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Case Report

Metagenomic Analysis of the Outdoor Dust Microbiomes: A Case Study from Abu Dhabi, UAE

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Abstract: Outdoor dust covers a shattered range of microbial agents from land over transportation, human microbial flora, which includes pathogen and commensals, and airborne from the environment. Dust aerosols are rich in bacterial communities that have a major impact on human health and living environments. In this study, outdoor samples from roadside barricades, safety walls, and fences (18 samples) were collected from Abu Dhabi, UAE and bacterial diversity was assessed through a 16S rRNA amplicon next generation sequencing approach. Clean data from HiSeq produced 1,099,892 total reads pairs for 18 samples. For all samples, taxonomic classifications were assigned to the OTUs (operational taxonomic units) representative sequence using the Ribosomal Database Project database. Analysis such as alpha diversity, beta diversity, differential species analysis, and species relative abundance were performed in the clustering of samples and a functional profile heat map was obtained from the OTUs by using bioinformatics tools. A total of 2814 OTUs were identified from those samples with a coverage of more than 99%. In the phylum, all 18 samples had most of the bacterial groups such as *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. Twelve samples had *Propionibacteria acnes* and were mainly found in RD16 and RD3. Major bacteria species such as *Propionibacteria acnes*, *Bacillus persicus*, and *Staphylococcus captis* were found in all samples. Most of the samples had *Streptococcus mitis*, *Staphylococcus capitis*. and *Nafulsella turpanensis* and *Enhydrobacter aerosaccus* was part of the normal microbes of the skin. *Salinimicrobium* sp., *Bacillus alkalisediminis*, and *Bacillus persicus* are halophilic bacteria found in sediments. The heat map clustered the samples and species in vertical and horizontal classification, which represents the relationship between the samples and bacterial diversity. The heat map for the functional profile had high properties of amino acids, carbohydrate, and cofactor and vitamin metabolisms of all bacterial species from all samples. Taken together, our analyses are very relevant from the perspective of out-door air quality, airborne diseases, and epidemics, with broader implications for health safety and monitoring.

Keywords: dust microbiomes; metagenomics; microbial diversity; pollution; GIS

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1. Introduction

Airborne particles containing living or dead organisms, or their derivatives are referred to as bioaerosols. Bacteria, fungi, viruses, plant pollen parts, algae, spores, and bacterial endotoxins, and fungal mycotoxins form a large group of bioaerosols [1]. These inhalable particles with diameters of 10µm and smaller can enter the lungs and cause PM10 toxicity, resulting in adverse chronic health effects [2]. Such microbial aerosols found in both indoor

and outdoor dust are considered as a major source of harmful biological agents and their toxicity or pathogenicity depends on the components present in the dust [3]. Dust exposure has been implicated with different ailments such as allergies, asthma, chronic bronchitis, cancer, fibrosis, chronic obstructive lung diseases, bronchial hyperreactivity, organic dust toxic syndrome, and in irritating various mucous membranes present in the conjunctiva of the eye and skin [4]. Aside from public health, these airborne microorganisms play potential roles in meteorological processes such as clouds and snow formation, and in agriculture, they aid in the dispersion or deposition of phytopathogens on plant surfaces [5]. The main source for microbial aerosol transport across a large distance can be attributed to desert dust. Atmospheric dust loads are concentrated in the "Global Dust Belt", the broad region extending from the west coast of North Africa, through the Middle East, and into Central Asia [6]. We previously reported several studies pertaining to the dust characteristics observed in the United Arab Emirates as the region is characterized by a large expansion of deserts and frequent dust storms. Our earlier studies estimated the regional trend of aerosol size distribution over the Arabian Gulf region, the various seasonal impacts on the air qualities in industrial areas of the Arabian Gulf region, and the assessment pertaining to heavy metals in roadside dust along the Abu Dhabi-Al. Ain National Highway [7–9]. A recently concluded study [10] reported potential pathogenic-relevant bacteria in the dust event related samples, suggesting that the desert dust serves as a "vehicle for the global transport of pathogenic microbes as they can be carried over very long distances". However, their release can be influenced by temperature, humidity, radiation, and biological properties [11,12]. Although there are many reports available on the outdoor air-quality (AQ) of the UAE in terms of the particulate matter and heavy metal, no comprehensive study has been conducted on the nature of microbial consortia present in the outdoor air in this region. In this study, we investigated the microbial communities and their taxonomic composition of outdoor dust samples collected alongside the Abu Dhabi-Liwa Highway. DNA isolated from the collected samples were subjected to the 16S ribosomal RNA gene and 18S ribosomal RNA gene sequencing to better comprehend the level of microbial diversity and abundance in the samples. The identified microbial consortia were then matched to human microbiomes and many other publicly available data sources. Information on taxonomy from our study may shed more light on the health effects of specific groups of microorganisms, which might help in explaining the varied molecular patterns observed with the activity of the pathogen and allergens [9,10] across different taxa in the future. Moreover, constant monitoring of microbial aerosols through dust particles is highly relevant, especially in recent times due to high number of airborne disease outbreaks and related to the COVID-19 pandemic.

2. Materials and Methods

2.1. Sampling

The UAE is located in a dust hotspot that contributes to the arid climate. The selection of sampling sites was based on accessibility, intersections, topography, and susceptibility to strong wind impact, close proximity to farmlands, and trees/shrubs that act as wind breaks, and our spots covered a distance of 130 km (Figure 1). There were 18 dust samples swabbed from roadside barricades, safety walls, and fences by using a sterile spatula around 50 cm^2. Location coordinates for these dust samples were acquired using a S750 GPS instrument, which were processed and rectified in Microsoft Excel and ArcGIS 10.8 software. All collected dust samples were transported to the laboratory in an ice box with a cold gel pack.

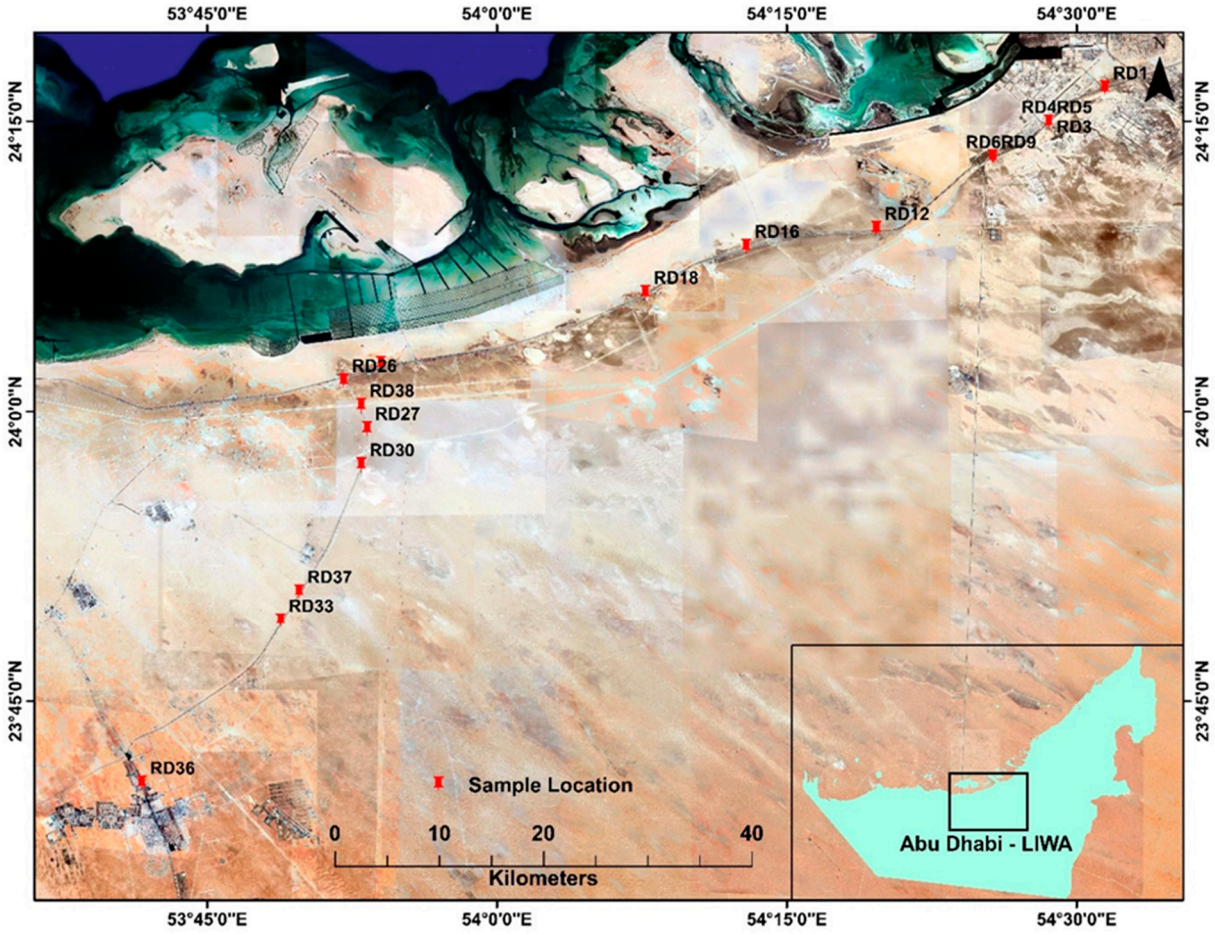


Figure 1. Location map of the surface roadside dust sample collection spots numbered as RD1-6, RD9, RD12, RD16, RD18, RD21, RD 26-27, RD30, RD33, and RD36-38.

2.2. DNA Extraction and Quantification

DNA extraction were collected for 18 dust sand samples by using the NucleoSpin Soil Extraction Kit (MACHERE-NAGEL, Düren, Germany). Dust samples of 500 mg were weighed into MN bead tube type A, 700 µL of SL1 was added, 150 µL Enhancer SX was added, and then horizontally vortexed for 5 min at RT. The DNA from the lysed samples was purified by following the manufacturer's instructions. DNA was eluted by 50 µL of the kit elution buffer and quantified by using Nanodrop (Thermo Scientific, Waltham, MA, USA).

2.3. Polymerase Chain Reaction (PCR) and Sequencing

Extracted DNA samples were sent to BGI Genomics, Hong Kong for 16S amplicon sequencing. A total of 30 ng of each DNA template and the 16S rRNA fusion primers (16S-V3-V4) were added for PCR. All PCR products were purified by Agencourt AMPure XP beads, dissolved in elution buffer, and eventually labeled to finish the library construction. Library size and concentration are detected by Agilent 2100 Bioanalyzer. Qualified libraries were sequenced on a HiSeq platform according to their insert size.

2.4. Bioinformatics Analysis

Raw data were filtered to obtain the high-quality clean data, after which the clean reads that could overlap each other were merged to tags and further clustered to OTUs. Taxonomic classifications were assigned to the OTU representative sequence using a database. OTU representative sequences were aligned against the database for taxonomic annotation by the Ribosomal Database Project classifier (v2.2) software (sequence identity was set as

0.6). Analyses such as alpha diversity, beta diversity, differential species analysis, network and model prediction were carried based on the OTU profile table and taxonomic annotation results. Taxonomic analysis of the OTU representative sequences was carried out by the RDP classifier Bayesian algorithm to identify the composition of the microbial structure. The abundances of species on seven levels (phylum, class, order, family, genus, species) were calculated after annotation.

Heat maps were generated by clustering the samples based on species abundance in the samples (at all seven levels) to reveal the similarity among samples. The software R (v3.1.1) package 'gplots' was used to complete the clustering method and 'Euclidean' distance. The predicted KEGG function abundance of the bacterial community was obtained by PICRUST2. The function used KO ID as the name, which represents a specific functional gene, and then obtained the three levels of metabolic pathway information according to the KEGG database, and finally, the abundance table of each level. The software used was PICRUSt2 v2.3.0-b,R (v3.4.10).

3. Results

We conducted a comprehensive sampling based on the accessibility, intersections, topography, and susceptibility to strong wind impact, close proximity to farmlands, and trees/shrubs that act as wind breaks in a 130 km roadside area of the Liwa-region in Abu Dhabi Emirate of the UAE (Figure 1). The collected 18 dust samples were subjected to DNA isolation and library preparation after quality control (QC) analysis. In total, we obtained 1,099,892 read pairs for 18 samples after the sequencing. We identified 2814 OTUs from a total of 1,099,892 reads in the dust samples with 25 known and other phyla, 15 known and other classes, 19 known and other orders, 25 known and others families, 27 known and other genera, and 20 predominant and other species. After slicing and filtering the double ended reads, 1,097,476 clean tags were generated. Each sample was produced at an average of 60,970 clean tags and an average of 418 tag length. With a coverage of more than 99%, a total of 2814 OTUs were identified from those samples. Major OUTs were found in sample RD36 as 487, RD33 as 417, RD2 as 260, and RD21 as 201 (Figure 2). Out of all the samples, RD33 and RD36 had above 1000 OTUs. All other samples had OTUs less than 50, and overall only two OTUs were found to be shared (Figure 2). The two shared OTUs found were *Corynebacterium tuberculostearicum* and *Propionibacterium acnes*.

Aside from the analysis of the major OTUs, we also performed species relative abundance and visualized them as phyla and species bar plots (Figure 3). In the phylum, all 18 samples had most of the bacterial groups such as *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. The *Firmicutes* phylum had all the Gram-positive bacteria and Bacteriodetes had all the Gram-negative bacteria. The RD1 sample was collected from the highway fence and it had a greater percentage of the Cyanobacteria unclassified family GpI (glucose-6-phosphate isomerase) and less of Actinobacteria and the others when compared to all of the samples. As per the OTU taxonomy, sample RD38 showed the *Cyanobacteria* species level identification as *Prochlorococcus_marinus*. Species were classified into 'others' if their relative abundance was less than 0.5%. Out of 18 samples, 12 samples had *Propionibacteria acnes* and were mainly found in RD16 and RD3. Major bacteria species such as *Propionibacteria acnes*, *Bacillus persicus*, and *Staphylococcus captis* were found in all samples. *Mycoplasma pirum* was found mainly in samples RD2, RD5, and RD9. Most of the samples had *Streptococcus mitis*, *Staphylococcus capitis*, and *Nafulsella turpanensis* and *Enhydrobacter aerosaccus* is part of the normal flora of the skin. *Salinimicrobium* sp., *Bacillus alkalisedimins*, and *Bacillus persicus* are halophilic bacteria that are normally found in sediments.

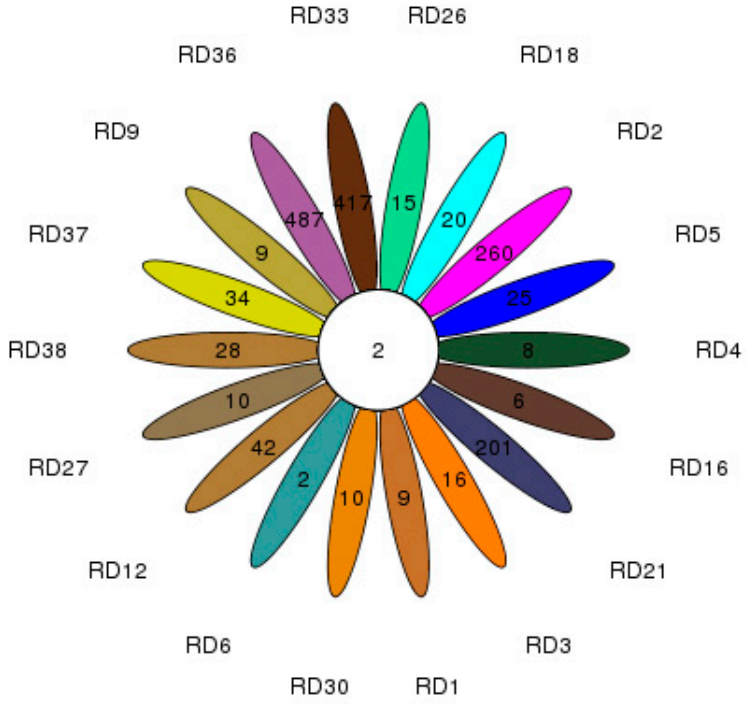
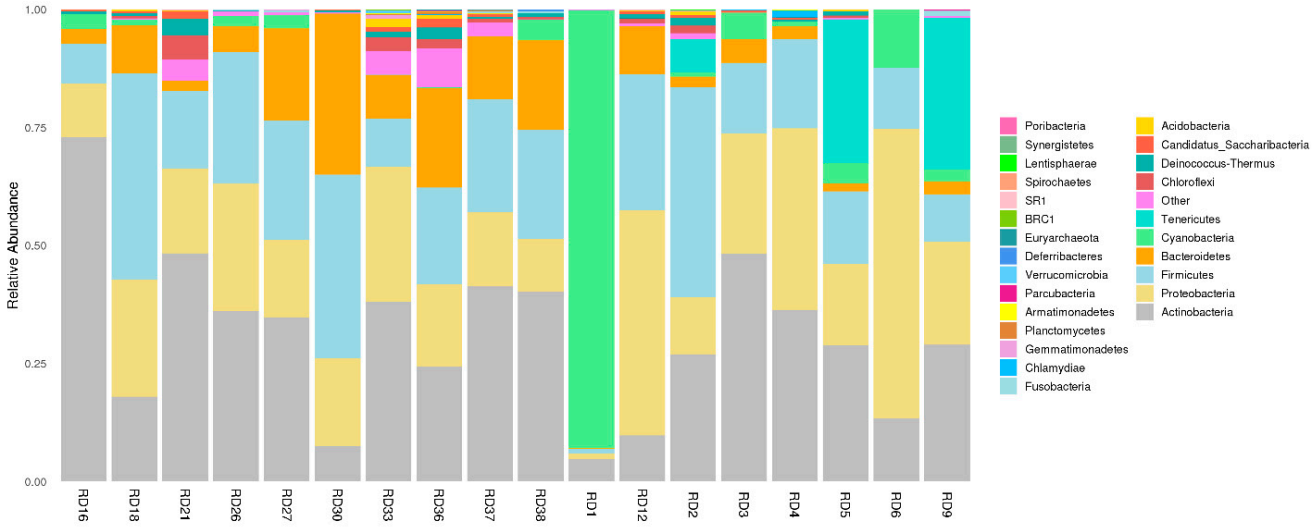


Figure 2. Core-Pan OTU plot. Similar and different OTUs of the 18 samples. The middle circle indicates the number of shared OTUs in these samples or groups and the ellipse OUT side the middle circle indicates the number of OTUs that are peculiar in only this sample or group.

Heat map of horizontal clusters indicate the similarity of certain species among different samples (Figure 4). It can be inferred that samples were more likely to be similar to each other when a closer distance/shorter branch length is shown in the graph. Relative abundance values were normalized through log-transformation. Additionally, if any sample's relative abundance value was 0, then the value will be replaced by half of the minimum abundance value of all samples. Based on this heat map, the identified bacterial species were not infectious and microbial flora of the environment. In horizontal clustering, RD16 and RD9 were high in similarity and these were both similar to RD1 and least similar to RD33 in terms of the functional profile. The functional profile heat map describes the biological and molecular ability of bacterial species in each sample (Figure 5). Bacterial species from all samples had high properties of harboring metabolic pathways such as amino acids, carbohydrate as well cofactor and vitamins as core metabolic signatures. All of the sampled bacterial species did not show environmental adaptation, infectious disease spreading, and an endocrine hormonal effect.

(**a**) Phylum



(**b**) Species

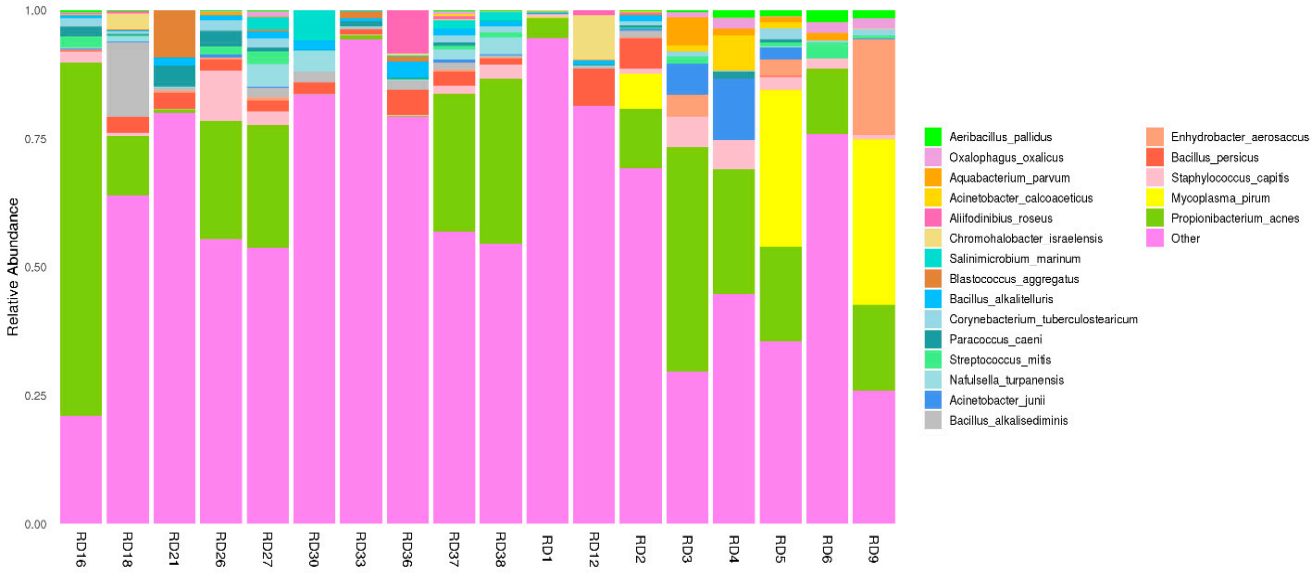


Figure 3. Bar plot shows the composition and proportion of species in each sample/group. Species were classified into 'others' if their relative abundance was less than 0.5%. Composition of the bacterial structure for phylum (**a**) and species (**b**).

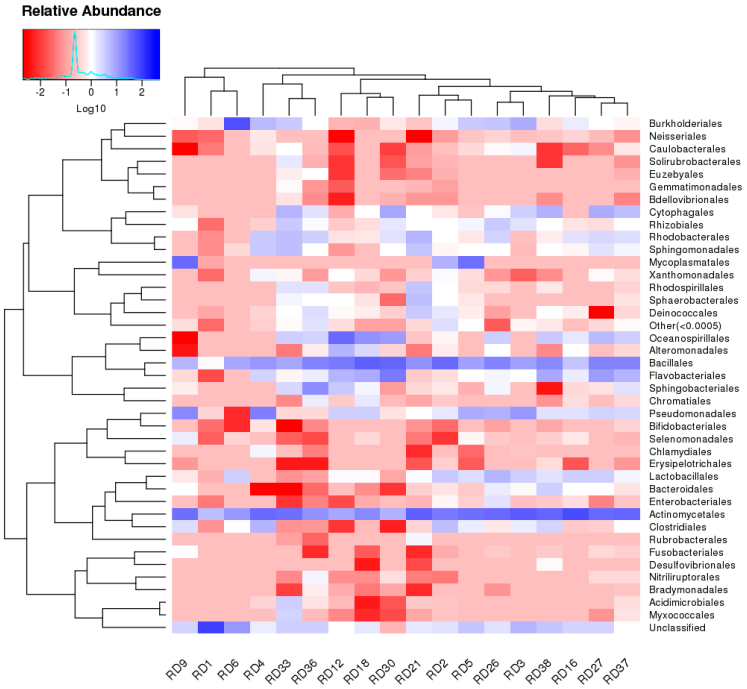


Figure 4. Order heatmap. Heatmap showing the bacterial taxa (order level) with relative abundance obtained by 16S rRNA amplicon sequencing. Horizontal clustering shows the range of similarity among the 18 samples in order abundance. Vertical clustering shows the bacterial phylogenetic similarity found in all samples.

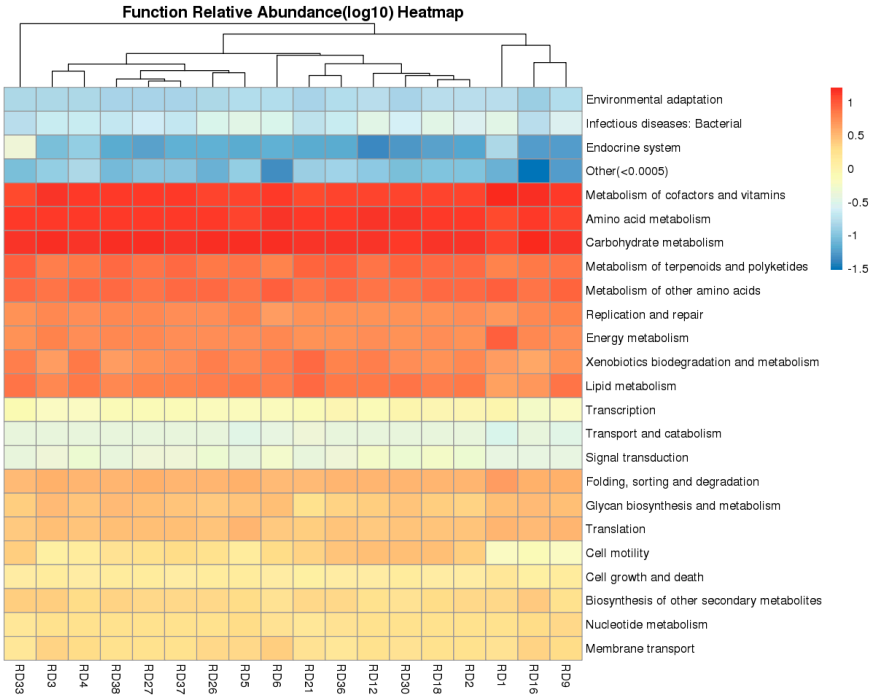


Figure 5. Functional profile heat map. Heatmap of the predicted function profile. Longitudinal clusters indicate the similarity of all of the predicted functions among different samples, and the horizontal clustering indicates the similarity of certain predicted functions among different samples, where the closer the distance and the shorter the branch length, the more similar the predicted function between the samples. Relative abundance values were log transformed for normalization. If the relative abundance of certain species is 0, half of the minimum abundance value will substitute for it. Functions whose abundance values are less than 0.5% will be combined into others.

4. Discussion

Dust samples analyzed from the reads of the 16S rRNA genes showed that the majority of the bacteria belonged to the phyla *Actinobacteria, Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. These listed phyla have been previously reported to be found at high relative abundances in aquatic and desert environments and are in good accordance with the results published by Liu et al. [11,12]. Actinobacteria are usually soil-borne bacterial species, while bacterial species belonging to Firmicutes are tailored to adapt to the dry and high temperature environment of the desert [13]. The wall less bacteria, with reduced genome belonging to phylum *Tenericutes*, was also observed in our analyzed samples. *Tenericutes* is phylogenetically related to Firmicutes and is known to inhabit various ecological niches [14]. Aside from these, the library also detected significant amounts of the sequences of phyla *Chloroflexi*, *Acidobacteria*, Actinobacteria, *Deinococcus-Thermus*, another characteristic feature of the investigated community. *Acidobacteria*, considered an oligotrophic phylum [15], was also well represented. These results are similar to the phylogenetic diversity of microbial communities in Lake Baikal [16]. Members belonging to the phylum *Chloroflexi* are associated with organic matter fermentation and with the zone of active methanogenesis [17]. The results from the metagenomic study conducted provide new insights into the species composition, relative abundance, and dynamics of the bacterial communities within the Abu Dhabi Emirate. The relative abundance of the major phyla present in the bacterial microbial communities are represented in the heat map (Figure 4). These heatmaps were used to visualize patterns in the bacterial communities found in the outdoor dust samples. We observed two shared OTUs of *Corynebacterium tuberculostearicum* and *Propionibacterium acnes*. Both are Gram-positive bacteria residing as human skin commensals and can behave as opportunistic pathogens, depending on the strain and environmental conditions [18]. At the species level, the majority of samples showed *Propionibacterium acnes* followed by *Mycoplasma pirum*, *Staphylococus capitis*, *Bacilus persicus*, *Enhydrobacter aerosaccus*. *Enhydrobacter aerosaccus* is the only species from the genus Enhydrobacter and halophilic Gram-positive bacterium such as *Bacilus persicus* was also identified in our analyzed sample [19,20]. *Mycoplasma pirum* lacks a cell wall, cell membrane, resists antibiotics, and was found to affect immunocompromised patients [21]. The majority of Streptococcus species are pathogenic and are implicated in the etiology of bacteremia, sepsis, and pneumonia [22]. Whilst the study was limited by its sample size and the geographical extent covered, it is of immense important in public health as some of them can act as "microbial hazards" for highway maintenance operatives and vehicle restraint operatives. Actinobacteria are ubiquitous in nature and abundant in the soil surface. It was reported that they can be considered as a bioaerosol when they are within a 0.3 m radius (breathing zone) of a worker's nose and mouth [23]. Phylum *Bacteroidetes* are usually 'friendly commensals in the gut" but can be "opportunistic pathogens" [24]. Another bacterial phylum identified was from the Firmicutes genera (e.g., *Bacillus*). Several studies have pointed out that this group of Gram-negative, endotoxin-producing bacteria can trigger chronic bronchitis, hypersensitivity pneumonitis, organic dust toxic syndrome, asthma, and allergic sensitization [25]. The phylum *Proteobacteria* includes a variety of pathogenic bacteria such as Escherichia, Salmonella, Vibrio, and Helicobacter and are considered as possible "microbial signatures of disease". They also include free-living bacteria that help in nitrogen fixation [26,27]. The functional profile heat map generated from all bacterial species in the study revealed that carbohydrate metabolism, amino acid metabolism, and metabolism of the cofactor and vitamins represented the most abundant pathways.

5. Conclusions and Outlook

Our preliminary data from bacterial communities suggest that bacterial communities in the outdoor samples were not related to infectious agents. These results from the UAE samples represent bacterial communities thriving in an arid desert climate and embodies the best way to map human microbial exposomes, improve pathogen surveillance in public

health decision-making in the UAE as well as their role in regulating atmospheric processes and global climate change [28,29]. The study is aligned with the UAE Vision 2021 [30], as the country is well-known for implementing extensive health system reforms and its commitment to maintaining air quality. It is noteworthy to mention that the UAE is not the only country in the region that faces dust storms but other neighboring countries in the gulf have a similar geo-climatic position regarding sand and dust storms. Researchers in the region can profit from this pilot study in analyzing the indoor as well as outdoor microbiomes with similar protocols adopted in this study. Likewise, a broader consortium from GCC countries about the study of outdoor dust microbiomes at different locations and different seasons of the years would be an interesting extension of the current work. Whereas microbes are not the only entity that can cause health effects in dusty ambiences, the particulate nature of the dust, the amount of heavy metals as well other organic and inorganic irritants are equally detrimental when it comes to assessing the health effects. Therefore, we advocate a holistic approach in future study that encompasses most of the underlying factors associated with dust effects, with better resolution in terms of the sample size, better representation in terms of the sampling locations, and various time-periods to consider the seasonal variations. This integrated approach will unfold a comprehensive inference of the health effects of outdoor dust environments.

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