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Differential Expression of Metabolic Genes and Diversity of Soil Bacterial Community in Alluvial and Rocky Soil

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***Abstract*:** Microbes in soil primarily function to regulate nutrient cycle and maintain soil fertility. Extracellular enzymes produced by microorganisms breakdown organic material (for example, dead plants) into accessible nutritional components such as carbon (C), nitrogen (N), and phosphorus (P). This study showed that different soil ecology has distinct bacterial genera dominance and metabolic pathway to maintain soil nutrient cycle. The soil obtained from two agro climates zones/regions, i.e., Ralegan-Siddhi, Maharashtra (S1), and BBA University, Lucknow (S2), shows highly variable climatic conditions. Metagenomics tools used for prediction showed, *Fusobacterium* predominates in rocky soil, while *Flavisolibacter* predominates in alluvial soil whereas metabolic gene profiling shows dominance of the soil bacteria mainly for nitrogen and sulphur metabolism. Metabolic genes involved in the nitrogen cycle are more abundant in soil samples collected from alluvial soil, while genes involved in the sulphur cycle are more abundant in rocky soil. This study suggests that microbial diversity depends on the environment and functions in response to their surroundings.

***Keywords*:** Microbial diversity, Rocks, Metagenomics and metabolic pathway

Microorganisms are thought to be a major driving force in all the major nutrient cycles on earth, and this is especially true in the case of deserts, where plants are limited or non-existent. Microbes create enzymes that aid in the maintenance of various nutrient cycles in soil (Cavicchioli et al 2019). Hydrolases and oxidases are enzymes that breakdown substrates and release nutrients into the soil (Chukwuma et al 2020). Urease is an enzyme that catalyzes the breakdown of urea and is linked to microbial N uptake, 1,4-glucosidase is a hydrolytic enzyme that decomposes polysaccharides and is generated by soil bacteria and Phosphatases, both acidic and alkaline, are enzymes involved in the acquisition of phosphorus, and they cleave PO_4^{3-} from phosphorus-containing organic molecules (Wei et al 2018, Lasa et al 2019 and Svane et al 2020). The enzyme arylsulfatase hydrolyzes the organic sulphate esters catalyzes releasing SO^2 for plant use (Sekaran et al 2018). Numerous natural (i.e., geographical, physio-geological, or physicochemical properties of soils and anthropogenic (agricultural practices, waste discharge and mining) factors can influence microbial enzyme activities and production. Sun et al (2020) reported that soil pH, AP content, and the activities of the four enzymes were the most important factors influencing the soil bacterial community structure. Furthermore, as subsidence increased, so did soil nutrient content, enzyme activity, bacterial richness, and evenness (coal mining subsidence is classified according to geological hazards, groundwater contamination, and landscape damage) (Jing et al 2018). Climate, microbial biomass, and microbial community makeup, according to Waldrop, might influence the maximum rates of soil enzyme activity, potentially altering decomposition and nutrient mineralization rates in soils (Ali et al 2021). Phetcharat et al (2018) demonstrated the effect of inorganic fertilizers on the change of bacterial communities connected to the reservoir rock. The development of new molecular techniques in environmental microbiology over the last few decades has increased interest in understanding microbial systems' complexity, diversity and activity.Still, metabolic activity in the Rocky plateau zone remains unexplored (Wiseschart and Pootanakit 2020). In present used the metagenomics technique study to investigate bacterial enzyme activity in semi-arid rocky plateaus and arid alluvial soil. The current state of capabilities for accessing and predicting the catalytic potential of microbial communities, to capitalize on the use of biological activities for bio catalysis is discussed.

MATERIAL AND METHODS

Sample collection, DNA extraction and analysis: Soil samples were collected in sterile bags from the Ralegan-Siddhi site in the district of Ahmednagar, Maharashtra (S1), and the Net house of BBA University in Lucknow (S2). Commercially available kits were used to extract microbial DNA from samples. Amplification was performed using

QIAGEN and 40 ng of extracted DNA, as well as 10pM forward and reverse primers of the 16S rRNA V3-V4 region for bacterial and TAQ Master mix. For the amplification, we employed denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds, elongation at 72°C for 2 minutes, and final extension at 72°C for 10 minutes. It was placed on hold at 4°C until the sequencing was completed. To prepare the sequencing libraries, Ampure beads were used to eliminate unwanted primers from each sample's amplicons, and an additional 8 cycles of PCR were done using Illumina barcoded adapters. Ampere beads were used to purify the libraries, and the Qubitds DNA high sensitivity assay kit was used to quantify them. Illumina Miseq with 2x300PE v3 sequencing kit was used for the sequencing. The KEGG database (https://www.genome. jp/kegg/) was used as reference data to align the representative sequences, and PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) 1.1.0 analysis tool was used to perform detailed investigations at the genus level from single reads.

RESULTS AND DISCUSSION

In the present study used a metagenomics tool to understand the diversity of bacterial genera and functional gene annotation in two different geological soils i.e., rocky soil from Ralegan-Siddhi (S1) and alluvial from BBAU net house (S2). In the S1 sample, *Fusobacterium* is the most dominant genus, accounting for 20% of the top ten, followed by *Prevotella*, *Leptotrichia*, *Capnocytophaga* and *Neisseria* whereas in the S2 sample, *Flavisolibacter* (16%) was dominant followed by *Clostridium*, *Bacillus*, *Pseudomonas* and *Prevotella*. *Fusobacterium* and *Prevotella* are common in both soil samples and are among the top ten bacterial genera (Fig. 1). indicating vast diversity in the bacterial genera of present in soils, and rocky soil.

The PICRUSt 1.1.0 analysis tool and the KEGG database (https://www.genome.jp/kegg/) analyzed the microbial communities' functional gene annotation. Both soil samples show a higher abundance of genes involved in metabolic pathways, the S1 sample has a greater abundance of bacterial communities contributing to the N2 cycle, followed by the Sulphur cycle, than the S2 sample. The Ammonia oxidation (80.5 %), dehalogenation (40.9 %), nitrite reducer (37.7 %), nitrogen fixation (25.3 %), sulfate reducer (48.2 %), sulfide oxidizer (27.3 %), and lignin degrader have relatively higher abundance in soil samples (0.4 %) as compared to rocky soil. The pathways involved in chitin degradation account for 19.6 percent of the S2 sample, followed by Xylan degrader, aromatic hydrocarbon degradation. Atrazine metabolism, and sulphur oxidizer. The bacterial community involved in degradation pathways dominates the S2 sample, whereas the bacterial community involved in the Nitrogen and Sulphur cycle dominates the S1 sample (Fig. 2). The bacterial community present in the S2 sample are active in the nitrogen cycle compared to rocky soil and on other S1 community are most active in the sulphur metabolic pathway for which low availability of nitrogen and a good source of sulphur mineral in rocky soil is one of the reasons. Jia et al (2020) observed that N enrichment has either a positive, neutral or negative impact on soil microbial biomass in ecosystems. In soil and aquatic ecosystem, the significance of bacteria in the nitrogen cycle has been widely studied, but

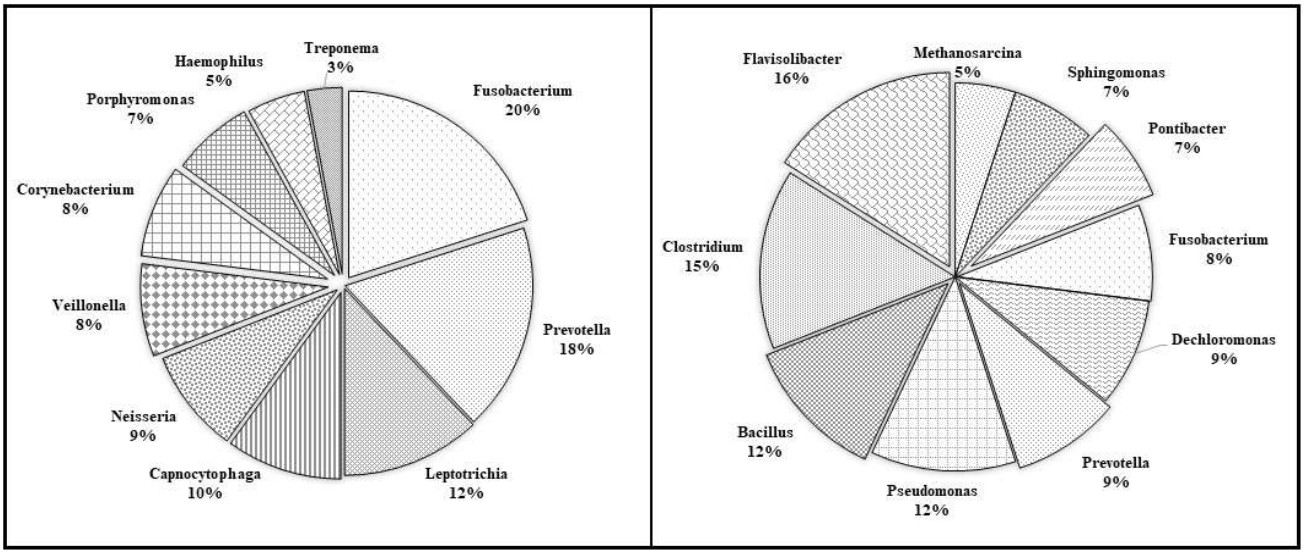

Fig. 1. Bacterial genera predicted in metagenomics analysis. (a) Rocky soil from Ralieh-gan siddhi, Maharashtra and (b) Alluvial soil from BBAU, Lucknow

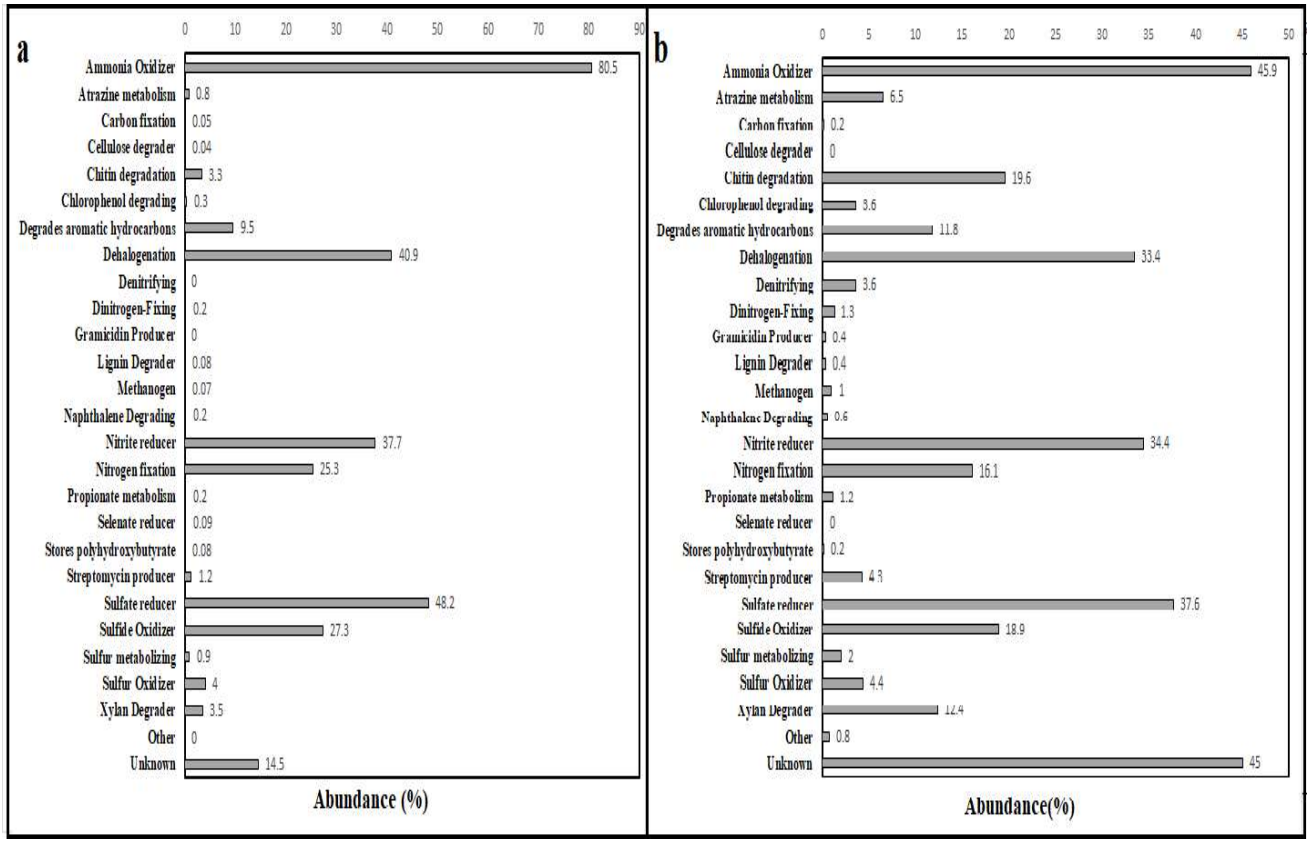

Fig. 2. Abundance of bacterial metabolic gene in different soil sample. (a) Rocky soil from Ralieh-gan siddhi, Maharashtra and (b) Alluvial soil from BBAU, Lucknow

knowledge of cave sediment is limited., Zhu et al (2019) concluded that microorganisms in oligotrophic conditions can acquire both energy and nutrients through nitrogen cycling activities. This research uncovered the majority of the nitrogen cycle genes. The presence of hydroxylamine oxidase genes shows that a crucial ammonia oxidizing bacterium is present. Lithochemotrophy may be a survival strategy for the bacterial communities on the rocks' sediments, as evidenced by the presence of ammonia oxidizing bacteria and sulfur-oxidizing bacteria in chemolithotrophic rocks (Flood et al 2021). Autotrophic nitrification has been discovered in *Nitrospira* and *Nitrosospira* indicating a CO2-fixation-coupled ammonia oxidation process in the studied ecosystems (Alfreider et al 2018).

CONCLUSION

Soil from Ralegan-Siddhi in Maharashtra and at BBAU in Lucknow were substantially different in every way, and metagenomics data revealed a significant difference in bacterial community diversity in both samples. The organisation of the bacterial population is influenced by environmental factors, and they express their metabolic activity accordingly. Understanding microbial diversity and metabolic gene prediction can aid in comprehending the environment from which they were isolated, as well as forecasting soil microbial ecology succession in evolution and microbe relationships.

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