)
		Pseudomonadaceae	<i>Pseudomonas</i> (76)
	Thiotrichales	Francisellaceae	<i>Francisella</i> (1)
		Piscirickettsiaceae	<i>Methylolphaga</i> (1)
		Thiotrichaceae	<i>Beggiotoa</i> (41)
	Vibrionales	Vibrionaceae	<i>Vibrio</i> (4)
	Xanthomonadales	Sinobacteraceae	<i>Sinobacter</i> (1)
		Xanthomonadaceae	<i>Dyella</i> (63), <i>Lysobacter</i> (5), <i>Rhodanobacter</i> (26), <i>Stenotrophomonas</i> (5), <i>Wohlfartimonas</i> (5), <i>Xanthomonas</i> (36), <i>Xylella</i> (9)
	Unclassified	Unclassified	<i>Candidatus Carsonella</i> (6), <i>Methylolobomonas</i> (39), <i>Methylonatrum</i> (3), <i>Solimonas</i> (8), Unclassified (3911)
Firmicutes	Bacilli	Alicyclobacillaceae	<i>Alicyclobacillus</i> (2337), Unclassified (2)
		Bacillaceae	<i>Amphibacillus</i> (18), <i>Anaerobacillus</i> (3), <i>Anoxybacillus</i> (382), <i>Bacillus</i> (16655), <i>Geobacillus</i> (949), <i>Halobacillus</i> (430), <i>Lysinibacillus</i> (279), <i>Marinococcus</i> (18), <i>Natronobacillus</i> (18), <i>Oceanobacillus</i> (209), <i>Terribacillus</i> (4), <i>Virgibacillus</i> (87)
		Listeriaceae	<i>Brochothrix</i> (1), <i>Listeria</i> (4)
		Paenibacillaceae	<i>Aneurinibacillus</i> (264), <i>Brevibacillus</i> (1422), <i>Cohnella</i> (2), <i>Paenibacillus</i> (2955), <i>Thermobacillus</i> (8)

			Pasteuria (12)
		Pasteuriaceae	<i>Kurthia</i> (71), <i>Planococcus</i> (71), <i>Planomicrobium</i> (872), <i>Sporosarcina</i> (26), <i>Ureibacillus</i> (337)
		Sporolactobacillaceae	<i>Sporolactobacillus</i> (9)
		Staphylococcaceae	<i>Staphylococcus</i> (66)
		Thermoactinomycoetaceae	<i>Thermoactinomyces</i> (2166), <i>Laceyella</i> (629), <i>Mechercharimyces</i> (4), <i>Thermoflavomicrobium</i> (7)
		Unclassified	<i>Alkalilactibacillus</i> (4), <i>Exiguobacterium</i> (26), <i>Gemella</i> (63), <i>Pullulanibacillus</i> (12), Unclassified (1)
		Aerococcaceae	<i>Abiotrophia</i> (16), <i>Aerococcus</i> (3)
		Carnobacteriaceae	<i>Carnobacterium</i> (2), <i>Granulicatella</i> (11), <i>Trichococcus</i> (10)
		Enterococcaceae	<i>Enterococcus</i> (18), <i>Tetragenococcus</i> (9)
		Lactobacillaceae	<i>Lactobacillus</i> (276)
		Leuconostocaceae	<i>Leuconostoc</i> (3)
		Streptococcaceae	<i>Lactococcus</i> (3), <i>Streptococcus</i> (14)
		Clostridiaceae	<i>Alkaliophilus</i> (121), <i>Butyricoccus</i> (8), <i>Caloramator</i> (17), <i>Clostridium</i> (2297), <i>Oxobacter</i> (26), <i>Tepidimicrobium</i> (6)
		Clostridiales Family XI. Incertae	<i>SedisAnaerococcus</i> (4), <i>SedisFinegoldia</i> (1), <i>SedisHelcococcus</i> (7), <i>SedisPeptoniphilus</i> (1855), <i>SedisSedimentibacter</i> (3), <i>SedisSoehngenia</i> (2), <i>SedisSporanaerobacter</i> (871), <i>SedisTissierella</i> (1009), <i>SedisFusibacter</i> (1)
		Clostridiales Family XII. Incertae	
		Clostridiales Family XIV. Incertae	<i>SedisAnaerobranca</i> (16)
		Clostridiales Family XVII. Incertae	<i>SedisSulfobacillus</i> (18), <i>SedisThermoerobacter</i> (7)
Clostridia		Clostridiales	



		Clostridiales Family XVIII. Incertae	<i>SedisSymbiobacterium</i> (156)
		Eubacteriaceae	<i>Acetobacterium</i> (8), <i>Eubacterium</i> (70)
		Heliobacteriaceae	<i>Heliobacillus</i> (12), <i>Heliobacterium</i> (871), <i>Heliophilum</i> (19), <i>Heliorestis</i> (8)
		Lachnospiraceae	<i>Anaerostipes</i> (52), <i>Butyrivibrio</i> (13), <i>Hespellia</i> (8), <i>Lachnospira</i> (16), <i>Pseudobutyrvibrio</i> (1), <i>Robinsoniella</i> (3), <i>Roseburia</i> (10), Unclassified (99)
		Peptococcaceae	<i>Candidatus Sulforudis</i> (9), <i>Dehalobacter</i> (32), <i>Desulfotobacterium</i> (321), <i>Desulfonipora</i> (268), <i>Desulfosporosinus</i> (160), <i>Desulfotomaculum</i> (1647), <i>Pelotomaculum</i> (41), <i>Thermincola</i> (17), Unclassified (111)
		Peptostreptococcaceae	<i>Peptostreptococcus</i> (108)
		Ruminococcaceae	<i>Acetivibrio</i> (96), <i>Ethanoligenens</i> (21), <i>Faecalibacterium</i> (68), <i>Ruminococcus</i> (2119), Unclassified (51)
		Syntrophomonadaceae	<i>Syntrophomonas</i> (10), <i>Syntrophothermus</i> (7)
		Unclassified	<i>Blautia</i> (4), <i>Epulopiscium</i> (39), Unclassified (78)
		Halanaerobiaceae	<i>Halarsenatibacter</i> (2), <i>Halothermothrix</i> (1)
	Halanaerobiales	Thermoanaerobacteraceae	<i>Ammonifex</i> (1), <i>Caldanaerobacter</i> (251), <i>Caldanaerobius</i> (282), <i>Moorella</i> (1), <i>Thermacetogenium</i> (3), <i>Thermoanaerobacter</i> (51)
		Thermoanaerobacterales Family III. Incertae	<i>SedisCaldicellulosiruptor</i> (184)
		Thermodesulfobiaceae	<i>Thermodesulfobium</i> (10)
		Unclassified	Unclassified (28)
		Erysipelotrichaceae	<i>Erysipelothrix</i> (1), Unclassified (7)
Erysipelitrichi	Erysipelotrichales		

	Negativivcutes	Selenomonadales	Acidaminococcaceae	<i>Acidaminococcus</i> (128), <i>Phascolarctobacterium</i> (8)
			Veillonellaceae	<i>Dialister</i> (7), <i>Megamonas</i> (4), <i>Megasphaera</i> (70), <i>Mitsuokella</i> (5), <i>Pectinatus</i> (7), <i>Selenomonas</i> (85), <i>Sporomusa</i> (115), <i>Veillonella</i> (28)
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	<i>Bacteroides</i> (125)
			Porphyromonadaceae	<i>Barnesiella</i> (2), <i>Butyrivimonas</i> (2), <i>Odoribacter</i> (2), <i>Parabacteroides</i> (59), <i>Porphyromonas</i> (6)
			Prevotellaceae	<i>Paraprevotella</i> (1), <i>Prevotella</i> (98)
			Rickenellaceae	<i>Alistipes</i> (16), <i>Rickenella</i> (130)
	Cytophagia	Cytophagales	Cyclobacteriaceae	<i>Cyclobacterium</i> (49)
			Cytophagaceae	<i>Cytophaga</i> (201), <i>Flexibacter</i> (567), <i>Hymenobacter</i> (230), <i>Marinoscillum</i> (4), <i>Microscilla</i> (7)
			Flammeovirgaceae	<i>Flammeovirga</i> (2), <i>Flexithrix</i> (261), <i>Persicobacter</i> (4)
	Flavobacteria	Flavobacteriales	Flavobacteriaceae	<i>Aquimarina</i> (4), <i>Arenibacter</i> (63), <i>Capnocytophaga</i> (1), <i>Cellulophaga</i> (1), <i>Chryseobacterium</i> (14), <i>Dokdonia</i> (5), <i>Elizabethkingia</i> (87), <i>Empedobacter</i> (22), <i>Flavobacterium</i> (1496), <i>Gaetbulibacter</i> (1), <i>Gramella</i> (8), <i>Leeuwenhoekeiella</i> (4), <i>Myroides</i> (20), <i>Riemerella</i> (21), <i>Robiginitalea</i> (45), <i>Zumongwangia</i> (6), Unclassified (3)
		Unclassified	Unclassified	Unclassified (2)
	Sphingobacteria	Sphingobacteriales	Rhodothermaceae	<i>Rhodothermus</i> (21), <i>Salinibacter</i> (3)
			Saprospiraceae	<i>Sprospira</i> (3)
			Sphingobacteriaceae	<i>Pedobacter</i> (681), <i>Sphingobacterium</i> (964), Unclassified (6381)
			Unclassified	<i>Chitinophaga</i> (12362), <i>Terrimonas</i> (15688)

Verrucomicrobia	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified	<i>Caandidatus Amoebophilus</i> (634), <i>Proloxibacter</i> (3), Unclassified (14) <i>Opitutus</i> (216), Unclassified (15) <i>Chthoniobacter</i> (5895) <i>Methylacidiphilum</i> (5) <i>Akkermansia</i> (18), <i>Prostheco bacter</i> (344), <i>Rubritalea</i> (6) Unclassified (2889)
	Opitutae	Unclassified	Unclassified	Opitutaceae	Unclassified	Unclassified (15)
	Spartobacteria	Unclassified	Methylacidophilales	Unclassified	Methylacidophilaceae	
	Unclassified	Verrucomicrobiales	Verrucomicrobiales	Verrucomicrobiaceae	Verrucomicrobiaceae	
	Verrucomicrobiae			Verrucomicrobia subdivision 3	Verrucomicrobiaceae	
	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified (15)
Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexales	Chloroflexaceae	Chloroflexaceae	<i>Chloroflexus</i> (29), <i>Roseiflexus</i> (21)
	Dehalococcoidetes	Herpetosiphonales	Herpetosiphonales	Oscillochloridaceae	Oscillochloridaceae	<i>Oscillochloris</i> (132)
	Ktedonobacteria	Unclassified	Unclassified	Herpetosiphonaceae	Herpetosiphonaceae	<i>Herpetosiphon</i> (2222)
	Thermomicrobia	Ktedonobacterales	Unclassified	Unclassified	Unclassified	<i>Dehalococcoides</i> (1)
		Unclassified	Unclassified	Ktedonobacteraceae	Ktedonobacteraceae	<i>Ktedonobacter</i> (4200)
		Sphaerobacterales	Sphaerobacterales	Sphaerobacteraceae	Sphaerobacteraceae	Unclassified (2556)
		Thermomicrobiales	Thermomicrobiales	Thermomicrobiaceae	Thermomicrobiaceae	<i>Sphaerobacter</i> (9)
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetales	Planctomycetaceae	Planctomycetaceae	<i>Thermomicrobium</i> (2) <i>Blastopirellula</i> (130), <i>Isosphaera</i> (1065), <i>Pirellula</i> (388), <i>Planctomyces</i> (218), Unclassified (59)
				Unclassified	Unclassified	<i>Candidatus Kuenenia</i> (1)
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetales	Brachyspiraceae	Brachyspiraceae	<i>Brachyspira</i> (241)
				Leptispiraceae	Leptispiraceae	<i>Leptospira</i> (1497)
				Spirochaetaceae	Spirochaetaceae	<i>Spirochaeta</i> (81), <i>Treponema</i> (1)
Cyanobacteria	Gloeobacteria	Gloeobaterales	Gloeobaterales	Unclassified	Gloeobacteraceae	<i>Gloeobacter</i> (36)
	Unclassified	Chroococcales	Chroococcales	Unclassified	Unclassified	<i>Aphanothece</i> (8), <i>Chamaesiphon</i> (5), <i>Cyanothece</i> (7), <i>Microcystis</i> (14), <i>Synechococcus</i> (22)
		Nostocales	Nostocales	Microchaetaceae	Microchaetaceae	<i>Tolythrix</i> (12)
				Nostocaceae	Nostocaceae	<i>Anabaena</i> (81), <i>Anabaenopsis</i> (3), <i>Aphanizomenon</i> (1), <i>Cylindrospermopsis</i> (2), <i>Cylindrospermum</i> (18),

			<i>Dolichospermum</i> (13), <i>Nodularia</i> (29), <i>Nostoc</i> (336), <i>Trichormus</i> (12), <i>Calothrix</i> (167) <i>Scytonema</i> (21)
	Rivulaceae		
	Scytonemataceae		
Oscillatoriales	Unclassified		<i>Arthrospira</i> (1), <i>Geitlerinema</i> (270), <i>Leptolyngbya</i> (110), <i>Lyngbya</i> (11), <i>Microcoleus</i> (126), <i>Oscillatoria</i> (22), <i>Phormidium</i> (3), <i>Pseudanabaena</i> (2), <i>Spirulina</i> (4), <i>Symploca</i> (21), <i>Chroococcidiopsis</i> (1), <i>Pleurocapsa</i> (5)
Prochlorales	Prochlorococcaceae		<i>Prochlorococcus</i> (2)
Stigonematales	Unclassified		<i>Fischerella</i> (17)
Unclassified	Unclassified		<i>Acaryochloris</i> (2), Unclassified (1)

#### 4.23 Bacterial diversity in the sample AH-5

The domain Bacteria was dominated by the phylum Actinobacteria (19.6%) followed by phylum Acidobacteria (12.03%) and thirdly by Proteobacteria (11.6%). Phylum Actinobacteria was composed majorly of genus *Thermoleophilum* occupying 24 per cent of the phylum. The acidobacterial phylum being the second most abundant phylum in the sample was less diverse than the less abundant Proteobacteria. The phylum Proteobacteria had a majority of the genus *Candidatus Koribacter* falling under unclassified class, order and family. The phylum Proteobacteria had its majority in the class Deltaproteobacteria with the abundant genus being unclassified. A notable population of *Bacillus* occupying almost 37 per cent of the phylum Firmicutes was also observed from the sample AH5. The genus-level diversity of the bacterial population has been enlisted Table 28.

The genus *Thermoleophilum* was observed to constitute 24 per cent of the actinobacterial population, being the dominant genera in the mentioned phylum. The class Deltaproteobacteria was found to be the dominant class in the phylum Proteobacteria, with the major portion of it being unclassified. Among the various genera of the phylum Firmicutes, *Bacillus* occupied 59 per cent of the total population in the phylum.

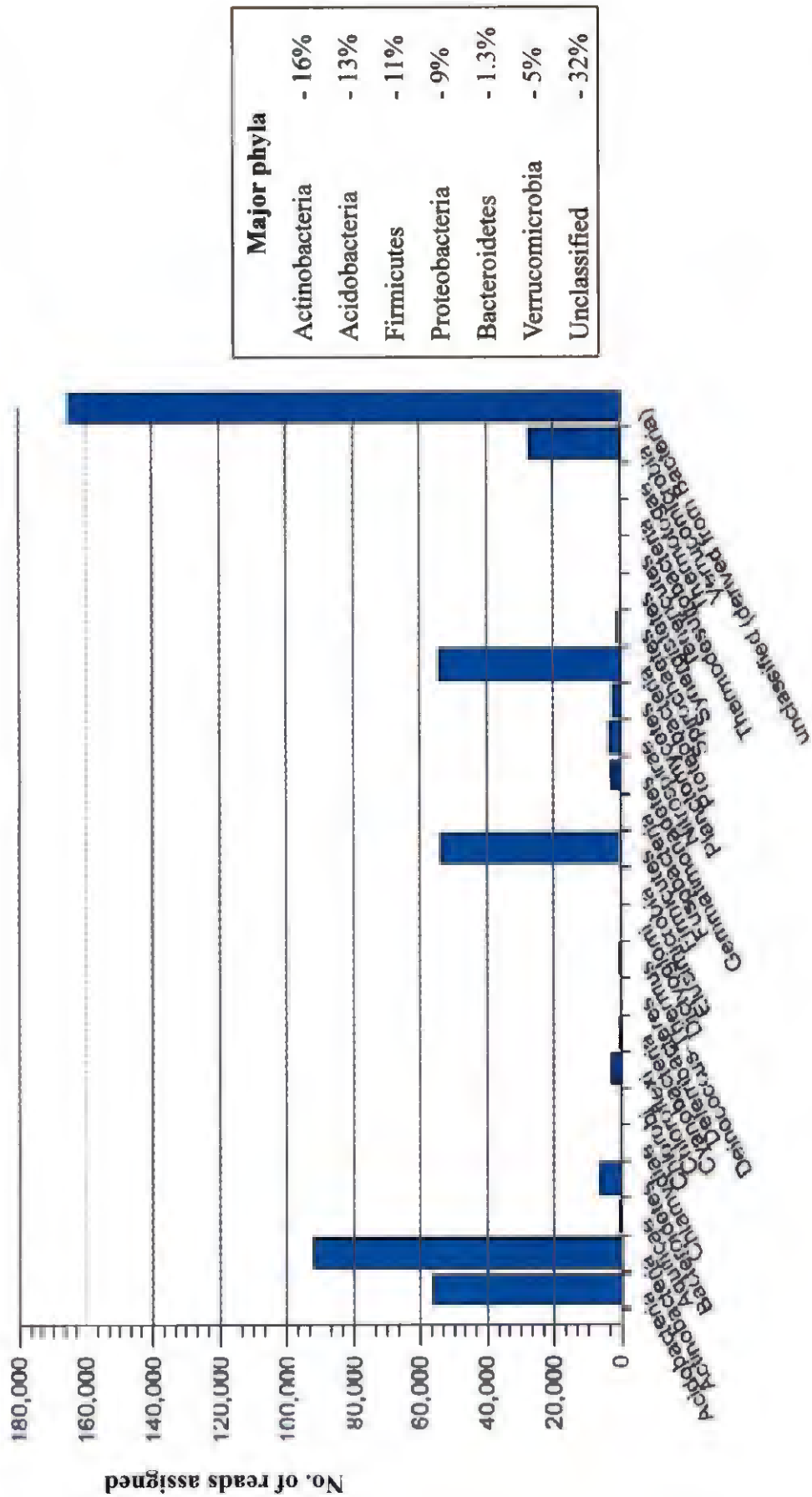


Plate 14. Phylum-level bacterial diversity in the sample AH-5 obtained using MG-RAST pipeline

Table 28. Genus-level taxonomic assemblage of bacterial diversity from 10 predominant phyla in the sample AH-5

Phylum	Class	Order	Family	Genus		
Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	<i>Acidimicrobium</i> (1041), <i>Acidithiomicrobium</i> (171)		
			Actinomycetales	<i>Acidothermus</i> (411)		
					Actinomycetaceae	<i>Actinobaculum</i> (22), <i>Actinomyces</i> (28), <i>Arcanobacterium</i> (8), <i>Mobiluncus</i> (4)
					Actinopolysporaceae	<i>Actinopolyspora</i> (1)
					Actinosynnemataceae	<i>Actinokineospora</i> (85), <i>Actinosynema</i> (9), <i>Lechevalieria</i> (11), <i>Lentzea</i> (94), <i>Saccharothrix</i> (15)
					Beutenbergiaceae	<i>Beutenbergia</i> (416)
					Brevibacteriaceae	<i>Brevibacterium</i> (587)
					Catenulisporaceae	<i>Catenulispora</i> (134)
					Cellulomonadaceae	<i>Cellulomonas</i> (787)
					Corynebacteraceae	<i>Corynebacterium</i> (532)
					Dermabacteraceae	<i>Brachybacterium</i> (249)
					Dermacoccaceae	<i>Dermacoccus</i> (14), <i>Kyococcus</i> (14)
					Dermatophilaceae	<i>Dermatophilus</i> (89)
					Dietziaceae	<i>Dietzia</i> (17)
					Frankiaceae	<i>Frankia</i> (2157)
					Geodermatophilaceae	<i>Geodermatophila</i> (178)
					Glycomycetaceae	<i>Glycomyces</i> (9), <i>Stackebrandtia</i> (15)
					Gordoniaceae	<i>Gordonia</i> (765)
					Intrasporangiaceae	<i>Intrasporangium</i> (196), <i>Janibacter</i> (453), <i>Serinicoccus</i> (84), <i>Terrabacter</i> (578), <i>Tetrasphaera</i> (131)
					Kineosporiaceae	<i>Kineococcus</i> (74)
			Microbacteriaceae	<i>Agreia</i> (7), <i>Agrococcus</i> (45), <i>Agromyces</i> (63), <i>Candidatus Aquiluna</i> (3), <i>Candidatus Rhodoluna</i> (1), <i>Clavibacter</i> (19), <i>Cryobacterium</i> (99), <i>Curtobacterium</i> (2), <i>Frigoribacterium</i> (1), <i>Glaciibacter</i> (19), <i>Leifsonia</i> (63), <i>Leucobacter</i>		



		(4), <i>Microbacterium</i> (469), <i>Mycetocola</i> (50), <i>Okibacterium</i> (5), <i>Pseudoclavibacter</i> (3), <i>Rathayibacter</i> (22), <i>Subtercola</i> (2)
Micrococcaceae		<i>Arthrobacter</i> (3228), <i>Kocuria</i> (103), <i>Micrococcus</i> (36), <i>Nesterenkonia</i> (43), <i>Renibacterium</i> (48), <i>Rothia</i> (845)
Micromonosporaceae		<i>Actinoplanes</i> (399), <i>Catenuloplanes</i> (194), <i>Dactylosporangium</i> (489), <i>Micromonospora</i> (3868), <i>Polymorphospora</i> (3), <i>Salinispora</i> (234), <i>Verucosipora</i> (437)
Mycobacteriaceae		<i>Mycobacterium</i> (3824)
Nakamurellaceae		<i>Nakamurella</i> (49)
Nocardiaceae		<i>Nocardia</i> (332), <i>Rhodococcus</i> (1189), <i>Smaragdicoscus</i> (137)
Nocardiodiaceae		<i>Aeromicrobium</i> (145), <i>Kribbella</i> (2449), <i>Nocardioides</i> (3598), <i>Pimelobacter</i> (348), <i>Nocardioopsis</i> (427), <i>Streptomonospora</i> (7), <i>Thermobifida</i> (17)
Promicromonosporaceae		<i>Cellulosimicrobium</i> (276), <i>Isoptericola</i> (6), <i>Promicromonospora</i> (299), <i>Xylanimicrobium</i> (7), <i>Xylanimonas</i> (1)
Propionibacteriaceae		<i>Microbunatus</i> (110), <i>Propionibacterium</i> (57), Unclassified (751)
Pseudonocardiaceae		<i>Amycolatopsis</i> (955), <i>Kibdelosporangium</i> (35), <i>Kutzneria</i> (1), <i>Prauserella</i> (36), <i>Pseudonocardia</i> (3184), <i>Saccharomonospora</i> (168), <i>Saccharopolyspora</i> (6425), <i>Thermobispora</i> (24), <i>Thermocrispum</i> (13)
Rarobacteraceae		<i>Rarobacter</i> (175)
Sanguibacteraceae		<i>Sanguibacter</i> (4)
Segniliparaceae		<i>Segniliparus</i> (2)
Streptomycetaceae		<i>Kitasatospora</i> (97), <i>Streptacidiphilus</i> (9), <i>Streptomyces</i> (2899)
Streptosporangiaceae		<i>Microbispora</i> (80), <i>Microtetraspora</i> (26), <i>Nonomuraea</i> (132), <i>Planobispora</i> (1),

				<i>Planomonospora</i> (34), <i>Streptosporangium</i> (1253), <i>Thermopolyspora</i> (1)
			Thermomonosporaceae	<i>Actinoallomurus</i> (1793), <i>Actinocoralitia</i> (221), <i>Actinomadura</i> (1748), <i>Spirillospora</i> (4), <i>Thermomonospora</i> (2579), Unclassified (1061)
			Tsakamurellaceae	<i>Tsakamurella</i> (12)
			Williamsiaceae	<i>Williamsia</i> (2287)
			Unclassified	<i>Tropheryma</i> (25), Unclassified (3)
		Bifidobacteriales	Bifidobacteriaceae	<i>Aeriscardovia</i> (1), <i>Alloscardovia</i> (1), <i>Bifidobacterium</i> (61), <i>Gardnerella</i> (30), <i>Metascardovia</i> (6)
			Unclassified	<i>Turicella</i> (3)
		Coriobacteriales	Coriobacteriaceae	<i>Atopobium</i> (3773), <i>Collinsella</i> (78), <i>Cryptobacterium</i> (9), <i>Eggerthella</i> (13), <i>Enterorhabdus</i> (13), <i>Gordonibacter</i> (323), <i>Slackia</i> (12)
			Rubrobacterales	<i>Rubrobacter</i> (783)
			Thermoleophilales	<i>Thermoleophilum</i> (22064)
			Unclassified	Unclassified (47)
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Acidobacterium</i> (8965), <i>Terriglobus</i> (340)
	Solibacteres	Solibacterales	Solibacteraceae	<i>Candidatus Solibacter</i> (13458)
	Unclassified	Unclassified	Unclassified	<i>Candidatus Koribacter</i> (33568)
Proteobacteria	Alphaproteobacteria	Caulobacteriales	Caulobacteraceae	<i>Asiccacaulis</i> (6), <i>Brevundimonas</i> (10), <i>Caulobacter</i> (7), <i>Phenylobacterium</i> (206)
			Bartonellaceae	<i>Bartonella</i> (3)
		Rhizobiales	Beijerinckiaceae	<i>Beijerinckia</i> (3), <i>Chelatococcus</i> (3), <i>Methylocapsa</i> (6), <i>Methylocella</i> (5)
			Bradyrhizobiaceae	<i>Afpia</i> (7), <i>Balneimonas</i> (35), <i>Bosea</i> (25), <i>Bradyrhizobium</i> (1657), <i>Nirobacter</i> (59), <i>Rhodoblastus</i> (3), <i>Rhodopseudomonas</i> (139), Unclassified (1826)
			Brucellaceae	<i>Ochrobactrum</i> (18)
			Hyphomicrobiaceae	<i>Blastochloris</i> (183), <i>Devosia</i> (1), <i>Hyphomicrobium</i> (80), <i>Rhodomicrobium</i> (26), <i>Rhodoplanes</i> (4)

			Methylobacteriaceae	<i>Methylobacterium</i> (336)
			Methylocystaceae	<i>Methylocystis</i> (16), <i>Methylophila</i> (1), <i>Methylosinus</i> (18), Unclassified (77)
			Phyllobacteriaceae	<i>Aminobacter</i> (2), <i>Chelatiavorans</i> (19), <i>Mesorhizobium</i> (273), <i>Phyllobacterium</i> (1), <i>Pseudoaminobacter</i> (1)
			Rhizobiaceae	<i>Agrobacterium</i> (2), <i>Candidatus Liberibacter</i> (3), <i>Rhizobium</i> (104), <i>Sinorhizobium</i> (6)
			Rhodobiaceae	<i>Affella</i> (2), <i>Rhodobium</i> (5), <i>Roseospirillum</i> (1)
			Unclassified	Unclassified (61)
			Xanthobacteraceae	<i>Azorhizobium</i> (1), <i>Xanthobacter</i> (3)
		Rhodobacterales	Rhodobacteraceae	<i>Pannonibacter</i> (55), <i>Rhodobacter</i> (1), <i>Rhodothermalassium</i> (5), <i>Rhodovulum</i> (1), Unclassified (2)
			Unclassified	Unclassified (8)
		Rhodospirillales	Acetobacteraceae	<i>Acetobacter</i> (7), <i>Gluconoacetobacter</i> (7), <i>Gluconobacter</i> (5), <i>Granulibacter</i> (78), <i>Kozakia</i> (3), <i>Neoaesa</i> (1), <i>Swaminathania</i> (1)
			Rhodospirillaceae	<i>Azospirillum</i> (51), <i>Dechlorospirillum</i> (1), <i>Magnetospirillum</i> (6), <i>Rhodospirillum</i> (3), <i>Rhodovibrio</i> (3), <i>Roseospira</i> (3), <i>Telmatospirillum</i> (2), <i>Tistrella</i> (3), Unclassified (155)
			Anaplasmataceae	<i>Anaplasma</i> (2), <i>Ehrlichia</i> (1)
			Rickettsiaceae	<i>Orientia</i> (16), <i>Rickettsia</i> (1)
			Unclassified	<i>Candidatus Odysella</i> (4)
		Sphingomonadales	Erythrobacteraceae	<i>Erythrobacter</i> (2), <i>Erythromicrobium</i> (1), <i>Porphyrobacter</i> (2)
			Sphingomonadaceae	<i>Blastomonas</i> (3), <i>Novosphingobium</i> (200), <i>Sphingobium</i> (176), <i>Sphingomonas</i> (559), <i>Sphingopyxis</i> (10), <i>Sphingosinicella</i> (2), <i>Zymomonas</i> (1)
		Unclassified	Unclassified	Unclassified (5870)

Betaproteobacteria	Burkholderiales	Alcaligenaceae	<i>Achromobacter</i> (5), <i>Alcaligenes</i> (8), <i>Azohydromonas</i> (13), <i>Bordetella</i> (2), <i>Derrxia</i> (2), <i>Pelistega</i> (1), <i>Pigmentiphaga</i> (1)
		Burkholderiaceae	<i>Burkholderia</i> (1485), <i>Candidatus Glomeribacter</i> (6), <i>Cupriavidus</i> (156), <i>Pandoraea</i> (3), <i>Paucimonas</i> (3), <i>Polynucleobacter</i> (5), <i>Ralstonia</i> (163)
		Commonadaceae	<i>Acidovorax</i> (25), <i>Brachymonas</i> (7), <i>Comamonas</i> (19), <i>Felomonas</i> (15), <i>Pseudacidovorax</i> (1), <i>Variovorax</i> (126), <i>Verminephrobacter</i> (19), <i>Xenophilus</i> (34), Unclassified (179)
		Oxalobacteraceae	<i>Collimonas</i> (34), <i>Duganella</i> (14), <i>Herbaspirillum</i> (13), <i>Herminimonas</i> (1), <i>Janthinobacterium</i> (32), <i>Massilia</i> (1124), <i>Oxalicibacterium</i> (12), <i>Telluria</i> (87)
		Unclassified	<i>Aquicola</i> (2), <i>Leptothrix</i> (7), <i>Roseateles</i> (6), <i>Rubrivivax</i> (8), <i>Sphaerotilus</i> (3), <i>Thiobacter</i> (3), <i>Thiomonas</i> (4), Unclassified (2332)
		Gallionellales	<i>Sideroxydans</i> (1)
		Hydrogenophilales	<i>Thiobacillus</i> (2)
		Methylophilales	<i>Methylotenera</i> (1)
		Neisseriales	<i>Aquaspirillum</i> (8), <i>Chromobacterium</i> (1), <i>Laribacter</i> (2), <i>Neisseria</i> (3), <i>Stenoxybacter</i> (1)
		Nitrosomonadales	<i>Nitrosomonas</i> (18), <i>Nitrosospira</i> (468), <i>Nitrosovibrio</i> (52), Unclassified (234)
		Unclassified	Unclassified (69)
		Rhodocyclales	<i>Aromatoleum</i> (1), <i>Azonexus</i> (1), <i>Dechloromonas</i> (1), <i>Georgfuchsia</i> (1), <i>Sterolibacterium</i> (16), <i>Thauera</i> (2), Unclassified (183)
		Unclassified	<i>Candidatus Trembalaya</i> (3), Unclassified (6946)
		Deltaproteobacteria	<i>Bacteriovorax</i> (4)
		Bdellovibrionales	<i>Bdellovibrio</i> (6)
Desulfobacterales	<i>Desulfatibacillum</i> (4), <i>Desulfobacter</i> (3), <i>Desulfobacterium</i> (2), <i>Desulfobotulus</i> (45), <i>Desulfocella</i> (4), <i>Desulfococcus</i> (37),		

			<i>Desulfofaba</i> (2), <i>Desulfofrigus</i> (4), <i>Desulfonema</i> (231), <i>Desulfosarcina</i> (1), Unclassified (1)	
	Desulfobulbaceae		<i>Desulfobulbus</i> (7), <i>Desulfohopatus</i> (4), Unclassified (2)	
Desulfovibrionales	Desulfohalobiaceae		<i>Desulfohalobium</i> (1), <i>Desulfonatronovibrio</i> (139)	
	Desulfomicrobiaceae		<i>Desulfomicrobium</i> (3)	
	Desulfonatronumaceae		<i>Desulfonatronum</i> (32)	
	Desulfovibrionaceae		<i>Desulfovibrio</i> (3646)	
	Unclassified		Unclassified (20)	
Desulfurellales	Desulfurellaceae		<i>Desulfurella</i> (2)	
Desulfuromonadales	Desulfuromonadaceae		<i>Desulfuromonas</i> (3), <i>Desulfuromusa</i> (1)	
	Geobacteraceae		<i>Geoalkalibacter</i> (178), <i>Geobacter</i> (98)	
Myxococcales	Pelobacteraceae		<i>Malonomonas</i> (34), <i>Pelobacter</i> (19)	
	Cystobacteraceae		<i>Cystobacter</i> (280), <i>Melittangium</i> (51), <i>Stigmatella</i> (46)	
	Haliangiaceae		<i>Haliangium</i> (5)	
	Myxococcaceae		<i>Anaeomyxobacter</i> (187), <i>Corallocooccus</i> (275), <i>Myxococcus</i> (458)	
	Polyangiaceae		<i>Chondromyces</i> (14), <i>Sorangium</i> (39)	
	Syntrophobacteraceae		<i>Desulfohabdus</i> (2), <i>Desulfovirga</i> (3), <i>Syntrophobacter</i> (2), <i>Thermodesulfohabdus</i> (1)	
Unclassified	Unclassified		<i>Desulfocaldus</i> (1), Unclassified (15925)	
Epsilonproteobacteria	Campylobacteraceae		<i>Arcobacter</i> (1), <i>Campylobacter</i> (57), Unclassified (1)	
	Nautiliaceae		<i>Caminibacter</i> (10)	
Gammaproteobacteria	Unclassified		<i>Nitratiruptora</i> (49), Unclassified (89)	
	Acidithiobacillales	Acidithiobacillaceae	<i>Acidithiobacillus</i> (43)	
	Aeromonadales	Aeromonadaceae	<i>Oceanimonas</i> (1)	
	Alteromonadales	Succinivibrionaceae		<i>Ruminobacter</i> (3), <i>Succinimonas</i> (2)
		Alteromonadaceae		<i>Marinobacterium</i> (1)
		Idiomarinaceae		<i>Idiomarina</i> (1)
	Shewanellaceae		<i>Shewanella</i> (36)	

		Chromatiales	Chromatiaceae	<p><i>Halochromatium</i> (1), <i>Lamprocystis</i> (1), <i>Marichromatium</i> (1), <i>Nitrosococcus</i> (2), <i>Thiocapsa</i> (6), <i>Thiorhodovibrio</i> (37)</p> <p><i>Alkalispirillum</i> (2), <i>Ectothiorhodosinus</i> (1), <i>Ectothiorhodospira</i> (5), <i>Halorhodospira</i> (1), <i>Nitrococcus</i> (2), <i>Thioalkalivibrio</i> (1), <i>Thiohalospira</i> (8)</p> <p><i>Halothiobacillus</i> (1)</p> <p><i>Buchnera</i> (7), <i>Candidatus Hamiltonella</i> (39), <i>Citrobacter</i> (5), <i>Enterobacter</i> (2), <i>Erwinia</i> (1), <i>Escherichia</i> (5), <i>Klebsiella</i> (8), <i>Pantoea</i> (5), <i>Pectobacterium</i> (1), <i>Photorhabdus</i> (1), <i>Raouliella</i> (1), <i>Serratia</i> (7), <i>Yersinia</i> (1), Unclassified (1457)</p> <p><i>Coxiella</i> (8)</p> <p><i>Legionella</i> (18)</p> <p><i>Methylabacter</i> (4), <i>Methylocaldum</i> (1), <i>Methylococcus</i> (2), <i>Methylohalobius</i> (3), <i>Methylomicrobium</i> (1), <i>Methylothermus</i> (7)</p> <p><i>Halomonas</i> (184), <i>Zymobacter</i> (1)</p> <p><i>Haemophilus</i> (1)</p> <p><i>Acinetobacter</i> (9), <i>Moraxella</i> (4)</p> <p><i>Pseudomonas</i> (30)</p> <p><i>Dyella</i> (7), <i>Ignatzschineria</i> (1), <i>Lysobacter</i> (22), <i>Sienotrophomonas</i> (11), <i>Wohlfahrtiimonas</i> (3), <i>Xanthomonas</i> (55), <i>Xylella</i> (10)</p> <p><i>Candidatus Carsonella</i> (1), <i>Methylohalomonas</i> (134), <i>Methylostratum</i> (7), <i>Thiohalomonas</i> (1), <i>Thiohalorhabdus</i> (1), Unclassified (2156)</p> <p>Unclassified (1350)</p>
		Enterobacteriales	<p>Halothiobacillaceae</p> <p>Enterobacteriaceae</p>	
		Legionellales	<p>Coxiellaceae</p> <p>Legionellaceae</p>	
		Methylococcales	Methylococcaceae	
		Oceanospirillales	Halomonadaceae	
		Pasteurellales	Pasteurellaceae	
		Pseudomonadales	<p>Moraxellaceae</p> <p>Pseudomonadaceae</p>	
		Xanthomonadales	Xanthomonadaceae	
		Unclassified	Unclassified	
	Unclassified	Unclassified	Unclassified	
	Bacilli	Bacillales	<p>Alicyclobacillaceae</p> <p>Bacillaceae</p>	
Firmicutes			<p><i>Alicyclobacillus</i> (1052), Unclassified (1)</p> <p><i>Amphibacillus</i> (12), <i>Anaerobacillus</i> (6), <i>Anoxybacillus</i> (637), <i>Bacillus</i> (31495), <i>Geobacillus</i> (263), <i>Halobacillus</i> (147), <i>Lysinibacillus</i> (939), <i>Marinococcus</i> (16), <i>Natronobacillus</i> (16), <i>Oceanobacillus</i> (288),</p>	



		<i>Ornithinibacillus</i> (17), <i>Terribacillus</i> (11), <i>Virgibacillus</i> (184)
	Listeriaceae	<i>Listeria</i> (15)
	Paenibacillaceae	<i>Aneurinibacillus</i> (199), <i>Brevibacillus</i> (360), <i>Cohnella</i> (78), <i>Paenibacillus</i> (2003), <i>Thermobacillus</i> (9)
	Pasteuriaceae	<i>Pasteuria</i> (3)
	Planococcaceae	<i>Jeotgalibacillus</i> (4), <i>Kurthia</i> (89), <i>Planomicrobium</i> (150), <i>Sporosarcina</i> (272), <i>Ureibacillus</i> (41), <i>Viridibacillus</i> (5)
	Sporolactobacillaceae	<i>Sporolactobacillus</i> (28)
	Staphylococcaceae	<i>Staphylococcus</i> (60)
	Thermoactinomycetaceae	<i>Thermoactinomyces</i> (71), <i>Thermo flavimicrobium</i> (2)
	Unclassified	<i>Alkalitactibacillus</i> (7), <i>Exiguobacterium</i> (35), <i>Gemella</i> (9), <i>Pullulanibacillus</i> (5)
	Aerococcaceae	<i>Abiotrophia</i> (9)
	Carnobacteriaceae	<i>Alkalibacterium</i> (2), <i>Carnobacterium</i> (2), <i>Granulicatella</i> (16), <i>Trichococcus</i> (14)
	Enterococcaceae	<i>Enterococcus</i> (22), <i>Melissococcus</i> (1), <i>Tetragenococcus</i> (4)
	Lactobacillaceae	<i>Lactobacillus</i> (135)
	Leuconostocaceae	<i>Oenococcus</i> (1), <i>Weisella</i> (1)
	Streptococcaceae	<i>Streptococcus</i> (17)
	Clostridiaceae	<i>Alkaliphilus</i> (62), <i>Butyricoccus</i> (9), <i>Caloramator</i> (12), <i>Clostridium</i> (3154), <i>Oxobacter</i> (13), <i>Tepidimicrobium</i> (6)
	Clostridiales Family XI. Incertae	<i>Sedis Anaerococcus</i> (3), <i>Sedis Helcococcus</i> (23), <i>Sedis Peptoniphilus</i> (791), <i>Sedis Sedimentibacter</i> (5), <i>Sedis Sporaerobacter</i> (1660), <i>Sedis Tissierella</i> (1755), <i>Sedis unclassified</i> (1)
	Clostridiales Family XII. Incertae	<i>Sedis Fusibacter</i> (1)
	Clostridiales Family XIV. Incertae	<i>Sedis Anaerobranca</i> (6)
Clostridia	Clostridiales	







	Unclassified	Unclassified	Unclassified	<i>Candidatus Amoebophilus</i> (236), <i>Candidatus Cardinium</i> (3), <i>Protixibacter</i> (2), Unclassified (41)
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	<i>Leptospirillum</i> (7), <i>Nitrospira</i> (3177), <i>Thermodesulfobivrio</i> (7)
Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexaceae	<i>Chloroflexus</i> (502), <i>Roseiflexus</i> (22)
			Oscillochloridaceae	<i>Oscillochloris</i> (22)
	Dehalococcoidetes	Herpetosiphonales	Herpetosiphonaceae	<i>Herpetosiphon</i> (635)
	Ktedonobacteria	Unclassified	Unclassified	<i>Dehalococcoides</i> (8)
		Ktedonobacterales	Ktedonobacteraceae	<i>Ktedonobacter</i> (790)
	Thermomicrobia	Unclassified	Unclassified	Unclassified (692)
		Sphaerobacterales	Sphaerobacteraceae	<i>Sphaerobacter</i> (51)
Gemmatimonadetes	Gemmatimonadetes	Thermomicrobiales	Thermomicrobiaceae	<i>Thermomicrobium</i> (2)
Planctomycetes	Planctomycetacia	Planctomycetales	Gemmatimonadaceae	<i>Gemmatimonas</i> (2715)
			Planctomycetaceae	<i>Blastopirellula</i> (131), <i>Isosphaera</i> (1643), <i>Pirellula</i> (196), <i>Planctomyces</i> (148), Unclassified (177)
			Unclassified	<i>Candidatus Kuenenia</i> (14)

#### 4.24 Bacterial diversity in the sample CH-5

The bacterial diversity in the sample CH-5 was found to be majorly composed of phylum Proteobacteria occupying 23 per cent of the bacterial population followed by Bacteroidetes (14%), Actinobacteria (12%), Firmicutes (10%) and Acidobacteria (8%), with other phyla occupying only a small fraction of the population. The phylum Proteobacteria was found to harbor more of the class Gammaproteobacteria. The most abundant known genus in the phylum Proteobacteria was observed to be *Rhodanobacter* occupying 17 per cent of the phylum. Other notable genera include *Pseudomonas* (7%), *Burkholderia* (6%), *Nitrosospira* (3%), *Bradyrhizobium* (1%) and *Geobacter* (1%). The second abundant phylum Bacteroidetes was found to be dominant in genus *Pedobacter* (18%) followed by 17 per cent of *Sphingobacterium*, 15 per cent *Flavobacterium* and 14 per cent *Terrimonas*. The phylum had notable populations of genera *Chitinophaga* (8%), *Cytophaga* (2%), *Alistipes* (2%) etc.

The phylum Actinobacteria was the third largest phylum and it was composed of 22 per cent of the genus *Arthrobacter*, 7 per cent of *Nocardioides* and 7 per cent *Thermoleophilum*. The phylum also had an array of other prominent bacterial genera like *Atopobium*, *Pseudonocardia*, *Mycobacterium*, *Frankia*, *Streptomyces*, *Williamsia*, *Corynebacterium* etc in notable proportion. The phylum Firmicutes was dominated by the genus *Bacillus* occupying 60 per cent of the population, with other genera like *Ruminococcus*, *Clostridium* and *Paenibacillus* occupying 8, 5 and 6 per cent of the population in the phylum Firmicutes. The phylum Acidobacteria was found to be less diverse with the majority of 58 per cent *Acidobacterium*, followed by 40 per cent of *Candodatus Koribacter* and 1 per cent *Terriglobus*. The genus-level bacterial diversity of the 10 most abundant phyla from the sample CH-5 has been provided in Table 29.

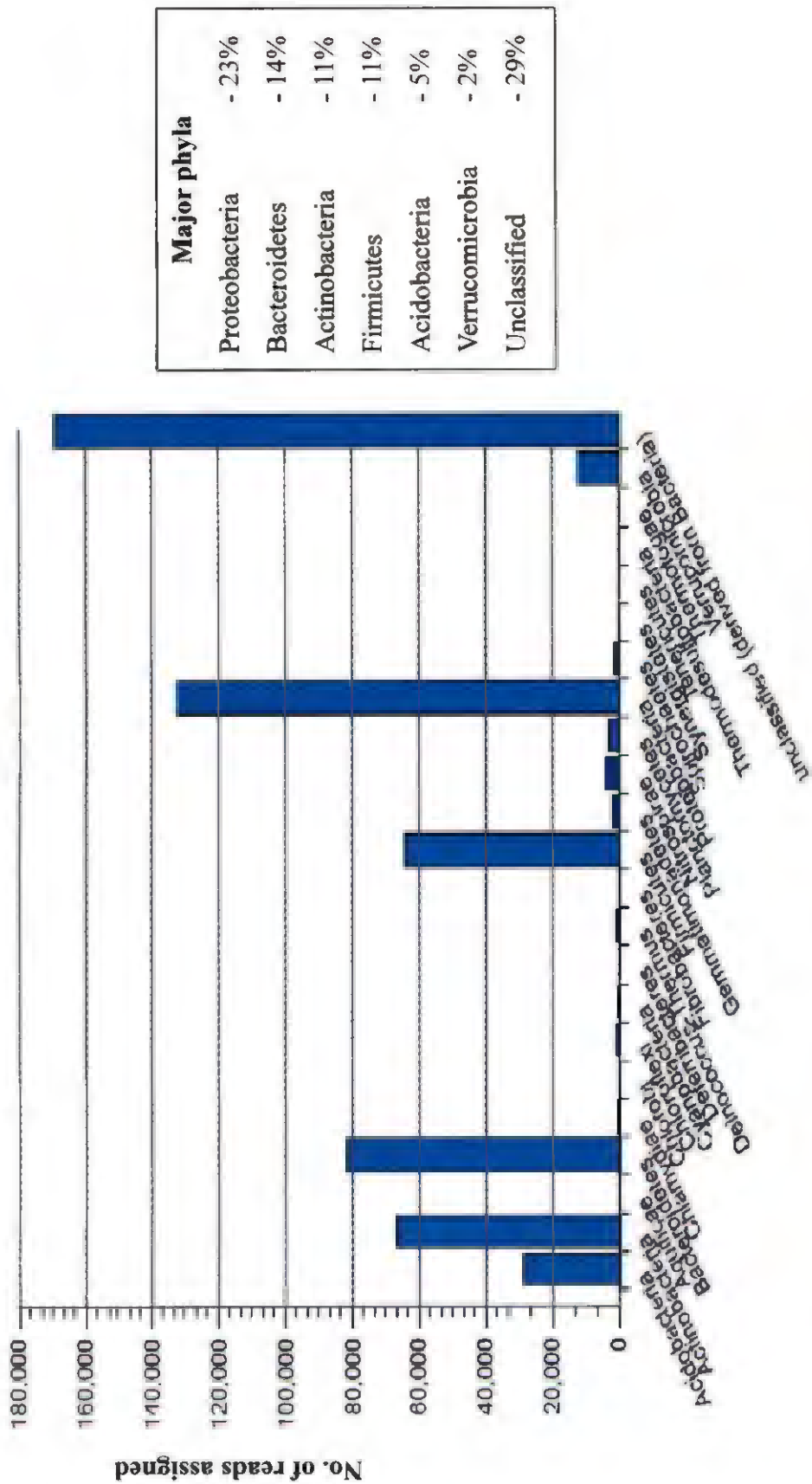


Plate 16. Phylum-level bacterial diversity in the sample CH-5 obtained using MG-RAST pipeline

**Table 29. Genus-level taxonomic assemblage of bacterial diversity from 10 predominant phyla in the sample CH-5**

Phylum	Class	Order	Family	Genus
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Asiccacaulis</i> (148), <i>Brevundimonas</i> (37), <i>Caulobacter</i> (23), <i>Phenyllobacterium</i> (223)
		Rhizobiales	Bartonellaceae	<i>Bartonella</i> (18)
			Beijerinckiaceae	<i>Beijerinckia</i> (5), <i>Chelatococcus</i> (1), <i>Methylocapsa</i> (8), <i>Methylocella</i> (7)
			Bradyrhizobiaceae	<i>Afipia</i> (72), <i>Baineiomonas</i> (72), <i>Blastobacter</i> (1), <i>Bosea</i> (46), <i>Bradyrhizobium</i> (1975), <i>Nitrobacter</i> (126), <i>Rhodoblastus</i> (3), <i>Rhodopseudomonas</i> (213), <i>Unclassified</i> (52)
			Brucellaceae	<i>Mycoplana</i> (1), <i>Ochroactrum</i> (38)
			Hypomicrobiaceae	<i>Blastochloris</i> (289), <i>Hyphomicrobium</i> (148), <i>Rhodomicrobium</i> (6), <i>Rhodoplanes</i> (16)
			Methylobacteriaceae	<i>Methylobacterium</i> (566)
			Methylocystaceae	<i>Methylocystis</i> (16), <i>Methylolpila</i> (1), <i>Methylosinus</i> (5), <i>Unclassified</i> (236)
			Phyllobacteriaceae	<i>Aminobacter</i> (21), <i>Chelativorans</i> (18), <i>Hoeflea</i> (1), <i>Mesorhizobium</i> (628), <i>Parvibaculum</i> (2), <i>Phyllobacterium</i> (16)
			Rhizobiaceae	<i>Agrobacterium</i> (14), <i>Candidatus Liberibacter</i> (1), <i>Ensifer</i> (3), <i>Rhizobium</i> (1082), <i>Sinorhizobium</i> (29)
			Rhodobiaceae	<i>Afifella</i> (5), <i>Rhodobium</i> (3)
			Unclassified	<i>Unclassified</i> (165)
			Xanthobacteraceae	<i>Azorhizobium</i> (2), <i>Xanthobacter</i> (1)
		Rhodobacterales	Rhodobacteraceae	<i>Paracoccus</i> (2), <i>Rhodobacter</i> (2), <i>Rhodotalassium</i> (1), <i>Rhodovulum</i> (7), <i>Ruegeria</i> (1), <i>Thioclava</i> (3), <i>Unclassified</i> (11)
			Unclassified	<i>Unclassified</i> (4)
		Rhodospirillales	Acetobacteraceae	<i>Acetobacter</i> (16), <i>Acidiphilum</i> (6), <i>Asaia</i> (6), <i>Gluconacetobacter</i> (38), <i>Gluconobacter</i> (23), <i>Granulibacter</i> (114), <i>Kozakia</i> (24), <i>Neosaisa</i> (6), <i>Roseococcus</i> (2), <i>Rubritepida</i> (13)



			Rhodospirillaceae	<i>Azospirillum</i> (43), <i>Magnetospirillum</i> (6), <i>Rhodocista</i> (1), <i>Rhodospirillum</i> (5), <i>Rhodovibrio</i> (4), Unclassified (203)
			Anaplasmataceae	<i>Anaplasma</i> (1), <i>Ehrlichia</i> (4)
			Holosporaceae	Unclassified (4)
			Rickettsiaceae	<i>Orientia</i> (36), <i>Rickettsia</i> (9), Unclassified (1)
			Unclassified	<i>Caedibacter</i> (1), <i>Candidatus Midichloria</i> (1), <i>Candidatus Nicolleia</i> (1), <i>Candidatus Odysseella</i> (8)
		Sphingomonadales	Erythrobacteraceae	<i>Erythrobacter</i> (6), <i>Erythromicrobium</i> (69), <i>Porphyrobacter</i> (26)
			Sphingomonadaceae	<i>Blastomonas</i> (11), <i>Novosphingobium</i> (21), <i>Sphingobium</i> (488), <i>Sphingomonas</i> (861), <i>Sphingopyxis</i> (93), <i>Sphingosinicella</i> (4), <i>Zymomonas</i> (6)
		Unclassified	Unclassified	Unclassified (9970)
		Burkholderiales	Alcaligenaceae	<i>Achromobacter</i> (38), <i>Alcaligenes</i> (16), <i>Azohydromonas</i> (29), <i>Bordetella</i> (9), <i>Oligella</i> (3), <i>Pelistega</i> (1), <i>Pigmentiphaga</i> (1), <i>Taylorella</i> (1), Unclassified (4)
			Burkholderiaceae	<i>Burkholderia</i> (8542), <i>Candidatus Glomeribacter</i> (10), <i>Cupriavidus</i> (681), <i>Fandoraea</i> (37), <i>Paucimonas</i> (4), <i>Polynucleobacter</i> (4), <i>Ralstonia</i> (1012), Unclassified (14)
			Comamonadaceae	<i>Acidovorax</i> (164), <i>Brachymonas</i> (9), <i>Caldimonas</i> (1), <i>Comamonas</i> (125), <i>Delftia</i> (7), <i>Pelomonas</i> (36), <i>Simplicispira</i> (1), <i>Variovorax</i> (553), <i>Verminephrobacter</i> (32), <i>Xenophilus</i> (47), Unclassified (447)
			Oxalobacteraceae	<i>Collimonas</i> (11), <i>Duganella</i> (20), <i>Herbaspirillum</i> (242), <i>Janthinobacterium</i> (34), <i>Massilia</i> (129), <i>Oxalibacterium</i> (137), <i>Oxalobacter</i> (3), <i>Telluria</i> (1)
		Unclassified	Unclassified	<i>Aquinola</i> (7), <i>Leptothrix</i> (79), <i>Mitsuaria</i> (27), <i>Roseateles</i> (3), <i>Rubrivivax</i> (28), <i>Sphaerotilus</i> (38), <i>Thiobacter</i> (10), <i>Thiomonas</i> (8), Unclassified (2443)
		Betaproteobacteria		





			Myxococcaceae	<i>Anaeromyxomonas</i> (51), <i>Corallocooccus</i> (138), <i>Myxococcus</i> (237)
			Nannocystaceae	<i>Nannocystis</i> (3)
			Polyangiaceae	<i>Chondromyces</i> (17), <i>Sorangium</i> (26)
	Syntrophobacterales		Syntrophobacteraceae	<i>Desulforhabdus</i> (2), <i>Desulfovirga</i> (19), <i>Syntrophobacter</i> (3), <i>Thermodesulforhabdus</i> (1)
	Unclassified		Unclassified	<i>Spirobacillus</i> (3), Unclassified (4964)
	Campylobacterales		Campylobacteraceae	<i>Arcobacter</i> (67), <i>Campylobacter</i> (93), <i>Sulfurospirillum</i> (1), Unclassified (4)
			Helicobacteraceae	<i>Helicobacter</i> (18)
			Nautiliaceae	<i>Caminibacter</i> (12), <i>Nautilia</i> (1)
	Unclassified		Unclassified	<i>Nitratiruptor</i> (1), Unclassified (68)
	Acidithiobacillales		Acidithiobacillaceae	<i>Acidithiobacillus</i> (1)
	Aeromonadales		Aeromonadaceae	<i>Aeromonas</i> (3), <i>Oceanimonas</i> (2)
			Succinivibrionaceae	<i>Ruminobacter</i> (1), <i>Succinomonas</i> (1)
	Alteromonadales		Alteromonadaceae	<i>Marinobacterium</i> (2)
			Shewanellaceae	<i>Shewanella</i> (48)
	Chromatiales		Chromatiaceae	<i>Chromatium</i> (1), <i>Halochromatium</i> (1), <i>Lamprocystis</i> (1), <i>Marichromatium</i> (1), <i>Nitrosococcus</i> (13), <i>Thiocapsa</i> (10), <i>Thiorhodovibrio</i> (5)
			Ectothiorhodospiraceae	<i>Alkalispirillum</i> (2), <i>Ectothiorhodosinus</i> (1), <i>Ectothiorhodospira</i> (5), <i>Halorhodospira</i> (4), <i>Thioalkalivibrio</i> (11), <i>Thiohalospira</i> (1), Unclassified (3)
			Halothiobacillaceae	<i>Halothiobacillus</i> (18)
	Enterobacteriales		Enterobacteriaceae	<i>Buchnera</i> (4), <i>Buttiauxella</i> (6), <i>Candidatus</i> <i>Hamiltonella</i> (6), <i>Citrobacter</i> (18), <i>Cronobacter</i> (10), <i>Edwardsiella</i> (24), <i>Enterobacter</i> (51), <i>Erwinia</i> (6), <i>Escherichia</i> (7), <i>Klebsiella</i> (104), <i>Kluverera</i> (3), <i>Leclercia</i> (1), <i>Morganella</i> (2), <i>Pantoea</i> (75), <i>Pectobacterium</i> (3), <i>Photorhabdus</i> (9), <i>Providencia</i> (1), <i>Raoultella</i> (1), <i>Salmonella</i> (3), <i>Serratia</i> (11), <i>Trabulsiella</i> (1), <i>Wigglesworthia</i> (1), <i>Xenorhabdus</i> (1), <i>Yersinia</i> (2), Unclassified (374)
	Legionellales		Coxiellaceae	<i>Coxiella</i> (11), <i>Rickettsiella</i> (3)



			Cytophagaceae		<i>Cytophaga</i> (1380), <i>Dyadobacter</i> (40), <i>Flectobacillus</i> (6), <i>Flexibacter</i> (465), <i>Hymenobacter</i> (696), <i>Marinoscillum</i> (3), <i>Microscilla</i> (2), <i>Spirosoma</i> (43), <i>Sporocytophaga</i> (12)
			Flammeovirgaceae		<i>Flammeovirga</i> (2), <i>Flexithrix</i> (98), <i>Persicobacter</i> (2)
			Flavobacteriaceae	Flavobacteriales	<i>Arenibacter</i> (58), <i>Cellulophaga</i> (22), <i>Chryseobacterium</i> (11), <i>Coenonia</i> (4), <i>Dokdonia</i> (17), <i>Elizabethkingia</i> (343), <i>Empedobacter</i> (12), <i>Flavobacterium</i> (12023), <i>Gaetbulibacter</i> (1), <i>Gramella</i> (24), <i>Leeuwenhoekella</i> (9), <i>Myroides</i> (5), <i>Polaribacter</i> (1), <i>Riemerella</i> (60), <i>Robiginitalea</i> (183), <i>Salegentibacter</i> (1), <i>Tenacibaculum</i> (71), <i>Zunongwangia</i> (10)
			Unclassified	Unclassified	Unclassified (11)
			Rhodothermaceae	Sphingobacteriales	<i>Rhodothermus</i> (23), <i>Salinibacter</i> (19)
			Saprosiraceae		<i>Saprosira</i> (8)
			Sphingobacteriaceae		<i>Pedobacter</i> (15061), <i>Sphingobacterium</i> (13919), Unclassified (13783)
			Unclassified		<i>Chitinophaga</i> (6317), <i>Terrimonas</i> (11765)
			Unclassified		<i>Candidatus Amoebophilus</i> (2173), <i>Candidatus Cardinium</i> (7), <i>Prolixibacter</i> (14), Unclassified (454)
			Acidimicrobiaceae	Acidimicrobiales	<i>Acidimicrobium</i> (103), <i>Acidithiomicrobium</i> (218)
			Acidothermaceae	Actinomycetales	<i>Acidothermus</i> (190)
			Actinomycetaceae		<i>Actinobaculum</i> (43), <i>Actinomyces</i> (21), <i>Arcanobacterium</i> (12), <i>Mobiluncus</i> (268)
			Actinosynnemataceae		<i>Actinoikineospora</i> (11), <i>Actinosynnema</i> (2), <i>Lechevalieria</i> (6), <i>Lentzea</i> (17), <i>Saccharothrix</i> (1)
			Beutenbergiaceae		<i>Beutenbergia</i> (265)
			Brevibacteriaceae		<i>Brevibacterium</i> (468)
			Catenulisporaceae		<i>Catenulispora</i> (44)
			Cellulomonadaceae		<i>Actinotalea</i> (8), <i>Cellulomonas</i> (150), <i>Oerskovia</i> (1)
			Corynebacteriaceae		<i>Corynebacterium</i> (1644)
			Dermabacteraceae		<i>Brachybacterium</i> (40)
			Dermacoccaceae		<i>Dermacoccus</i> (5), <i>Kytococcus</i> (35)
Actinobacteria	Actinobacteria	Actinobacteria	Actinobacteria	Actinobacteria	
			Unclassified	Unclassified	
			Sphingobacteria	Sphingobacteriales	
			Flavobacteria	Flavobacteriales	
			Flavobacteria	Flavobacteriales	

Dermatophilaceae	<i>Dermatophilus</i> (57)
Dietziaceae	<i>Dietzia</i> (21)
Frankiaceae	<i>Frankia</i> (2638)
Geodermatophilaceae	<i>Geodermatophilus</i> (260)
Glycomycetaceae	<i>Glycomyces</i> (3), <i>Stackebrandtia</i> (6)
Gordoniaceae	<i>Gordonia</i> (507), <i>Skermania</i> (1)
Intrasporangiaceae	<i>Intrasporangium</i> (323), <i>Serinococcus</i> (42), <i>Terrabacter</i> (651), <i>Tetrasphaera</i> (307)
Jonesiaceae	<i>Jonesia</i> (1)
Kineosporiaceae	<i>Kineococcus</i> (37)
Microbacteriaceae	<i>Agreia</i> (18), <i>Agrococcus</i> (88), <i>Agromyces</i> (137), <i>Candidatus Aquiluna</i> (8), <i>Candidatus Rhodoluna</i> (2), <i>Clavibacter</i> (660), <i>Cryobacterium</i> (115), <i>Curtobacterium</i> (665), <i>Glaciibacter</i> (48), <i>Leifsonia</i> (1598), <i>Leucobacter</i> (28), <i>Microbacterium</i> (3444), <i>Mycetocola</i> (10), <i>Okibacterium</i> (11), <i>Plantibacter</i> (2), <i>Pseudoclavibacter</i> (42), <i>Rathayibacter</i> (30), <i>Salinibacterium</i> (391), <i>Subtercola</i> (488), Unclassified (1)
Micrococcaceae	<i>Arthrobacter</i> (14801), <i>Kocuria</i> (30), <i>Micrococcus</i> (120), <i>Nesterenkonia</i> (15), <i>Renibacterium</i> (143), <i>Rothia</i> (181)
Micromonosporaceae	<i>Actinoplanes</i> (377), <i>Catenuloplanes</i> (53), <i>Couchioplanes</i> (3), <i>Dactylosporangium</i> (142), <i>Micromonospora</i> (1415), <i>Polymorphospora</i> (1), <i>Salinispora</i> (75), <i>Verrucosipora</i> (239)
Mycobacteriaceae	<i>Mycobacterium</i> (3390)
Nakamurellaceae	<i>Nakamurella</i> (238)
Nocardiaceae	<i>Nocardia</i> (191), <i>Rhodococcus</i> (1382), <i>Smaragdicoscus</i> (61)
Nocardioideaceae	<i>Aeromicrobium</i> (27), <i>Kribbella</i> (1950), <i>Nocardioides</i> (4690), <i>Pimelobacter</i> (162), <i>Nocardiosis</i> (243), <i>Streptomonospora</i> (5), <i>Thermobifida</i> (5)
Promicromonosporaceae	<i>Cellulosimicrobium</i> (59), <i>Isoptricola</i> (2), <i>Promicromonospora</i> (23)

			Propionibacteriaceae	<i>Microtunatus</i> (64), <i>Propionibacterium</i> (25), Unclassified (161)
			Pseudonocardiaceae	<i>Amycolatopsis</i> (471), <i>Kutzneria</i> (16), <i>Prauserella</i> (36), <i>Pseudomonocardia</i> (3121), <i>Saccharomonospora</i> (37), <i>Saccharopolyspora</i> (1038), <i>Streptoalloeichus</i> (10), <i>Thermobispora</i> (19), <i>Thermocrispum</i> (3)
			Rarobacteraceae	<i>Rarobacter</i> (67)
			Sanguibacteraceae	<i>Sanguibacter</i> (15)
			Segniliparaceae	<i>Segniliparus</i> (15)
			Streptomycetaceae	<i>Kitatospora</i> (86), <i>Streptacidiphilus</i> (7), <i>Streptomyces</i> (2077)
			Streptosporangiaceae	<i>Microbispora</i> (22), <i>Microtetrastroma</i> (1), <i>Nonomuraea</i> (37), <i>Planomonospora</i> (10), <i>Streptosporangium</i> (384)
			Thermomonosporaceae	<i>Actinoallomurus</i> (336), <i>Actinocorallia</i> (54), <i>Actinomadura</i> (399), <i>Spirillospora</i> (1), <i>Thermomonospora</i> (366), Unclassified (244)
			Tsukamurellaceae	<i>Tsukamurella</i> (19)
			Unclassified	<i>Tomitella</i> (5), <i>Tropheryma</i> (13), Unclassified (1)
			Williamsiaceae	<i>Williamsia</i> (2061)
		Bifidobacteriales	Bifidobacteriaceae	<i>Aeriscardovia</i> (2), <i>Alloiscardovia</i> (1), <i>Bifidobacterium</i> (92), <i>Gardnerella</i> (25)
			Unclassified	<i>Turicella</i> (4)
		Coriobacteriales	Coriobacteriaceae	<i>Atopobium</i> (2464), <i>Collinsella</i> (17), <i>Cryptobacterium</i> (1), <i>Eggerthella</i> (22), <i>Enterorhabdus</i> (22), <i>Gordonibacter</i> (250), <i>Slackia</i> (43)
			Rubrobacteraceae	<i>Rubrobacter</i> (397)
			Thermoleophilaceae	<i>Thermoleophilum</i> (4643)
			Unclassified	Unclassified (59)
		Bacillales	Alicyclobacillaceae	<i>Alicyclobacillus</i> (860)
			Bacillaceae	<i>Amphibacillus</i> (185), <i>Anaerobacillus</i> (112), <i>Anoxybacillus</i> (516), <i>Bacillus</i> (38721), <i>Geobacillus</i> (281), <i>Halobacillus</i> (33), <i>Lysinibacillus</i> (1528), <i>Marinococcus</i> (2), <i>Natronobacillus</i> (4)
Firmicutes		Bacilli		







			Eubacteriaceae	<i>Acetobacterium</i> (4), <i>Eubacterium</i> (24)
			Heliobacteriaceae	<i>Heliobacillus</i> (9), <i>Heliobacterium</i> (39), <i>Heliophilum</i> (24), <i>Heliorestis</i> (3)
			Lachnospiraceae	<i>Anaerostipes</i> (4), <i>Butyrivibrio</i> (4), <i>Cellulosilyticum</i> (24), <i>Hespellia</i> (6), <i>Lachnospira</i> (28), <i>Robinsoniella</i> (1), <i>Roseburia</i> (4), Unclassified (44)
			Peptococcaceae	<i>Candidatus Desulforudis</i> (6), <i>Dehalobacter</i> (9), <i>Desulfotobacterium</i> (127), <i>Desulfonispota</i> (267), <i>Desulfosporosinus</i> (39), <i>Desulfotomaculum</i> (516), <i>Pelotomaculum</i> (5), <i>Thermincola</i> (3), Unclassified (11)
			Peptostreptococcaceae	<i>Peptostreptococcus</i> (23)
			Ruminococcaceae	<i>Acetivibrio</i> (95), <i>Ethanoligenens</i> (4), <i>Faecalibacterium</i> (27), <i>Ruminococcus</i> (5004), Unclassified (42)
			Syntrophomonadaceae	<i>Syntrophomonas</i> (1), <i>Syntrophothermus</i> (1)
			Unclassified	<i>Blautia</i> (5), <i>Eputopiscium</i> (7), Unclassified (92)
		Thermoanaerobacterales	Thermoanaerobacteraceae	<i>Caldanaerobacter</i> (2), <i>Caldanaerobius</i> (174), <i>Moorella</i> (30), <i>Thermoanaerobacter</i> (13)
			Thermoanaerobacterales Family III. Incertae	<i>SedisCaldicellulosiruptor</i> (88)
			Thermodesulfobiaceae	<i>Thermodesulfobium</i> (3)
			Unclassified	Unclassified (1)
		Erysipelotrichales	Erysipelotrichaceae	<i>Erysipelotrix</i> (1), Unclassified (5)
		Selenomonadales	Acidaminococcaceae	<i>Phascotartarobacterium</i> (6)
			Veillonellaceae	<i>Dialister</i> (6), <i>Megamonas</i> (3), <i>Megasphaera</i> (36), <i>Mitsuokella</i> (1), <i>Pectinatus</i> (2), <i>Selenomonas</i> (59), <i>Sporomusa</i> (88), <i>Veillonella</i> (20)
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Acidobacterium</i> (16740), <i>Terriglobus</i> (353)
	Solibacteres	Solibacterales	Solibacteraceae	<i>CandidatusSolibacter</i> (11570)
Verrucomicrobia	Opitutae	Puniceococcales	Puniceococcaceae	<i>Coralimargarita</i> (2)
		Unclassified	Opitutaceae	Unclassified (50)
	Spartobacteria	Unclassified	Unclassified	<i>Chthoniobacter</i> (4805)
	Unclassified	Methylacidiphilales	Methylacidiphilaceae	<i>Methylacidiphilum</i> (22)

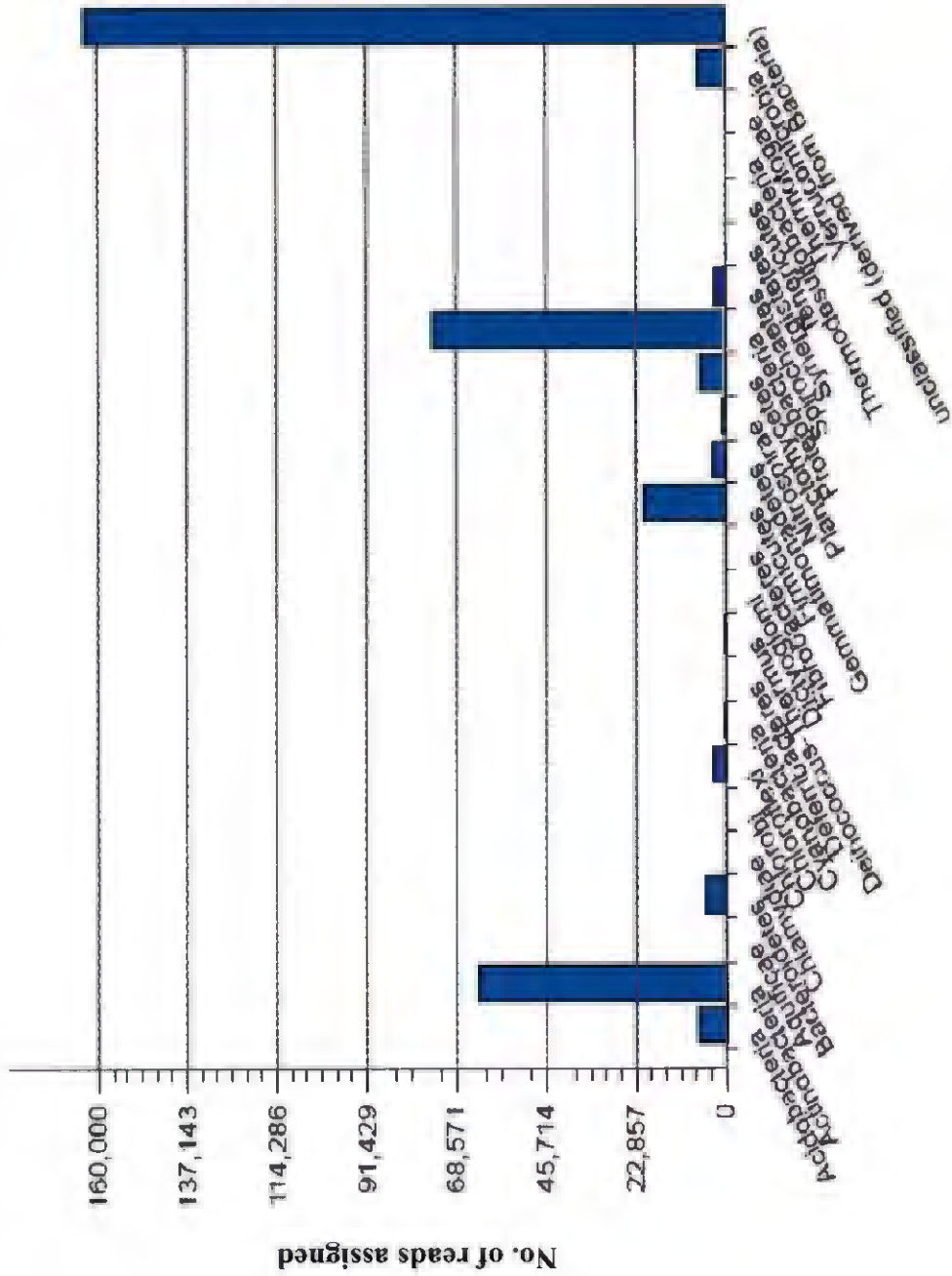
	Unclassified	Unclassified	Unclassified (70)
	Verrucomicrobiae	Verrucomicrobiales	Unclassified (3)
			Unclassified (3950)
			<i>Akkermansia</i> (5), <i>Prostheco bacter</i> (2322), <i>Rubritalea</i> (76), Unclassified (162), <i>Verrucomicrobium</i> (5)
Nitrospirae	Nitrospira	Nitrospirales	<i>Leptospirillum</i> (3), <i>Nitrospira</i> (4030), <i>Thermodesulfobivrio</i> (2)
Planctomycetes	Planctomycetacia	Planctomycetales	<i>Blastopirellula</i> (151), <i>Isosphaera</i> (1114), <i>Pirellula</i> (775), <i>Planctomyces</i> (466), <i>Rhodopirellula</i> (5), Unclassified (205)
Gemmatimonadetes	Gemmatimonadetes	Unclassified	<i>Candidatus Kuenenia</i> (1)
Spirochaetes	Spirochaetes	Gemmatimonadales	<i>Gemmatimonas</i> (1884)
		Spirochaetales	<i>Brachyospira</i> (182)
			<i>Leptonema</i> (1), <i>Leptospira</i> (1374)
			<i>Spirochaeta</i> (37), <i>Treponema</i> (1)

#### 4.25 Bacterial diversity in the sample CH-7

The bacterial diversity in the completely healthy rhizosphere soil sample CH-7 was analysed and the dominant phylum was observed to be Proteobacteria being 21 per cent of total bacterial population. The second most abundant phylum was found to be Actinobacteria (18%), followed by Firmicutes (6%) and other phyla like Acidobacteria, Verrucomicrobia and Planctomycetes each constituting 2 per cent of the bacterial population and the phylum Bacteroidetes being 1 per cent of bacterial population.

The phylum Proteobacteria was dominated by class Deltaproteobacteria (39% of Proteobacteria) followed by Gammaproteobacteria. The majority of the phylum remained unclassified, while some genera like *Pseudomonas*, *Burkholderia*, *Ralstonia*, *Desulfovibrio*, *Myxococcales* etc constituted only a minor portion of the population. In the phylum Actinobacteria, genus *Arthrobacter* occupied 33 per cent, followed by *Thermoleophilum* (12%). Other genera like *Atopobium*, *Nocardioides*, *Williamsia*, *Mycobacterium*, *Desulfotomaculum* and *Ruminococcus* were found to occupy a notable proportion in the population.

The phylum Firmicutes was found to be dominated by genus *Clostridium* (28%), followed by *Bacillus* (18%). The phylum Bacteroidetes, being a minority was observed to be composed of 31 per cent *Flavobacterium*, 28 per cent *Terribacter*, 9 per cent *Chitinophaga* and various other genera in trace percentages. The genus-level bacterial diversity in the 10 abundant phyla in the sample CH-7 has been provided in Table 30.



Major phyla	
Proteobacteria	- 18%
Actinobacteria	- 16%
Firmicutes	- 5%
Acidobacteria	- 2%
Verrucomicrobia	- 2%
Bacteroidetes	- 1%
Unclassified	- 46%

Plate 17. Phylum-level bacterial diversity in the sample CH-7 obtained using MG-RAST pipeline

**Table 30. Genus-level taxonomic assemblage of bacterial diversity from 10 predominant phyla in the sample CH-7**

Phylum	Class	Order	Family	Genus
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	<i>Brevundimonas</i> (6), <i>Caulobacter</i> (14), <i>Phenyllobacterium</i> (40)
		Rhizobiales	Bartonellaceae Beijerinckiaceae  Bradyrhizobiaceae  Brucellaceae Hyphomicrobiaceae Methylocystaceae  Phyllobacteriaceae  Rhizobiaceae  Rhodobiaceae Xanthobacteraceae Unclassified Hyphomonadaceae Rhodobacteraceae	<i>Bartonella</i> (2) <i>Beijerinckia</i> (1), <i>Chelatococcus</i> (26), <i>Methylocella</i> (1) <i>Afpia</i> (5), <i>Balneimonas</i> (1), <i>Bosea</i> (25), <i>Bradyrhizobium</i> (307), <i>Nitrobacter</i> (4), <i>Rhodopseudomonas</i> (21), Unclassified (4) <i>Ochrobactrum</i> (14) <i>Blastochloris</i> (36), <i>Hyphomicrobium</i> (231) <i>Hansschlegelia</i> (2), <i>Methylocystis</i> (17), <i>Methylosinus</i> (1), Unclassified (72) <i>Aminobacter</i> (10), <i>Chelativorans</i> (6), <i>Mesorhizobium</i> (331), <i>Phyllobacterium</i> (10) <i>Agrobacterium</i> (1), <i>Candidatus Liberibacter</i> (1), <i>Ensifer</i> (37), <i>Rhizobium</i> (78), <i>Sinorhizobium</i> (2) <i>Affella</i> (3), <i>Rodobium</i> (2) <i>Ancylobacter</i> (9), <i>Azorhizobium</i> (4), <i>Xanthobacter</i> (1) Unclassified (133) <i>Hirschia</i> (2), <i>Maricaulis</i> (1) <i>Jannaschia</i> (1), <i>Loktanella</i> (1), <i>Pannonibacter</i> (4), <i>Paracoccus</i> (2), <i>Rhodobacter</i> (1), <i>Rhodovulum</i> (8), <i>Roseovarius</i> (1), <i>Thioclava</i> (1), Unclassified (7) <i>Acetobacter</i> (1), <i>Acidiphilium</i> (1), <i>Ascia</i> (2), <i>Gluconacetobacter</i> (3), <i>Gluconobacter</i> (1), <i>Granulibacter</i> (32), <i>Kozakia</i> (1), <i>Roseococcus</i> (3), <i>Rubritepida</i> (1) <i>Azospirillum</i> (55), <i>Magnetospirillum</i> (1), <i>Rhodospirillum</i> (1), <i>Rhodovibrio</i> (4), <i>Telmatospirillum</i> (1), Unclassified (160)
		Rhodospirillales	Acetobacteraceae	<i>Rubritepida</i> (1)
		Rickettsiales	Rhodospirillaceae Anaplasmataceae Rickettsiaceae	<i>Azospirillum</i> (55), <i>Magnetospirillum</i> (1), <i>Rhodospirillum</i> (1), <i>Rhodovibrio</i> (4), <i>Telmatospirillum</i> (1), Unclassified (160) <i>Anaplasma</i> (2), <i>Wolbachia</i> (1) <i>Orientia</i> (3)





	Desulfobacterales	Desulfobacteraceae	<i>Desulfatibacillum</i> (185), <i>Desulfobacter</i> (2), <i>Desulfobacterium</i> (1), <i>Desulfobotulus</i> (18), <i>Desfocella</i> (3), <i>Desulfococcus</i> (56), <i>Desulfofaba</i> (2), <i>Desulfonema</i> (90), <i>Desulforegula</i> (19), <i>Desulfosarcina</i> (1), <i>Desulfotignum</i> (1), Unclassified (2) <i>Desulfobulbus</i> (15), <i>Desulfurivibrio</i> (2), Unclassified (3)
	Desulfovibrionales	Desulfohalobiaceae	<i>Desulfohalobium</i> (2), <i>Desulfonatronovibrio</i> (287)
		Desulfomicrobiaceae	<i>Desulfomicrobium</i> (1)
		Desulfonatronumaceae	<i>Desulfonatronum</i> (15)
		Desulfovibrionaceae	<i>Desulfovibrio</i> (565)
		Unclassified	Unclassified (74)
	Desulfurellales	Desulfurellaceae	<i>Desulfurella</i> (1)
	Desulfuromonadales	Desulfuromonadaceae	<i>Desulfuromonas</i> (8)
		Geobacteraceae	<i>Gealkalibacter</i> (221), <i>Geobacter</i> (38)
		Pelobacteraceae	<i>Pelobacter</i> (13)
	Myxococcales	Cystobacteraceae	<i>Cystobacter</i> (201), <i>Melittangium</i> (5), <i>Stigmatella</i> (12)
		Haliangiaceae	<i>Haliangium</i> (10)
		Myxococcaceae	<i>Anaeromyxobacter</i> (72), <i>Corallococcus</i> (82), <i>Myxococcus</i> (130)
		Nannocystaceae	<i>Nannocystis</i> (23)
		Polyangiaceae	<i>Chondromyces</i> (31), <i>Sorangium</i> (48)
		Unclassified	<i>Enhygromyxa</i> (78)
	Syntrophobacterales	Syntrophaceae	<i>Desulfobacca</i> (6)
		Syntrophobacteraceae	<i>Desulfoglaeba</i> (2), <i>Desulforhabdus</i> (3), <i>Desulfovirga</i> (5), <i>Syntrophobacter</i> (1)
		Unclassified	Unclassified (23136)
Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	<i>Arcobacter</i> (3), <i>Campylobacter</i> (21)
	Nautiliales	Nautiliaceae	<i>Caminibacter</i> (42), <i>Nautilia</i> (14)
	Unclassified	Unclassified	<i>Nitratiruptor</i> (6), Unclassified (122)
Gammaproteobacteria	Acidithiobacillales	Acidithiobacillaceae	<i>Acidithiobacillus</i> (26)
	Aeromonadales	Succinivibrionaceae	<i>Ruminobacter</i> (10), <i>Succinimonas</i> (272)
	Alteromonadales	Alteromonadaceae	<i>Marinobacter</i> (1)



			Idiomarinaceae	<i>Idiomarina</i> (2)
			Shewanellaceae	<i>Shewanella</i> (47)
Chromatiales			Chromatiaceae	<i>Halochromatium</i> (19), <i>Marichromatium</i> (4), <i>Nitrosococcus</i> (1), <i>Thiocapsa</i> (4), <i>Thiorhodovibrio</i> (36), Unclassified (2)
			Ectothiorhodospiraceae	<i>Alkalispirillum</i> (3), <i>Ectothiorhodospira</i> (10), <i>Halorhodospira</i> (4), <i>Nitrococcus</i> (5), <i>Thioalkalivibrio</i> (4), <i>Thiohalospira</i> (17), <i>Thiorhodospira</i> (1), Unclassified (1)
Enterobacteriales			Halothiobacillaceae	<i>Halothiobacillus</i> (4)
			Enterobacteriaceae	<i>Buchnera</i> (9), <i>Candidatus Hamiltonella</i> (50), <i>Candidatus Ishikawaella</i> (1), <i>Citrobacter</i> (8), <i>Enterobacter</i> (2), <i>Erwinia</i> (1), <i>Escherichia</i> (9), <i>Klebsiella</i> (11), <i>Pantoea</i> (2), <i>Photorhabdus</i> (14), <i>Serratia</i> (12), <i>Xenorhabdus</i> (1), Unclassified (1626)
Legionellales		Coxiellaceae	<i>Coxiella</i> (97)	
Methylococcales			Legionellaceae	<i>Fluoribacter</i> (1), <i>Legionella</i> (18)
			Methylococcaceae	<i>Methylocaldum</i> (3), <i>Methylohalobius</i> (3), <i>Methylothermus</i> (2), Unclassified (1)
Oceanospirillales			Alcanivoracaceae	<i>Alcanivorax</i> (2), <i>Kangiella</i> (1)
			Halomonadaceae	<i>Candidatus Portiera</i> (5), <i>Halomonas</i> (125), Unclassified (1)
Pasteurellales			Oceanospirillaceae	<i>Marinomonas</i> (1), <i>Oceanospirillum</i> (1)
			Pasteurellaceae	<i>Aggregatibacter</i> (1), <i>Avibacterium</i> (1), <i>Gallibacterium</i> (25), <i>Haemophilus</i> (3), <i>Mannheimia</i> (1)
Pseudomonadales			Moraxellaceae	<i>Acinetobacter</i> (9), <i>Moraxella</i> (1)
			Pseudomonadaceae	<i>Azotobacter</i> (19), <i>Pseudomonas</i> (858)
Thiotrichales			Piscirickettsiaceae	<i>Cycloclasticus</i> (1), <i>Methylophaga</i> (2), <i>Piscirickettsia</i> (1)
Vibrionales			Thiotrichaceae	<i>Beggiatoa</i> (5), <i>Thiothrix</i> (3)
			Vibrionaceae	<i>Vibrio</i> (1)
Xanthomonadales			Sinobacteraceae	<i>Sinobacter</i> (1)

Actinobacteria	Actinobacteria		Xanthomonadaceae	<i>Aquimonas</i> (8), <i>Dyella</i> (6), <i>Lysobacter</i> (59), <i>Pseudoxanthomonas</i> (1), <i>Rhodanobacter</i> (4), <i>Stenotrophomonas</i> (8), <i>Xanthomonas</i> (8), <i>Xylella</i> (3)
		Unclassified	Celerinatantimonadaceae	<i>Celerinatantimonas</i> (1)
			Unclassified	<i>Methylolhalomonas</i> (81), <i>Methylonatrimum</i> (12), <i>Thiohalomonas</i> (1), <i>Thiohalorhabdus</i> (1), Unclassified (10623)
		Acidimicrobiales	Acidimicrobiaceae	<i>Acidimicrobium</i> (248), <i>Acidithiomicrobium</i> (197)
		Actinomycetales	Acidothermaceae	<i>Acidothermus</i> (313)
			Actinomycetaceae	<i>Actinomyces</i> (10), <i>Arcanobacterium</i> (11), <i>Mobiluncus</i> (2)
			Actinopolysporaceae	<i>Actinopolyspora</i> (1)
			Actinosynnemtales	<i>Actinokineospira</i> (7), <i>Lechevalieria</i> (29), <i>Lentzea</i> (10)
			Beutenbergiaceae	<i>Beutenbergia</i> (34)
			Brevibacteriaceae	<i>Brevibacterium</i> (486)
			Catenulisporaceae	<i>Catenulispora</i> (19)
			Cellulomonadaceae	<i>Actinotalea</i> (6), <i>Cellulomonas</i> (136)
			Corynebacteriaceae	<i>Corynebacterium</i> (415)
			Dermabacteraceae	<i>Brachybacterium</i> (79)
			Dermacoccaceae	<i>Kytococcus</i> (2)
			Dermatophilaceae	<i>Dermatophilus</i> (51)
			Dietziaceae	<i>Dietzia</i> (14)
			Frankiaceae	<i>Frankia</i> (526)
			Geodermatophilaceae	<i>Geodermatophilus</i> (431)
			Glycomycetaceae	<i>Glycomyces</i> (3), <i>Stackebrandtia</i> (49)
			Gordoniaceae	<i>Gordonia</i> (442)
			Intrasporangiaceae	<i>Janibacter</i> (50), <i>Serinicoccus</i> (14), <i>Terrabacter</i> (145), <i>Tetrasphaera</i> (182)
			Kineosporiaceae	<i>Kineococcus</i> (12)
			Microbacteriaceae	<i>Agreia</i> (5), <i>Agrococcus</i> (36), <i>Agromyces</i> (988), <i>Candidatus Aquiluna</i> (5), <i>Candidatus Rhodoluna</i> (3), <i>Clavibacter</i> (18), <i>Cryobacterium</i> (108), <i>Curtobacterium</i> (14), <i>Frigoribacterium</i> (87),

		<p><i>Glaciibacter</i> (13), <i>Leifsonia</i> (73), <i>Microbacterium</i> (494), <i>Mycetocola</i> (14), <i>Okibacterium</i> (2), <i>Pseudoclavibacter</i> (3), <i>Rathayibacter</i> (13), <i>Salinibacterium</i> (22)</p> <p><i>Arthrobracter</i> (18111), <i>Kocuria</i> (52), <i>Micrococcus</i> (75), <i>Nesterenkonia</i> (22), <i>Renibacterium</i> (42), <i>Rothia</i> (321)</p> <p><i>Actinoplanes</i> (131), <i>Catenuloplanes</i> (211), <i>Dactylosporangium</i> (181), <i>Micromonospora</i> (1118), <i>Polymorphospora</i> (4), <i>Salinispora</i> (44), <i>Verrucosisspora</i> (24)</p> <p><i>Mycobacterium</i> (2175)</p> <p><i>Nakamurella</i> (110)</p> <p><i>Nocardia</i> (91), <i>Rhodococcus</i> (879), <i>Smaragdificoccus</i> (50)</p> <p><i>Aeromicrobium</i> (99), <i>Kribbella</i> (1216), <i>Nocardioides</i> (3852), <i>Pimelobacter</i> (110)</p> <p><i>Nocardioopsis</i> (268), <i>Streptomonospora</i> (1), <i>Thermobifida</i> (4)</p> <p><i>Cellulostimicrobium</i> (51), <i>Isopetricola</i> (3), <i>Promicromonospora</i> (49), <i>Xylanimicrobium</i> (1)</p> <p><i>Microthunatus</i> (108), <i>Propionibacterium</i> (146)), Unclassified (353)</p> <p><i>Amycolatopsis</i> (240), <i>Prauserella</i> (4), <i>Pseudonocardia</i> (2159), <i>Saccharomonospora</i> (14), <i>Saccharopolyspora</i> (1473), <i>Thermobispora</i> (5), <i>Thermocrispum</i> (3)</p> <p><i>Rarobacter</i> (6)</p> <p><i>Segniliparus</i> (25)</p> <p><i>Kitasatospora</i> (41), <i>Streptomyces</i> (989)</p> <p><i>Microbispora</i> (88), <i>Microtetraspora</i> (3), <i>Nonomuraea</i> (115), <i>Planomonospora</i> (7), <i>Streptosporangium</i> (508)</p> <p><i>Actinoallomurus</i> (128), <i>Actinocoralia</i> (37), <i>Actinomadura</i> (543), <i>Spirillospora</i> (1), <i>Thermomonospora</i> (273), Unclassified (121)</p>
	Micrococcaceae	
	Micromonosporaceae	
	Mycobacteriaceae	
	Nakamurellaceae	
	Nocardiaceae	
	Nocardioidaceae	
	Nocardioseae	
	Promicromonosporaceae	
	Propionibacteriaceae	
	Pseudonocardiaceae	
	Rarobacteraceae	
	Segniliparaceae	
	Streptomycetaceae	
	Streptosporangiaceae	
	Thermomonosporaceae	

Firmicutes	Bacilli	Bacilli	Tsukamurellaceae	<i>Tsukamurella</i> (2)				
			Williamsiaceae	<i>Williamsia</i> (2702)				
			Bifidobacteriales	Unclassified	<i>Tropheryma</i> (5)			
				Bifidobacteriaceae	<i>Alloscardovia</i> (1), <i>Bifidobacterium</i> (27), <i>Gardnerella</i> (7)			
			Coriobacteriales	Coriobacteriaceae	<i>Atopobium</i> (1761), <i>Collinsella</i> (26), <i>Cryptobacterium</i> (1), <i>Eggerthella</i> (6), <i>Enterorhabdus</i> (12), <i>Gordonibacter</i> (226), <i>Slackia</i> (6)			
				Rubrobacterales	<i>Rubrobacter</i> (807)			
			Thermoleophilales	Thermoleophilaceae	<i>Thermoleophilum</i> (6677)			
				Unclassified	Unclassified (12)			
			Bacillales	Bacillales	Alicyclobacillaceae	<i>Alicyclobacillus</i> (691)		
					Bacillaceae	<i>Anoxybacillus</i> (103), <i>Bacillus</i> (3334), <i>Geobacillus</i> (45), <i>Halobacillus</i> (29), <i>Lysinibacillus</i> (154), <i>Natronobacillus</i> (2), <i>Oceanobacillus</i> (128), <i>Virgibacillus</i> (19)		
					Paenibacillaceae	<i>Aneurinibacillus</i> (34), <i>Brevibacillus</i> (33), <i>Paenibacillus</i> (590), <i>Thermobacillus</i> (1)		
					Pasteuriaceae	<i>Pasteuria</i> (6)		
					Planococcaceae	<i>Jeotgalibacillus</i> (2), <i>Kurthia</i> (428), <i>Planomicrobium</i> (17), <i>Sporosarcina</i> (5), <i>Ureibacillus</i> (11), <i>Viridibacillus</i> (7)		
						Sporolactobacillaceae	<i>Sporolactobacillus</i> (15)	
					Staphylococcaceae	<i>Macrococcus</i> (1), <i>Staphylococcus</i> (75)		
					Thermoactinomycetaceae	<i>Thermoactinomyces</i> (26), <i>Thermoflavomicrobium</i> (1)		
					Unclassified	<i>Exiguobacterium</i> (3), <i>Gemella</i> (1)		
					Lactobacillales	Lactobacillales	Carnobacteriaceae	<i>Alkalibacterium</i> (3), <i>Carnobacterium</i> (1), <i>Granulicatella</i> (2), <i>Trichococcus</i> (5)
							Enterococcaceae	<i>Enterococcus</i> (30)
							Lactobacillaceae	<i>Lactobacillus</i> (243)
Streptococcaceae	<i>Lactococcus</i> (4), <i>Streptococcus</i> (7)							
Clostridiales	Clostridiales	Clostridiaceae	<i>Alkaliphilus</i> (23), <i>Butyrivibrio</i> (77), <i>Caloramator</i> (6), <i>Clostridium</i> (5140), <i>Oxobacter</i> (57), <i>Sarcina</i> (1), <i>Tepidimicrobium</i> (21)					

				<i>SedisAnaerococcus</i> (1), <i>SedisHelcococcus</i> (6), <i>SedisPeptoniphilus</i> (414), <i>SedisSoehngenia</i> (2), <i>SedisSporanaerobacter</i> (1548), <i>SedisTissierella</i> (523) <i>Sedisfusibacter</i> (1)
		Clostridiales Family XI. Incertae		
		Clostridiales Family XII. Incertae		
		Clostridiales Family XIV. Incertae		<i>SedisAnaerobranca</i> (4)
		Clostridiales Family XVII. Incerate		<i>SedisSulfobacillus</i> (9), <i>SedisThermaerobacter</i> (12)
		Clostridiales Family XVIII. Incertae		<i>SedisSymbiobacterium</i> (16)
		Eubacteriaceae		<i>Acetobacterium</i> (12), <i>Eubacterium</i> (18)
		Heliobacteriaceae		<i>Heliobacillus</i> (4), <i>Heliobacterium</i> (82), <i>Heliophilum</i> (1), <i>Heliorestis</i> (6)
		Lachnospiraceae		<i>Acetitomaculum</i> (1), <i>Anaerostipes</i> (2), <i>Butyrivibrio</i> (2), <i>Cellulosilyticum</i> (3), <i>Hespelia</i> (5), <i>Lachnospira</i> (10), <i>Pseudobutyrvibrio</i> (1), <i>Roseburia</i> (15), <i>Syntrophococcus</i> (1), Unclassified (12)
		Peptococcaceae		<i>Candidatus Desulforudis</i> (12), <i>Dehalobacter</i> (6), <i>Desulfitobacterium</i> (384), <i>Desulfonisporea</i> (59), <i>Desulfosporosinus</i> (38), <i>Desulfotomaculum</i> (1152), <i>Pelotomaculum</i> (3), Unclassified (9)
		Peptostreptococcaceae		<i>Peptostreptococcus</i> (36)
		Ruminococcaceae		<i>Aceitvibrio</i> (11), <i>Eihanoligenens</i> (2), <i>Faecalibacterium</i> (12), <i>Ruminococcus</i> (997), Unclassified (8)
		Syntrophomonadaceae		<i>Syntrophomonas</i> (1), <i>Syntrophothermus</i> (1)
		Unclassified		<i>Blautia</i> (1), <i>Epulopiscium</i> (1), Unclassified (52)
		Thermoanaerobacteraceae		<i>Caldanaerobacter</i> (4), <i>Cadanaerobius</i> (176), <i>Carboxydotherrmus</i> (1), <i>Moorella</i> (32), <i>Thermacetogenium</i> (16), <i>Thermoanaerobacter</i> (19) <i>SedisCaldicellulosiruptor</i> (331)
		Thermoanaerobacterales Family III. Incertae		
		Thermodesulfobiaceae		<i>Coprothermobacter</i> (96), <i>Thermodesulfobium</i> (14)
		Erysipelotrichaceae		<i>Erysipelotrichix</i> (1), Unclassified (1)
Erysipelotrichi	Erysipelotrichales			

	Negativicutes	Selenomonadales	Acidaminococcaceae Veillonellaceae	<i>Acidaminococcus</i> (1), <i>Phascolarctobacterium</i> (38) <i>Dialister</i> (9), <i>Megamonas</i> (3), <i>Megashpaera</i> (112), <i>Mitsuokella</i> (4), <i>Pectinatus</i> (5), <i>Selenomonas</i> (18), <i>Sporomusa</i> (32), <i>Veillonella</i> (52)	
Verrucomicrobia	Opitutae	Puniciceocales	Puniciceococcaceae	<i>Coraliomargarita</i> (1)	
		Unclassified	Opitutaceae	<i>Opitutus</i> (342), Unclassified (44)	
		Unclassified	Unclassified	<i>Cthoniobacter</i> (1098)	
Verrucomicrobiae	Unclassified	Methylacidophilales	Methylacidophilaceae	<i>Methylacidiphilum</i> (44)	
		Verrucomicrobiales	Verrucomicrobia subdivision 3	Unclassified (2866)	
			Verrucomicrobiaeae	Verrucomicrobiaceae	<i>Akkermansia</i> (12), <i>Prostheco bacter</i> (508), <i>Rubritalea</i> (396), <i>Verrucomicrobium</i> (180), Unclassified (285)
			Unclassified	Unclassified	Unclassified (9)
Acidobacteria	Unclassified	Unclassified	Unclassified	Unclassified (73)	
	Acidobacteria	Acidobacteriales	Acidobacteriaceae	<i>Acidobacterium</i> (257)	
	Solibacteres	Solibacterales	Solibacteraceae	<i>Candidatus Solibacter</i> (3712)	
	Unclassified	Unclassified	Unclassified	<i>Candidatus Koribacter</i> (1865)	
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	<i>Blastopirellula</i> (134), <i>Isosphaera</i> (972), <i>Pirellula</i> (687), <i>Planctomyces</i> (571), <i>Rhodopirellula</i> (45), Unclassified (2877)	
			Unclassified	Unclassified	<i>Candidatus Kuenenia</i> (4)
			Bacteroidaeae	Bacteroidales	<i>Bacteroides</i> (1)
			Porphyromonadaceae	Porphyromonadales	<i>Barneisiella</i> (1), <i>Butyricimonas</i> (1), <i>Porphyromonas</i> (6)
Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	<i>Prevotella</i> (27)	
			Rikenellaceae	<i>Alistipes</i> (27), <i>Rikenella</i> (2)	
			Cyclobacteriaceae	Cyclobacterium (22)	
			Cytophagaceae	<i>Cytophaga</i> (219), <i>Dyadobacter</i> (5), <i>Flectobacillus</i> (7), <i>Flexibacter</i> (216), <i>Hymenobacter</i> (96), <i>Marinoscillum</i> (1), <i>Microscilla</i> (1), <i>Spirosoma</i> (6)	
Flavobacteria	Flavobacteriales	Flammeovirgaceae	Flammeovirgaceae	<i>Flexithrix</i> (135), <i>Persicobacter</i> (8)	
		Blattabacteriaceae	Blattabacterium (1)		
		Flavobacteriaceae	<i>Arenibacter</i> (9), <i>Capnocytophaga</i> (9), <i>Cellulophaga</i> (5), <i>Dokdonia</i> (1), <i>Elizabethkingia</i> (66),		



				<i>Empedobacter</i> (1), <i>Flavobacterium</i> (1444), <i>Gramella</i> (9), <i>Leeuwenhoekella</i> (1), <i>Mariniflexile</i> (1), <i>Myroides</i> (6), <i>Riemerella</i> (138), <i>Tenacibaculum</i> (55), <i>Zunongwangia</i> (1)
	Unclassified		Unclassified	Unclassified (41)
Spingobacteria	Spingobacteriales		Rhodothermaceae	<i>Rhodothermus</i> (99), <i>Salinibacter</i> (3)
			Saprosiraceae	<i>Saprosira</i> (17)
			Spingobacteriaceae	<i>Pedobacter</i> (21), <i>Spingobacterium</i> (83), Unclassified (31)
Unclassified	Unclassified		Unclassified	<i>Chitinophaga</i> (401), <i>Terrimonas</i> (1278)
			Unclassified	<i>Candidatus Amoebophilus</i> (61), <i>Candidatus Cardinium</i> (1), <i>Prolixibacter</i> (1), Unclassified (60)
Gemmatimonadetes	Gemmatimonadales		Gemmatimonadaceae	<i>Gemmatimonas</i> (2986)
Chloroflexi	Chloroflexales		Chloroflexaceae	<i>Chloroflexus</i> (753), <i>Roseiflexus</i> (11)
			Oscillochloridaceae	<i>Oscillochloris</i> (34)
	Herpetosiphonales		Herpetosiphonaceae	<i>Herpetosiphon</i> (1468)
Dehalococcoidetes	Unclassified		Unclassified	<i>Dehalococcoides</i> (1)
Ktedonobacteria	Ktedonobacterales		Ktedonobacteraceae	<i>Ktedonobacter</i> (380)
	Unclassified		Unclassified	Unclassified (129)
Thermomicrobia	Sphaerobacterales		Sphaerobacteraceae	<i>Sphaerobacter</i> (14)
	Thermomicrobiales		Thermomicrobiaceae	<i>Thermomicrobium</i> (59)
Spirochaetes	Spirochaetales		Brachyspiraceae	<i>Brachyspira</i> (25)
			Leptosiraceae	<i>Leptosira</i> (2128)
			Spirochaetaeaceae	<i>Borrelia</i> (1), <i>Spirochaeta</i> (223)



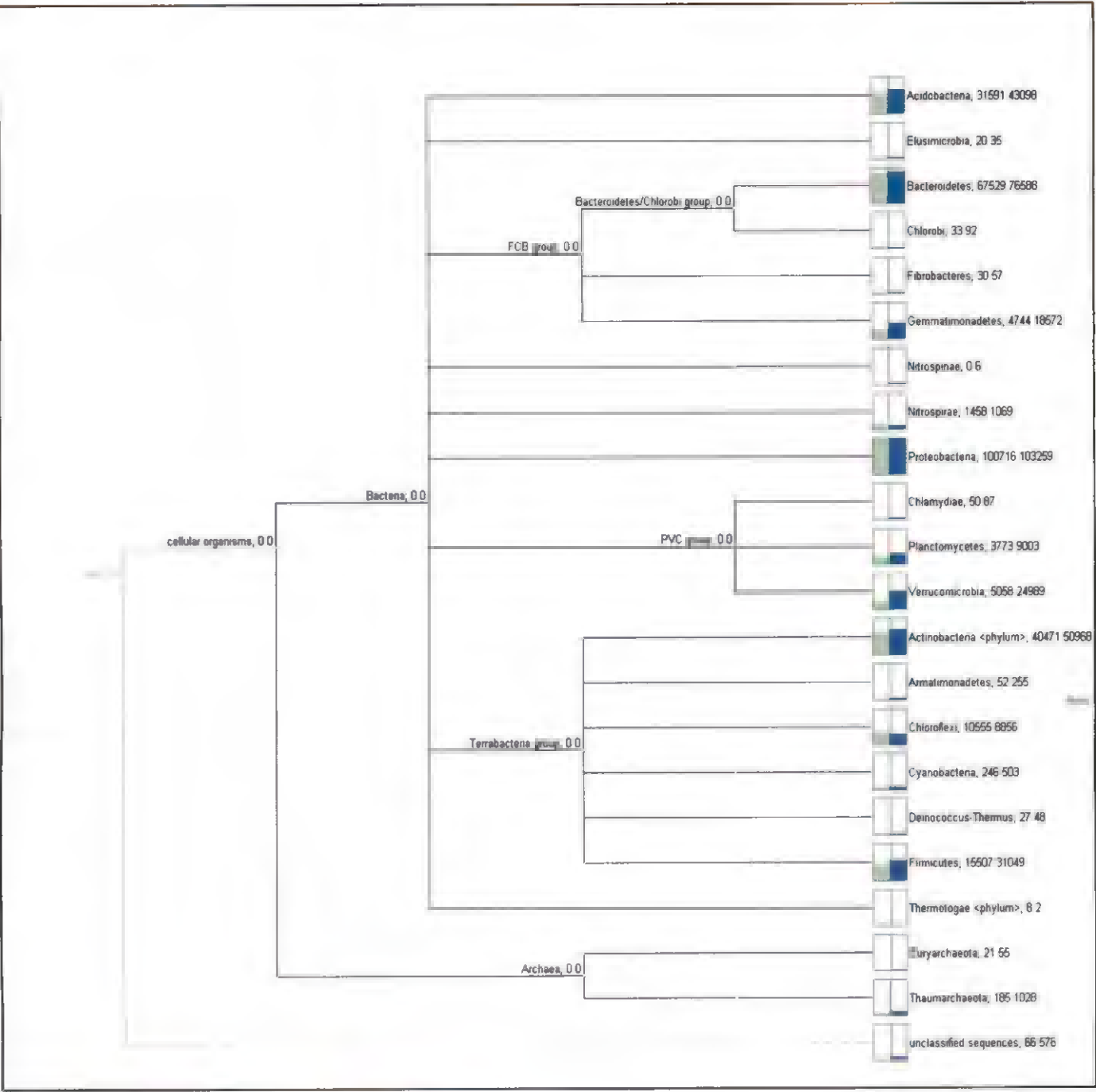
## 4.26 Comparative analysis of bacterial and archaeal diversity

A comparative analysis of predominant between the yellowing affected rhizosphere soil samples and its corresponding apparently healthy rhizosphere samples was done. A collective comparison between all the samples, which includes completely healthy rhizosphere soil samples including the aforementioned categories was also done.

### 4.26.1 Comparison between the samples YL-2 and AH-2

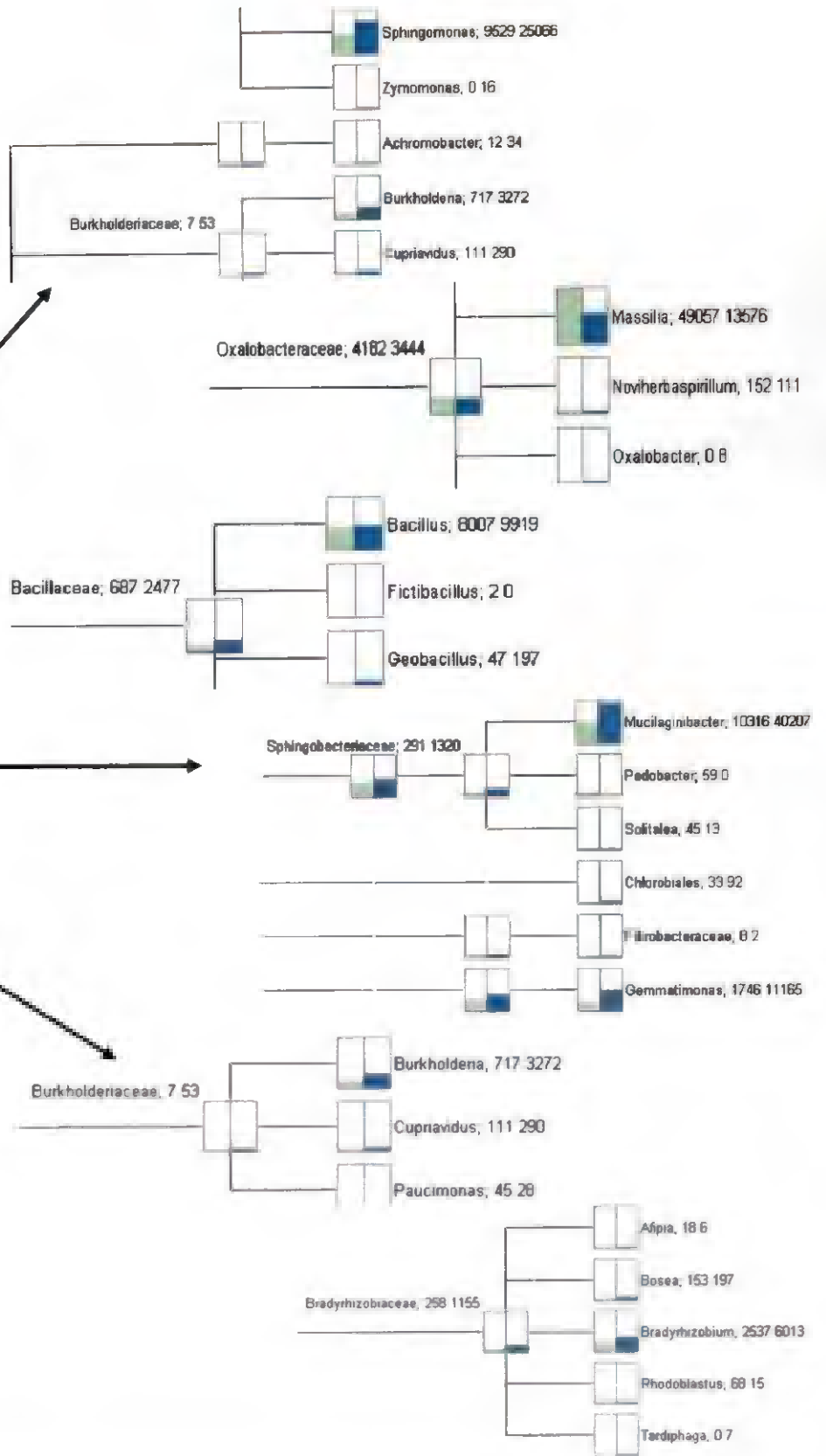
A comparison between the yellowing affected rhizosphere soil sample YL-2 and its corresponding apparently healthy rhizosphere soil sample AH-2 that bacterial population was comparatively high in the sample AH-2. The population of the phyla Acidobacteria, Bacteroidetes, Proteobacteria, Actinobacteria, Verrucomicrobia, Firmicutes, Gemmatomonadetes and Cyanobacteria was found to be higher in the sample AH-2, while the population of the phylum Chloroflexi was higher in the sample YL-2 compared to AH-2. Within the Bacteroidetes phylum, the class Chitinophagia was found to be high in YL-2, while the class Sphingobacteria was higher in the sample AH-2. The classes Alphaproteobacteria and Deltaproteobacteria of the phylum Proteobacteria was found to be high in the sample AH-2, while the population of Betaproteobacteria was high in the sample YL-2.

The population of the genera *Sphingomonas* and *Burkholderia* was found to be high in the sample AH-2, while the population of *Massilia* was observed to be significantly high in the sample YL-2. A slightly increased population of class Gammaproteobacteria in YL-2 was also reflected in the genus *Pseudomonas*, with a slight but not significant increase in its population in YL-2. Both the samples had high number of genus *Arthrobacter* with a slightly increased proportion in AH-2. The population of *Bacillus* was also found to be higher in the sample AH-2. The archaeal population was also found to be slightly high in the sample AH-2.



YL-2
  AH-2

**Plate 18. Comparative analysis of YL-2 and AH-2 at phylum-level using MEGAN**



**Plate 19. Comparative analysis of YL-2 and AH-2 at genus-level using MEGAN**

#### 4.26.2 Comparison between the samples YL-7 and AH-7

A comparison of bacterial and archaeal population between the sample YL-7 and AH-7 was done and an increase in the population of bacteria was found in the sample YL-7. The sample AH-7 was found to harbor an increased population of the phyla Acidobacteria, Proteobacteria, Planctomycetes, Gemmatimonadetes, Verrucomicrobia and Chloroflexi. The population of Actinobacteria was found to be very high in the sample YL-7 when compared to AH-7. The population of Bacteroidetes, Nitrospirae and Firmicutes were also found to be higher in the sample YL-7.

The class Acidobacteria and Solibacteres of phylum Acidobacteria was found to be high in the sample AH-7 compared to YL-7. The class Chitinophagia of phylum Bacteroidetes was found to be high in the sample AH-7, while the population of Sphingobacteria was higher in the sample YL-7. The classes Alpha, Beta and Deltaproteobacteria of phylum Proteobacteria was higher in the sample AH-2, while that of Gammaproteobacteria was comparatively higher in the sample YL-7.

Among the prominent bacterial genera, the genus *Methylobacterium* was observed to be significantly high in the sample YL-7, while an increased population of *Sphingomonas* was observed in the sample AH-7. The population of *Burkholderia* and *Massilia* was found to be higher in the sample AH-7. An increased population of the genus *Pseudomonas* and *Rhodanobacter* was found in the sample YL-7. A significantly high population of the genus *Williamsia* of phylum Actinobacteria was observed in the sample YL-7. The population of the genus *Bacillus* of phylum Firmicutes was also high in the sample YL-7 compared to AH-7. The proportion of archaeobacteria was found to be high in the sample YL-7.

#### 4.27 Comparison of prokaryotic diversity among all the samples

The highest population of bacteria was recorded in the sample YL-7 followed by AH-7, while the lowest bacterial population was recorded in the sample CH-7. The

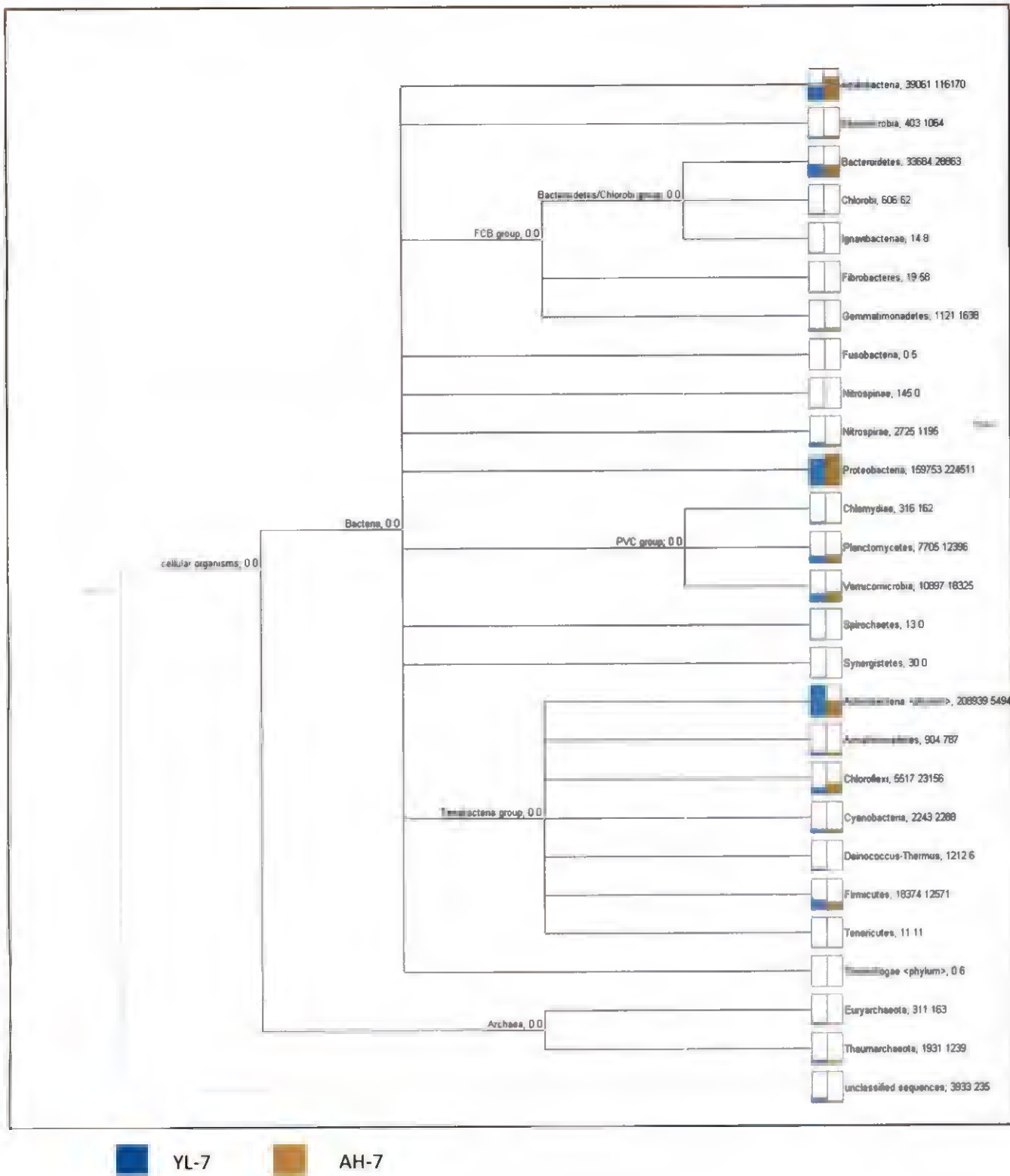
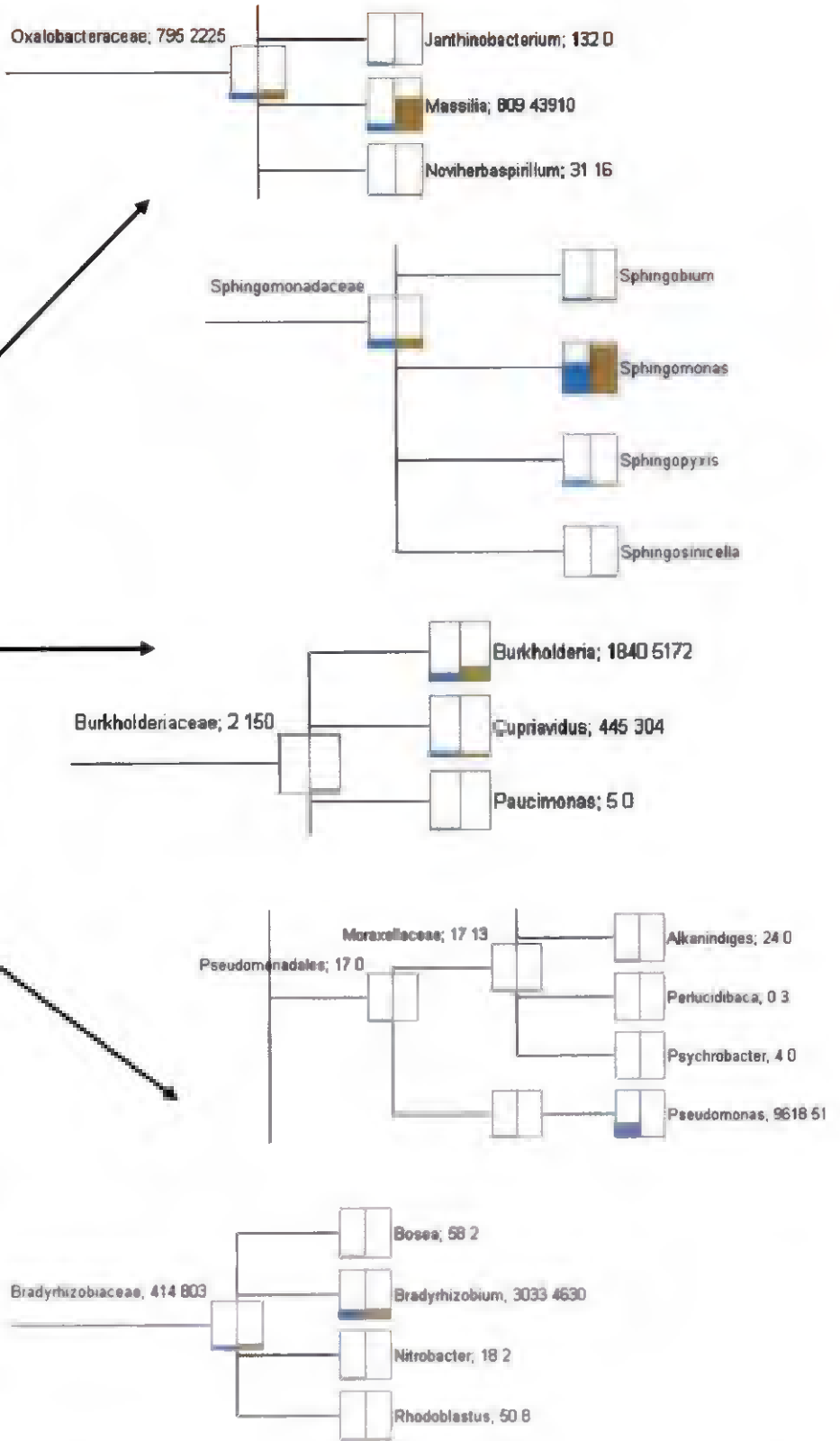
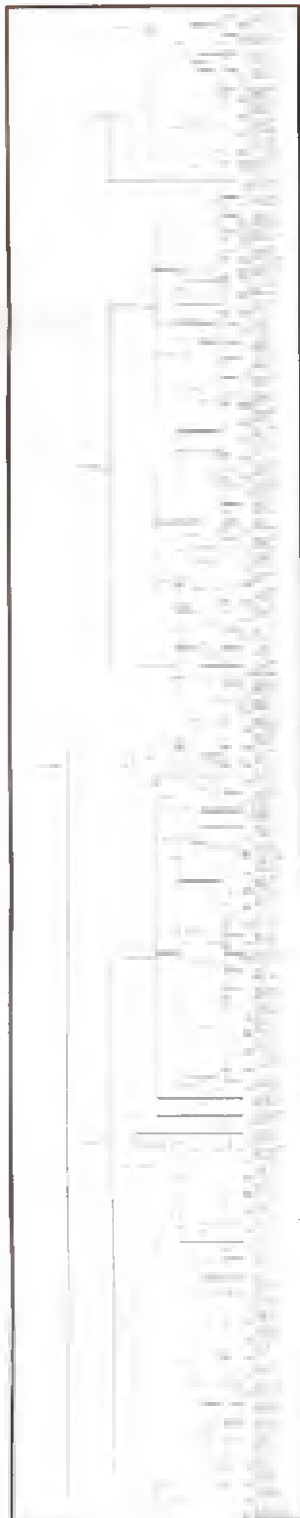


Plate 20. Comparative analysis of YL-7 and AH-7 at phylum-level using MEGAN



**Plate 21. Comparative analysis of YL-7 and AH-7 at genus-level using MEGAN**

highest population of Acidobacteria and Proteobacteria was recorded in the sample AH-7. The highest population of Actinobacteria was recorded in the sample YL-7. The classes Alphaproteobacteria and Betaproteobacteria had its highest population in the sample AH-7, while the highest population of the sample CH-5.

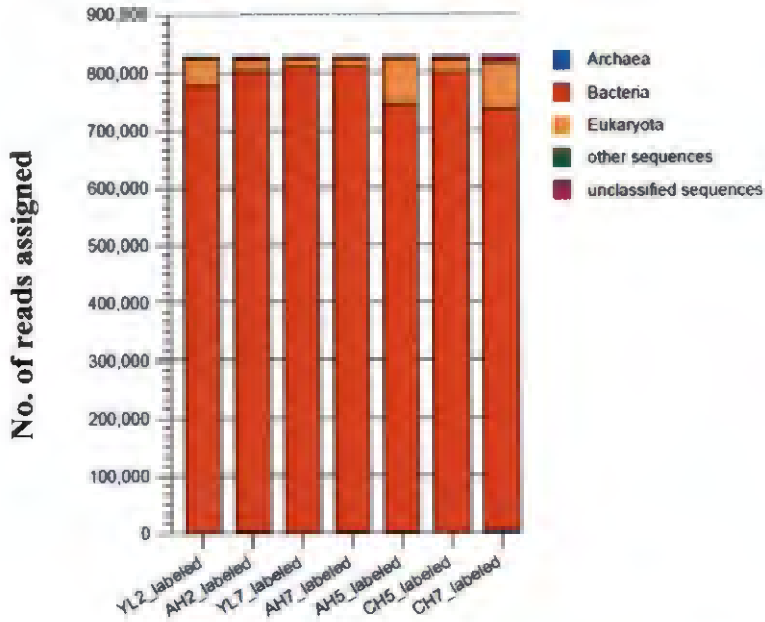
The comparison of prominent genera among the samples was done and the highest population of *Pseudomonas* was recorded in the sample CH-5. The population of the genus *Williamsia* was observed to be significantly high in the sample YL-7. The highest number of genus *Massilia* was recorded in the sample YL-2 and the highest population of the genus *Sphingomonas* was recorded in the sample AH-7. The population of the genus *Methylobacterium* was found to be notably high in the sample YL-7 and the population of *Arthrobacter*, *Bacillus*, *Gemmatimonas* and *Mucilaginibacter* was highest in the sample AH-2.

**Table 31. Number of taxa at each taxonomic level**

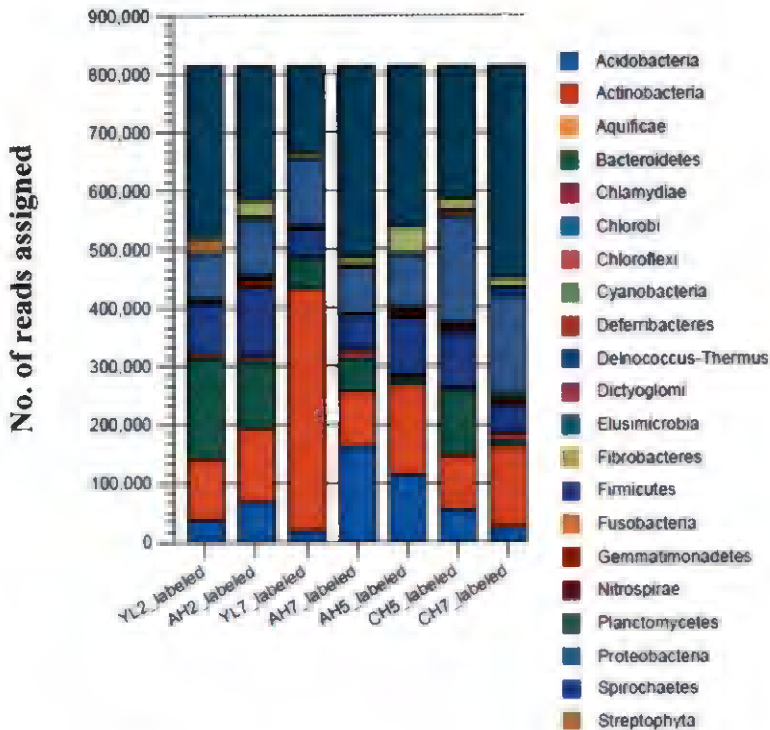
Sample	No. of taxa				
	Phylum	Class	Order	Family	Genus
YL-2	24	46	104	239	652
AH-2	26	49	107	243	716
YL-7	23	43	97	225	654
AH-7	26	48	104	242	688
AH-5	25	48	105	236	646
CH-5	24	44	102	237	725
CH-7	25	47	101	233	635

The diversity was compared using the number of taxa at each level of the taxonomic classification from phylum to genus. The more the number, the more was the diversity. The YL rhizospheres were found to harbor more diversity than its corresponding AH rhizosphere. The CH samples showed an intermediary value. Even



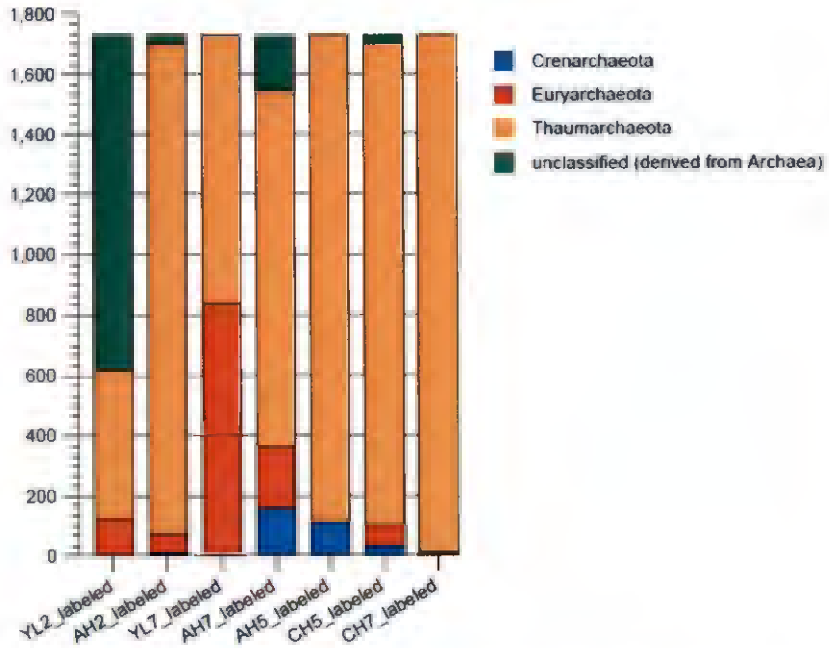


**Plate 22.a. Domain-level comparison of all the samples using MG-RAST**

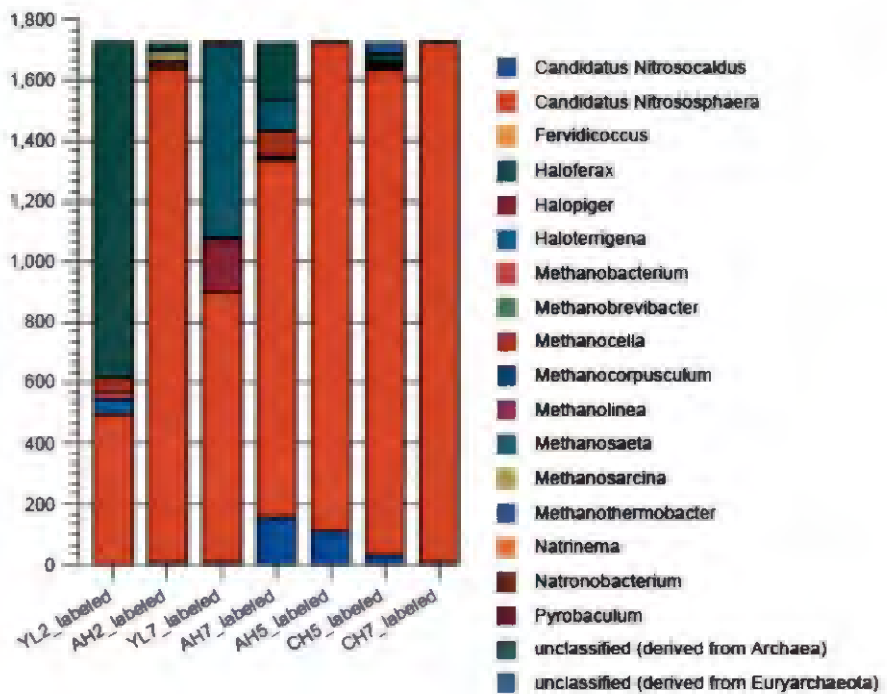


**Plate 22. b. Phylum-level comparison of all the samples using MG-RAST**

**Plate 22. Comparison of bacterial diversity of all samples using MG-RAST**



**Plate 23.a. Phylum level comparison of archaeal diversity from all samples**



**Plate 23. b. Genus-level comparison of archaeal diversity from all samples**

**Plate 23. Comparison of archaeal diversity of all samples using MG-RAST**

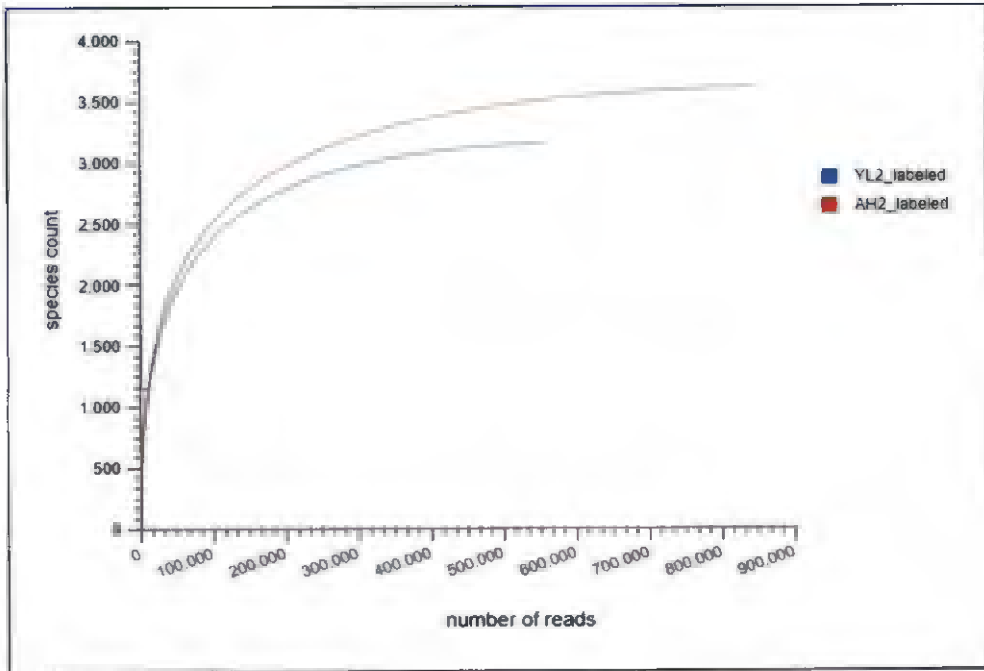
though, the samples AH-2 and AH-7 had the same number of phyla, the diversity in further levels were found to be different (Table 31).

The diversity of the prokaryotes from all the samples was analysed using the diversity indices viz, Shannon-Weaver index and Simpson index. The indices were used to rank the samples and to compare them based its diversity. The diversity indices has been provided in the table 32.

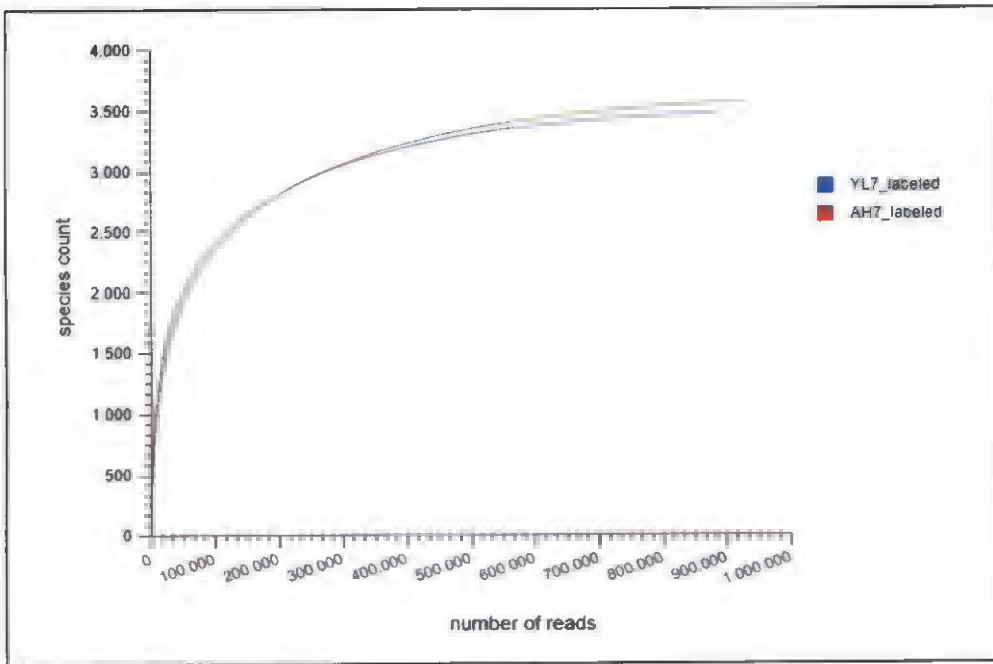
**Table 32. Diversity indices of domain bacteria and archaeobacteria at phylum level**

Diversity indices	Range	YL-2	AH-2	YL-7	AH-7	AH-5	CH-5	CH-7
Shannon-Weaver index (H)	$0 - \ln^*S$	1.74	1.95	1.43	1.72	1.82	1.85	1.61
Simpson index (D)	$1 - S$	4.45	5.78	2.96	4.05	4.82	5.19	3.49

The highest value of Shannon-Weaver index (H) was recorded in the sample AH-2, which indicates that the rhizosphere of that palm had the ability to accommodate a wide range of prokaryotic phyla compared to its corresponding yellowing affected sample YL-2. The same scenario was observed in the case of AH-7, which had a higher value of H compared to the sample YL-7. The least value of H was recorded in the sample YL-7 indicating the inability of the rhizosphere to support a wide range of microorganisms. The maximum value of Simpson index (D) was recorded in the sample AH-2, which indicated the richness of phyla in the sample and the least value was recorded in YL-7 indicating the less number of phyla in the sample compared to others.



**Plate 24.a. Rarefaction analysis of YL-2 and AH-2 using MG-RAST pipeline**



**Plate 24.b. Rarefaction analysis of YL-7 and AH-7 using MG-RAST pipeline**

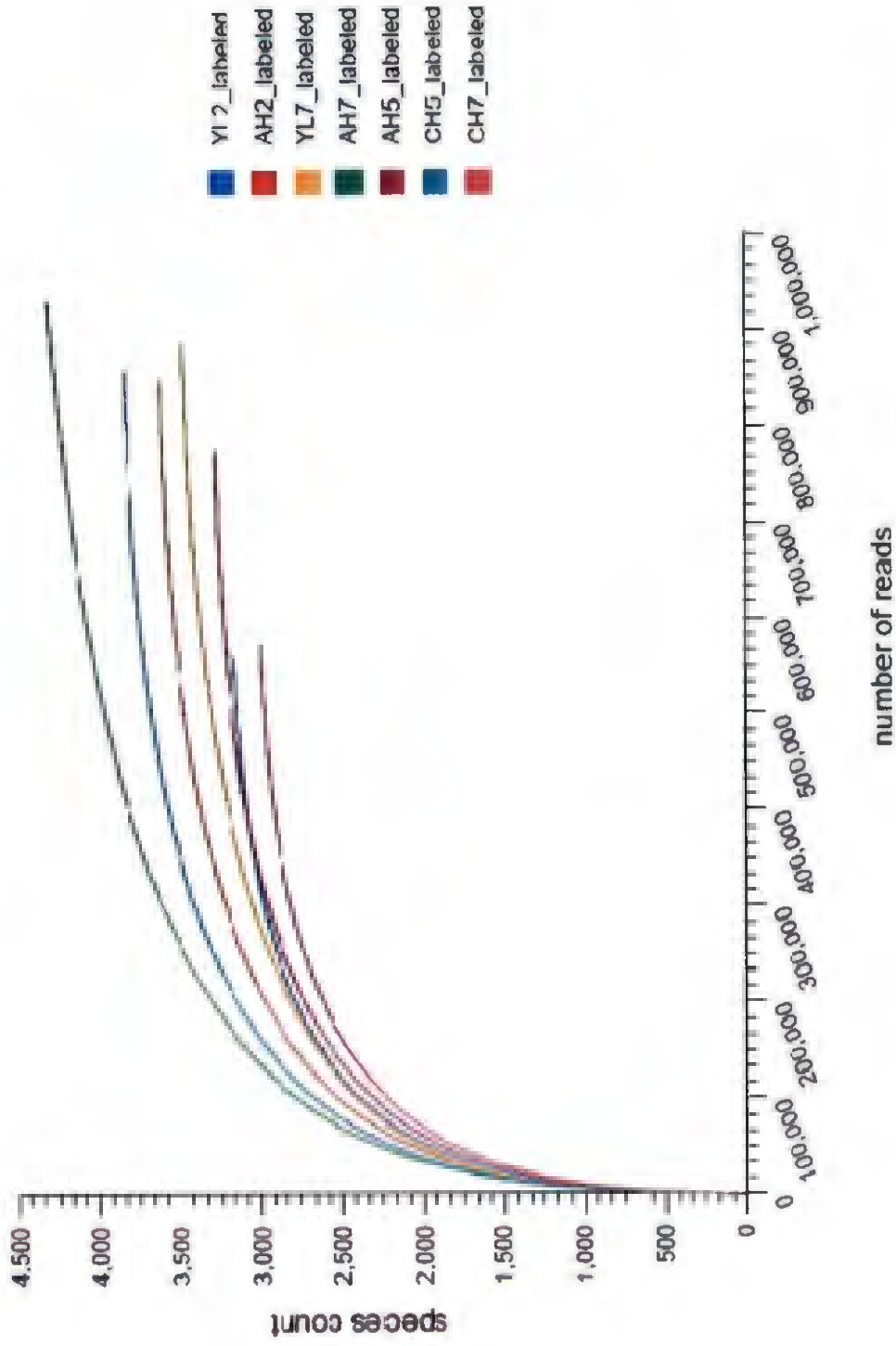


Plate 24.c. Rarefaction curve to compare the bacterial diversity among the samples

Plate 24. Rarefaction analysis

#### **4.28 Submission of sequences in NCBI database**

The metagenomic DNA sequences were submitted in the Sequence Read Archive (SRA) for the accessibility by the public domain. The sequences of the samples YL-2, AH-2 and CH-5 were submitted in the SRA portal under the bioaccession numbers SAMN08005381, SAMN08005832 and SAMN08005833 respectively.

## DISCUSSION



## 5. DISCUSSION

Arecanut cultivation in India has always been of great importance due to its commercial and cultural significance. The cultivation has been restricted to a few states, even though the nation leads in the cultivation in the global scenario. A 70 per cent increase in the area under arecanut cultivation in the past two decades has contributed to the expansion of the crop into converted paddy lands. This has ultimately invited many constraints, the major one being stagnation of productivity due to intricacies in climate, soil and crop management practices (CPCRI, 2015). Yellowing of arecanut, vernacularly known as 'Kattuveezhcha' in Malayalam, has emerged to be one of the most detrimental diseases affecting arecanut palms in various parts of Kerala and Karnataka. Yellowing of arecanut was observed to have a high rate of spread causing an accelerated decrease in the yield.

Two types of yellowing symptoms are observed in arecanut, which can be differentiated by the symptom expression on leaves. Arecanut yellow leaf disease (YLD) is associated with yellowing of leaves starting from the tips of leaflets in two or three leaves of the outermost whorl and gradually extended to the middle of the lamella with a clear demarcation between green and yellow region. In advanced stages, yellowing extends to the middle whorl, the trunk becomes tapering, with closely bunched, puckered leaves (Muddumadiah *et al.*, 2014). Analysis of 16S rRNA sequencing revealed the presence of phytoplasma in symptomatic palms, but not in asymptomatic ones (Manimekalai *et al.*, 2010).

The second type of yellowing in arecanut is found in low lying areas and converted paddy fields (Jacob *et al.*, 2014). Symptoms include yellowing of the tips of leaflets of a few leaves in the outermost whorls. Leaf tips dry up, leaves becomes shortened and the crown size is reduced. The symptoms were found to be expressed after the onset of monsoon and a reduction in the symptoms were observed with the reduction in rainfall. This disease can be clearly distinguished from YLD by the absence of green and yellow demarcation on leaflets.

The present investigation was undertaken with an intention to widen the understanding about the association of bacteria with yellowing affected and healthy arecanut palms in Wayanad district. Yellowing affected and healthy arecanut plantations were identified with the help of officials from the Department of Agriculture, Kalpetta and scientists from Regional Agricultural Research Station, Ambalavayal. The yellowing affected plantations were located in the converted paddy fields in Meenangadi and Kakkavayal, while the completely healthy plantations were located in Ambalavayal and Kolagappara areas. The yellowing affected plantations were either low lying areas with high water table or converted paddy lands. The elevation of such plantations ranged from 2439 to 2516 ft above MSL (Table 4). Healthy plantations were located at a higher elevation ranging from 2640 to 2991 ft above MSL. A series of earlier studies on the etiology of the disease also indicated the incidence of yellowing to be high in low lying areas where high water table was observed during the monsoon (Rawther, 2000).

In diseased plantations, based on the occurrence of yellowing symptoms on leaves, two categories of palms were identified: yellowing affected (YL) and apparently healthy (AH). Apparently healthy palms were the ones that did not show any symptom of yellowing, but situated in a diseased plantation. A third category of completely healthy (CH) palms were identified from healthy plantations, which did not show any symptoms of yellowing.

Rhizosphere soil samples were collected from the active root zone of seven palms from each category for physico-chemical and biological characterization. Physico-chemical analysis included bulk density, pH, EC, organic carbon, N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B and Al. A significantly low value of soil pH was observed in the case of YL, when compared to that of AH and CH (Table 7). Bulk density, EC and organic carbon failed to show any significant difference among the three categories of samples, but the average value of organic carbon was higher in CH than the other two categories. Soil has always been the major source and utilizer of organic carbon in

the environment, hence forming the most dynamic platform for the growth of plants and microorganisms (Tate, 1995). A previous study concerning the nutrient recycling in arecanut plantations with laterite soils with acidic pH revealed that the healthy arecanut soil ecosystem to be high in organic carbon and microbial activity (Balasimha and Rajagopal, 2004). The role of microorganisms in the contribution to the organic carbon in the soil is also considered to be significant, as they not only mediate the decomposition process, but also contribute themselves to form a part of the organic carbon content (Schmidt *et al.*, 2011). Another report also indicated that the organic carbon content was high (2.45 to 2.47%) in arecanut plantations, irrespective of whether high or low yielding (Sujatha and Bhat, 2012).

Among the values of nutrients, Fe and Cu showed significantly higher value in the yellowing affected soil samples. Even though not significant, the concentration of aluminium was higher in YL compared to AH and CH. The concentrations of other nutrients showed no significant differences, most probably due to the heterogeneity among the samples within a category. Previous studies in Vittal region of Karnataka State indicated above optimum levels of micronutrients like Cu, Zn, Fe, Mn, and B in both high and low yielding arecanut plantations (Sujatha and Bhat, 2012). The nutritional imbalance prominent in the low lying lateritic soils with high manganese and iron content were suspected to be the cause of the yellowing syndrome (UAS, 1990).

The content of nutrients in index leaves of all the three categories of palms was also assessed. There was no significant variation in the concentration of any nutrient other than Fe. The concentration of Fe was significantly higher in the category YL (Table 8). The yellowing of other crops like paddy, especially in low-lying areas in the tropics with flooded condition, where the ferric ions are easily converted into soluble ferrous form with the help of soil microflora are very common (Audebert, 2006). Other crops like banana cultivated in paddy lands were also found to be prone to iron toxicity (Suresh, 2005).

The concentration of Ca, though not statistically significant, was found to be higher in CH, followed by AH and then YL. This was exactly an opposite scenario when compared to the concentration of Ca in soil. In soil, a gradation was observed in the Ca concentration from CH to AH to YL. This might indicate the hindrance of Ca uptake by the presence of high Fe and Al in the soil. The uptake of Ca was found to be hindered by the precipitation of Fe and Al in the root tips of plants in acidic soils (Clarkson and Sanderson, 1971; Rorison, 1973). A negative correlation in the Fe concentration and occurrence of yellowing symptom in arecanut was earlier reported by Jacob (2007).

The rhizosphere soil samples were also characterized for biological properties including microbial biomass carbon and population of culturable microflora. Fumigation-extraction method proposed by Vance *et al.*, (1987) was preferred for biomass carbon estimation, as it is considered to provide more accurate values compared to that obtained by direct count method, which may also include necromass values (Schnurer *et al.*, 1985). Microbial biomass carbon is a measure of the weight of microorganisms in soil. It is also used as an indicator of soil quality and depends heavily on management. In the present investigation, microbial biomass carbon did not vary significantly among the samples. The values were generally high in CH when compared to AH and YL. A previous study stated that soil with less pH failed to support acid-intolerant bacteria but supported only acid-tolerant bacteria, which in fact lessened the microbial biomass carbon. An increase in the microbial biomass carbon was observed after liming to elevate the soil pH near neutral to increase the population of acid-intolerant bacteria (Neale *et al.*, 1997). Earlier reports indicated that soil microbial biomass carbon would only show trends in the long term. It may be equivalent to any other soil chemical indicator, in the short-term. However, it changes more quickly than other indicators like soil organic carbon (Hargreaves *et al.*, 2003).

Population of different culturable microflora including bacteria, fungi, actinomycets, N-fixers, P-solubilizers, fluorescent pseudomonads, *Bacillus* and

*Trichoderma* was estimated in all the rhizosphere soil samples. No significant variation in the population of any of these groups was observed, except *Bacillus*. A significantly higher population of *Bacillus* was found in CH, compared to that of AH and YL (Table 12). The presence of *Bacillus*, *Arthrobacter*, *Micrococcus* and *Pseudomonas* in the rhizosphere of arecanut palms has been reported earlier (Bopaiah and Bhat, 1981). Population of *Bacillus* in AH and YL were on par, though AH recorded a higher population. The significant decrease in the population of *Bacillus* spp. in YL might be linked with the soil properties like low pH and high concentration of Fe and Cu.

A slight increase in the population of *Trichoderma* in the AH samples was detected, even though the population between the treatments were not significant. The fungal population was majorly composed of *Trichoderma* spp. and other notable genera like *Aspergillus* spp. and *Fusarium* spp. were also observed. A study conducted by Bopaiah in the year 1990 to analyse the microbiological profile of arecanut rhizosphere revealed the predominance of the aforementioned fungal genera in arecanut palm rhizosphere. Apart from biological control of plant pathogens, *Trichoderma* spp. are considered to mediate availability of phosphorous, iron, magnesium and manganese by the secretion of organic acids thereby decreasing the soil pH (Vinale *et al.*, 2008).

The population of actinomycetes did not show any significant difference among the three categories of samples. The population of phosphate-solubilizers and fluorescent pseudomonads were also observed to be non-significant among the three categories of samples, even though a high average value was observed in CH. The population of nitrogen-fixers were also found to be non-significant with the highest average in AH.

Microbial population and biomass C are two indicators of soil microflora. Even though not significant, a slight increase in the microbial population, in general, was observed in CH, as compared to AH and YL. This corresponded to microbial biomass carbon, which was also highest in CH. The concentration of Fe and Al was found



lowest in CH. A previous report indicated that the presence of Fe and Al hydroxides had an inhibitory effect on the biodegradation of organic matter, especially organic acids, as it formed a protective barrier. It was also observed that the microorganisms possessed the capacity to utilize metal-organic complexes as well as the non-complex forms of organic acids for its metabolism (Jones, 1998).

During sample collection, it was learnt from the farmers that no organic or inorganic amendments were applied in diseased plots, whereas organic manures were annually applied in the healthy gardens. This difference must have led to nutritional and biological imbalance in YL and AH, which might have contributed to the altered rhizosphere microflora. It also emphasizes the need for application of manures in a perennial crop like arecanut, with a high rate of nutrient recycling, to maintain the microbial population and thus, soil health. A systematic practice to improve the health of arecanut palms and to reduce the intensity of yellowing might help the farmers, as most of the plantations are being abandoned after the incidence of yellowing in Wayanad.

Rhizosphere microorganisms play an important role in maintaining the health of plants. The root exudates secreted by the plants are found to mediate the chemoatxis and engineering the rhizosphere microflora (Chaparro *et al.*, 2014). The root exudates of arecanut palms were found to be composed of sugars, amino acids and other organic acids, while phenolic compounds were not detected. The quantity of the root exudates were also found to differ according to soil conditions and cropping pattern (Nagaraja, 1988). Since the rhizosphere microflora is highly dependent on the root exudates, a similar shift in the population dynamics can be observed in parallel with the variation in the release of root exudates with soil conditions and cropping pattern.

The rhizosphere microflora of a plant is considered to be designed as a result of the selective pressures exerted by the biological processes and abiotic factors in the soil (Nunes *et al.*, 2009). The studies on physiological aspects showed variation in the

osmotic potential and chlorophyll content (Jacob, 2007). The change in the physiology of the plant along with the abiotic aspects concerned with the yellowing of arecanut was hypothesized to shape a selective rhizosphere microflora.

After the enumeration of soil microorganisms using dilution plate method, 27 predominant bacterial isolates were obtained, which were purified and used for the study of plant growth promoting activities. These isolates were tested for the production of IAA, HCN, siderophore and ammonia and phosphate solubilization (Table 13). Ten bacterial isolates exhibited the capacity of phosphate solubilization and seven were able to synthesize IAA. The siderophore and HCN producing capacities were found to be very less accounting to only one for siderophore production and two for HCN production. The siderophore producing bacterial isolate was obtained from the sample YL-4, which had sufficient level of iron concentration, but was found to be the sample with lowest iron concentration among the YL category. A previous study reported that Fe-stress-induced system can trigger the production of siderophores in bacteria (Crowley *et al.*, 1991). The isolates AH4FP and YL4NF were identified to be *Burkholderia cenocepacia* and *Caulobacter flavus* respectively. Plant Growth Promoting Rhizobacteria (PGPR) are considered as bacteria prevalent in the rhizosphere region of a plant, capable of improving the growth, nutrient utilization and productivity of the plant (Saharan and Nehra, 2011). The presence of increased number of phosphate solubilizers was detected in arecanut rhizosphere and it was observed to be positively correlated with the arbuscular mycorrhizal population (Ambili *et al.*, 2012). A whole genome sequencing analysis of PGPR from arecanut rhizosphere discovered the presence of IAA producing genomes and presence of phosphate solubilizing bacterial population in the arecanut rhizosphere. (Gupta *et al.*, 2014). The bacterial isolates obtained from the arecanut rhizosphere were found to be able to tolerate low pH in the same study.

As the soil is considered to be a reservoir of microbial resources and 99 per cent of them are unculturable, enumeration of the total bacterial population is not possible



using dilution plate method. Hence, in the present investigation, a culture-independent method using the high-diversity soil DNA for unravelling the total bacterial diversity in the arecanut rhizosphere was done using the three categories: YL, AH and CH. For this purpose, a culture-independent tool called metagenomics was employed. It helps to assess the microbial composition by avoiding the plate count anomaly and provides information of almost 95-99 per cent of the microbial composition of the sample that remain unculturable (Nichols, 2007). The usage of metagenomic methods for the study of microbial population could be used to link the phylogenetic information with the environmental functions (Riesenfeld *et al.*, 2004).

Obtaining good quality metagenomic DNA is the major challenge in metagenomic studies. Basically, there are two methods for DNA isolation: direct and indirect. The difference in the two methods is that cells are directly lysed in the soil itself in the first method, whereas in the second method, the cells are first isolated from the soil and then DNA is extracted. In the present study, direct method of DNA isolation by soft lysis, illustrated by Siddhapura *et al.*, 2010 was initially employed for the metgenomic DNA extraction. Both the long and short procedures were employed to extract the soil DNA. It was found that the DNA obtained was contaminated with higher proportion of impurities like humic acid. The protocol lacked any clean-up or filtration step for removal of humic acid components, which could act as PCR inhibitors. Therefore, MN Nucleospin soil DNA isolation kit was used, which included a combined physical and chemical DNA lysis step followed by filtering of impurities after binding the DNA to the inhibitor removal column. The good quality DNA was then eluted using suitable buffers. The metagenomic DNA obtained was of good quality, devoid of PCR inhibitors as evidenced by a crisp, intact band on agarose gel, and produced 16S rDNA amplicons when compared to the DNA obtained using the direct method.

The lysis method employed in the MN Nucleospin soil DNA isolation kit included the usage of lysis buffers in combination with mechanical lysis using ceramic

beads. This type of combinational lysis was found to be highly efficient in extracting DNA from Gram positive and archaeal cells that are hard to lyse and other cells residing in the inaccessible soil pockets, without shearing the Gram negative DNA (Frostegard *et al.*, 1999). The bead beating method was found to extract high quantity of DNA from samples. While extracting metagenomic DNA, harsh protocols are recommended for samples abundant in Gram positive bacteria and gentle lysis protocol for the samples abundant in Gram negative bacteria. It was also reported that the extraction of DNA from Firmicutes, preferred enzymatic lysis. Even though many protocols are available for the extraction of metagenomic DNA, commercial kits were found to be efficient in providing optimum lysis and removal of PCR-inhibitors and other contaminants (Keisam *et al.*, 2016). To avoid the biases involved in the manual protocols of metagenomic DNA extraction, MN Nucleospin soil DNA isolation kit was employed. A previous study conducted in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara by Ashwini (2016) revealed that the direct method of DNA extraction failed to obtain archaeal sequences, but the usage of MN Nucleospin soil DNA isolation kit in the current investigation provided optimum lysis to obtain the archaeal diversity from the soil samples.

In order to study the community composition of any environmental sample, 16S rRNA gene sequence is generally used. This gene which encodes the 16S ribosomal RNA, is 1500 bp in size and is composed of nine variable regions interspersed between conserved regions. Woese (1987) reported that 16S rRNA gene is the best molecular chronometer with greater resolving power than other oligonucleotides used earlier for cataloguing purposes. 16S rRNA gene was used to classify bacteria based on the difference in the hyper-variable regions V3 and V4 by designing specific primers to amplify the mentioned hyper-variable regions. The 16S rDNA sequences generated using Illumina sequencing was found to be an effective tool in analyzing the diversity and taxonomic assemblage in environmental metagenomes (Logares *et al.*, 2014). The method of classification is considered to be an efficient one even though only 17 per

cent of the sequences are assigned to known bacterial genera. The phylum level diversity has also been analysed and it was previously reported that soil ecosystem was composed of almost 32 bacterial phyla and uncertainties prevailed with the assigning of sequences to known and unknown taxa of Proteobacteria (Janssen, 2006). The importance of the hyper-variable V3 and V4 regions was identified in a study conducted by Claesson *et al.*, (2010), who found that V3/V4 and V4/V5 combinations provided high simulation coverage and accuracy compared to the other combinations. In the present investigation also, the hyper-variable V3 and V4 regions were used for investigating the diversity of bacteria in the rhizosphere of the three categories of arecanut palms by metagenomics.

Metagenomics heavily depends on Next Generation Sequencing (NGS), which is a high throughput sequencing technology. A massive amount of data is generated from NGS, which is analysed using various bioinformatics tools. The NGS technology being applicable in the comprehensive study of diversity has enabled it to be used in various fields including the microbial exploration from intestinal tracts, rumen and soil. However, management of large amount of data produced by NGS processes and the need for the application of highly complex computational algorithms for the study has always been a major drawback of NGS technology (Scholz *et al.*, 2012). Several platforms are available for NGS, which includes 454, Illumina and Ion Torrent. Illumina workflow involves amplification of V3-V4 regions using suitable primers, ligation of adapters and dual indices, followed by library quantification. The denatured library was then subjected to MiSeq sequencing.

The sequences were obtained in Fastq format. The quality of the Fastq sequences were analysed using the parameters like base quality score distributions, average base content and GC distribution. The sequences were trimmed and filtered to remove mismatches, chimeras and singletons to obtain total number of Operational Taxonomic Units (OTUs).

The number of OTUs from all the samples were analysed and it was found that the AH samples had comparatively more number of OTUs than their corresponding YL samples. However, the sample CH-5 had higher number of OTUs than the YL samples, but CH-7 was found to have an intermediate number when compared to the other samples. Operational Taxonomic Unit is generally referred to as a group of related organisms. In the metagenomic sense, an OTU is described as a cluster of 16S gene sequence variant with a minimum of 97 per cent identity threshold at genus level. An increased identity threshold at 98 or 99 per cent is considered to be suitable for species level classification. The total number of OTUs was measured to compare the bacterial population among the samples.

The taxonomical classification of the OTUs thus obtained was done using QIIME programme, which stands for Quantitative Insight Into Microbial Ecology developed and hosted by Knight and Caporaso labs. This site acts as an open source pipeline to graphically represent the bacterial composition provided as raw sequence (Caporaso *et al.*, 2010). In the present study, PyNASt programme, which is a sequence aligner was employed by QIIME to align the reads against the Greengenes core set, that provides annotated, chimera-filtered 16S rRNA sequences (DeSantis *et al.*, 2006).

The tool MG-RAST (Metagenomic analysis by Rapid Annotation using Subsystem Technology) hosted by Argonne Laboratory was used to taxonomically characterize the OTUs using RDP (Ribosomal Database Project) database. RDP is an rRNA sequence database containing ribosomal information of bacterial, archaeal and fungal sequences (Maidak *et al.*, 1994). The taxonomic characterization was also studied using MEGAN (MEtaGenome ANalyzer) that employs NCBI taxonomy to classify the bacterial diversity provided in the input file. The taxonomical composition was studied and graphical representations were obtained using the above mentioned tools (Plate 11).

In the present investigation, among the nine metagenomic DNA samples, two (YL5 and CH2) failed to produce V3 and V4 amplicons and hence, these were eliminated from further studies. This might be due to the presence of traces of PCR inhibitors like humic acid or phenolic compounds. The presence of humic acids and PCR inhibitors were observed in a previous study and it was also observed that the humic acid content was successfully removed using commercial cleaning kits, while the inhibitors were not separated efficiently using the same (Desai and Madamwar, 2007).

The sequences were deciphered using various *in silico* tools. The eukaryotic sequences present in the data obtained due to the amplification of mitochondrial DNA were removed using the filter provided by the *in silico* tools. In the following discussion, the details of individual samples are given, followed by a comparison of bacterial taxa in YL and the corresponding AH sample.

The sample YL-2 had 97 per cent of its total reads belonging to the domain bacteria, 3 per cent eukaryota, and 0.01 per cent archaea. The most abundant bacterial phyla in the sample YL-2 was in the order Bacteroidetes, Actinobacteria, Firmicutes and Proteobacteria, while 36 per cent of sequences belonged to the unclassified phyla (Plate 11a). The Bacteroidetes population in the sample YL-2 was majorly composed of the genus *Terrimonas* (40%), followed by *Chitinophaga* (28%). The phylum Actinobacteria was majorly composed of *Arthrobacter* (41%), followed by *Thermoleophilum* (10%) and the phylum Firmicutes had a comparatively huge proportion of *Bacillus* (59%) and *Clostridium* (8%). The phylum Proteobacteria was dominant in the class Betaproteobacteria with the genus *Massilia* (11%) being the abundant known genus.

A similar proportion of bacteria (97%) was present in the sample AH-2 with 2 per cent eukaryota and 0.2 per cent archaea. The major phyla in AH-2 was found to be Actinobacteria, Firmicutes and Bacteroidetes respectively. About 28 per cent of the



sequences were assigned to the unclassified phyla in this sample (Plate 11c). The sample AH2 had a dominant population of the phylum Actinobacteria majorly composed of *Arthrobacter* (45%) followed by *Thermoleophilum* (7%). An almost similar trend in the Firmicutes population with 58 per cent of *Bacillus* population and 10 per cent *Clostridium* was observed. The primary habitat for the genera *Bacillus* and *Clostridium* was found to be soil and plant ecosystem as most of them are capable to survive as saprophytes. The wide adaptation of the genera was found to enable them in the suppression of plant pathogens (Govindasamy *et al.*, 2010). A growth promotion study in cucumber revealed that *Clostridium* sp. applied to the rhizosphere were found to be comparatively slow growing and able to suppress fungal growth in the vicinity, which was hypothesized to be the direct control exerted by the bacterium on the fungi or indirect effect by the activation anti-fungal native bacteria by the applied *Clostridium* sp. (Polyanskaya *et al.*, 2002). The population of the genus *Massilia* was found to be high in AH-2. The bacterial genus *Massilia* was found to possess PGP characters like IAA and siderophore production, antagonism against *Phytophthora infestans* and cellulose degradation, as they are found in high numbers in plant rhizosphere due to its copiotrophic nature (Ofek *et al.*, 2012).

In the Bacteroidetes phylum, the population of *Terrimonas* (28%) dominated followed by *Chitinophaga* (18%). The genera *Arthrobacter* and *Thermoleophilum* of phylum Actinobacteria were found to be useful in bioremediation of pesticide contaminated soil and capable to degrade a wide range of hydrocarbons (Qingyan *et al.*, 2008; Shivilata and Sathyanarayana, 2015). The genus *Arthrobacter* found in Antarctic soils were found to have psychrotolerant abilities and to possess metabolic adaptability by utilizing a range of carbon sources (Dsouza *et al.*, 2015).

A comparative analysis between the samples YL-2 and AH-2 was done and an increased population of the phyla Acidobacteria, Gemmatimonadetes, Proteobacteria, Planctomycetes, Verrucomicrobia, Actinobacteria and Firmicutes were observed in the sample AH-2. The population of Actinobacteria and Firmicutes were high in the sample

AH-2, while that of Bacteroidetes was high in the sample YL-2. The phyla Nitrospirae, Chloroflexi, Chlorobi and Fibrobacteres were higher in the sample YL2 with only a slight difference in the population. An increased proportion of unclassified bacteria was found in the sample YL-2, while the proportion was comparatively less in that of AH-2 (Plate 12).

The reason behind significantly increased population of the genera *Edaphobacter* in AH-2 and *Flavisolibacter* YL-2 could not be justified, as the exact role in the rhizosphere was not known. The population of *Mucilaginibacter* was found to be very high in the sample AH-2. The genus *Mucilaginibacter* was isolated from the peat soil amended with cellulose, but the *in vitro* studies failed to prove cellulolytic ability (Lopez-Mondejar *et al.*, 2016). The population of the genera *Bradyrhizobium*, *Sphingomonas*, *Burkholderia* and *Bacillus* was high in AH-2, while that of *Massilia* was high in the sample YL-2 (Plate 13).

The metagenomic analysis of the sample YL-7 was done and 99 per cent of the sequence belonged to bacterial domain, 1 per cent eukaryotic and 0.03 per cent archaeal domain. The abundance of bacterial phyla was in the order Actinobacteria (52%), Proteobacteria (14%), Bacteroidetes (6%), Firmicutes (5%) and Acidobacteria (2%) (Plate 11b). The unclassified phyla occupied almost 18 per cent of the total sequences. The phylum Actinobacteria was composed majorly of the genus *Williamsia* (59%), followed by *Gordonia* and *Brevibacterium* (both 10%) and *Microbacterium* (8%). The genus *Microbacterium* was reported to possess PGP characters like IAA, siderophore production, N mobilization and P-solubilization (Ma *et al.*, 2011). The phylum Proteobacteria was majorly composed of the class Alphaproteobacteria (49%) and the genus *Methylobacterium* (33%) was observed to be the most abundant genus. The majority in the phylum Bacteroidetes was the genus *Terrimonas* (16%) followed by *Prevotella* (14%) and *Flavobacterium* (11%). *Prevotella* was found to be of medically importance, as they were found to be an active member of oral microflora (Takahashi, 2005). The phylum Firmicutes was majorly composed of the genus *Bacillus* (49%),



while other genera like *Paenibacillus*, *Clostridium* and *Ruminococcus* shared 4, 5 and 6 per cent of the population. The genus *Williamsia* abundant in the sample YL-7 was reported to be abundant in oil-contaminated ecosystem (Yassin *et al* 2007). The genus *Williamsia* was also reported to have the capacity to degrade PCB (Polychlorinated Biphenyl) and were found to be present in root ecosystem of pine (Leigh *et al.*, 2006).

Almost 98 per cent of the sequence belonged to bacterial domain, with 1 per cent eukaryotic and 0.09 per cent archaeal sequences of the sample AH-7. The most abundant phylum in the sample was Acidobacteria (17%), followed by Actinobacteria (12%) and thirdly Proteobacteria (10%). The unclassified phyla occupied almost 42 per cent of the bacterial population (Plate 11e). The phylum Acidobacteria was less diverse with three genera composing the whole of the phylum. The phylum Actinobacteria was majorly composed of the genus *Thermoleophilum* (19%) followed by *Arthrobacter* (14%) and 10 per cent of genus *Williamsia*. The phylum Proteobacteria was dominant in the class Betaproteobacteria (41%) with 15 per cent *Burkholderia*. Other genera like *Bradyrhizobium*, *Pseudomonas*, *Methylobacterium* etc. were found in minor proportions.

A comparative analysis between the samples YL-7 and AH-7 was done and the phyla Acidobacteria, Proteobacteria, Planctomycetes, Verrucomicrobia and Chloroflexi was abundant in the sample AH-7 compared to YL-7. The population in the phylum Actinobacteria was tremendously high in YL-7 compared to AH-7, while the phyla Bacteroidetes, Chlorobi, Nitrospirae and Firmicutes was slightly higher in the sample AH-7. The proportion of unclassified phyla was found to be very high in the sample AH-7 compared to that of YL-7 (Plate 14).

Among the prominent genera, a few genera namely *Edaphobacter* and *Flavisolibacter* with unknown functions were prevalent in the apparently healthy rhizosphere. These genus *Edaphobacter* has been reported to be very difficult to culture in the laboratory (Koch *et al.*, 2008) and this could be the reason for its unknown

functions in soil. The population of *Mucilaginibacter*, *Methylobacterium*, *Pseudomonas*, *Williamsia* and *Brevibacter* was highly pronounced in the sample YL-7. *Methylobacterium* was also found to occupy a notable population in the arecanut rhizosphere and was found to have endophytic association which resulted in increased photosynthetic activity in tobacco (Andreote *et al.*, 2009). The population of *Pseudomonas* was found to be high in YL-7 than that in AH-7. The genus *Pseudomonas* always had significant role as PGPR and has always been one of the most exploited microorganism in agriculture. Apart from phytohormone production, suppression of plant pathogens by phloroglucinol production reported by Raajimakers and Weller, (1998). The genus was also found to provide Induced Systemic Resistance in the host plants by the production of 2,3-butanediol was also reported (Bakker *et al.*, 2007). Apart from *Pseudomonas*, other bacterial genera namely *Serratia* and *Bacillus* was found to elicit Induced Systemic Resistance in plants (Bakker *et al.*, 2013). The number of *Bradyrhizobium* OTUs in the samples AH-2 and AH-7 were found to be high when compared to their yellowing affected counterparts. Apart from the diazotrophic capabilities and nodulation properties, *Bradyrhizobium* was reported to possess PGP activities like phytohormone production, siderophore production, phosphate solubilization and antagonistic activities in non-host or non-legume rhizospheres (Antoun *et al.*, 1998). The bacteria with predatory nature like *Bdellovibrio* was also found to be high in the sample AH7, in which the number of OTUs were also found to be higher than that of YL7 (Plate 15).

The sample AH5 was left out without its respective yellowing affected metagenomic data due to PCR inhibition. The sample AH5 was analysed for its bacterial diversity and the most abundant bacterial phyla was found to be Actinobacteria (19%), followed by Acidobacteria (12%) and thirdly by Proteobacteria (11%), while the unclassified phyla occupied 32 per cent of the bacterial population (Plate 11d). The genus *Thermoleophilum* (24%) was found to be abundant in the phylum Actinobacteria. The phylum Acidobacteria was composed of three genera and

hence it was less diverse. The class Betaproteobacteria with a majority of unclassified genera was found to be the most abundant class in phylum Proteobacteria. The proportion of unclassified genera from all the classes of the phylum Proteobacteria was found to be high. Other notable genera included *Burkholderia*, *Desulfovibrio* and *Massilia*.

The CH samples being collected from entirely different region of Wayanad, showed different results in the soil physico-chemical analysis and the culturable microflora enumeration, because of this reason, the CH samples were considered as a separate category. The completely healthy samples studied for its bacterial diversity revealed that both the samples were dominant in the phylum Proteobacteria. The sample CH5 was majorly composed of Proteobacteria (23%) followed by Bacteroidetes (14%) and then Actinobacteria (12%) (Plate 11f). The sample CH7 on the other hand had 20 per cent of its bacterial population composed of Proteobacteria, followed by 17 per cent by Actinobacteria. The unclassified phyla contributed to 29 per cent of the bacterial population in the sample CH-5, while it was 46 per cent in the sample CH-7 (Plate 11g). The population of Bacteroidetes accounted only 1 per cent of the bacterial population. An abundance of Gammaproteobacteria was observed in the sample CH-5, while in CH-7, Deltaproteobacteria was the abundant class of the phylum Proteobacteria. The genera *Pedobacter*, *Sphingobacterium*, *Terrimonas* and *Chitinophaga* were predominant in Bacteroidetes phylum in the sample CH-5. Genus *Chitinophaga* was reported to be a free living saprophytic bacterium and a prolific degrader of plant derived polysaccharides (McKee and Brumer, 2015). It was reported to secrete diverse glycoside hydrolases for the utilization of complex polysaccharides. The genus *Arthrobacter* dominated in the phylum Actinobacteria of the sample CH-7. Apart from the previously mentioned soil activities, a few members of the genus *Arthrobacter* was reported to be capable of solubilizing phosphorus in the soil (Chen *et al.*, 2006).

The phylum Proteobacteria was found to be abundant in the AH samples than that of its corresponding YL samples. The population was also found to be high in the CH samples. Among all the bacterial phyla, the phylum Proteobacteria was found to be the most versatile, diverse and dominant one in rhizosphere ecosystems (Sanguin *et al.*, 2006). A diversity analysis of disease suppressive soil revealed the greater proportion of the phyla Proteobacteria and Firmicutes varying from 20 to 39 per cent of the population respectively was reported (Plate 16). The classes Gamma and Beta Proteobacteria of the phylum Proteobacteria, while the family Lactobacillaceae of Firmicutes, were found to play dynamic roles in disease suppression (Mendes *et al.*, 2011). The genus *Burkholderia* was also found to be notably important in the diversity of arecanut palm rhizosphere. Apart from the clinical and minor phytopathological roles of a few members of the genera, they are known for its capacity to produce antimicrobial agents, siderophore and elicit other PGP activities like nitrogen fixation, iron sequestration, phosphate solubilization and phytohormone production (Compant *et al.*, 2008). Genus like *Cellulosimicrobium* found in low proportion were also found to exhibit plant growth promoting activities like IAA production and P-solubilization. Other properties like cellulolytic and xylanolytic activities were also reported. (Chatterjee *et al.*, 2009; Bakalidou *et al.*, 2002).

Apart from these major phyla, Acidobacteria and Verrucomicrobia were also found to form an important portion of the arecanut microbiome. A possible hypothesis would be that the capacity of Acidobacteria to tolerate lower pH might have been the reason of its abundance in the samples, since the lateritic soils of Wayanad was found to be highly acidic. The samples AH-7 and CH-5 had comparatively higher number of Acidobacteria and the pH of the samples were 4.9 and 6.1 respectively. It was also reported that the competition in the rhizosphere was a matter of less concern for phylum Acidobacteria (Da Rocha *et al.*, 2013), while another study revealed that OTUs of Verrucomicrobia and Betaproteobacteria to be highly sensitive to the changing soil conditions like temperature (Campbell and Kirchman, 2013). The preference of

Acidobacteria to oligotrophic habitats was observed and the ratio of Proteobacteria to Acidobacteria was found to be higher in copiotrophic ecosystems, hence a selective increase in the Acidobacterial population was observed in the oligotrophic habitats (Lee *et al.*, 2008). The ratio of Proteobacteria to Acidobacteria for the samples AH2, AH5 and AH7 were 1.57, 0.9 and 0.58 respectively, while the ratio was higher than 4 in the case of completely healthy and yellowing affected rhizosphere soils indicating a minor possibility of elevated copiotrophic nature of the yellowing affected and healthy rhizosphere. Regardless of this ratio, the composition of bacterial taxa in whole might play a huge role in mediating the health of arecanut palms.

The unclassified taxa were found to be abundant in all the samples, the proportion was less in the domain level, but was found to be very high in phylum level. The percentage of unclassified genera particularly in the phylum Proteobacteria was found to be very high than any other bacterial phyla. The population of the unclassified phyla was found to be high in the AH samples compared that of its corresponding YL samples. A random pattern was observed in the population of unclassified taxa in the CH samples. This might indicate that the majority of the soil bacteria are undiscovered and the activity of these bacteria are still unknown.

The archaeal population in the samples were found to follow a random pattern, but an exceptionally high proportion was observed in AH-5 compared to other samples. The study of archaeal diversity from the samples revealed that the AH rhizospheres were abundant in the population of phylum Thaumarchaeota when compared to that of YL. The AH and CH rhizospheres were observed to harbor higher number of Thaumarchaeota than that by YL rhizospheres (Plate 17). The phylum was composed of only one genera *Candidatus Nitrososphaera* in all the seven samples. The members of phylum Thaumarchaeota was found to be active ammonia oxidizers and the production of oxidized nitrogen species as a result of the action of Thaumarchaeota was found to inhibit the growth of methanogens. This phenomenon was observed mainly in paddy fields (Ke *et al.*, 2014).



The total number of bacterial taxa at each taxonomic level was listed to compare the diversity of all the seven samples. A comparison between YL and the corresponding AH samples revealed that the number of taxa was high in the AH samples and hence the diversity. The number of taxa in the CH samples were also analysed, but it showed values intermediate to that of the other samples. The rarefaction analysis was also done using the MG-RAST pipeline and it was observed that the AH samples were more diverse than its corresponding YL samples (Plate 18a and b). The analysis of the samples was also done and the curve was found to be higher in AH-7, indicating the highest diversity (Plate 18c).

The analysis of the prokaryotic diversity including archaeal diversity from the seven samples using Shannon-Weaver and Simpson indices at phylum level indicated that the diversity was high in the apparently healthy rhizosphere when compared to that of their yellowing affected counterpart (Table 32). Shannon-Weaver index, being an information statistic index, defines the diversity in a sample, while Simpson index is considered to be a dominance index measuring the diversity with the priority given to the dominant taxa. The highly diverse bacterial assemblage in the apparently healthy rhizosphere might indicate that the healthy plants have the ability to support a wide range of bacterial taxa when compared to that of yellowing affected ones. The diversity in the sample CH-5 was found to be higher than that of YL, but intermediary to AH samples, while that of CH7 was found to be less than that of all the other samples.

From the present investigation, it is evident that, in general, the yellowing affected plantations were situated in low lying areas. The parameters like bulk density and EC was found to be not significant. The YL samples were of low pH, high Fe and Cu and non-significantly high Al, while AH and CH were found to possess slightly high pH and comparatively low Fe and Cu. Even though statistically not significant, the organic carbon content was found to be little high in the CH samples. Other nutrients were not found to play any significant role in expression of yellowing in arecanut. The increased Fe and low Ca concentration in YL leaf samples also indicated

the possibility of the hindrance of Ca uptake by high Fe and Al in the soil. The microbial biomass carbon was not significantly different among the three categories, however it showed a slightly high value in CH samples and the corresponding value to that of the culturable microflora was also observed. The culturable microflora was found to be not significantly different except *Bacillus* with a high population in CH. The population of *Trichoderma* was found to be more in the AH samples, even though it was not statistically significant.

The bacterial diversity analysis using metagenomics revealed that the population of Acidobacteria, Verrucomicrobia, Proteobacteria and Gemmatomonadetes to be high in AH samples than their corresponding YL samples. The phyla Bacteroidetes, Firmicutes and Actinomycetes followed a random trend in its population. The proportion of the unclassified phyla was also observed to follow a random pattern. An increased population of Thaumarchaeota of domain archaeobacteria was found to be high in the AH samples than their corresponding YL samples. The population of the genera *Edaphobacter*, *Flavisolibacter*, *Arthrobacter* and *Mucilaginibacter* was found to follow a random pattern, while that of *Sphingomonas*, *Burkholderia* and *Bradyrhizobium* was found to be high in AH samples than that of their corresponding YL samples. The population of *Massilia* was found to be high in the YL samples than that of their corresponding AH samples. The population of different members of the Bacillaceae family was also found to be dynamic in the rhizosphere of the three categories of palms. Genera like *Bacillus*, *Anoxybacillus*, *Oceanobacillus*, *Lysinibacillus*, *Brevibacillus* and *Paenibacillus* were observed in all the samples.

A difference in a few important bacterial taxa and one archaeal taxa was found to vary among the three categories of samples and this variation was hypothesized to be linked with the expression of yellowing of arecanut palms. To confirm this hypothesis, more samples are needed to be studied and a detailed investigation on the bacterial composition and ecological function in mediating the availability of nutrients



and improving plant health is needed to be done. The proper management of native microflora, emphasizing the multi-partite interactions to be used for improving the plant health should be done in future. The role of unclassified bacterial taxa needs to be found out to know whether they act as an aggravator of the condition or beneficiary in reducing the symptom expression of yellowing in arecanut. The role of the unclassified bacterial taxa can only be understood by doing whole metagenome sequencing to identify the presence of functional genes and comparing it with a suitable genomic database. The role of endophytic microorganisms was also reported to be very important in mediating plant health. Hence, a need for the study of endophytic microorganisms associated with arecanut palms along with the rhizosphere microorganisms as a continuum may be attempted in future, because these two cannot be considered independently. Nutrient management based on soil analysis and the enhancement of the native microflora using proper application of organic amendments to improve soil health may be considered to improve the health of the arecanut palms and reduce the expression of yellowing.

# SUMMARY

## 6. Summary

The study on “Metagenomic analysis of bacterial diversity in the rhizosphere of arecanut palms affected by yellowing in Wayanad” was carried out in the Department of Agricultural Microbiology and Radio-Tracer Laboratory, College of Horticulture, Vellanikkara, during the period 2015-2017. The objective of the study was to analyse the bacterial diversity in the rhizosphere of yellowing affected and healthy arecanut palms in Wayanad using metagenomics. The salient findings of the study are summarized below:

- Yellowing affected were collected from Meenangadi and Kakkavayal and healthy arecanut plantations from Ambalavayal and Kolagappara were identified with the help of the officials from the Department of Agriculture, Kalpetta and scientists from Regional Agricultural Research Station (RARS), Ambalavayal.
- Rhizosphere soil samples were collected from yellowing affected, apparently healthy palms and completely healthy arecanut palms. Seven samples from each category of yellowing affected, apparently healthy and completely healthy (YL, AH and CH respectively) was collected for further analysis. The coordinates and altitudes were noted. It was observed that the yellowing affected plantations were situated in converted paddy lands of lower elevations, while healthy plantations were situated at higher elevations.
- The index leaves (middle portion of the fourth leaf from the apex) from the arecanut palms were also collected and processed for the nutrient analysis.
- The soil samples were processed and subjected to physico-chemical analysis and it was found that the YL samples were significantly low in pH and high in the concentration of Fe and Cu. Even though statistically not significant, a higher percentage of organic carbon was detected in CH samples and the concentration of Al was found high in YL than AH and CH. A higher concentration of Ca was found in YL samples followed by AH and then CH.

- The leaf samples were also analysed for the nutrient content and a significantly increased concentration of Fe was found in YL samples. Even though not significant, a low concentration of Ca was detected in YL samples followed by AH and the CH.
- After the analysis of the physico-chemical properties of the soil and nutrient concentration in leaf samples, the analysis of the biological characteristics was done. It included microbial biomass carbon and population of culturable microflora. The microbial biomass carbon was estimated using fumigation-extraction method and it was found to be not significant among the three categories, but the average value was found to be high in CH.
- The microbial population was enumerated using dilution plate method and the population of bacteria, fungi, actinomycetes, N-fixers, P-solubilizers, fluorescent pseudomonads, *Bacillus* and *Trichoderma* was estimated. Even though not significant, a slight increase in the average population of bacteria and fungi was observed in CH samples and an increased population of *Trichoderma* in the AH samples were also detected. A significantly high population of *Bacillus* was found in CH samples.
- The microbial enumeration was done and 27 predominant bacterial isolates were obtained, which were then subjected to PGP characterization like IAA, HCN, siderophore and ammonia production and phosphate solubilization. Among the 27 isolates, 10 showed phosphate solubilization, seven showed IAA production, 15 showed ammonia production, one isolate showed siderophore production and two HCN production. The isolate AH4FP was found to show the highest P-solubilization and the isolate CH2BC showed highest IAA production after the quantification.
- After the analysis of microbial biomass C and enumeration of microbial population from the soil samples, the diversity of culturable and unculturable bacteria was analysed using a culture-independent method called as

metagenomics. The metagenomic DNA was extracted using MN Nucleospin soil DNA isolation kit, as the direct method experimented showed a high concentration of humic acids. The metagenomic DNA samples were subjected to qualitative and quantitative analysis using agarose gel electrophoresis and spectrophotometry. Nine samples were subjected for metagenomic analysis, in which two samples failed to produce V3-V4 amplicons most probably due to the presence of PCR-inhibitors. The rest of the seven samples were used for the analysis.

- The amplicons were subjected to various processes like PCR-cleanup and index PCR followed by library quantification and denaturation. It was then subjected to Illumina sequencing after loading into the MiSeq sequencer. The raw sequences were obtained in Fastq format.
- The sequences were subjected for qualitative analysis like determination of GC composition and base composition. It was further subjected to various filtration steps to remove mismatch reads, chimeric sequences and singletons to obtain the total number of OTUs. The total number of OTUs were observed and it was found that the AH samples had comparatively higher number of OTUs compared to that of YL. The number of OTUs in the CH samples were found to show an intermediate value to the other samples.
- The taxonomic assemblage of the bacterial diversity was analysed using the bioinformatics tools QIIME, which uses PyNAST programme to align the sequence against the Greengenes core set. The *in silico* tool, MG-RAST and MEGAN was also used to obtain the bacterial diversity by using RDP database and NCBI taxonomy respectively.
- The domain level comparison indicated that the AH samples had higher bacterial population than that of their corresponding YL samples. The archaeal population was also found to be higher in AH samples than the YL samples.

The bacterial and archaeal population in the CH samples were found to be intermediary to the other samples.

- The sample YL-2 was studied for its bacterial diversity and it was found that the major phyla included Bacteroidetes, Actinobacteria, Firmicutes, Proteobacteria, Acidobacteria and Verrucomicrobia. The unclassified phyla was found to occupy 36 per cent of the total population. The predominant genera in the sample was found to be *Citinophaga*, *Terrimonas*, *Arthrobacter*, *Thermoleophilum*, *Bacillus*, *Clostridium*, *Massilia*, *Burkholderia* and *Bradyrhizobium*. The genera *Bacillus*, *Clostridium* and *Massilia* was reported to sow PGP activities, while *Chitinophaga* was found to be a chitin destroyer.
- The sample AH-2 was analysed for bacterial diversity and the major phyla was found to be Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Acidobacteria and Verrucomicrobia. The unclassified phyla was found to occupy 28 per cent of the total population. The major genera observed were *Terrimonas*, *Arthrobacter*, *Thermoleophilum*, *Chitinophaga*, *Bacillus*, *Burkholderia*, *Ralstonia* and *Clostridium*.
- A comparison between the samples YL-2 and AH-2 was done and an increased population of the phyla Acidobacteria, Bacteroidetes, Gemmatimonadetes, Proteobacteria, Planctomycetes, Verrucomicrobia, Actinobacteria and Firmicutes were observed in the sample AH-2, while that of Bacteroidetes was found to be high in YL-2. The population of the genera *Sphingomonas*, *Mucilaginibacter*, *Bradyrhizobium*, *Burkholderia* and *Bacillus* was found to be high in AH-2, while that of *Massilia* was found to be high in YL-2. The genus *Bradyrhizobium* was found to enhance plant growth in non-host or non-legume plants also.
- The sample YL-7 was found to be dominant in phyla Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes, Acidobacteria and Verrucomicrobia. The unclassified phyla was found to be only 16 per cent of

the total bacterial population. The population of *Williamsia* of phylum Actinobacteria was found to be very high in the sample and it was reported to be present in oil contaminated sites and used for bioremediation of PCB contaminated soils. Other genera including *Gordonia*, *Brevibacter*, *Methylobacterium*, *Prevotella* and *Terrimonas* were also found to be prevalent in the sample.

- The sample AH-7 was found to be abundant in the phyla Acidobacteria, Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes and Verrucomicrobia. The unclassified phyla occupied 42 per cent of the total population. The major genera included *Arthrobacter*, *Thermoleophilum*, *Williamsia*, *Burkholderia*, *Pseudomonas*, *Bradyrhizobium* and *Methylobacterium*. The genus *Pseudomonas* was reported to exhibit PGP activities and elicit ISR in host plants, while *Methylobacterium* was also reported to act as an endophyte with plant growth promotion activities. The PGP activities of *Burkholderia* was also reported earlier.
- A comparison between YL-7 and AH-7 revealed an increased proportion of Acidobacteria, Proteobacteria, Planctomycetes and Verrucomicrobia in AH-7, while the population of Actinobacteria was found to be tremendously high in YL-7. The population of *Mucilaginibacter*, *Methylobacterium*, *Pseudomonas*, *Williamsia* and *Brevibacter* was observed to be very high in the sample YL-7, while that of *Burkholderia*, *Pseudomonas* and *Bradyrhizobium* was found to be high in AH-7.
- The sample AH-5 was analysed and it was left out without its corresponding YL sample. The abundant phyla was found to be Actinobacteria, Acidobacteria, Firmicutes, Proteobacteria, Bacteroidetes and Verrucomicrobia. The unclassified phyla occupied 32 per cent of the total population. The notable genera in the sample was found to be *Burkholderia*, *Desulfovibrio* and *Massilia*.



- The completely healthy samples (CH-5 and CH-7) were analysed for the bacterial diversity and it was found that the total bacterial population was intermediary to the other samples. The major phylum in both the samples was found to be Proteobacteria. The other major phyla in the sample CH-5 was found to be Bacteroidetes Actinobacteria, Firmicutes, Acidobacteria and Verrucomicrobia, while that of CH-7 was in the order Actinobacteria, Firmicutes, Acidobacteria, Verrucomicrobia and Bacteroidetes. The unclassified phyla was found to be 29 per cent in CH-5, while it was 46 per cent in CH-7. An abundance of class Gammaproteobacteria was observed in CH-5 and that of Betaproteobacteria was found in CH-7. The class Gammaproteobacteria was reported to be an active member of disease suppressive soils. The genera *Pedobacter*, *Sphingomonas*, *Terrimonas* and *Chitinophaga* was found to be abundant in CH samples.
- The abundance of phylum Proteobacteria in the healthy rhizospheres were observed and it was earlier reported that the members of the phylum to be an active part of soil microflora and known for their versatility in utilizing a wide range of organic compounds. The abundance of Acidobacteria was also observed and it was considered to be because of its preference for low pH, as the soil samples were found to be acidic in nature.
- The ratio of Proteobacteria to Acidobacteria was calculated from the samples and low values were obtained for AH samples, while it was higher than four in YL and CH samples. It was reported that higher the ratio, the more copiotrophic was the soil.
- The population of phylum Thaumarchaeota of domain archaeobacteria was also found to be high in the healthy rhizosphere and they were reported to be active ammonia oxidizers, especially important in paddy lands, where the population of methanogens are reduced by the oxidation of ammonia.

- The diversity of the bacterial population was analysed from all the samples using the total number of bacterial taxa at each taxonomic level and also diversity indices like Shannon-Weaver and Simpson indices at phylum level. It was found that the AH samples were found to be diverse than the corresponding YL samples and the diversity of CH samples were found to be intermediary to the other samples.
- The study was concluded by understanding the difference in soil properties and bacterial diversity among the three categories of soil samples. It was observed that more samples need to be studied, with the analysis of their functional and ecological role to find the bacterial composition variation and the expression of arecanut yellowing in Wayanad. A whole metagenome study of the arecanut rhizosphere should be done to understand the functions of unclassified taxa. A need for the study of endophytes in arecanut palms in relation with the rhizosphere microflora as a continuum was also identified in the current investigation. The manipulation of native microflora by enhancing the soil conditions and proper nutrient management after soil analysis could improve the health of the arecanut palms and reduce the incidence of yellowing up to a limit.

## REFERENCES

## REFERENCES

- Ahmad, F., Ahmad, I., and Khan, M.S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Res.* 163(2): 173-181.
- Aiyer, A.K.Y.N. 1966. *Field Crops of India* (6<sup>th</sup> Ed.). Bangalore Printing and Publishing Co. Ltd., Bangalore, 564p.
- Alabouvette, C. 1986. *Fusarium*-wilt suppressive soils from the Châteaurenard region: review of a 10-year study. *Agronomie* 6(3): 273-284.
- Alabouvette, C. 1999. *Fusarium* wilt suppressive soils: an example of disease-suppressive soils. *Australasian Plant Pathol.* 28(1): 57-64.
- Ambili, K., Thomas, G.V., Indu, P., Gopal, M., and Gupta, A. 2012. Distribution of arbuscular mycorrhizae associated with coconut and arecanut based cropping systems. *Agric. Res.* 1(4): 338-345.
- Andreote, F.D., Carneiro, R.T., Salles, J.F., Marcon, J., Labate, C.A., Azevedo, J.L., and Araújo, W.L. 2009. Culture-independent assessment of Rhizobiales-related Alphaproteobacteria and the diversity of *Methylobacterium* in the rhizosphere and rhizoplane of transgenic eucalyptus. *Microbial Ecol.* 57(1): 82-93.
- Antoun, H., Beauchamp, C.J., Goussard, N., Chabot, R., and Lalande, R. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). *Plant Soil* 204(1): 57-67.

- Ashwini, S.P. 2016. Metagenomics to assess bacterial diversity in the soil as influenced by organic and chemical inputs. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 90p.
- Audebert, A. 2006. Iron partitioning as a mechanism for iron toxicity tolerance in lowland rice. In: Audebert, A., Narteh, L.T., Kiepe, P., Millar, D., and Beka, B. (eds). *Iron Toxicity in Rice-Based System in West Africa*. WARDA, Cotonou, pp. 41-53.
- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., and Vivanco, J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Ann. Rev. Plant Biol.* 57: 233-266.
- Bakalidou, A., Kämpfer, P., Berchtold, M., Kuhnigk, T., Wenzel, M., and König, H. 2002. *Cellulosimicrobium variabile* sp. nov., a cellulolytic bacterium from the hindgut of the termite *Mastotermes darwiniensis*. *Int. J. Syst. Evolutionary Microbiol.* 52(4): 1185-1192.
- Bakker, P.A., Doornbos, R.F., Zamioudis, C., Berendsen, R.L., and Pieterse, C.M. 2013. Induced systemic resistance and the rhizosphere microbiome. *Plant Pathol. J.* 29(2): 136-143.
- Bakker, P.A., Pieterse, C.M., and Van Loon, L.C. 2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. *Phytopathology* 97(2): 239-243.
- Balasimha, D. and Rajagopal, V. 2004. *Arecanut*. Karavali Colour Cartons Ltd. Mangalore, 313p.
- Barea, J.M., Pozo, M.J., Azcon, R., and Azcon-Aguilar, C. 2005. Microbial co-operation in the rhizosphere. *J. Exp. Bot.* 56(417): 1761-1778.

- Bashan, Y. and Holguin, G. 1998. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (Plant growth-promoting bacteria) and PGPB. *Soil Biol. Biochem.* 30(8): 1225-1228.
- Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* 47(2): 151-163.
- Becker, P.M. and Stottmeister, U. 1998. General (Biolog GN) versus site-relevant (pollutant-dependent) sole-carbon-source utilization patterns as a means to approaching community functioning. *Can. J. Microbiol.* 44(10): 913-919.
- Bellary, S.M. and Patil, V.C. 2010. Agronomic practices adopted by arecanut farmers in Koppa and Sringeri taluks. *Karnataka J. Agric. Sci.* 18(3): 791-793.
- Berendsen, R.L., Pieterse, C.M., and Bakker, P.A. 2012. The rhizosphere microbiome and plant health. *Trends Plant Sci.* 17(8): 478-486.
- Berger, K.C. and Truog, E. 1939. Boron determination in soils and plants. *Ind. Eng. Chem. Anal. Ed.* 11(10): 540-545.
- Bertrand, H., Poly, F., Lombard, N., Nalin, R., Vogel, T.M., and Simonet, P. 2005. High molecular weight DNA recovery from soils prerequisite for biotechnological metagenomic library construction. *J. Microbiological Methods* 62(1): 1-11.
- Bhat, K.S. 1978. Agronomic research in arecanut - a review. *J. Plant. Crops* 6: 67-80.
- Bhat, K.S. and Khader, A.K.B. 1982. Crop Management. B. Agronomy. In: Bavappa, K.V.A., Nair, M.K., and Prem Kumar, T. (eds), *The Arecanut Palm*. CPCRI, Kasaragod, pp. 105-131.

- Bhat, R. and Sujatha, S. 2004. Recent advances in areca crop production. In: *Souvenir and proceedings of national workshop on Arecanut Production- Aspects and prospects*, Oct. 11-12, 2004. Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka, India, pp. 19-23.
- Bhat, R. and Sujatha, S. 2013. Establishing leaf nutrient norms for arecanut by boundary line approach. *J. Plant Nutr.* 36(6): 849-862.
- Bopaiah, B.M. 1990. Microbiological and enzyme activities profile in the root zone and interspace soils of coconut and arecanut palms. *J. Plant. Crops* 18(1): 50-54.
- Bopaiah, B. M. and Bhat, N. T. 1981. Effect of application of manures and fertilizers on rhizosphere microflora in arecanut palm. *Plant Soil* 63: 497-499.
- Campbell, B.J. and Kirchman, D.L. 2013. Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. *ISME J.* 7(1): 210-220.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., and Huttley, G.A. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7(5): 335-336.
- Cappuccino, J.C. and Sherman, N. 1992. *Microbiology: A Laboratory Manual*, Wesley Publication, New York, 179p.
- Cavaglieri, L., Orlando, J., and Etcheverry, M. 2009. Rhizosphere microbial community structure at different maize plant growth stages and root locations. *Microbiological Res.* 164(4): 391-399.



- Chandramohanam, R. and Nair, B.P. 1985. Frequency of occurrence and intensity of yellow leaf disease of arecanut (*Areca catechu* L.). In: Bhat, K.S. and Nair, R.C.P (eds), *Arecanut Research and Development*. CPCRI, Kasargod, pp. 104-106.
- Chaparro, J.M., Badri, D.V., and Vivanco, J.M. 2014. Rhizosphere microbiome assemblage is affected by plant development. *ISME J.* 8(4): 790.
- Chaparro, J.M., Sheflin, A.M., Manter, D.K., and Vivanco, J.M. 2012. Manipulating the soil microbiome to increase soil health and plant fertility. *Biol. Fertil. Soils* 48(5): 489-499.
- Chatterjee, S., Sau, G.B., and Mukherjee, S.K. 2009. Plant growth promotion by a hexavalent chromium reducing bacterial strain, *Cellulosimicrobium cellulans* KUCr3. *World J. Microbiol. Biotechnol.* 25(10): 1829-1836.
- Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., and Young, C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* 34(1): 33-41.
- Chowdappa, P. and Balasimha, D. 1992. Non-stomatal inhibition of photosynthesis in arecanut palms affected with yellow leaf disease. *Indian Phytopathol.* 45: 312-315.
- Chowdappa, P., Iyer, R., and Gunasekaran, M. 2002. Plant Pathology Research at CPCRI. Central Plantation Crop Research Institute, Kasaragod, Kerala, India, 113p.
- Clarkson, D.T. and Sanderson, J. 1971. Inhibition of the uptake and long-distance transport of calcium by aluminium and other polyvalent cations. *J. Exp. Bot.* 22(4): 837-851.

- Classen, A.T., Boyle, S.I., Haskins, K.E., Overby, S.T., and Hart, S.C. 2003. Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soils. *FEMS Microbiol. Ecol.* 44(3): 319-328.
- Claesson, M.J., Wang, Q., O'sullivan, O., Greene-Diniz, R., Cole, J.R., Ross, R.P., and O'toole, P. W. 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Res.* 38(22): 200.
- Compant, S., Nowak, J., Coenye, T., Clément, C., and AitBarka, E. 2008. Diversity and occurrence of *Burkholderia* spp. in the natural environment. *FEMS Microbiol. Rev.* 32(4): 607-626.
- Cook, R.J. and Baker, K.F. 1983. *The Nature and Practice of Biological Control of Plant Pathogens*. American Phytopathological Society, New York, 539p.
- CPCRI [Central Plantation Crops Research Institute]. 2000. *Annual Report 1999-2000*. Central Plantation Crops Research Institute, Kasaragod, 160p.
- CPCRI [Central Plantation Crops Research Institute]. 2013. *Annual Report 2012-2013*. Central Plantation Crops Research Institute, Kasaragod, 166p.
- CPCRI [Central Plantation Crops Research Institute]. 2015. *Annual Report 2014-2015*. Central Plantation Crops Research Institute, Kasaragod, 152p.
- Crowley, D.E., Wang, Y.C., Reid, C.P.P., and Szaniszlo, P.J. 1991. Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant Soil* 130(1): 179-198.
- Da Rocha, U.N., Plugge, C.M., George, I., Van Elsas, J.D., and Van Overbeek, L.S. 2013. The rhizosphere selects for particular groups of Acidobacteria and Verrucomicrobia. *PLoS One* 8(12), p.e82443.

- Da Rocha, N.U., Van Overbeek, L., and Van Elsas, J.D. 2009. Exploration of hitherto-uncultured bacteria from the rhizosphere. *FEMS Microbiol. Ecol.* 69(3): 313-328.
- Daniel, R. 2005. The metagenomics of soil. *Nat. Rev. Microbiol.* 3(6): 470-478.
- De Leij, F.A.A.M., Whipps, J.M., and Lynch, J.M. 1994. The use of colony development for the characterization of bacterial communities in soil and on roots. *Microbial Ecol.* 27(1): 81-97.
- DeLong, E.F. 1998. Everything in moderation: archaea as 'non-extremophiles'. *Curr. Opinion Genet. Dev.* 8(6): 649-654.
- Desai, C. and Madamwar, D. 2007. Extraction of inhibitor-free metagenomic DNA from polluted sediments, compatible with molecular diversity analysis using adsorption and ion-exchange treatments. *Bioresource Technol.* 98(4): 761-768.
- DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., and Andersen, G.L. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72(7): 5069-5072.
- Dsouza, M., Taylor, M.W., Turner, S.J., and Aislabie, J. 2015. Genomic and phenotypic insights into the ecology of *Arthrobacter* from Antarctic soils. *BMC Genomics* 16(1): 36.
- FAO [Food and Agriculture Organization] 2017. FAOSTAT homepage [online]. Available: <http://faostat3.fao.org/home/index.html#DOWNLOAD> [07-03-2017].
- Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Quantification of bias related to

the extraction of DNA directly from soils. *Appl. Environ. Microbiol.* 65(12): 5409-5420.

Garbeva, P.V., Van Veen, J.A., and Van Elsas, J.D. 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Ann. Rev. Phytopathol.* 42: 243-270.

Gardener, B.B.M. and Weller, D.M. 2001. Changes in populations of rhizosphere bacteria associated with take-all disease of wheat. *Appl. Environ. Microbiol.* 67(10): 4414-4425.

Garland, J.L. and Mills, A.L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl. Environ. Microbiol.* 57(8): 2351-2359.

George, M.V., Mathew, J., and Nagaraj, B. 1980. Indexing the yellow leaf disease of arecanut. *J. Plant. Crops* 8: 82-85.

Govindasamy, V., Senthilkumar, M., Magheshwaran, V., Kumar, U., Bose, P., Sharma, V., and Annapurna, K. 2010. *Bacillus* and *Paenibacillus* spp.: potential PGPR for sustainable agriculture. In: *Plant growth and health promoting bacteria*, Springer, Berlin, Heidelberg, pp. 333-364.

Graham, J.H., Hodge, N.C., and Morton, J.B. 1995. Fatty Acid methyl ester profiles for characterization of glomalean fungi and their endomycorrhizae. *Appl. Environ. Microbiol.* 61(1): 58-64.

Griffiths, B.S., Ritz, K., Ebbelwhite, N., and Dobson, G. 1998. Soil microbial community structure: effects of substrate loading rates. *Soil Biol. Biochem.* 31(1): 145-153.

- Gupta, A., Gopal, M., Thomas, G.V., Manikandan, V., Gajewski, J., Thomas, G., Seshagiri, S., Schuster, S.C., Rajesh, P., and Gupta, R. 2014. Whole genome sequencing and analysis of plant growth promoting bacteria isolated from the rhizosphere of plantation crops coconut, cocoa and arecanut. *PloS One* 9(8): p.e104259.
- Handelsman, J. 2004. Metagenomics: application of genomics to uncultured microorganisms. *Microbiol. Mol. Boil. Rev.* 68(4): 669-685.
- Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J., and Goodman, R.M. 1998. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem. Biol.* 5(10): 245-249.
- Hargreaves, P.R., Brookes, P.C., Ross, G.J.S., and Poulton, P.R. 2003. Evaluating soil microbial biomass carbon as an indicator of long-term environmental change. *Soil Biol. Biochem.* 35(3): 401-407.
- Hart, M.G.R. 1961. A turbidimetric method for determining elemental sulphur. *Analyst* 86(1024): 472-475.
- Hesse, P.R. 1971. *A Textbook of Soil Chemical Analysis*. Cambridge University Press, 520p.
- Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T., and Nelson, E.B. 2000. Methods for assessing the composition and diversity of soil microbial communities. *Appl. Soil Ecol.* 15(1): 25-36.
- Hirsch, A.M., Bauer, W.D., Bird, D.M., Cullimore, J., Tyler, B., and Yoder, J.I. 2003. Molecular signals and receptors: controlling rhizosphere interactions between plants and other organisms. *Ecology* 84(4): 858-868.

- Hoper, H. and Alabouvette, C. 1996. Importance of physical and chemical soil properties in the suppressiveness of soils to plant diseases. *Eur. J. Soil Biol.* 32(1): 41-58.
- Hornby, D. and Bateman, G.L. 1997. Potential use of plant root pathogens as bioindicators of soil health. In: Pankhurst, C., Doube, B.M., and Gupta, V.V.S.R. (eds), *Biological Indicators of Soil Health*. CAB International, Wallingford, UK, pp. 179-200.
- Huson, D.H., Auch, A.F., Qi, J., and Schuster, S.C. 2007. MEGAN analysis of metagenomic data. *Genome Res.* 17(3): 377-386.
- Ibekwe, A.M. and Kennedy, A.C. 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. *FEMS Microbiol. Ecol.* 26(2): 151-163.
- ICAR-CPCRI [Indian Council of Agricultural Research-Central Plantation Crops Research Institute] 2015. *Vision 2050*. Central Plantation Crops Research Institute, Kasaragod, 88p.
- Jackson, M.L. 1958. *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs. 498p.
- Jackson, M. L. 1973. *Soil Chemical Analysis* (2<sup>nd</sup> Ed.). Prentice hall of India, New Delhi, 498p.
- Jacob, D. 2007. Nutritional management of yellowing in arecanut. Ph.D (Ag) thesis, Kerala Agricultural University, Thrissur. 259p.
- Jacob, D., George, M., and John, P.S. 2014. Effect of nutrient management on leaf chlorophyll and productivity of yellowing affected arecanut (*Areca catechu* L.). *Int. J. Agric. Sci.* 10(1): 36-41.
- Janssen, P.H. 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl. Environ. Microbiol.* 72(3):1719-1728.

- Janvier, C., Villeneuve, F., Alabouvette, C., Edel-Hermann, V., Mateille, T., and Steinberg, C. 2007. Soil health through soil disease suppression: which strategy from descriptors to indicators? *Soil Biol. Biochem.* 39(1): 1-23.
- Jayman, T.C.Z. and Sivasubramaniam, S. 1974. The use of ascorbic acid to eliminate interference from iron in the aluminon method for determining aluminium in plant and soil extracts. *Analyst* 99(1178): 296-301.
- John, N. and Thankavel, M. 2015. *In vitro* screening of siderophore producing bacteria from Arabian Sea off the coast of Thiruvananthauram. *Indian J. Appl. Res.* 5 (12): 164-166.
- Jones, D.L. 1998. Organic acids in the rhizosphere - a critical review. *Plant Soil* 205(1): 25-44.
- Kauffmann, I.M., Schmitt, J., and Schmid, R.D. 2004. DNA isolation from soil samples for cloning in different hosts. *Appl. Microbiol. Biotechnol.* 64(5): 665-670.
- Kelly, J.J., Haggblom, M., and Tate, R.L. 1999. Changes in soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. *Soil Biol. Biochem.* 31(10): 1455-1465.
- Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H., and Trevors, J.T. 2004. Methods of studying soil microbial diversity. *J. Microbiological Methods* 58(2): 169-188.
- Ke, X., Lu, Y. and Conrad, R., 2014. Different behavior of methanogenic archaea and Thaumarchaeota in rice field microcosms. *FEMS Microbiol. Ecol.* 87(1): 18-29.
- Keisam, S., Romi, W., Ahmed, G., and Jeyaram, K., 2016. Quantifying the biases in metagenome mining for realistic assessment of microbial ecology of naturally fermented foods. *Sci. Rep.* 6: 1-12.



- Khandige, K.S., Patel, G.J., and Bavappa, K.Y.A. 1957. Preliminary observation on the yellow leaf disease of arecanut palm. *Arecanut J.* 8(2): 61-62.
- Ko, T.W.K., Stephenson, S.L., Bahkali, A.H., and Hyde, K.D. 2011. From morphology to molecular biology: can we use sequence data to identify fungal endophytes? *Fungal Diversity* 50(1): 113.
- Koch, I.H., Gich, F., Dunfield, P.F., and Overmann, J. 2008. *Edaphobacter modestus* gen. nov., sp. nov., and *Edaphobacter aggregans* sp. nov., acidobacteria isolated from alpine and forest soils. *Int. J. Syst. and evolutionary Microbiol.* 58(5): 1114-1122.
- Koshy, P.K., Sosamma, V.K., and Nair, C.R. 1975. Preliminary studies on *Radopholus similis* (Cobb, 1893) Thorne, 1949 infesting coconut and arecanut palms in south India. *Indian J. Nematology* 5(1): 26-35.
- Lakshmanan, V., Selvaraj, G., and Bais, H.P. 2014. Functional soil microbiome: below ground solutions to an aboveground problem. *Plant Physiol.* 166(2): 689-700.
- Lee, S.H., Ka, J.O., and Cho, J.C. 2008. Members of the phylum Acidobacteria are dominant and metabolically active in rhizosphere soil. *FEMS Microbiol. Lett.* 285(2): 263-269.
- Leigh, M.B., Prouzova, P., Mackova, M., Macek, T., Nagle, D.P., and Fletcher, J. S. 2006. Polychlorinated biphenyl (PCB)-degrading bacteria associated with trees in a PCB-contaminated site. *Appl. Environ. Microbiol.* 72(4): 2331-2342.
- Lindsay, W.L. and Norvell, W.A. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* 42(3): 421-428.

- Liu, F.P., Liu, H.Q., Zhou, H.L., Dong, Z.G., Bai, X.H., Bai, P., and Qiao, J.J. 2014. Isolation and characterization of phosphate-solubilizing bacteria from betel nut (*Areca catechu*) and their effects on plant growth and phosphorus mobilization in tropical soils. *Biol. Fertil. Soils* 50(6): 927-937.
- Logares, R., Sunagawa, S., Salazar, G., Cornejo-Castillo, F. M., Ferrera, I., Sarmiento, H., Hingamp, P., Ogata, H., Vargas, C., Lima-Mendez, G., and Raes, J. 2014. Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities. *Environ. Microbiol.* 16(9): 2659-2671.
- Lopez-Mondejar, R., Zuhlke, D., Becher, D., Riedel, K., and Baldrian, P. 2016. Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. *Sci. Rep.* 6: 252-263.
- Ma, Y., Prasad, M.N.V., Rajkumar, M., and Freitas, H. 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. *Biotechnol. Adv.* 29(2): 248-258.
- Maidak, B.L., Larsen, N., McCaughey, M.J., Overbeek, R., Olsen, G.J., Fogel, K., Blandy, J., and Woese, C.R. 1994. The ribosomal database project. *Nucleic Acids Res.* 22(17): 3485-3487.
- Manimekalai, R., Kumar, R.S., Soumya, V.P., and Thomas, G.V. 2010. Molecular detection of phytoplasma associated with yellow leaf disease in areca palms (*Areca catechu*) in India. *Plant Dis.* 94(11): 1376-1376.
- Massoumi, A. and Cornfield, A.H. 1963. A rapid method for determining sulphate in water extracts of soils. *Analyst*, 88(1045): 321-322.
- McDougald, D., Rice, S.A., Weichart, D., and Kjelleberg, S. 1998. Nonculturability: adaptation or debilitation? *FEMS Microbiol. Ecol.* 25(1): 1-9.

- McKee, L.S. and Brumer, H. 2015. Growth of *Chitinophaga pinensis* on plant cell wall glycans and characterisation of a glycoside hydrolase family 27  $\beta$ -l-arabinopyranosidase implicated in arabinogalactan utilisation. *PLoS One* 10(10): p.e0139932.
- Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A., and Raaijmakers, J.M. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332(6033): 1097-1100.
- Mendes, R., Garbeva, P., and Raaijmakers, J.M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.* 37(5): 634-663.
- Menon, R. 1959. *Cercospora arecae* Menon sp. novo. *Arecanut J.* 10: 108-109.
- Menon, R. 1960. Serological tests on Yellow leaf disease of arecanut. *Arecanut J.* 11: 12-13.
- Menon, R. 1961. Biochemical studies on the yellow leaf disease on arecanut palms. *Arecanut J.* 12: 16-21.
- Menon, R. 1963. Transmission of yellow leaf disease. *J. Pytopath.* 48: 82-88.
- Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., and Wilkening, J. 2008. The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinforma.* 9(1): 386.
- Micallef, S.A., Shiaris, M.P., and Colon-Carmona, A. 2009. Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. *J. Exp. Bot.* 60(6): 1729-1742.

- Miller, K.M., Ming, T.J., Schulze, A.D., and Withler, R.E. 1999. Denaturing gradient gel electrophoresis (DGGE): a rapid and sensitive technique to screen nucleotide sequence variation in populations. *Biotechniques* 27(5): 1016-1031.
- Mocali, S. and Benedetti, A. 2010. Exploring research frontiers in microbiology: the challenge of metagenomics in soil microbiology. *Res. Microbiol.* 161(6): 497-505.
- Mohapatra, A.R. and Bhat, N.T. 1975. Note on the association of high aluminium content of root with the yellow leaf disease of arecanut. *Indian J. Agric. Sci.* 45: 380-382.
- Mohapatra, A.R. Bhat, N.T., and Harishkumar, P. 1976. Yellow leaf disease of arecanut - soil fertility studies. *Arecanut Spices Bull.* 8: 27-31.
- Mohapatra, A.R. and Bhat, N.T. 1982. Crop management. A. Soils and manures. In: Bavappa, K.V.A., Nair, M.K., and Kumar, P.T. (eds), *The Arecanut Wm.* CPCRI, Kasaragod, pp. 97-104.
- Muddumadiah, C., Kumar, S., Manimekalai, R., and Rao, G.P. 2014. Detection and characterization of 16SrI-B phytoplasmas associated with yellow leaf disease of arecanut palm in India. *Phytopathogenic Mollicutes* 4(2): 77-82.
- Nagaraja. K.B. 1988. Biochemical charectarization of root region soil from areca based cropping systems. *Current Sci.* 57: 1078-79.
- Nair, R.B. 1964. *Carvalhoia arecae* Miller and Chinaa major pest of *Areca catechu* L. *Arecanut J*, 15: 57-59.
- Nambiar, K.K. 1949. *A survey of arecanut crop in Indian union.* Indian Central arecanut committee, Calicut, Kerala, India, 76 p.

- Nayar, R. 1971. Etiological agent of yellow leaf disease of *Areca catechu*. *Plant Dis. Reporter*. 55: 170-171
- Nayar, R. 1976. Yellow leaf disease of arecanut: Virus pathological studies. *Arecanut Spices Bull.* 8: 25-26.
- Nayar, R. and Selsikar, C.E. 1978. Mycoplasma like organisms associated with yellow leaf disease of *Areca catechu* L. *Eur. J. For. Pathol.* 8: 125-126.
- Neale, S.P., Shah, Z., and Adams, W.A. 1997. Changes in microbial biomass and nitrogen turnover in acidic organic soils following liming. *Soil Biol. Biochem.* 29(9): 1463-1474.
- Nichols, D. 2007. Cultivation gives context to the microbial ecologist. *FEMS Microbiol. Ecol.* 60(3): 351-357.
- Nihorimbere, V., Ongena, M., Smargiassi, M., and Thonart, P. 2011. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnol. Agron. Soc. Environ.* 15(2): 327-337.
- Nunan, N., Wu, K., Young, I.M., Crawford, J.W., and Ritz, K. 2002. In situ spatial patterns of soil bacterial populations, mapped at multiple scales, in an arable soil. *Microbial Ecol.* 44(4): 296-305.
- Nunes da Rocha, U., Van Overbeek, L., and Van Elsas, J.D. 2009. Exploration of hitherto-uncultured bacteria from the rhizosphere. *FEMS Microbiol. Ecol.* 69(3): 313-328.
- Nüsslein, K. and Tiedje, J.M. 1999. Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. *Appl. Environ. Microbiol.* 65(8): 3622-3626.

- Ofek, M., Hadar, Y., and Minz, D. 2012. Ecology of root colonizing *Massilia* (Oxalobacteraceae). *PloS one* 7(7): p.e40117.
- Olsen, S.R. 1954. *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. United States Department of Agriculture, Washington. 19p.
- Overbeek, R., Begley, T., Butler, R.M., Choudhuri, J.V., Chuang, H.Y., Cohoon, M., de Crécy-Lagard, V., Diaz, N., Disz, T., Edwards, R., and Fonstein, M. 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. *Nucleic Acids Res.* 33(17): 5691-5702.
- Pace, N.R. 1996. New perspectives on the natural microbial world: molecular microbial ecology. *ASM News* 62: 463-470.
- Panhwar, Q.A., Othman, R., Rahman, Z.A., Meon, S., and Ismail, M.R. 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. *Afr. J. Biotechnol.* 11(11): 2711-2719.
- Pillai, R.S.N. and Murthy, K.N. 1973. Altitude and areca cultivation. *Arecanut and Spices Bull.* 4:12-13.
- Piper, C.S. 1966. *Soil and Plant Analysis*. Hans Publishers, Bombay, 384p.
- Polyanskaya, L.M., Vedina, O.T., Lysak, L.V., and Zvyagintsev, D.G. 2002. The growth-promoting effect of *Beijerinckia mobilis* and *Clostridium* sp. cultures on some agricultural crops. *Microbiology* 71(1): 109-115.
- Ponnamma, K.N., Rajeev G., and Solomon J.J. 1991. Detection of mycoplasma-like organisms in *Proutista moesta* (Westwood) a putative vector of yellow leaf disease of arecanut. *J. Plant. Crops* 19(1): 63-65.

- Purushothama, C.R.A., Ramanayaka, J.G., Sano, T., Casati, P., and Bianco, P.A. 2007. Are phytoplasmas the etiological agent of yellow leaf disease of *Areca catechu* in India? *Bulletin Insectology* 60(2): 161-162.
- Qingyan, L.I., Ying, L.I., Xikun, Z.H.U., and Baoli, C.A.I. 2008. Isolation and characterization of atrazine-degrading *Arthrobacter* sp. AD26 and use of this strain in bioremediation of contaminated soil. *J. Environ. Sci.* 20(10): 1226-1230.
- Raaijmakers, J.M. and Weller, D.M. 1998. Natural plant protection by 2, 4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. *Mol. Plant-Microbe Interactions* 11(2): 144-152.
- Raaijmakers, J.M., Weller, D.M., and Thomashow, L.S. 1997. Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. *Appl. Environ. Microbiol.* 63(3): 881-887.
- Ramappa, B.T. 2013. Economics of areca nut cultivation in Karnataka - a case study of Shivamogga District. *J. Agric. Vet. Sci.* 3: 50-59.
- Ramaswamy, M., Nair, S., Soumya, V.P., and Thomas, G.V. 2013. Phylogenetic analysis identifies a '*Candidatus Phytoplasma oryzae*'-related strain associated with yellow leaf disease of areca palm (*Areca catechu* L.) in India. *Int. J. Syst. Evolutionary Microbiol.* 63(4): 1376-1382.
- Rawther, T.S.S. 1976. Yellow leaf disease of arecanut: Symptomatology, bacterial and pathological studies. *Arecanut Spices Bull.* 9:22-24.
- Rawther, T.S.S. 2000. Origin, distribution and spread. In: Nampoothiri, K.U.K., Ponnamma, K.N., and Chowdappa, P. (eds), *Arecanut yellow leaf disease*. CPCRI, Kasaragod, India, pp. 1-4.



- Rawther, T.S.S. and Abraham, K.J. 1972. Effect of application of macro and micro nutrients and irrigation on the incidence of yellow leaf disease of arecanut. *J. Plant. Crops* 1: 127-128.
- Riesenfeld, C.S., Schloss, P.D., and Handelsman, J. 2004. Metagenomics: genomic analysis of microbial communities. *Ann. Rev. Genet.* 38: 525-552.
- Robe, P., Nalin, R., Capellano, C., Vogel, T.M., and Simonet, P. 2003. Extraction of DNA from soil. *Eur. J. Soil Biol.* 39(4): 183-190.
- Rondon, M.R., Goodman, R.M., and Handelsman, J. 1999. The Earth's bounty: assessing and accessing soil microbial diversity. *Trends Biotechnol.* 17(10): 403-409.
- Rorison, I.H. 1973. The effect of extreme soil acidity on the nutrient uptake and physiology of plants. In: Dost, H. (ed.) *Acid sulphate soils*. Proceedings of the International Symposium, Wageningen, 254p.
- Saharan, B.S. and Nehra, V. 2011. Plant growth promoting rhizobacteria: a critical review. *Life Sci. Med. Res.* 21(1): 30.
- Sanguin, H., Herrera, A., Oger-Desfeux, C., Dechesne, A., Simonet, P., Navarro, E., Vogel, T.M., Moëgne-Loccoz, Y., Nesme, X., and Grundmann, G. L. 2006. Development and validation of a prototype 16S rRNA-based taxonomic microarray for Alphaproteobacteria. *Environ. Microbiol.* 8(2): 289-307.
- Schloss, P.D. and Handelsman, J. 2005. Metagenomics for studying unculturable microorganisms: cutting the Gordian knot. *Genome Biol.* 6(8): 229.
- Schmeisser, C., Steele, H., and Streit, W.R. 2007. Metagenomics, biotechnology with non-culturable microbes. *Appl. Microbiol. Biotechnol.* 75(5): 955-962.
- Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A., and

- Nannipieri, P. 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478(7367): 49-56.
- Schnürer, J., Clarholm, M., and Rosswall, T. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Biol. Biochem.* 17(5): 611-618.
- Scholz, M.B., Lo, C.C., and Chain, P.S. 2012. Next generation sequencing and bioinformatic bottlenecks: the current state of metagenomic data analysis. *Curr. Opinion Biotechnol.* 23(1): 9-15.
- Schramm, A., Larsen, L.H., Revsbech, N.P., Ramsing, N.B., Amann, R., and Schleifer, K.H. 1996. Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. *Appl. Environ. Microbiol.* 62(12): 4641-4647.
- Seliskar, C.E. and Wilson, C.I. 1981. Yellow diseases of trees. In: Maromorosch, K. and Raychaudhuri, S.P. (eds), *Mycoplasma Diseases of Trees and Shrubs*. Academic Press, New Delhi, pp. 43-44.
- Sharma, P.K., Capalash, N., and Kaur, J. 2007. An improved method for single step purification of metagenomic DNA. *Mol. Biotechnol.* 36(1): 61-63.
- Shendure, J. and Ji, H. 2008. Next-generation DNA sequencing. *Nat. Biotechnol.* 26(10): 1135-1145.
- Shivakumara, B.S., Niranjana, K.S., Chandru, P., and Renukaswamy, N.S. 2014. Status and surveillance of Yellow Leaf Disease of arecanut in Sringeritaluk of Chikmagalore district, Karnataka. *Mysore J. Agric. Sci.* 48(2): 199-203.
- Shivlata, L. and Satyanarayana, T. 2015. Thermophilic and alkaliphilic Actinobacteria: Biology and potential applications. *Frontiers Microbiol.* [e-

journal]6.Available:<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4585250/>  
.[8-July-2017].

- Shtark, O.Y., Borisov, A.Y., Zhukov, V.A., Provorov, N.A., and Tikhonovich, I.A. 2010. Intimate associations of beneficial soil microbes with host plants. In: *Soil Microbiology and Sustainable Crop Production*, Springer, Netherlands, pp. 119-196.
- Siddhapura, P.K., Vanparia, S., Purohit, M.K., and Singh, S.P. 2010. Comparative studies on the extraction of metagenomic DNA from the saline habitats of Coastal Gujarat and Sambhar Lake, Rajasthan (India) in prospect of molecular diversity and search for novel biocatalysts. *Int. J. Biol. Macromolecules* 47(3): 375-379.
- Simon, A. and Sivasithamparam, K. 1989. Pathogen-suppression: a case study in biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. *Soil Biol. Biochem.* 21(3): 331-337.
- Simon, C. and Daniel, R. 2011. Metagenomic analyses: past and future trends. *Appl. Environ. Microbiol.* 77(4): 1153-1161.
- Sims, J.T. and Johnson, G.V. 1991. *Micronutrients in Agriculture* (2<sup>nd</sup> Ed.). Soil Science Society of America, Madison, 476p.
- Sorek, R. and Cossart, P. 2010. Prokaryotic transcriptomics: a new view on regulation, physiology and pathogenicity. *Nat. Rev. Genet.* 11(1): 9-16.
- Srinivasan, N. 1982. Significance of damaged chlorophyll system associated with yellow leaf disease of arecanut. In: *Proceedings of the Fifth Annual Symposium on Plantation Crops*. Central Plantation Crops Research Institute, Kasargod, Kerala, pp. 555-560.

- Steffan, R.J., Goksoyr, J., Bej, A.K., and Atlas, R.M. 1988. Recovery of DNA from soils and sediments. *Appl. Environ. Microbiol.* 54(12): 2908-2915.
- Sujatha, S. and Bhat, R. 2012. Impacts of vermicompost and nitrogen, phosphorus, and potassium application on soil fertility status in arecanut grown on a laterite Soil. *Commun. Soil Sci. Plant Anal.* 43(18): 2400-2412.
- Sundararaju, P. and Koshy, P.K. 1986. Effect of different nematicides and neem oil cake in the control of *Radopholus similis* in yellow leaf disease affected arecanut palms. *Indian J. Nematology* 16(1): 44-47.
- Suresh, S. 2005. Characteristics of soils prone to iron toxicity and management-A review. *Agric. Rev.* 26(1): 50-58.
- Sutton, S. 2011. Accuracy of plate counts. *J. Validation Technol.* 17(3): 42-46.
- Swaminathan, A. 2002. Isozyme variation in *Areca catechu* L. and allied species. M.Sc (Ag) thesis, Kerala Agricultural University, Thrissur. 169p.
- Takahashi, N. 2005. September. Microbial ecosystem in the oral cavity: metabolic diversity in an ecological niche and its relationship with oral diseases. In: *International Congress Series. Elsevier.* 1284: 103-112.
- Tate III, R.L. 1995. *Soil microbiology*. John Wiley and Sons Publishing Company, New York. 508p.
- Tebbe, C.C. and Vahjen, W. 1993. Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast. *Appl. Environ. Microbiol.* 59(8): 2657-2665.
- Theron, J. and Cloete, T.E. 2000. Molecular techniques for determining microbial diversity and community structure in natural environments. *Crit. Rev. Microbiol.* 26(1): 37-57.

- Thomas, G.V., Krishanakumar, H. P., Bhat, M. R., and Balasimha, D. 2011. *Areca nut Based Cropping/Farming Systems*. Central Plantation Crops Research Institute, Kasaragod, 138p.
- Tiedje, J.M., Asuming-Brempong, S., Nüsslein, K., Marsh, T.L., and Flynn, S.J. 1999. Opening the black box of soil microbial diversity. *Appl. Soil Ecol.* 13(2): 109-122.
- Torsvik, V., Daae, F.L., Sandaa, R.A., and Ovreas, L. 1998. Novel techniques for analysing microbial diversity in natural and perturbed environments. *J. Biotechnol.* 64(1): 53-62.
- Torsvik, V., Goksoyr, J., and Daae, F.L. 1990. High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* 56(3): 782-787.
- Torsvik, V., Salte, K., Sorheim, R., and Goksoyr, J. 1990. Comparison of phenotypic diversity and DNA heterogeneity in a population of soil bacteria. *Appl. Environ. Microbiol.* 56(3): 776-781.
- Turner, T.R., James, E.K., and Poole, P.S. 2013. The plant microbiome. *Genome Biol.* 14(6): 209.
- UAS [University of Agricultural Sciences]. 1990. *Investigations on Yellow Leaf Disease of Areca nut 1985-1990*. University of Agricultural Sciences, Bangalore, 48p.
- Uren, N.C. 2007. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In: *The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*. Marcel Dekker, New York, pp. 1-21.
- Van Bruggen, A.H. C. and Semenov, A.M. 2000. In search of biological indicators for soil health and disease suppression. *Appl. Soil Ecol.* 15(1): 13-24.

- Van Der Heijden, M.G., Bardgett, R.D., and Van Straalen, N.M. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* 11(3): 296-310.
- Van Elsas, J.D. and Wolters, A. 1995. Polymerase chain reaction (PCR) analysis of soil microbial DNA. In: *Molecular Microbial Ecology Manual*. Springer, Netherlands, pp. 235-244.
- Vance, E.D., Brookes, P.C., and Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19(6): 703-707.
- Vijaya Kumar, B.G., Veerappadevaru, G., Balasimha, D., Abdul Khader, K. B., and Ranganna, G. 1991. *J. Plant. Crops* 19: 33-36.
- Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Woo, S.L., and Lorito, M. 2008. *Trichoderma*-plant-pathogen interactions. *Soil Biol. Biochem.* 40(1): 1-10.
- Voget, S., Leggewie, C., Uesbeck, A., Raasch, C., Jaeger, K.E., and Streit, W.R. 2003. Prospecting for novel biocatalysts in a soil metagenome. *Appl. Environ. Microbiol.* 69(10): 6235-6242.
- Walkley, A. and Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* 37(1): 29-38.
- Watanabe, F.S. and Olsen, S.R. 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO<sub>3</sub> extracts from soil. *Soil Sci. Soc. Am. J.* 29(6): 677-678.
- Weischer, B. 1967. Types of losses caused by nematodes. In: *Proceedings of the FAO Symposium on Crop Losses*, Rome, pp. 181-187.

- Weller, D.M. 2006. Disease suppressive soils. *IOBC wprs Bull.* 29(2): 173p.
- Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M., and Thomashow, L.S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Ann. Rev. Phytopathol.* 40(1): 309-348.
- Wilmes, P. and Bond, P.L. 2006. Metaproteomics: studying functional gene expression in microbial ecosystems. *Trends Microbiol.* 14(2): 92-97.
- Woese, C.R. 1987. Bacterial evolution. *Microbiological Rev.* 51(2): 221-271.
- Wolf, B. 1974. Improvements in the azomethine-H method for the determination of boron. *Commun. Soil Sci. Plant Anal.* 5(1): 39-44.
- Workneh, F., Van Bruggen, A.H.C., Drinkwater, L.E., and Shennan, C. 1993. Variables associated with corky root and *Phytophthora* root rot of tomatoes in organic and conventional farms. *Phytopathology* 83(5): 581-589.
- Yadava, R.B.R., Vellaichamy, K., and Mathai, C.K. 1972. Role of nutritional elements and their deficiency symptoms with reference to arecanut. *Arecanut Spices Bull.* 3: 4-7.
- Yassin, A.F., Young, C.C., Lai, W.A., Hupfer, H., Arun, A.B., Shen, F.T., Rekha, P.D., and Ho, M. J. 2007. *Williamsia serinedens* sp. nov., isolated from an oil-contaminated soil. *Int. J. Syst. Evolutionary Microbiol.* 57(3): 558-561.
- Young, C.C., Burghoff, R.L., Keim, L.G., Minak-Bernero, V., Lute, J.R., and Hinton, S.M. 1993. Polyvinylpyrrolidone-agarose gel electrophoresis purification of polymerase chain reaction-amplifiable DNA from soils. *Appl. Environ. Microbiol.* 59(6): 1972-1974.



# ANNEXURES

## Annexure I

### **Equipment used in the present study**

Sterilization of culture media - Equitron SLEFA and NatSteel horizontal autoclave

Incubation of cultures - GeNei OS-250

Centrifugation - Eppendorf 5804R and SPINWIN MC-02

pH of culture media and buffers - Eutech pH Tutor

Visualization of agarose gel

Photomicrography - Leica ICC50

Storage of microbial cultures and metagenomic DNA

## Annexure II

### Chemicals used in direct method of metagenomic DNA extraction by soft lysis

#### 1. Extraction buffer

A. 100 mM Tris HCl	-	10 ml
1M Tris HCl (pH – 8.0)	-	1 ml
Distilled water	-	100 ml
B. 100mM EDTA	-	0.372 g
C. 1.5M NaCl		
1M NaCl	-	0.75 ml
Distilled water	-	100 ml

#### 2. Lysis buffer

A. 20% SDS	-	0.8 g
B. Lysozyme	-	20 mg/ml
C. Proteinase K	-	10 mg/ml
D. N-lauroyl sarcosine	-	10 mg/ml
E. 1% CTAB	-	4 g

#### 3. Phenol : Chloroform : Isoamyl alcohol (25:24:1)

To 25 parts of phenol, 24 parts of chloroform, 1 part of isoamyl alcohol was added and mixed properly. The mixture was stored in refrigerator before use.

#### 4. Chloroform : Isoamyl alcohol (24:1)

To 24 parts of chloroform, 1 part of isoamyl alcohol was added and mixed properly. The mixture was stored in refrigerator before use.

#### 5. Potassium acetate 7.5 M

A. Potassium acetate	-	20.412 g
B. Distilled water	-	50 ml

6. Chilled ethanol (70%)

To 70 parts of absolute ethanol, 30 parts of double distilled water was added.

7. Sterile distilled water - 20-50  $\mu$ l

### Annexure III

#### Chemicals used in direct method of metagenomic DNA extraction by direct lysis

##### 1. Extraction buffer

A. 200mM Tris (pH - 8.0)	-	0.2 ml
B. 25 mM EDTA (pH – 8.0)	-	0.5 ml
C. 250 mM NaCl	-	0.375 ml
D. 0.5% SDS	-	0.005 ml

##### 2. Phenol : Chloroform : Isoamyl alcohol (25:24:1)

To 25 parts of phenol, 24 parts of chloroform, 1 part of isoamyl alcohol was added and mixed properly. The mixture was stored in refrigerator before use.

##### 3. Ice-cold isopropanol

Equal volume of isopropanol

##### 4. 70 per cent chilled ethanol

## Annexure IV

### Materials used for agarose gel electrophoresis

#### 1. 6x Loading/tracking dye

Bromophenol blue	-	0.25 %
Xylene cyanol	-	0.25 %
Glycerol	-	30 %

The dye was prepared and kept in refrigerator at 4<sup>0</sup>C

#### 2. Ethidium bromide (Intercalating agent)

The dye was prepared as a stock solution of 10 mg/ml in water and was stored at room temperature in a dark bottle.

#### 3. 50x TAE buffer (pH - 8.0)

Tris base	-	242.0 g
Glacial acetic acid	-	57.1 ml
0.5 M EDTA (pH - 8.0)	-	100 ml
Distilled water	-	1000 ml

The solution was prepared and stored at room temperature

## Annexure V

### Media used and composition

#### a) Actinomycete isolation media

Sodium caseinate	2.00 g
L-asparagine	0.10 g
Sodium propionate Dipotassium phosphate	0.50 g
Magnesium sulphate	0.10 g
Ferrous sulphate	0.001 g
Agar	20.00 g
Distilled water	1000 ml

#### b) Ashby's Mannitol agar

Mannitol	20.00 g
Dipotassium phosphate	0.20 g
Magnesium sulphate	0.20 g
Sodium chloride	0.20 g
Potassium sulphate	0.10 g
Calcium carbonate	5.00 g
Agar	20.00 g
Distilled water	1000 ml



**c) Jensen's agar**

Sucrose	20.00 g
Dipotassium phosphate	1.00 g
Magnesium sulphate	0.50 g
Sodium chloride	0.50 g
Ferrous sulphate	0.10 g
Sodium molybdate	0.005 g
Calcium carbonate	2.00 g
Agar	20.00 g
Distilled water	1000 ml

**d) Kenknight & Munaierers agar**

Dextrose	1.00 g
Monopotassium Dihydrogen phosphate	0.10 g
Sodium nitrate	0.10 g
Potassium chloride	0.10 g
Magnesium sulphate	0.10 g
Agar	20.0 g
Distilled water	1000 ml

**e) King's medium B base**

Proteose peptone	1.00 g
Dipotassium hydrogen phosphate	1.50 g
Magnesium sulphate heptahydrate	1.50 g
Agar	20.00 g
Glycerol	15 ml
Distilled water	1000 ml
pH	7.2 ± 0.2

**f) Martin's Rose Bengal agar**

Papaic digest of soybean meal	5.00 g
Dextrose	10.00 g
Monopotassium phosphate	1.00 g
Magnesium sulphate	0.50 g
Rose Bengal	0.05 g
Agar	20.00 g
Distilled water	1000 ml
pH	7.2 ± 0.2

**g) Nutrient agar**

Beef extract	3.00 g
Peptone	5.00 g
NaCl	5.00 g
Agar	20.00 g
Distilled water	1000 ml

**h) Pikovskaya's agar**

Glucose	10.00 g
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	5.00 g
(NH <sub>2</sub> ) <sub>4</sub> SO <sub>4</sub>	0.50 g
NaCl	0.20 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.10 g
KCl	0.20 g
Yeast extract	0.50 g
MnSO <sub>4</sub> .H <sub>2</sub> O	0.002 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.002 g
Distilled water	1000g
Agar	20.00 g
pH	7.0

**i) Potato Dextrose agar**

Potato infusion	200.00 g
Dextrose	20.00 g
Agar	20.00 g
Distilled water	1000 ml

**METAGENOMIC ANALYSIS OF BACTERIAL DIVERSITY IN  
THE RHIZOSPHERE OF ARECANUT PALMS AFFECTED BY  
YELLOWING IN WAYANAD**

*by*

**MAHESH MOHAN**

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**ABSTRACT OF THE THESIS**

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**KERALA, INDIA**

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## Abstract

Arecanut (*Areca catechu* L.), is a major masticatory crop that has become an integral part of the nation's culture as well as the economy. The crop is mainly cultivated in the Western Ghats and North-Eastern parts of the nation and Kerala occupies second position in the production of arecanut in India, after Karnataka. An increase in the area of cultivation has been observed but the productivity has been stagnant due to the intricacies concerned with soil, climate and nutrient imbalances. Among the challenges faced in arecanut cultivation, yellowing of arecanut palms has gained greater attention due to the crop loss inflicted by it. Various etiological reasons among which, nutrient imbalances and cultivation in low-lying areas stand to be the probable cause of the disease.

The objective of the present investigation was to analyse the bacterial diversity in the rhizosphere of yellowing affected and healthy arecanut palms in Wayanad district. Soil samples were collected from the rhizospheres of yellowing affected, apparently healthy (healthy palms in yellowing affected plantation) and completely healthy palms and analysed for their chemical and biological properties. The culturable microflora was enumerated using dilution plate method for bacteria, fungi, actinomycetes, nitrogen fixers, phosphate solubilizers, fluorescent pseudomonads, *Trichoderma* and *Bacillus*. The predominant bacterial isolates obtained from soil samples were screened for plant growth promotion activities like production of IAA, siderophore, HCN and ammonia and phosphate solubilization. A whole genome study on the arecanut rhizosphere revealed the presence of IAA producing genes in the bacterial metagenome and other reports indicating the activity of phosphate solubilizers indicated the abundance of PGPR in the arecanut rhizosphere. The bacterial diversity in the rhizosphere of the three categories of palms was analysed by metagenomic library construction and sequencing of V3-V4 regions on the 16S rRNA gene, using Next Generation Sequencing (NGS) technology. The sequences thus obtained were

analysed for the Operational Taxonomic Units (OTUs) present using the MG RAST server and MEGAN.

The analysis of soil physico-chemical parameters revealed that an increased proportion of Fe and Al in the yellowing affected soils might have hindered the uptake of other nutrients like calcium and magnesium. The culturable microflora and microbial biomass carbon analysis indicated a slight increase in the rhizosphere of apparently healthy and completely healthy arecanut palms than that of the yellowing affected ones. A significant increase in the population of *Bacillus* was observed in the completely healthy arecanut palm rhizospheres.

The predominant bacterial phyla in the arecanut rhizosphere included Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes and Firmicutes. The phyla Proteobacteria, Acidobacteria and Verrucomicrobia were found to be dominant in the apparently healthy arecanut palm rhizospheres when compared to the yellowing affected counterpart. The class Alphaproteobacteria was found to be abundant in the apparently healthy rhizosphere with the other classes namely Beta, Gamma and Epsilon Proteobacteria showing no pattern of abundance. The abundance of genera *Bradyrhizobium*, *Sphingomonas* and *Burkholderia* were observed in the apparently healthy rhizosphere soil samples. An increased population of *Pseudomonas*, *Bacillus* and *Nitrospira* in one of the completely healthy soil sample was identified, while an increased proportion of *Massilia* in one of the yellowing affected rhizosphere was recorded. Bacterial diversity in the rhizosphere of apparently healthy palms was found to be higher than that of the yellowing affected palms as indicated by Shannon-Weaver and Simpson indices.

The results indicated that an increased population of the aforementioned bacterial taxa might have a significant role in maintaining the health of arecanut palms and reducing the yellowing symptoms by mediating nutrient recycling and plant growth promotion. Further studies to engineer the rhizosphere microflora might help in reducing the yellowing incidence in the near future.

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