

## Abstract

Microbial communities within the deep Earth crust play critical role in major biogeochemical activities of this planet. It is of great importance in understanding the biosphere within large igneous provinces having limited nutrient resources. The present study aims to explore the microbial diversity and function within the deep terrestrial crystalline basaltic and granitic horizons and aquifer therein of the Koyna–Warna region known for Reservoir Triggered Seismicity. Core samples, collected from granitic, basaltic and transition zones at different depths (60–1490 m) were used for detailed geo-microbiological investigation. Presence of different prerequisites of life such as water, nutrients and amicable environment within the extreme and oligotrophic igneous provinces of deep subsurface Deccan traps was studied through different geochemical analysis. Geochemical data of the rock samples revealed low organic carbon content (4–48 mg/kg) and characteristic mafic-felsic minerals containing high silica and iron. Exploration of bacterial and archaeal diversity through deep sequencing of bacterial V4 and archaeal V4-V5 region of 16S rRNA gene was conducted. Bacterial communities were mainly dominated by *Alpha-*, *Beta-*, *Gamma- Proteobacteria*, *Actinobacteria* and *Firmicutes*. Archaeal communities in basaltic horizon were dominated by acidiphilic *Thermoplasmatales* members *Thermoplasmata* and *Ferroplasma*, hydrogenotrophic methanogen *Methanobacterium* along with *Sulfolobaceae* and *Halobacteriaceae*. Acetoclastic methanogen *Methansarcinales* and South African Gold mine crenarchaeotic group 1 (SAGMCG-1) were found to be the abundant archaeal taxa in organic carbon lean granitic samples. Microbial community composition was found to be governed by various geochemical characteristics specific to different rock horizons. Shotgun metagenome sequencing displayed that fermentation, denitrification, ammonification (assimilation and respiration) and sulphur oxidation are the major fuelling reactions in the subsurface ecosystem which links the carbon, nitrogen and sulphur cycles. Microbial community structure of surface and sub-surface (up to 1027 mbs) water from Deccan Traps was explored through sequencing of V4 region of 16S rRNA gene. Distinct difference in microbial community of surface and subsurface water was observed. *Comamonadaceae* and *Moraxellaceae* families were more abundant in groundwater samples whereas *Sporichthyaceae* and *Planctomycetaceae* members represented the major populations of surface water. Comparative analysis among microbial diversity and community function across different subsurface provinces (subterranean rock and water samples) revealed distinct

partitioning of microbial population. Microbial community response under pressure stress (25 and 50 MPa) was studied using basaltic water. A prominent shift in community structure was observed under different pressure and nutrient conditions which indicated presence of piezotolerant and piezophilic bacterial populations in the Deccan groundwater. Genomic inventories of different piezotolerant strains were assessed to understand the possible genomic repertoire responsible for pressure adaptation and tolerance. Overall results suggest that deep, aphotic oligotrophic Deccan subsurface at Koyna-Warna region is not only habitable for life but also supports and sustains diverse microbial community partitioned by horizon specific ambient milieu which have adapted/evolved under different extreme stresses.

**Keywords:** Deep Life, Koyna-Warna region, Geochemistry, Amplicon sequencing, Shotgun metagenome sequencing, Biogeochemical cycles, Pressure adaptation