# Microbiome analysis of rhizosphere of *Santalum album* grown in tropical dry evergreen forests India

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**Abstract**

Microbiome of the plant rhizosphere has great impact on plant health and results in high quality by products. Sandal is an evergreen, root parasite with slow growth plant. The present work investigated with the assemblage of the bacterial communities associated with *S. album* rhizosphere soil for metagenomic studies. 9,038 bacterial communities were identified using common and unique operational taxonomic units (OTUs) by QIIME pipeline (1.9.1). The aims were to highlight the importance of assessing the potential role of *Actinobacteria* using as a culture treated bio fertilizer which was basically having the capacity of inhibiting phytopathogens on rhizosphere soil which helps to improve sandal tree productivity. A field experiment was conducted to grow sandal saplings 1) Neem tree as host, 2) *Actinobacteria* as culture treatment without host and 3) bio fertilizers without host and treatment. Plant morphometric analysis were determined and compared for a one year and two years old tree. The results showed that the effect of *Actinobacteria* treated bio fertilizer on sandal sapling having good growth on height, basal grith and leaves. Height and basal grith significantly increased when it was treated with *Actinobacteria* when compared with rest of two experimental saplings. The highest growth rate shows the efficiency of *Actinobacteria* uptake on rhizosphere level. *Actinobacteria* can have the ability of promoting growth on one year and two years old sandal tree. Therefore, *Actinobacteria* treatment should be considered when treated bio fertilizer is applied to soil as fertilizer.

**Keywords** *Santalum album*, rhizosphere soil, microbiome, High-throughput sequencing, QIIME (1.9.1), *Actinobacteria.*

## 1. Introduction

Soil microorganisms have an important role on soil fertility and plant health. The differing physical, chemical and biological properties of the root associated soil, compared with those root free bulk soils are responsible for changes in microbial diversity and for increased numbers and activity of microorganisms in the micro-environment of rhizosphere (Velmourougane et al. 2017).In general, parasites uptake nutrient from their host for their growth, if a parasitic plant that has directly attaches to another plant via a haustorium which has a specialized structure that forms a morphological and physiological link between the parasite and host (Yoshida et al. 2016).

*Santalum album* L. (Sandalwood) is a commercially and culturally important plant species belonging to the family Santalaceae and the genus *Santalum*. There are around 18 sandalwood species of genus *Santalum* (Baldovini et al. 2011). Among various Santalum species, Indian sandalwood (*S. album*), which is also sometimes referred as east Indian Sandalwood, stands out for its highly valued oil and aromatic wood (Arunkumar et al. 2016). However, there is lack of knowledge on the ecology and the distribution of this commercially valued species due to inadequate research (Subasinghe 2013). *Santalum album L.* is an important semiparasitic tree whose roots join to host roots, through haustoria, to acquire water and nutrients. Due to hemiparasitic nature, finding suitable host species is a limitation in establishment of sandalwood plantations (Teixeira et al. 2016). In India, the area under sandalwood is decreasing fast due to illegal collection and its difficulty in plantation establishment (Rocha et al. 2014). Due to over harvesting
in its natural habitat in India, it is listed as vulnerable by the International Union for Conservation of Nature (IUCN) and is placed in Appendix II of CITES. Owing to the immense value and its declining population in its natural habitats, opportunity for plantation of sandalwood to satisfy its demand is huge (Jones, 2008).

The biogeographical patterns exhibited by soil microbial communities has grown significantly. This aspect of biodiversity, besides being fundamentally important for our understanding of the forces shaping the bacterial biosphere (Fierer et al. 2007) has practical value because it can provide a scientific basis for modern agriculture. With the development of sequencing technology, metagenomic analysis involves within the application of bioinformatics tools to review the genetic material from environmental, unculturable microorganisms. Considering its successful natural establishment at Sivaganga, this study aimed to identify the host species and enumerate environmental factors supporting its microbial population in the study area. The rhizosphere microbiome of *S. album* has focused on basic 16S rRNA sequencing to evaluate the bacterial diversity and distinguishes thousands of organisms. There are three primary phases in metagenomic data processing: 1) assembly, 2) annotation, and 3) statistical analysis (Thomas et al. 2011). We also provided the analysis of soil physico-chemical properties and the microbiome analysis to understand the soil – microbes relationship between soil nutrients which involves in the growth of beneficial microorganisms. Furthermore, the genus of *Actinobacter* shows highly responsive on plant growth promotion. This will provide opportunity for the cultivation of sandalwood tree and benefits economically. In some literatures they reported that after 3–4 years, the host is no longer necessary for its growth and development. Our aim to grow the one year and two years old sandal saplings without any host. According to our results, we focused on *Actinobacteria* culture for the

growth and development on one year and two years old sandal saplings in RBL nursery, Bharathidasan University, Tamil Nadu.

## Materials and Methods

### 2.1. Sample Collection and processing

*S. album* rhizosphere soil were collected from V.Pudur, Sivaganga District at 9.9726° N, 78.5661° E, Tamil Nadu, India on December 2020 (Fig.1), collected sample was carried out under aseptic procedures were separated in the laboratory and stored at −20 °C.

### 2.2. Soil Physiochemical Properties

A comprehensive study of soil physicochemical and biological properties was analyzed. Soil moisture content was measured by the weighing method. The soil pH was determined using a calibrated pH meter by the microelectrode method (Zhang et al. 1999). The EC (Electrical Conductivity) (Hardie and Doyle, 2012) and organic carbon were determined (Walkley and Black, 1934; Piper, 1966). The available nitrogen was determined by kjeldal method (Subbiah and Asija, 1956) available phosphorus (Tale and Ingole, 2015) by spectrophotometer and potassium by flame emission method (Jackson, 1973). Available sulphur in the soil was extracted using 0.15% CaCl**2** solution (Williams and Steinbergs, 1959). Exchangeablecalcium and exchangeable magnesium by EDTA titration method (Raij, 1966). General soil physical profiles of texture, structure, color and density (Blake and Hartze, 1986), soil porosity, moisture content and water holding capacity (Sankaram, 1966) were determined.

### 2.3. DNA Extraction

DNA extraction using extraction buffer (10 ml) was mixed with sandal rhizosphere soil (5g) on iceand glass beads were added. The mixture was sonicated using a high intensity ultrasonic processor(Vibra Cell) with a standard 13mm horn solid probe for 150 seconds. The sample was cooled in iceand the sonication repeated. Sodium dodecyl sulphate (SDS) was added (3 ml; 20 %) and blendingcontinued for a further 5 sec. The sample was incubated at 65°C for 1 hr. transferred to centrifuge bottles (50 ml) and centrifuged at 6000g for 10 min. The supernatant was collected, and the soil pellet re-extracted with further extraction buffer (100 ml), incubation at 65ºC for 10 minutes and centrifugation as above.10 ml supernatants were transferred to centrifuge tubes (15 ml) containinga half-volume of polyethylene glycol (5 ml; 30%), and incubated at room temperature for 2 hrs. Samples were centrifuged (8000rpm for 20 min) and the partially purified nucleic acid pellet resuspended in 100 µl of TE. Potassium acetate (400 µl; 7.5 M) was added to a final concentrationof 0.5 M. Samples were transferred to ice for 5 min then centrifuged (8000 rpm, 45 min) at 4°C toprecipitate proteins and polysaccharides. The aqueous phase 0.5ml was extracted with phenol/chloroform and chloroform/isoamyl alcohol and DNA was precipitated by adding 2 volume(1ml) isopropanol. It was incubated at -20°C for overnight, DNA was pelleted by centrifugation (8000 rpm for 20 min) and resuspended in TE (50 µl) (Yeates et al. 1998).

### 2.4. HTS, Library preparation and Data Quality Optimization

A Qubit 2.0 fluorometer was used to measure DNA samples (Invitrogen, Carlsbad, CA, USA). AMetaVxTM library preparation kit was used to produce amplicons from 40–50 ng of DNA

(GENEWIZ, Inc., South Plainfield, NJ, USA). The hypervariable V3 and V4 regions of bacterial 16S rRNA were chosen for amplicon sequencing, followed by taxonomic analysis (Caporaso et al. 2012; Gilbert et al. 2014). GENEWIZ created a set of proprietary primers aiming at the relatively conserved areas of the V3 and V4 hypervariable regions of the 16S rRNA of bacteria. The agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) was used to confirm library quality control, and the Qubit 2.0 Fluorometer was used to quantify it. Adopted the manufacturer's recommendations such as DNA libraries were multiplexed and put intoan Illumina MiSeq device (Illumina, San Diego, CA, USA). The MiSeq control softwarepackage (MCS) integrated in the MiSeq instrument was used to sequence using a 2300 pairedend (PE) configuration; image analysis and base calling were accomplished using the MCS (Caporaso et al. 2010). The metagenome sequence of *S. album* associated bacterial community was submittedin NCBI with the accession number of bioproject PRJNA832090.

**2.5. Statistical and Bioinformatics Analysis**

Sequence analysis was performed using the Vsearch (1.9.6) (Westcott et al. 2015). To determine operational taxonomic units (OTUs), the 16S rRNA gene sequences were trimmed to a average length of 393 bp, sorted by using Cutadapt (v1.9.1). Operational Taxonomic Units were taxonomically annotated following a Basic Local Alignment Search Tool (BLAST) analysis against the Unite Database of each identified representative bacterial sequence done in QIIME software. Alpha diversity analysis was carried out to find the complexity of species diversity for each sample using 6 indices, which include observed species, Chao1 (Chao, 1987), Shannon (Shannon, 1948), Simpson (Simpson, 1949), abundance-based coverage estimator (ACE) (Chao and Lee, 1992) and Good's coverage (Good, 1953). Indices calculation for all the samples was done using QIIME and visualized in R software (Caporaso et al. 2010). Community richness was identified with Chao indices richness estimator of the total number of species in ecology (http://www.mothur.org/wiki/Ace). An index that uses Chao 1 algorithm to estimate the OTU number of samples commonly used in ecology to assess the total number of species. (http://www.mothur.org/wiki/Chao). The Shannon index commonly used to reflect the diversity index a for the estimation of microbial diversity (http://www.mothur.org/wiki/Shannon) and Simpson index (http://www.mothur.org/wiki/Simpson) indices were used for the identification ofcommunity diversity in all the samples. To characterize the sequencing depth and coverage, the Good's coverage (http://www.mothur.org/wiki/Coverage) was used. Rank abundance curve performed by using R packages and OTU clustered by Vsearch (1.9.6) (Rand, 1971; Hubert, and Arabie, 1985).

RDP classifier (Cole et al. 2014) Bayesian algorithm was used to classify the OTU representative sequences of 97% similarity level, and the community composition of each samplewas analyzed and summarized at all levels. Taxon assignment was performed using the QIIME (v1.9.1). For each OTU cluster, a representative sequence was screened to perform taxonomic annotation. Rank Abundance Curve (RAC) is used to analyze diversity (MacArthur, 1957; Whittaker, 1965) by R packages based on the results of OTU analysis. Rank-abundance curve reflects both species abundance and species uniformity. Species uniformity is reflected by the shape of the curve. The rarefaction curve (Heck et al. 1975) is a useful tool to characterize the species composition of a sample and predicting the abundance of species in a

sample. It efficientlydeals with the increase of detected species due to the increase in sample size. The observed numbers of OTUs were plotted against the number of extracted sequences by QIIME (1.9.1).

### 2.6. Experimental site and plantation establishment

The study was carried out during one year old (2020-2021) and two years old (2020–2022) sandalwood plantations on RBL nursery field, Bharathidasan University located inTiruchirappalli district, Tamil Nadu, India 10°40′29″N 78°44′39″E. The climate of the experimental site is semi hot, receiving 387 mm annual rainfall distributed with dryer June - September, wetter January - February, hotter April- May, and cooler October - December. The soil of the study area was low fertile, red soil, with pH (5.4). During summer, the plantation was provided with protective irrigation through the drip irrigation system. The sandal sapling considered in the study were one year and two years old with *Actinobacter*ia culture treatments, bio fertilizers (without treatment) without any host and neem tree as host for morphology analysis.

### 2.7. Treatment and experimental design

The one- and two-year-old sandal tree were taken for the study. For first experiment *Santalum album* seeds were first soaked in *Actinobacteria* culture for 12 hrs at room temperature. Then the seeds were sown in soil in a RBL nursery, Bharathidasan University. For the second experiment only bio fertilizers are used for the growth. After seed germination and the seedlings developed at least six leaves, 12 cm tall seedlings were transplanted into grow bags (12x12 cm) filled with a mixture of green manure and vermicompost and placed in a greenhouse set up. After the growth of 1-month seedlings, they are moved to the field. As the seedlings grew to over 35 cm in height, the seedlings are directly transferred to the RBL nursery field soil. For the third experiment after sandal sapling plantation on the soil neem tree was planted at the side of sandal sapling which was taken as their host for growth. Compared to all three saplings they show differences in their height, girth and number of leaves present in the one year and two-year-old sandal tree.

## 3. Results

### 3.1. Soil Physicochemical Profiles

The soil sample was collected upto 15cm depth and analyzed for available macronutrients and soilphysical parameters. The physiochemical properties of the rhizosphere soil sample were presentedin Table 1. The soil was red in color that has gravelly clay texture. The bulk density values were recorded as 1.34 Mg $m^{-3}$. The water holding capacity of soil was recorded as 15.6%. The moisturecontent of the soil was recorded as 13.7%. The pH range was recorded as 5.4. The EC value was recorded as 0.14ds $m^{-1}$. The organic carbon content was recorded as 6.3%. The availablenitrogen content recorded as 204.0 kg $ha^{-1}$. The available phosphorus content was recorded as 11.00 kg $ha^{-1}$. Available potassium content was recorded as 72.0 kg $ha^{-1}$. Available sulphur contentwas recorded as 5.9 mg $kg^{-1}$. Calcium content was recorded as 9.35 kg $ha^{-1}$. Magnesium was recorded as 3.86kg $ha^{-1}$.

### 3.2. Sequencing Data

A total of 7,878,805 bases numbers with a length of 235 bp were obtained by sequencing 16S rRNA from *S. album* rhizosphere soil sample. A total PE reads of 16,741 numbers with 393 bp average length from *S. album* rhizosphere soil sample. Filtration of raw reads of 16S rRNAwere filtered using QIIME quality filters, followed by OTU identification, clustering and analysis (Fig. 2).

### 3.3. Operational Taxonomic Unit (OTU) cluster and species annotation

QIIME pipeline (1.9.1) was used to analyses the bacterial diversity in the *S. album* obtained fromthe sivaganga forest. 16S rRNA sequences number were obtained having a combined length of 200-500 base pairs (bp), and 16S rRNA V3-V4 gene profiles were generated from the *S. album* isto evaluate the diversity and abundance of bacterial association, 448 OTUs were identified. Operational Taxonomic Units identification was done with Vsearch software (1.9.6). Based on ≥97% of sequence similarity, all the effective tags were clustered into OTUs. For each OTU cluster, a representative sequence was screened to perform taxonomic annotation. OTUs were taxonomically annotated following a BLAST analysis against the Unite Database of each identified representative bacterial sequence done in QIIME software.

### 3.4. Diversity Index, Microbial Composition and Statistical analysis

The community compositions of annotated microbiome indicated that there were 5 *Kingdoms*, 15 *Phylas*, 37 *Classes*, 62 *Orders*, 75 *Families*, 53 *Genus*, and 8 *Species* in the rhizosphere soilof *S. album*. Alpha diversity refers to the diversity within a particular sample individually, and it is usually represented by the microbial species (i.e., species richness) enumerated in *S. album*. Alpha diversity analysis was done using Shannon, Simpson and Chao indices, Rarefaction curves, Rank abundance and good's coverage for 16S rRNA sample. Alpha diversity consists of plots displaying Shannon, Simpson, and Chao indices, built using 16S rRNA samples (Fig. 3).

#### 3.4.1. Rarefaction Curve

In order to determine whether the sample size is enough and to calculate the species abundance, the rarefaction curve is frequently employed in biodiversity and community surveys. As a result, when the sample size is adequate, the rarefaction curve can forecast the species abundance in addition to determining whether the sample size is adequate. Qiime created the rarefaction curve using random sampling (1.9.1) (Fig. 4).

#### 3.4.2. Rank-Abundance Curve

The number of valid sequences in each OTU of a given sample was first calculated, and all the OTUs were then ranked in descending order based on their relative abundance (number of valid sequences). The result was then plotted, with OTU ranking on the X axis and the number of sequences in the OTU on the Y axis. The OTU relative abundance in % might alternatively be the Y axis. The length of the curve on the X axis reflects the number of species present. More species are present if the X axis is stretched. The curve's shape reflects the uniformity of the species. The higher the species homogeneity, the smoother the curve. (Fig. 5).

### 3.4.3. Taxonomy Diversities

The abundant phyla identified in *S. album* using 16S rRNA data were *Actinobacteria* (23.4%), *Proteobacteria* (14.9%), *Acidobacteria* (7.1%), *Chloroflexi* (4.1%), *Firmicutes* (2.3%), *Nitrospirae* (1.9%), *Gemmatimonadetes* (1.9%), *OD1* (0.1%), *Armatimonadetes* (0.1%),*Planctomycetes* (0.09%), *Bacteroidetes* (0.08%), *WS3* (0.05%), *Cyanobacteria* (0.04%), *Spirochaetes* (0.03%). The top 3 phyla present in *S. album* are *Actinobacteria, Proteobacteria, Acidobacteria*. The top 15 distribution of the abundant classifications of *S. album* rhizosphere soil sample at phylum level (Fig. 6).

The top 20 bacterial genera of 16S rRNA databased analysis were *Acetobacter* (2.3%),*Lactobacillus* (1.9%), *Pseudonocardia* (1.3%), *Kribbella* (0.5%), *Iamia* (0.36%), *Streptomyces*(0.34%),*Virgisporangium* (0.30%), *Mannheimia* (0.29%), *Mycobacterium*(0.29%), *Bradyrhizobium* (0.27%), *Rhodoplanes* (0.25%), *Agromyces* (0.25%), *Bacillus* (0.22%),*Mesorhizobium* (0.22%), *Balneimonas* (0.19%), *Kaistobacter* (0.18%), *Nitrospira* (0.18%),*Candidatus* (0.17%), *Cellulomonas* (0.17%), *Pasteurella* (0.15%) and *Pseudomonas* (0.08%). Thetop 3 genera present in *S. album* are *Acetobacter, Lactobacillus, Pseudonocardia*. Species distribution using 16S rRNA sequence databased analysis were *halophobica* (0.7%), *ochraceum* (0.3%), *multocida* (0.1%), *aureum* (0.07%), *vinacea* (0.05%), *marinus* (0.04%), *scabrisporus* (0.04%). *halophobica* were the most abundant species present in *S. album* rhizosphere soil. The distribution of the abundant classifications of *S. album* rhizosphere soil sample at species levels (Fig. 7).

### 3.4.4. Treatment and untreated sandal sapling morphology analysis

The one year and two years old sandal saplings were taken for the study. Three types of sandal plantation were done. At first *Santalum album* seeds were first soaked in *Actinobacteria* culture. For second sapling plantation only with biofertilizers and for the third experiment, the sapling was planted with host (neem). The seeds were sown in soil in a RBL nursery, Bharathidasan University. After seed germination saplings were placed in a greenhouse set up. After 1 month, the seedlings are grown up to 30 - 40 cm in height, then the seedlings are directly transferred to the RBL nursery field soil. After 1 year and 2 years, treated and untreated sapling without host and with host (neem) sandal sapling shows some morphological variations in their growth. According to three saplings, the culture treated sandal sapling shows healthy with consistent growth during morphological studies. The sandal sapling was grown in a glasshouse in Bharathidasan University, Trichy with 10°40′29″N 78°44′39″E. Table 2 gives the details of field treated with culture and untreated sapling morphology. Table 3 gives the details of field sapling morphology with host (neem) studied in this current experiment.

## 4. Discussion

Present study needs to focused on *S. album* microbiome because of *S. album* natural habitat population has experienced substantial decrease due to extensive exploitation for commercial purposes (Teixeira et al. 2016). The conservation status of *S. album* alone has been on alevel of vulnerability as listed by International Union for Conservation of Nature and Natural Resources (IUCN) after the assessment of conservation status held in Viet Namin 1998. The growth of sandalwood is closely affected by its growth environment, especially the soil conditions and host plant because of its hemiparasitic nature (Liu et al. 2009; Ouyang et al.

2016; Teixeira et al. 2016). Sandalwood requires fertile soil with good drainage to exhibit optimum growth. The presence of a host plant for sandalwood has been proven to be essential in the root association through haustoria formation for supplying water and other available nutrientsto improve morphological growth (Deepa and Yusuf, 2016). Sandalwood develops strategy by modifying the roots to form haustorium when in physical contact with the roots of the host plants. The formed haustorium would connect sandalwood to the host plant, in terms of anatomically, morphologically and physiologically. That connection thus would allow the flow of water and nutrients from the host plant to the parasite plant (Teixeira et al. 2016). Within a year since sandalwood was grown with the host plants, it would manage to form the haustorium between their roots (Lu et al. 2014). Haustoria formed under root association between sandalwood and host plant showed varied growth performance of sandalwood. The presence of host plants in sandalwood cultivation is advisory because of their role as sources of K, P, Ca, and Na, even N, and C (Lu et al. 2013; Teixeira et al. 2016).

The present study was revealed on *S. album* species which are geographically distributed inS.Pudur, Sivaganga of Tamilnadu, India. To understand the plant growth promoting and pathogenic microbes, which are surrounded around the soil, we need to study the *S. album* rhizosphere microbiome community. Bacteria are the most abundant of all the rhizospheric microbiota, and many are known to promote plant growth (Antoun et al. 2005; Van Loon, 2007). The present study made an effort to understand the diverse and complex bacterial communities present in the rhizospheric soils of *S. album*. 16S rRNA sequence data revealed 446OTUs assigned to different bacterial species colonizing the rhizosphere of *S. album* species studied.

Analysis of these OTUs showed that *Acidobacteria* has the most significant number of rhizobacterial communities present in *S. album* phyla were studied here. Besides this, alpha diversity analysis also predicted for *S. album* sample. Phylum level distribution studies identified the dominance of *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Firmicutes*, *Nitrospirae*, *Gemmatimonadetes*, *OD1*, *Armatimonadetes*, *Planctomycetes*, *Bacteroidetes*, *WS3*, *Cyanobacteria* and *Spirochaetes*. Many of them were reported to be present in the *S. album* rhizosphere (Bing et al. 2022). The 16S rRNA gene sequence analysis had helped to identify the top twenty genera (*Acetobacter, Lactobacillus, Pseudonocardia, Kribbella, Iamia, Streptomyces, Virgisporangium, Mannheimia, Mycobacterium, Bradyrhizobium, Rhodoplanes, Agromyces, Bacillus, Mesorhizobium, Balneimonas, Kaistobacter, Nitrospira, Candidatus, Cellulomonas* and *Pasteurella*).

Out of these 20 genera, most of the genera were found to be capable of fix nitrogen in *S. album* and otherplants. *Acetobacter* is plant growth promoting Rhizobacteria (PGPR) provided a significant increase in shoot and root length and biomass (Majeed et al. 2015). In our study *Acetobacter* genera shows high abundance for sandal tree growth. *Azotobacter* genus is involvedin atmospheric nitrogen fixation in different crops (Jiménez et al. 2011). It is hard to identify andclassify most bacteria in culture because of their morphological similarities. But, culture- independent methods, such as 16S rRNA sequencing, are highly efficient, cost-effective and provide accurate identification and classification of rhizobacteria. More recently, strains of *Bacillus, Pseudomonas, Glomus* and others have been commercialized. The use of bacterial taxa in plant production has been reviewed previously for *Bacillus (*Borriss, 2011), *Pseudomonas* (Santoyo et al. 2012; Sivasakthi et al. 2014), *Actinobacteria* (Shivlata et

al. 2017) and *Lactobacillus* (Lamont et al. 2017). In addition, *Acetobacter* was also been shown to enhance crop production (Babalola, 2010).

In our previous studies, we observed the occurrence of Proteobacteria, *Acidobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Bradyrhizobium* and *Gemmatimonas* in the *S. album* rhizosphere (Bing et al. 2022). Some genera, such as *Bacillus* and *Pseudomonas* known for their plant growth promoting (Ma et al. 2010; Ma et al. 2011; Wani et al. 2010) and nitrogen fixing properties, were found to be enriched in the *S. album* rhizosphere. Community composition analysis of 16S rRNA sequence data helped to track phylum and genus level distribution of rhizobacteria of *S. album* species studied. The characterization of these bacteria colonizing the *S. album* rhizosphere will be beneficial to improve *S. album* tree productivity. Diversity among these bacteria was revealed by alpha analyses. Genera *Bacillus, Pseudomonas* and *Streptomyces* are well known for plant growth and plant disease suppression activities in othercrops (Amna et al. 2020; Chandra et al. 2020; Jiao et al. 2021). Comparedto previous studies *Acidobacteria, Actinobacteria* and *Firmicutes* are higher abundance at generalevel in *S. album* microbiome (Bing et al. 2022). Overall, the dominant genera identified in this study are known to fix atmospheric nitrogen, facilitating to plant growth.

A sandal tree can grow without the need of the host for their growth. To prove this concept we tried our experiment into three parts which was discussed above in the experimental setups. The available potassium (K) and phosphorus (P) plays important role in Bagaldhara plantations for enhancing the height and girth growth of sandal seedlings (Das et al. 2018). According to the previous report we have done our experiment without host. Instead of providing host, we applied bio fertilizers for their growth. Compared to sapling growth with host, using of only bio fertilizers shows good growth in morphology when compared to with host. Chemical elicitors are used as a treatment in one year and two years old sandal saplings to analysis the growth parameters (Yuan Li et al. 2021). Instead of using chemical elicitors our experiment carried out with *Actinobacteria* culture for their growth promotion. Compared with overall experiments culture treated saplings shows good morphology results for both one and two year old sandal saplings.

## 5. CONCLUSIONS

The rhizosphere microbial community is an important player in the plant-soil ecosystem. The approach used in this work let us recover a total of 9,038 communities. The sequencing data analysis of the V3-V4 region on the Illumina showed distributed over 15 phyla, 53 genera, and 8 species. Our study is to establish a *S. album* rhizosphere having the highest relative abundance of five major phyla, *Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi* and *Firmicutes* was found to be the dominant flora in the rhizosphere soil of *S. album* during growth. The top-ranked communities are concerning as growth promotion in *S. album*. The genera identified in this work are part of the microbiome of *S. album* identified in other research works. Filling the significant knowledge gap on soil nutrients and microbiota of *S. album* interactions is critical for exploiting these beneficial microbes for sustainable sandal tree cultivation. According to the soil nutrients morphology analysis report, the more organic carbon and macronutrients (N, P, and K) the soil contains, the better the sandalwood seedlings develop (both in height and girth) and survive without the host. Actinobacteria culture treated sandal sapling show higher height and girth when compared to soil nutrients. As per our report

proves that a sandal sapling can grow without host when it is treated with culture for their growth.

**Author contributions**

NSB performed the lab work under the supervision of VRK. MD did the statistical analysis. All authors contributed to interpret the results, write and revise the manuscript critically. All authors agreed with the final version of the manuscript.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data availability**

Data will be made available on request.

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**Table 1**
**Soil physicochemical properties of *Santalum album*.**

| S.No | Properties | Values |
|---|---|---|
| 1. | pH | 5.4 |
| 2. | Bulk Density | 1.34 Mg $m^{-3}$ |
| 3. | Water Holding Capacity | 15.6 % |
| 4. | Moisture Content | 13.7% |
| 5. | Electrical Conductivity | 0.14ds $m^{-1}$ |
| 6. | Organic Carbon | 6.3% |
| Available Nitrogen | | 91.6 mg/kg |
| Available Phosphorus | | 11.00 kg $ha^{-1}$ |
| Available Potassium | | 72.0 kg $ha^{-1}$ |
| 10. | Available Sulphur | 5.9 mg $kg^{-1}$ |
| 11. | Calcium | 9.35 kg $ha^{-1}$ |
| 12. | Magnesium | 3.86kg $ha^{-1}$ |

**Table 2**
**Growth Data of Sandal tree with host (neem) as on March 2021 and 2022.**

| S. No. | Year of Plantation | With Host (Neem Tree) | | |
|---|---|---|---|---|
| | | Height (cm) | Basal Grith (cm) | No. of.Leaves |
| 1. | 2020-2021 | 156 | 8 | 4 |
| 2. | 2020-2022 | 150 | 11 | 6 |

**Table 3**

**Growth Data of Sandal tree with *Actinobacteria* culture treated seeds + Bio fertilizers without host plantation as on March 2021 and 2022.**

| S. No. | Year of Plantation | *Actinobacteria* treatment (Sandal Seeds + Bio fertilizers) | | |
|---|---|---|---|---|
| | | Height (cm) | Basal Grith (cm) | No. of.Leaves |
| 1. | 2020-2021 | 214 | 20 | 8 |
| 2. | 2020-2022 | 196 | 16 | 10 |

**Table 4**

**Growth Data of common Bio fertilizers applied on sandal tree without host as on March 2021 and 2022.**

| S. No. | Year of Plantation | Bio fertilizers | | |
|---|---|---|---|---|
| | | Height (cm) | Basal Grith (cm) | No. of.Leaves |
| 1. | 2020-2021 | 180 | 8 | 6 |
| 2. | 2020-2022 | 176 | 6 | 8 |

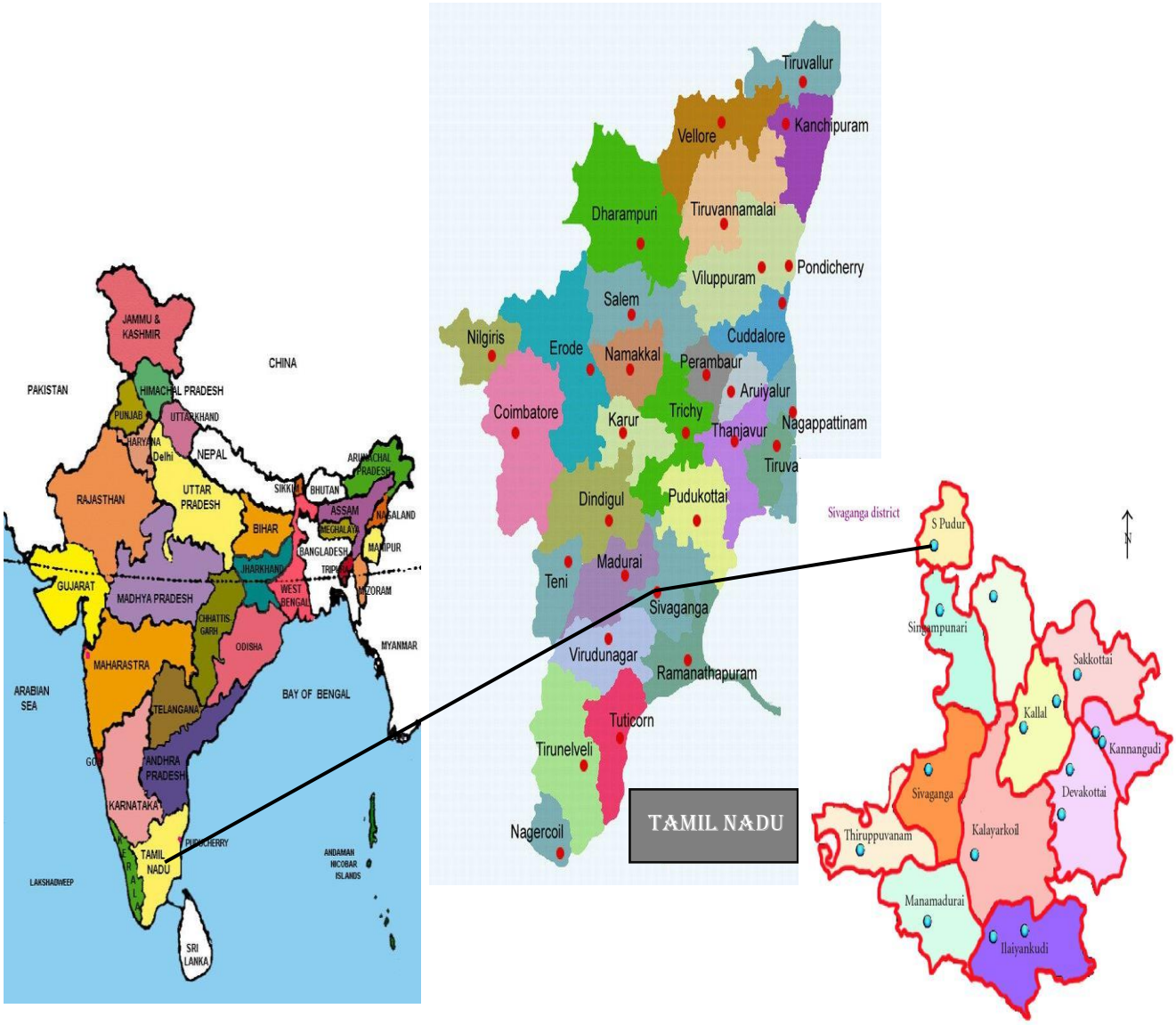


**Fig. 1.** Location map of S.Pudhur in Sivaganga District, Tamilnadu

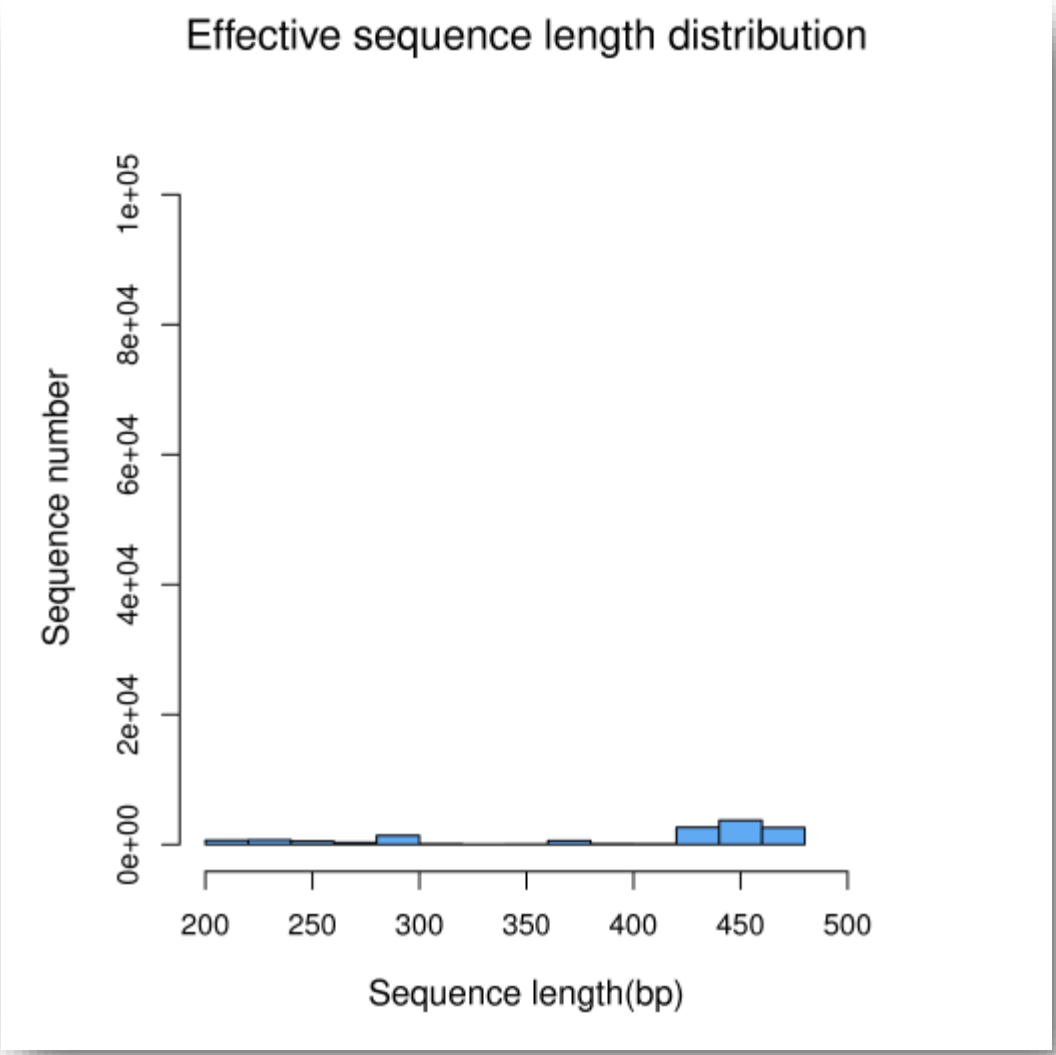


**Fig. 2.** Sequence length distribution for *S. album* rhizosphere soil.

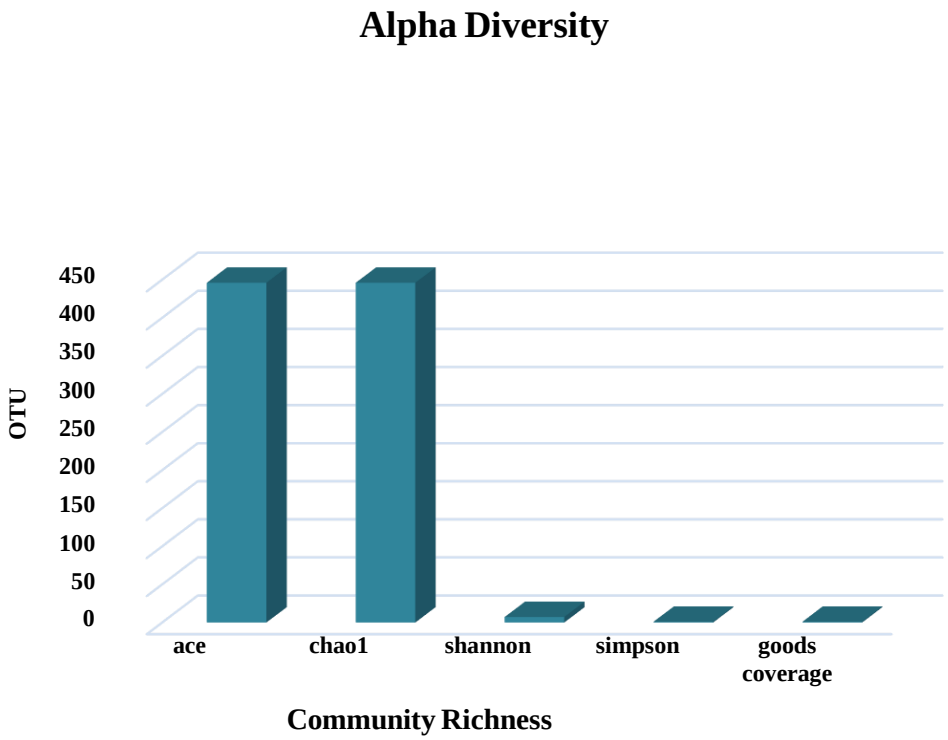


**Fig. 3.** Alpha diversity for *S. album* rhizosphere soil sample.

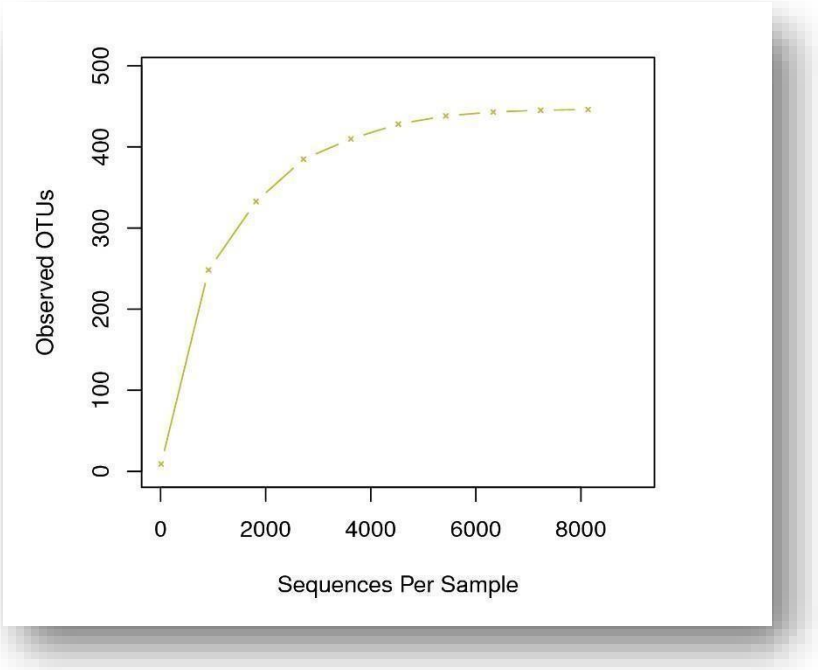


**Fig. 4.** Rarefaction curve.

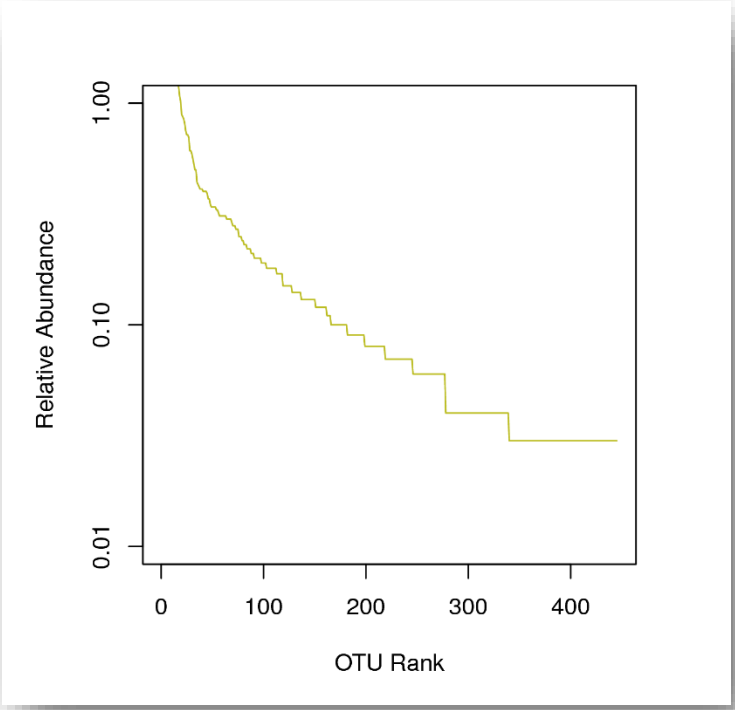


**Fig. 5.** Rank abundance curves

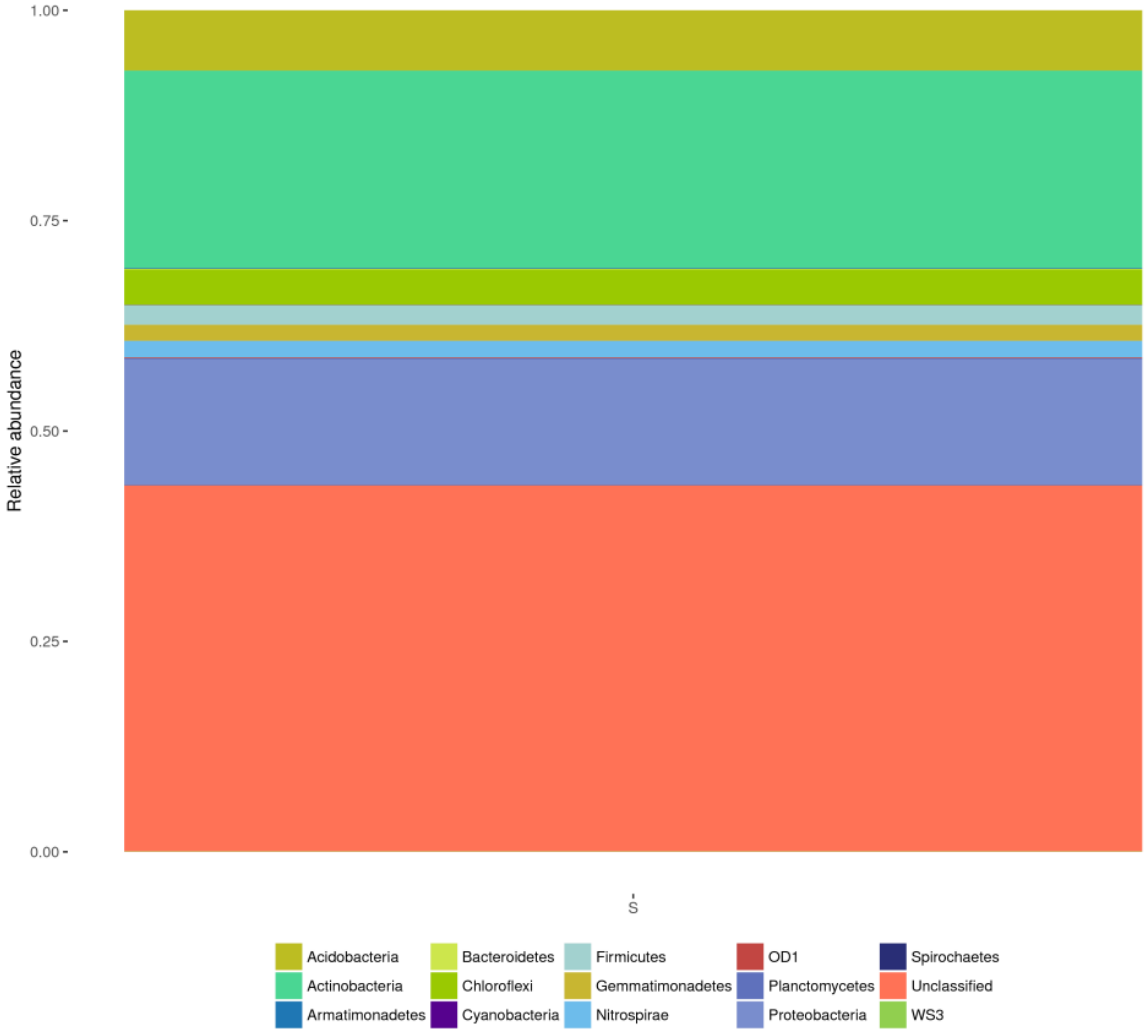


**Fig. 6.** The relative abundances of *Santalum album* at *Phylum* levels.

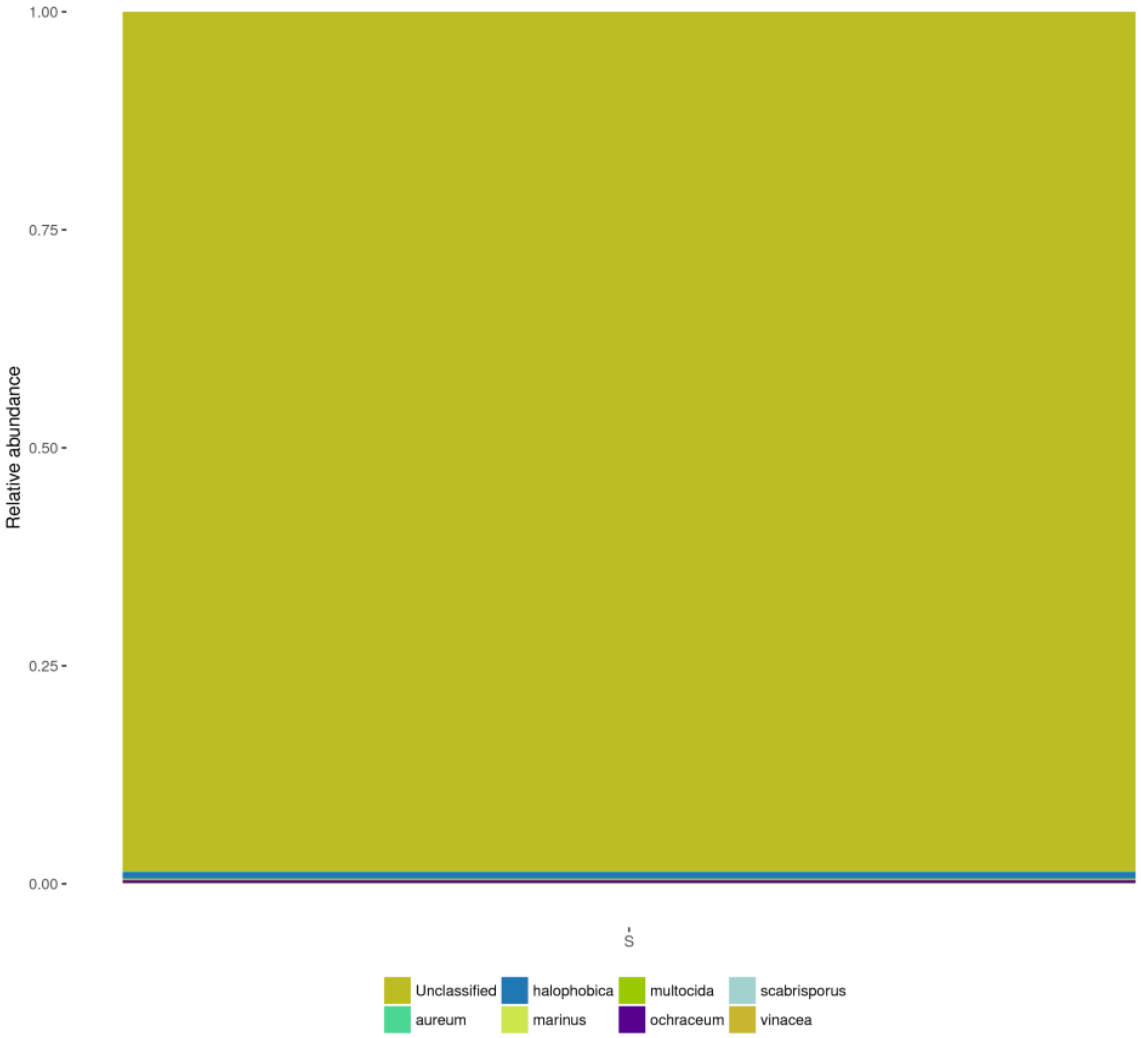


**Fig. 7.** The relative abundances of *Santalum album* at *Species* levels.