Unveiling of metal-tolerance bacterial consortia in chromite mine by metagenomic approaches

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Abstract

Heavy metal-contaminated soils are a widespread problem in mine field environments. To gain insights on the structural and functional diversity of bacterial community present in chromium contaminated mine soil obtained from Sukinda Chromite mine of Odisha (India), whole metagenomic analysis was performed by using Illumina HiSeq platform. Chromite mine soils contain high concentrations of heavy metals such as Mn, Fe, Cu besides chromium and low concentrations of organic carbon, available nitrogen, phosphorus and potassium. The bacterial community living in that hostile environment was correlated with the inherent abilities of these strains to be involved in the reduction of the major heavy metal Cr(VI). Our results showed that *Proteobacteria* (66.45%) were found to be the most abundant in the study area, followed by *Actinobacteria* (17.32%), *Bacteroidetes* (4.65%). The Keto Encyclopedia of Genes and Genomes (KEGG) functional category has 228543 predicted functions, 20% of which were involved in cellular metabolic functions, 5.21% in genetic information processing and 5.13% in environmental and information processing, while SEED functional category has 112542 predicted functions. Of which 11.73% are involved in carbohydrate metabolism with 13202 hits, followed by 10.57% amino acids and derivatives with 11899 hits, 8.24% protein metabolism with 9282 hits, 6.88% DNA metabolism with 7746 hits with and 5.94% cofactors, vitamins, prosthetic group, pigments with 6687 hits. The isolated bacterial consortia exhibited visible growth up to a Cr concentration of 800 mg L^{-1}. The results presented in this study have important implications for understanding bacterial diversity in chromium-contaminated mine soils and their role in exhibiting Cr(VI) resistance.

Introduction

L *and use change* is one of the important factors of global change, affecting many vital ecological properties and functions, which can change at the ecosystem scale resulting in changes in ecological functions during the conversion of native habitats[1,2]. Soil horizon, the most diverse environments on earth, harbours thousands of different microbial species inhabiting in each gram of soil[3]. Soil microorganisms are playing important role in ecosystem managements such as, soil formation, nutrient cycling, promoting plant health and soil erosion control[4,5,6]. However, these soil microbial communities or micro-environment are dynamic and can change across agricultural practices and environmental gradients[7]. Extensive mining activities alter the land use of the mine area and are one of the most pressing environmental concerns. Continuous mining activities resulted in exposure to the environment with high concentrations of heavy metals (Cr, Co, Ni, Cd, Cu, U and Zn) and resulted in various health hazards. Heavy metal contamination has been reported to decrease microbial composition, microbial biomass, respiration, enzyme activity and carbon utilization in soils contaminated with Cd[8], U[9,10], Zn and Cd[11], Zn[12], Pb and Cu[13] and other heavy metals [14,15].

Mine area micro-environments can be protected through effective bioremediation options, the bioremediation of these metal pollutants can be explored by exploiting the biodegradation property of microorganisms hindering that hostile environment. In this context, metagenomics is being used to detect

lethal bacteria from contaminated soil and water bodies for potential application in bioremediation programme. The identification of these novel genes and enzymes associated with the degradation of toxic heavy metals and the discovery of new metabolic pathways will be useful for bioremediation and industrial application.

Exposed chromium (Cr) due to extensive mining activity and various industrial processes pollutes both the soil and water bodies around the mining area. Chromium is available in several ionic forms (-2 to +6), the hexavalent form (Cr^{6+}) being highly soluble and toxic in contrast to its other ionic forms. In addition, hexavalent chromium (Cr^{6+}) toxicity is thought to be involved with the generation of free radicals and reactive oxygen species (ROS) due to its depletion inside cells. The accumulation of chromium in contaminated sites poses many challenges on the problem of soil quality, crop yield and other health hazards to animal and human beings through the food chain[16,17]. Considering the importance of metal tolerant microorganisms from the contaminated environment, several culture-based studies were conducted to isolate Cr^{6+} tolerant bacteria from chromite mine soil/water and evaluate their metal tolerance[18,19,20,21,22,23].

The present study investigates metagenomics insight into bacterial community structure and functions in heavy metal-contaminated chromite mine soil Sukinda, *Odisha*, India by using Illumina HiSeq sequencing platform and their role in conferring chromate resistance. Knowledge of soil microbial communities of native Sukinda chromite mine areas can provide valuable information for the identification of bacterial communities and its functional characteristics useful for bioremediation programme.

Materials And Methods

Ethics statement

The sampling areas in Sukinda chromite mines, Odisha, India were not coming under protected area and did not need any special permission for the studies. The location is not privately-owned or protected in any way and the field studies did not involve endangered or protected species.

Collection of Samples

Soil samples were collected from a depth of 10-20 cm without any vegetation from multiple sites of *in situ* mines of chromite Sukinda, Odisha, India (Latitude 21° 00’ 07” to 21° 02’ 46” N; Longitude 85° 44’ 12” to 85° 47’ 22” E) which represent high level of chromium contamination [21,22]. Homogeneity mixtures were generated by the multiple individual soils sample and transferred to laboratory on 4°C in sterilized sealed plastic bags and further stored at -80 °C for whole metagenomic profiling, soil physico-chemical properties characterization and heavy metal concentration detection.

Soil physico-chemical analysis of the metal contaminated soil of chromite mine

The physicochemical properties of the soil samples were estimated at the beginning of the experiment in order to have a basic idea of the quality of the soil samples prevailing in the chromite mine area. Soil parameters were analysed based on different methods for heavy metal concentration (PinAAcle 900F, Perkin Elmer, USA), pH, EC, organic carbon (Flash, 2000; Thermo Scientific, USA), available N, P, and K[24,25,26].

Evaluation of bacterial consortia for Cr (VI) tolerance and reduction

In nature, Cr is available in several ionic forms (-2 to +6). Several abiotic and biotic factors are influencing the oxidation and reduction of Cr in the environment. Chromium tolerance studies were carried out using agar plate supplemented with varying concentration of Cr (VI) ranging from 100 to 900 mgL^{-1}, incubated at 35 °C for 48 h. The plates were analysed for the number of bacterial colonies after 48 hours of incubation. Morphologically distinct colonies were picked up and preserved in NA slants/vials under refrigerated (4 °C) conditions.

The role of native microbial consortia for reduction of Cr(VI) into less toxic form was analyzed using diphenylcarbazide (DPC) method for Cr(VI) reduction studies[27]. The chromium reduction was estimated by inoculated the 24 hr old grown culture in 100 ml of LB broth containing 50 mg L^{-1} of Cr(VI) as $K_2Cr_2O_7$ at 30 °C, precipitated the concentrated Cr(VI)-DPC complex in the supernatant at the absorbance of 540 nm using spectrophotometer at various time intervals. Further, the percentage reduction of Cr(VI) was calculated by using the following formula:

$$\text{Cr(VI) reduction (\%)} = A - BB \times 100$$

Where A- absorbance of control; B- absorbance of sample.

DNA extraction from contaminated soil sample

DNA from the collected homogeneity mixtures of the multiple individual *in situ* soil samples was isolated using Power Soil® DNA Isolation Kit (MO-BIO, USA). The equal concentration (= 200 µg) of environmental DNA from the 10 different homogeneity mixtures of soil samples were pooled, to form a composite genetic pool representing total DNA composition for the site. The quality and quantity of DNA were checked both through NanoDrop™ 2000 Spectrophotometer (Thermo Scientific).Further, using Qubit® 2.0 Fluorometer (Thermo Fisher Scientific) the exact concentration of dsDNA present in the extracted DNA was checked. DNA having absorption ratio A260/A280 greater than 1.9 was considered for further analysis.

Illumina sequencing

DNA integrity and size distribution were further analyzed in Agilent High Sensitivity DNA Kit on Bio-analyzer 2100 (Agilent, Santa Clara, CA, USA) and samples having clear intact peak were used for library preparation, which reduced amplification bias and improving the quality of sequencing data. One paired-

end (PE) DNA library was generated from the pooled total gDNA (1µg) for metagenomics sequencing on Illumina HiSeq 1000 platform. The library was constructed according to the Next Ultra DNA Library Preperation Kit of New England Biolabs, Inc. (NEB) followed by manufacturer instruction. Briefly, The DNA is fragmented and end repaired using Next End Prep Enzyme Mix followed by poly-A tailing by Blunt/TA Ligase Master Mix and adapter ligation by Next Ligation Enhancer and amplification by Next Q5 Hot Start HiFi PCR Master Mix. Further, the prepared library was purified by magnetic beads, quantified using Qubit fluorometer (Invitrogen) and quality cheeked on High Sensitivity chip (Agilent, Cat # 5067–4626) on Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, USA). Sequencing was done in one lane to generate **2x250**bp PE reads.

Quality controls and *de novo* assembly

The raw sequencing reads generated by Illumina HiSeq 1000 platform were processed with Cutadapt (version - 1.8.1) to checked for various quality controls, i.e., filtering of high-quality reads based on the score value, removal of reads containing primer/ adaptor sequences and trimming of read length, base quality score distribution, sequence quality score distribution, average base content per read, GC distribution in the reads, PCR amplification issue, check for over represented sequences, adapter contamination to retain only high-quality reads.

The *de novo* assembly of the adapter trimmed fastq files was carried out on a server with 48 cores processor and 256 GB random access memory using massively distributed metagenome assembler, coupled with Ray Communities called "*Ray Meta assembler (v2.3.1)*" which profiles microbiomes based on uniquely-colored k-mers, followed by the assembly algorithm to create scaffolds. N50 contigs were kept as good assembly while bad or mis assemblies were removed from the result. Assembly was performed with default k-mer length (31-size) using de-bruijn graph algorithm. In-house PERL and Python codes were used to parse the fastq files for the downstream analysis.

Taxonomic annotation of metal tolerance bacterial consortia

High-quality reads were projected for downstream analysis by SILVA pipeline[28]. Briefly, each read was aligned using the SILVA Incremental Aligner (SINA SINA v1.2.10 for ARB SVN (revision 21008)[29] against the SILVA SSU rRNA SEED and quality controlled[28]. Reads shorter than 50 aligned nucleotides and reads with more than 2% of ambiguities, or 2% of homopolymers, respectively, were excluded from further processing. Putative contaminations and artefacts, read with a low alignment quality (50 alignment identity, 40 alignment score reported by SINA), were identified and excluded from downstream analysis. After these initial steps of quality control, identical reads were identified (dereplication), the unique reads were clustered (OTUs), on a per sample basis, and the reference read of each OTU was classified. De-replication and clustering was done using VSEARCH (version 2.15.1; https: //github.com/torognes/vsearch)[30] applying identity criteria of 1.00 and 0.98, respectively. The classification was performed by BLASTn (2.2.30+; http://blast.ncbi.nlm.nih.gov/Blast.cgi)[31] with standard settings using the non-redundant version of the SILVA SSU Ref dataset as classification

reference (release 138.1; http://www.arb-silva.de). The classification of each OTU reference read was mapped onto all reads that were assigned to the respective OTU. This yields quantative information (number of individual reads per taxonomic path), within the limitations of PCR and sequencing technique biases, as well as, multiple rRNA operons. Reads without any or weak classifications, where the function "(% sequence identity + % alignment coverage)/2" did not exceed the value of 93, remain unclassified. These reads were assigned to the meta group "No Relative" in the SILVAngs fingerprint and Krona charts[32]. This method was first used in the publications[33,34].

ORF prediction and functional assignment

The MetaGeneAnnotator (MGA) were for the prediction of open reading frames (ORFs) from assembled contigs, which distinguished between coding regions and non-coding DNA. The predicted ORFs were taken for the functional annotation and taxonomic classification. The predicted ORFs were searched against the non-redundant (NCBI-nr) database using a high-throughput program DIAMOND (v0.7.9.58), which aligning a file of short reads against a protein reference database such as NR, at 20,000 times the speed of BLASTX, with high sensitivity. The functional annotation of the putative ORFs were further doen using MEGAN5 (MEtaGenome ANalyzer) software. MEGAN5 is a fast and easy tool for metagenomic data analysis and can be used to analyze and compare metagenomic and metatranscriptomic data, both at taxonomically and functional level.

Statistical analysis

Fisher's exact test[35] tool at Cenargen Bioinformatics platform was used to compare and find out the levels and significance of contig expression between generated libraries which had passed through quality control.

Results

Characterisation of heavy metals on Soil samples

Sample collected from active chromite mine soil of Sukinda contains various heavy metals apparently at a higher concentration such as Mn (5444 mg kg^{-1}), Fe (5418 mg kg^{-1}), Pb (4713 mg kg^{-1}), Cr (2993 mg kg^{-1}), Ni (2278 mg kg^{-1}), Co (1548 mg kg^{-1}). Other soil physico-chemical parameters analysed include pH (7.32), EC (0.32 ds/m), organic carbon (0.19%), N (81.53 mg ha^{-1}), P (62.35 mg ha^{-1}) and K (27.05 mg ha^{-1}) (Table.1).

Metal tolerance studies on bacterial consortia

The chromium tolerance studies on bacterial consortia were observed interestingly that, the bacterial consortia could sustain visible growth in the petriplate up to Cr concentration of 800 mg L^{-1}. Beyond this concentration, the numbers of colonies observed were substantially reduced. No growth was observed beyond 1000 mg L^{-1} Cr concentration.

Besides that, the bacterial consortia also showed their tolerance towards high concentrations (400 mg L^{-1}) of Fe, Mn, Pb, Co, and Ni. The bacterial strains of the community dwelling in the mine soil sample exhibited tolerance to Cr along with other heavy metals. This metal tolerant ability of these bacterial consortia may be due to the presence of inherent mechanisms evolved while growing in such metal contaminated environment.

De novo assembly and data availability

A total of 10,349,864 PE (5,174,932 from each end) raw sequence reads with each 250 bp length were generated using Illumina Hiseq 1000, encompassing about 2.1 GB of sequence data consisting of 5.2G bases data in fastq format. The raw reads produced have been deposited in the NCBI SRA database (accession number: SRP148083 https://www.ncbi.nlm.nih.gov/sra/?term=SRP148083). There were total 907,765 numbers of contigs generated after assembly of reads. After filtering the sequence data for low-quality reads at higher stringency and reads containing primer/adaptor sequence, resulted in a total of 780,534 (87%) high-quality sequence reads with N50 length of 239,701 (100-200 bps), 452,040 (201-300 bps), 52,530 (301-400 bps), 19,509 (401-500 bps) and 16,754 (>=500 bps) (more than 70% of bases in a read with more than 20 phred score and 36.21 % of the total reads were having phred score >=30).

Characterizations of metal tolerance bacterial consortia

Taxon abundance and their assignment to different functional categories are considered to be critical in whole metagenomic study. The taxonomic profiling of the metagenomic sample provided an insight into bacterial community structure of the chromium contaminated mine soil collected from Sukinda chromite mine. The taxonomic tree at phylum level and species level showed the hierarchy of comparative taxonomic abundance in the sample based on contig abundance (Fig.1). Bacteria were the most abundant among the three domains and accounted for more than 90% of the microbial population of the metagenome. Phylum level characterizations from SILVA databases showed soil samples included 39 phyla among Bacteria. Overall, the most abundant phyla comprised *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroides,* between both the groups (Fig.1). Phylum level taxonomic hits distribution of the mining residue sample showed that *Proteobacteria* were the most abundant (66.45%) followed by *Actinobacteria* (17.32%), *Bacteroidetes* (4.65%), *Firmicutes* (1.78%), *Nitrospirae* (1.47%) and Acidobacteria (1.375%) (Fig.2). Other phyla like Chloroflexi, Gemmatimonadetes, Planctomycetes, Thaumarchaeota, Basidiomycota and Deinococcus-Thermus constituted ~4% of the whole metagenome (Fig.2 and Fig.3). Among the Proteobacteria phyla, Alpha proteobacteria(25.47%) was found to be most abundant class followed by Betaproteobacteria (23.61%), Gammaproteobacteria (9.71%) and Deltaproteobacteria (7.72%). *Bradyrhizobium* was found to be the most predominant genus with 15796 hits followed by *Bdellovibrio* (9809), *Acinetobacter* (6457), *Reyranella* (5695), *Burkholderia* (5388) and *Pseudomonas* (5240). Bacteria like *Nitrospira*, *Sediminibacterium*, *Nocardioides*, *Propionibacterium* etc. were the other members as revealed from the metagenome of the chromite mine soil.

α-Diversity and rarefaction curve analysis

α-Diversity (Species richness) reflect the diversity of organisms in a sample with a single number which can be estimated from the distribution of the species-level classifications. Species richness and rarefaction curve was generated by comparing the species abundance based on number of leaves in the taxonomy and number of sequences occurred. The curves characterize the average number of different species annotations for the complete dataset. The curve is made for all taxa include Bacteria, Archaea, Eukaryote, Viruses, unclassified and other sequences. The plot (Fig. 4) showed the rarefaction curve of annotated species richness in the metagenome. The steep slope on the left of the curve represented a large portion of the species diversity existed and the curve pointing towards the right meant that a reasonable number of individuals were sampled; more intensive sampling is likely to yield few additional species. Initially, the sampling curve rose very rapidly but gradually levelled off indicating fewer new species per unit of individuals collected.

KEGG and SEED functional category hits distribution and analysis

KEGG pathway analysis was performed for each contig sequences by assigning KEGG Orthology (KO) numbers obtained from the known reference hits. Further, enzyme and pathway information were assigned to contigs based on KO. The pathway classifications were represented as bubbles in bubble plot shown in Fig.5 (b). Each bubble from the plot illustrated the distribution of different functional categories at the highest level supported by the functional hierarchies. The protein regulating the metabolic functions were higher in number which comprises about 20% of the whole metagenome (88470 reads) followed by the proteins regulating the genetic information processing which were 5.21% of the metagenome with 23036 hits and environment information processing 5.13% with 22671 hits. Further, proteins responsible for cellular processes (7277), human diseases (5106) and organismal systems (3831) constituted ~ 4% of the whole metagenome, while SEED functional category has 112542 predicted functions. Out of which 11.73% are involved in carbohydrate metabolism with 13202 hits followed by 10.57% amino acids and derivatives with 11899 hits, 8.24% protein metabolism with 9282 hits, 6.88 % DNA metabolism with 7746 hits and 5.94% cofactors, vitamins, prosthetic groups, pigments with 6687 hits are shown in Fig.5(a).

While analysis of enzyme level observed that, 903 sequences were involved in TonB-dependent receptor followed by Cobalt-zinc-cadmium resistance protein CzcA (866 sequences), Acriflavin resistance protein (792 sequences), Long-chain-fatty-acid--CoA ligase (607 sequences), Acyl-CoA dehydrogenase, short-chain specific (489 sequences), DNA polymerase III alpha subunit (419 sequences), acyl-CoA synthetase (411 sequences), ATP-dependent protease La Type I (359 sequences), Multimodular transpeptidase-transglycosylase (354 sequences), Adenylate cyclase (349 sequences), Excinuclease ABC subunit A (349 sequences), Chaperone protein DnaK (308 sequences), DNA-directed RNA polymerase beta' subunit (307 sequences) and Enoyl-CoA hydratase (305 sequences) etc.

Discussion

The Sukinda valley is rich in chromite deposits and has the largest open cast chromite ore mine in the world, with 98% availability of total proven chromite (chromium ore)(http://ospcboard.org/). The chromium industry includes the mining of chromite ore and the use and production of chromium in chemicals, metals, refractories, ferrochromium, and stainless steel. On one hand, minerals and mineral products are the main key to most industries and are mined in some form or the other in almost every country around the world. On the other hand, pollution from mines is a major health hazard (https://www.worstpolluted.org/) due to lack of proper environmental controls. A total of 60% of drinking water contains hexavalent chromium at levels exceeding double international standards, and the Indian Health Group estimates that 84.75% of deaths in mining areas - where regulations do not exist - are due to chromite-related diseases. Virtually no attempt has been made to clean up the contamination. The Sukinda valley is also on the list of most polluted cities in the world. Hence, remediating the Cr- polluted soil due to mining in Sukinda is very much essential. Bioremediation though *in situ* microbial resources may act as one of the powerful technique to address this issue. Therefore, isolated and identified strains of chromium-tolerant bacteria (CTBs) had great scope for their application in the bioremediation of toxic chromium ions in the presence of other metal ions, which need to be explored for their biotechnological applications. However, isolated and identified strains of chromium tolerant bacteria (CTBs) had great scope for their application in the bioremediation of toxic chromium ions in the presence of other metal ions, which need to be explored for their biotechnological applications. Metagenomic is one of the efficient molecular tools to unveil the potential of CTBs under *in situ* chromium site and it has potential to improve our knowledge by studying the genetic components of uncultured microorganisms[36,37,38,39]. Techniques of metagenomics have been used to trace soil microbes to its composition and to understand its functional mechanisms at the molecular level that allow the successful adaptation of microbes to mining environments. Taxonomic analyzes showed that *Proteobacteria* (66.45%) and *Actinobacteria* (17.32%) were the most abundant in the three domains (which account for more than 90% of the microbial population) in the "*micro-environment*" of the chromium mine, which are responsible for tolerance. This finding is in line with other studies on soil bacterial communities[40,41,42,43] in which the most dominant phylum found to be *Proteobacteria*. Among *Proteobacteria*, *α-proteobacteria*, *β-proteobacteria* was found to be the most abundant class. Studies have suggested that the abundant Proteobacteria and Actinobacteriaphyla correlated significantly with metal contaminated environments. The development of new genetic characteristics is the key to their successful survival in a changing environment[44,45].The microorganisms consortia *i.e, Alpha-proteobacteria*, *Beta-proteobacteria*, *Gamma-proteobacteria*, *Delta-proteobacteria*, *Bradyrhizobium*, *Bdellovibrio*, *Acinetobacter*, *Reyranella*, Burkholderia, *Pseudomonas*, *Nitrospira*, *Sediminibacterium*, *Nocardioides*, *Propionibacterium* etc. were adapt to micro-habitats and live together with more or less sharp boundaries, interacting with each other and with other parts of the soil biota in the micro-environment*"* of chromium mine.

Predictive functional profiling of bacterial communities in *in situ* chromite mine soils showed that "metabolism" was the most prominent category, with "*carbohydrate metabolism", "amino acid metabolism*", and "*DNA metabolism*" being the top three abundant pathways. "*Carbohydrate metabolism*" involves a complex series of enzymatic steps to convert external substrates into metabolic precursors

such as *acetyl-CoA, pyruvate* and *D-fructose-6-phosphate*, and involves a hydrolysis reaction in which polymers are converted to monomers are hydrolyzed[46,47]. The "*amino acid metabolism*" pathways commonly used by bacteria for osmosis, soils have been exposed to constant moisture stress, mainly in the mine field[48,49]. DNA metabolism in which cellular deoxyribonucleic acid (DNA) is maintained, and includes both DNA synthesis and degradation reactions involved in DNA replication and repair of damaged DNA due metal toxicity[50,51].

Enzyme level analysis found involvement of TonB-dependent receptor, cobalt-zinc-cadmium resistance protein CzcA, acriflavin resistance protein, long-chain-fatty-acid-CoA ligase, Acyl-CoA dehydrogenase, short-chain specific, DNA polymerase III alpha subunit, acyl-CoA synthetase, ATP-dependent protease La Type I, Multimodular transpeptidase-transglycosylase, Adenylate cyclase, Excinuclease ABC subunit A, Chaperone protein DnaK, DNA-directed RNA polymerase beta' subunit and Enoyl-CoA hydratase, which help in metals tolerant. i.e., TonB dependent outer membrane receptors are related to outer membrane proteins (OMPs) that play various roles in bacteria, so that they are involved in the transport of siderophore, heme, maltose, vitamin B12, nickel and other essential ions in the bacterial cell[52,53,54,55,56]. Nies,1992,[57] reported that C*obalt-zinc-cadmium resistance proteinCzc* from the *Alcaligenes eutrophus* a gram-negative multiple-metal-resistant bacterium, which encodes proteins required for $Co^{2+,}$ $Zn^{2+,}$ and Cd^{2+} efflux (CzcA : cation-proton antiporter, CzcB : cation-binding subunit, and CzcC: modifier protein) and regulation of the czc determinant (CzcD) and substrate specificity of the system from Zn^{2+} only to $Co^{2+,}$ Zn^{2+} and Cd^{2+} Similarly, *Acriflavin resistance protein* helps in tolerance of mutagenic agents such as acriflavine acridine orange, and ethidium bromide[58].

Conclusion

Taxonomic profiling of the soil metagenome of the chromine mine of Sukinda, Odisha revealed bacterial dominance over the other three domains in the contaminated site, where Proteobacteria were the most abundant phylum, followed by Actinobacteria and Bacteroidetes. The dominance of functional profiling i.e. *carbohydrate metabolism, amino acid metabolism and DNA metabolic pathways* confer resistance to chromate in bacterial communities of *in situ* chromite mines. Similarly the enzymatic profiling of bacterial species shows tolerance to mutagenic agents, which are resistance to chromate. Overall, The results reported to extend our current knowledge of microbial taxonomics in chromite mining areas. The data could be a relevant contribution in the exploration of natural bioremediation strategies in mining and surrounding areas.

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Tables

Table 1. Physico-chemical analyses of chromite mine soil, Sukinda, Odisha

Parameters	Value
pH	7.32±0.11a
EC (dS/m)	0.32±0.03a
Organic carbon (%)	0.19±0.01c
Avail. N (kg ha^{-1})	81.53±3.23c
Avail. P (kg ha^{-1})	62.35±4.21a
Avail. K (kg ha^{-1})	27.05±2.54c
Fe (mg kg^{-1})	5418.02±34.54a
Cr (mg kg^{-1})	2993.68±45.83a
Co (mg kg^{-1})	1548.50±23.65c
Ni (mg kg^{-1})	2278.01±21.22a
Cu (mg kg^{-1})	ND
Zn (mg kg^{-1})	225.79±10.23a
Pb (mg kg^{-1})	4713.01±31.21a
Cd (mg kg^{-1})	5.15±0.23a
Mn (mg kg^{-1})	5444.01±23.32b

Values represent means ± standard error (n=3). Different letters in the rows indicate significant difference among the treatments at Tukey HSD (p<0.05).

Figures

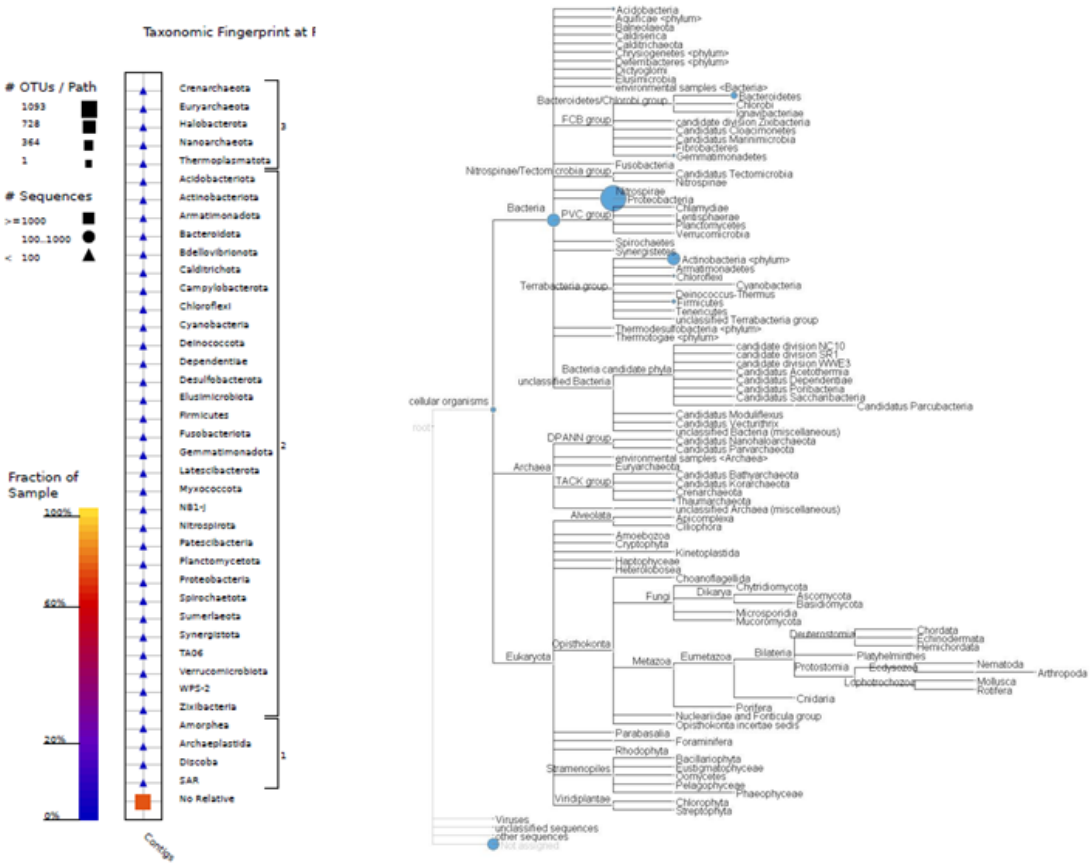

Figure 1

Phylogenetic tree showing divergence at phylum level

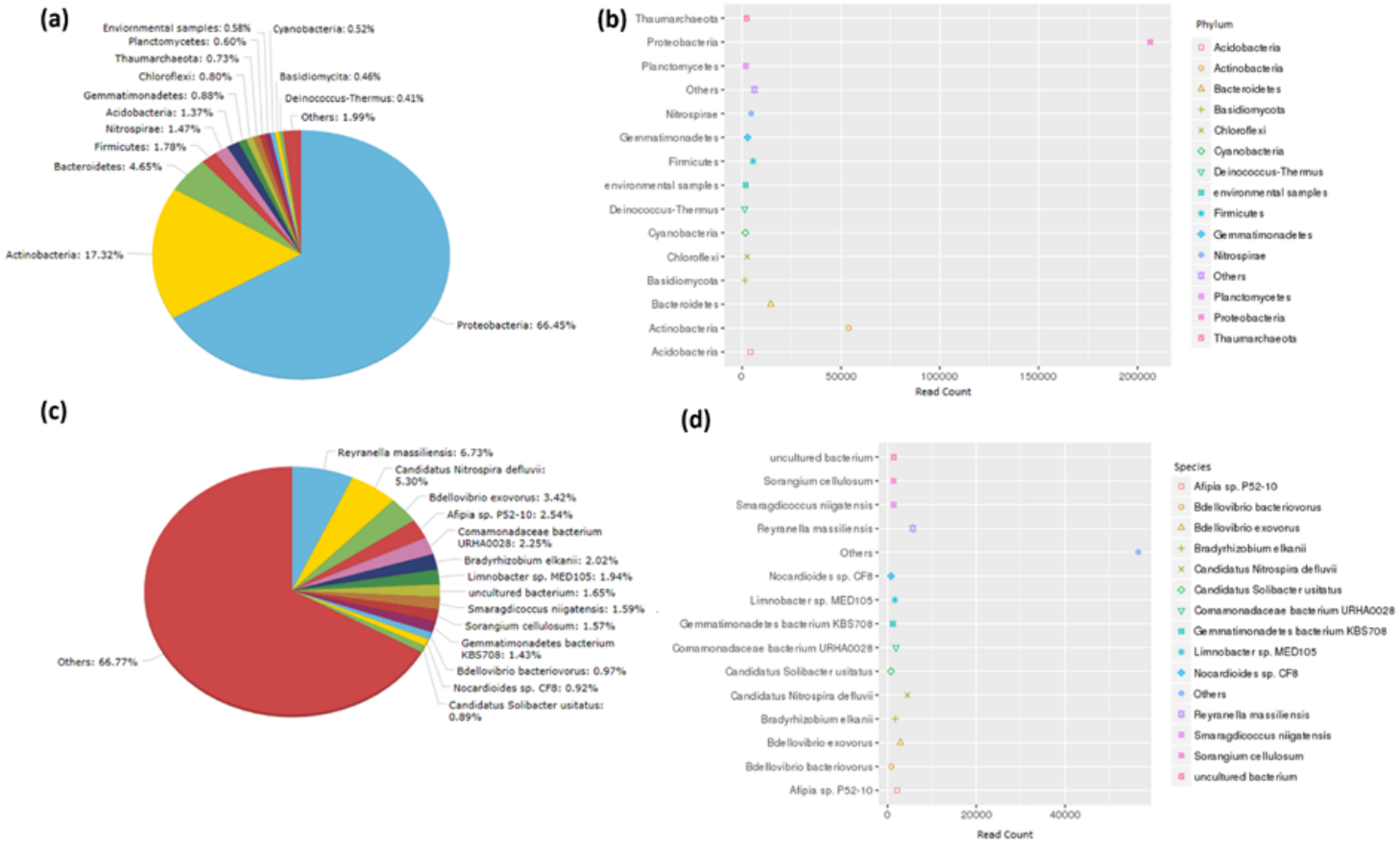

Figure 2

Taxonomy abundance in the sample. (a) The pie chart shows the percentage of microbiome at phylum level (b) The scatter plot represents the abundance level of phylum with respect to read count (c) The pie chart shows the percentage of various species reported in the sample (d) The scatter plot shows the read abundance at species level.

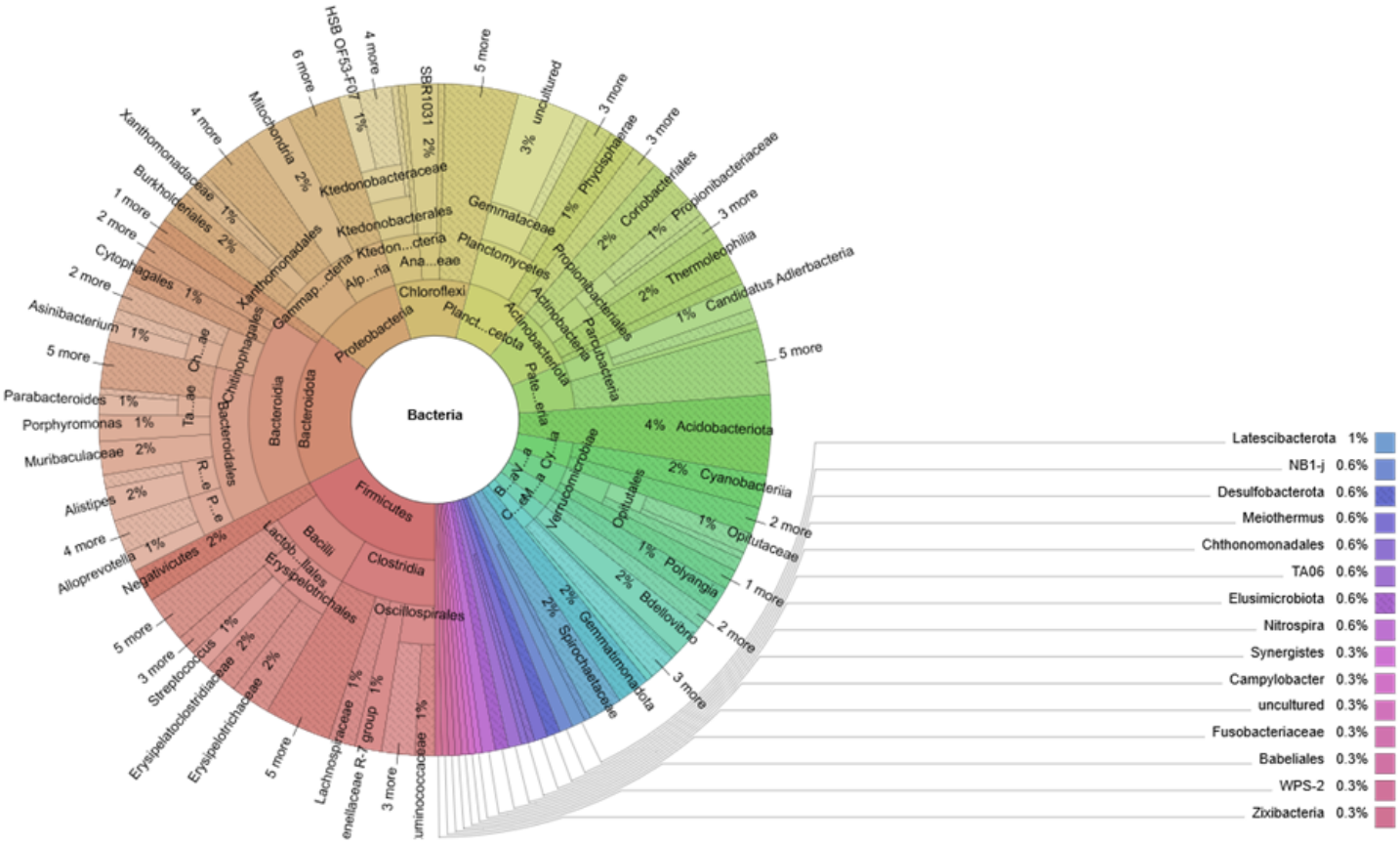

Figure 3

The Krona graph showing the relative abundance of annotated taxa in the sample

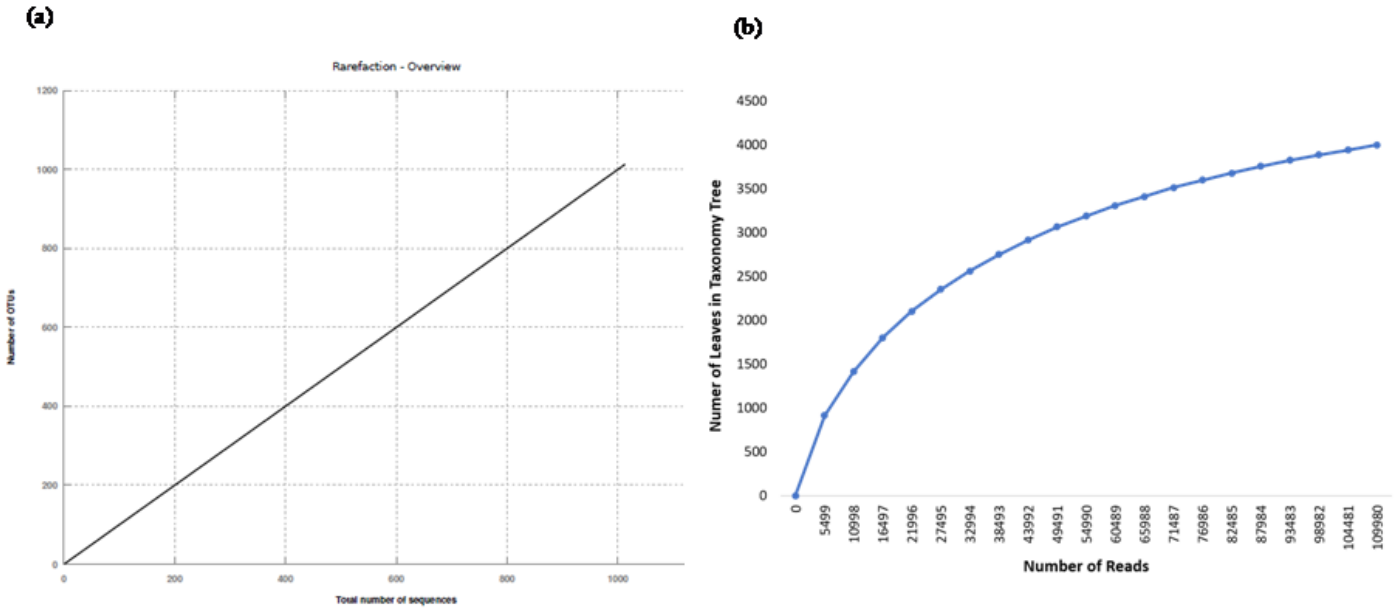

Figure 4

The alpha rarefaction studies. The plot (a) shows the number of observed taxonomic units (OTUs) of bacterial diversity while curve plot (b) shows the observed taxonomic unit of bacterial diversity distributed in in situ *soil sample*

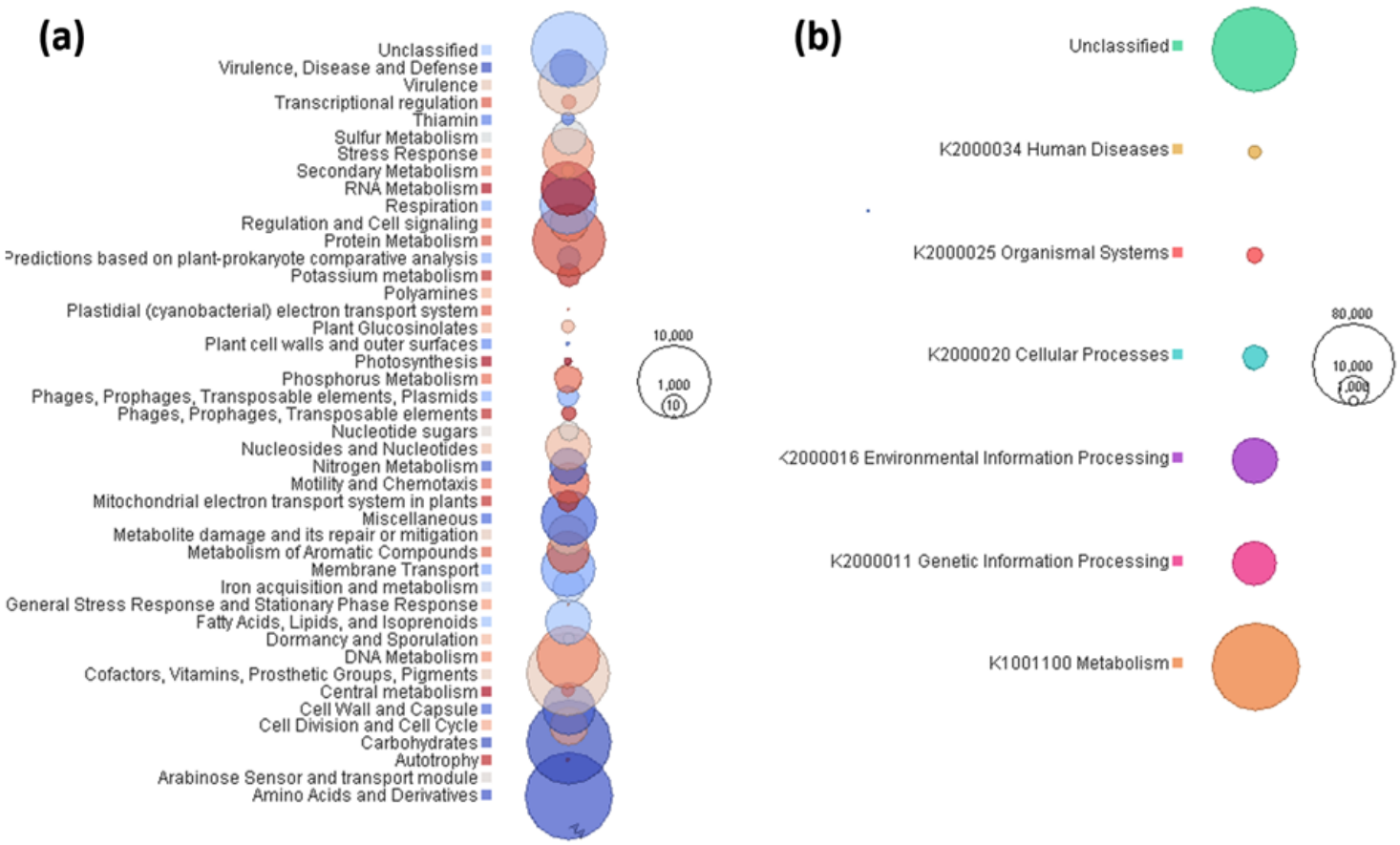

Figure 5

Bubble plot showing functional analysis of predicted ORFs obtained after metagenome assembly. Larger bubble size indicates more numbers of ORF assigned to the functional groups. The size of the circle is scaled logarithmically to represent the number of ORFs assigned directly to the functional categories.(a) Bubble plot shows the SEED based gene ontology (b) Primary pathway summary as a result of KEGG annotation.