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Exploring deep life underneath the crystalline granitic crust at Koyna, India through 3000 meter deep Koyna pilot borehole

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Scientific drilling of 3000 meter deep borehole at Koyna, India has created an unprecedented opportunity to explore microbial life from interior of the dark, granitic Earth crust of Archean eon. Here we present the first report on deep life communities residing within this extreme realm as studied through rock cores recovered from seven horizons (1680 - 3000 mbsl) and corresponding drilling fluids (DF). The igneous rock samples represented a progressively hot (55.6-73.9 ^{o}C), crystalline, organic carbon lean (maximum 0.0012%), alkaline (pH>9.0) environment with extremely low porosity (<0.2 %). Analysis of bentonite based DF indicated infusion of rock constituents during the coring, thus impacting its chemical properties significantly as evident from change in calcium, manganese, iron, sulfate concentrations and pH. Rock samples yielded considerably low concentration of DNA; together with qPCR data, a low biomass condition (estimated 10^2-10^3/g) was evidenced in these samples. DFs, on the other hand were microbially enriched (estimated 10^6-10^7cells/ml). High throughput 16S rRNA amplicon sequencing indicated that the rock microbiomes were dominated by *Gammaproteobacteria*, *Actinobacteria*, *Firmicutes*, *Alphaproteobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Deinococcus-Thermus* and *Chloroflexi*. Except *Gammaproteobacteria*, abundance of all other taxa increased with depth, indicating a strong change in community composition with increasing extremities. Among *Archaea*, phyla *Euryarchaeota* and *Thaumarchaeota* were represented by less abundant populations. Networks built on Spearman correlation suggested presence of strong tie ups among the thermophilic, extreme stress tolerant, anaerobic, fermentative, sulfate-reducing, N_2-fixing microorganisms. Members of *Cyanobacteria* were distributed into two different clades (*Oxyphotobacteria* and *Melanibacteria*) with evolutionary linkage to origin of oxygenic photosynthesis, which represented the integral parts of such networks indicating the potential biogeochemical significance of such ancient organisms. From evolutionary perspectives, this observation was extremely intriguing. Analysis of DF microbiomes showed predominance of *Firmicutes, Gammaproteobacteria, Actinobacteria* and *Euryarchaeota* with distinct change in their relative abundance over depths. Most abundant sequence reads from DF suggested strong lineages to extremophilic organisms (peizotolerant *Marinilactibacillus;* thermophilic, sulphur-respiring *Anaerobranca;* alkaliphilic, thiosulfate-reducing *Mongolitalea, etc.*) which could be well adapted in deep, dark, hot, alkaline crustal systems. Abundance of such microorganisms highlighted the importance of drilling fluid as a window to look into the deep life of crystalline Earth crust. In order to ascertain the cultivability and explore metabolic potential of rock dwelling microorganisms, a set of cultivation strategies was adopted using different electron acceptors (SO_4^{2-}, NO_3^-, Fe_3^+,CO_2) and energy donors including H_2andCH_4 and . Strict anaerobic, high temperature (50 ^{o}C and above) incubations allowed growth of *Micrococcus* and *Corynebacterium* as CH_4 metabolizing organisms, while *Ralstonia*, *Pseudomonas, Comamonas* grew with H_2 and CO_2. Enrichment of Fe_3^+ respiring *Lysinibacillus, Paracoccus, Exiguobacterium*; NO_3^- reducing *Skermanella*, *Kocuria*, *Sporosarcina, Actinotalea, Paracoccus*, and SO_4^{2-}/$S_2O_3^{2-}$ reducing *Anaerobranca* indicated existence of biogeochemically relevant, live microbial populations within the deep subsurface at Koyna. Further research will explore the details of the functional aspects of these extreme communities providing better insights in to the deep biosphere.