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Indigenous bacterial community of heavy metal tolerance in the rhizosphere soils of *Mimosa pudica* naturally growing on an ex-tin mining area

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ABSTRACT

Aims: The purpose of this research was to explore the composition and genomic functions of bacterial community inhabiting the rhizosphere of *Mimosa pudica*, which were naturally growing on tailing and non-tailing soils of an ex-tin mining area.
Methodology and results: DNA were extracted from rhizosphere soils and PCR targeting the hypervariable region V3-V4 was carried out by Illumina 16S metagenomic library. Libraries were sequenced using Illumina MiSeq. The Operational Taxonomic Units (OTUs) were assigned to 23 bacterial phyla, 72 classes, 165 orders, 248 families and 357 genera. The most represented and dominant phylum was Proteobacteria, with an average abundance value of 41.2%. The most represented genera included *Paraburkholderia*, *Bradyrhizobium*, *Bacillus*, *Candidatus*, *Acidothermus*, *Acidibacter* and *Nitrospira*. Non-tailing soils had more number and richness of species while the tailings had more diversity of species. The metagenomes accommodate suspected genes for heavy metal tolerance of microbes (As, Cr, Co, Zn, Ni, Cu, Cd, Fe and Hg) and microbial plant-growth-promoting traits for hyperaccumulator plants (synthesis of indole acetic acid (IAA), siderophore and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase; solubilization of phosphate and potassium and nitrogen fixation).
Conclusion, significance and impact of study: Bacteria and predicted genes discovered could be part of major factors influencing growth of *M. pudica* in heavy metal-contaminated soils. The study provides the first report and a basis into the bacterial community associated with *M. pudica* in ex-tin mining soils from the studied geographical location. The findings also provide fundamental knowledge on phytoremediation potential of heavy metal contaminated soils involving indigenous beneficial microbial populations.

Keywords: 16S amplicon metagenome, rhizosphere soil, *Mimosa pudica*, heavy metals tolerance, plant growth-promoting traits

INTRODUCTION

Several land areas worldwide that have been polluted with heavy metals are mainly attributed to industrial activities, including mining activities (Tiwari and Lata, 2018; Shah and Daverey, 2020). In former mining areas and mine tailing deposits, the principal issue is heavy metal contamination (Karaca *et al*., 2018) and the heavy metals resulting from mines can adversely affect the soil and groundwater (Ahn *et al*., 2020). Contamination of soils with heavy metals can have huge and considerable environmental effect, and if the heavy metals find their way into the food chain, this may also cause health issues to humans and animals (Mishra *et al*., 2017). The heavy metals are said to affect not only the contaminated area but also the surroundings because of their mobilization and transportation by water, groundwater and air (Karaca *et al*., 2018).

The rhizosphere, rhizoplane or internal tissues of host plants are generally colonized with microbial community, potentially benefiting their host plants. Earlier reports (Sbabou *et al.*, 2016; Yahaghi *et al*., 2018; Jian *et al*., 2019) have shown that the microbial community of hyperaccumulator and other metalliferous plants plays very important role in ensuring growth and survival of the host plants in soils of mining areas that are highly polluted with heavy metals. Information on the bacterial community's composition and structure would clarify their participation in enhancing the growth and survival of the host plants and finally promote heavy metals accumulation activity (Costa *et al*., 2015). Studies on microbial populations in various environments provide

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new insights into their structure and function, contributing to the discovery of new functional genes (Das *et al*., 2017; Gu *et al.*, 2017). The exploitation of diverse bacteria and their detoxification genes has been used to ameliorate the field of environmental bioremediation (Das *et al*., 2016).

In-dept insight into the populations of microbes controlling different activities and biogeochemical cycles in the natural system is very difficult due to their extreme diversity and uncultivated status (Das *et al*., 2017). Metagenomic research has created an unparalleled opportunity to analyze microbial communities' reaction and adaptation strategies to environmental toxicity (Das *et al*., 2017). Some studies on microbial communities from some environments including contaminated soils (Costa *et al*., 2014; Feng *et al*., 2018) have offered new insights into the structure of the microbial world, their work and pattern of evolution, and have contributed to the discovery of new species and genes (Dai *et al*., 2014; Das *et al*., 2017; Karthik *et al*., 2017). Metagenomics analysis provides an approach to disentangle the plant-associated microbial diversity of polluted environments, building a framework for understanding and implementing strategies for the remediation of such polluted sites using indigenous microbial populations (Malla *et al*., 2018).

Little information is available regarding the microbial population associated with the rhizosphere of metal accumulating plants, most particularly in the tropical regions (Lopez *et al*., 2019). Also, the structural and functional variations, and the effects of the root-associated microbiomes in relation to their host plants remain poorly understood (Luo *et al*., 2017). It is important to understand the relationships between host plants, related rhizosphere microorganisms and surrounding soil because the bacterial population of the rhizosphere will directly affect the mobility and availability of metals to plants (Lopez *et al*., 2019). There is an increasing interest in studying the microbial communities of metal tolerant plants (Khan *et al*., 2015; Lopez *et al*., 2019; Sun *et al*., 2019) and very few or none to explore the composition and diversity of bacteria associated with *M. pudica* in mining areas. *M. pudica* is metalliferous and said to house a number of plant growth-promoting bacteria (Klonowska *et al*., 2012; Felestrino *et al*., 2017) and is one of the plants endemic in the present study area.

Thus, the objective of this research was to analyze the community structure, diversity and to predict the genomic functions of the bacteria inhabiting the rhizosphere soil of *M. pudica* growing in tailing and non-tailing soils of ex-tin mining area contaminated with heavy metals. It also provides fundamental knowledge on phytoremediation potential of contaminated soils involving indigenous beneficial microbial populations.

MATERIALS AND METHODS

Sample collection

Rhizosphere soil samples of *M. pudica* were collected from an ex-tin mining area (latitude 5°389'N, longitude 101°19'E) which had been abandoned for more than 30 years in Perak, Malaysia. Plants were carefully pulled out from the soil with intact roots, and the soils attached to the roots were collected and combined together. Three samples were collected per site and combined as one (in each case) before DNA extraction for metagenomic analysis. These were the rhizosphere soils. The samples were collected from mine tailings (samples MRS1, MRS2 and MRS3) and non-tailings (samples MRS4, MRS5 and MRS6) (Abdullahi *et al.*, 2020a) in sterile plastic bags, and moved under cold conditions to the laboratory and immediately stored at –20 °C. The samples were collected at least 100 m apart from each other.

Total genomic DNA extraction from rhizosphere soil samples and QC

DNA extraction directly from the rhizosphere soils (300 mg of each sample) was done with a NucleoSpin® Soil DNA isolation kit (Macherey-Nagel, Germany) in accordance with the manufacturer's instructions, and the quality was checked on 1% agarose gel. NanoDrop Spectrophotometer was used to assess sample purity and concentration, and Fluorometer (Biotek FLX800) was further used for the sample quantification (dsDNA).

16S rRNA bacterial amplification, library construction, and sequencing

The PCR that targeted the V3-V4 hypervariable region was done using the Illumina 16S metagenomic library preparation method (Illumina, 2013). The quality and quantity of the amplicons were assessed using Tapestation 4200, Helixgreen and NanoDrop. All the samples passed the QC measurements and proceeded directly for the library preparation's 2nd PCR step. Using the Illumina 16S metagenomics library prep kit, the libraries were prepared, and their quality and quantity were determined using Agilent Tapestation 4200, qPCR, and Helixgreen. All the libraries passed the QC measurement and were pooled according to the Illumina's protocol and proceeded straight to sequencing using the MiSeq platform at 2x301PE format (Illumina). Quality assessment of paired-end reads was done using FASTQC (https://github.com/s-andrews/FastQC).

Bioinformatics and sequence data analysis

BBDuk of the BBTools package (https://sourceforge.net/projects/bbmap/) was used to remove sequence adaptors and low-quality reads from the paired end reads (Bushnell *et al*., 2017). The forward and reverse reads were merged using USEARCH v11.0.667 (https://www.drive5.com/usearch/) (Liu *et al.*, 2020). The downstream processing excluded sequences (sequenced on the MiSeq platform) that were shorter than 150 bp or longer than 600 bp (Abdullahi *et al*., 2020b). Reads were then aligned with the 16S rRNA database (SILVA Release 132) (Glöckner, 2019) and inspected for chimeric errors using VSEARCH v2.6.2 (Rognes *et al.*,

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Table 1: Sequence characteristics and α-diversity of bacteria from rhizosphere soil of *M. pudica* in an ex-tin mining site of tailing and non-tailing soils.

Sample	Type	Sequence no.	OTU	α-diversity			
				Chao1	ACE	Shannon	Simpson
MRS1	Tailing	9,995	957	923	922	5.945	0.995
MRS2	Tailing	26,105	1,306	1125	1149	6.171	0.996
MRS3	Tailing	9,199	942	915	921	5.810	0.993
MRS4	Non-tailing	28,389	1,213	1060	1064	5.919	0.992
MRS5	Non-tailing	27,898	1,308	1158	1183	6.062	0.992
MRS6	Non-tailing	23,572	1,285	1152	1163	5.683	0.975

2016). Readings were clustered *de novo* after the quality assessment measures into operational taxonomic units (OTUs) at 97% similarity using UPARSE v11.0.667 (Edgar, 2013) and rare OTUs with less than two reads (doubleton) which are sometimes spurious were also deleted from the downstream processing. From each OTU, a single representative sequence was randomly selected and Pynast (https://www.ncbi.nlm.nih.gov/pubmed/19914921) was used to align and create a phylogenetic tree against the SILVA Release 132 16S rRNA database. The taxonomic assignment of OTUs against the SILVA database was done using QIIME V1.9.1 (Caporaso *et al*., 2010). R package V3.6.1 was used for all statistical analyses (https://www.r-project.org/) (R Core Team, 2020).

Prediction of functional gene analysis

Functional gene prediction was made by PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) functional prediction (Douglas *et al.*, 2020). PICRUSt uses 16S rRNA genes to infer the functional content of the metagenome gene from phylogenetic knowledge and predictions for genes in databases like the Kyoto Encyclopedia of Genes and Genomes have been determined (Sibanda *et al.*, 2019; Douglas *et al.*, 2020). The output of PICRUSt consists of a table of functional gene counts as KEGG orthologs (KOs).

Accession number/Data deposition

The nucleotide sequences (bacterial metagenome data) were submitted in the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) with BioProject accession number PRJNA601794 (Abdullahi *et al*., 2020b).

RESULTS AND DISCUSSION

In this study, the 16S amplicon metagenomic analysis supplied information on the composition and diversity of bacteria in the rhizosphere soil of *M. pudica* on ex-tin mining soil, along with functional genes related to heavy metals tolerance and plant growth promotion.

Overview of 16S rRNA amplicon sequence, richness and diversity of the rhizosphere bacteria

After paired end joining and quality trimming, the total count of high-quality sequence reads obtained was 125,158 ranging from 9,199–28,389 per sample as presented in Table 1. The sample MRS4 had the highest sequence read count of 28,389 and MRS3 had the least of 9,199. The number of OTUs per sample varied from 942 to 1,308, where MRS5 had the highest and MRS3 the least. The richness and diversity of the bacterial community in the rhizosphere soil samples were estimated by computational analysis of alpha-diversity (Table 1). Chao1 and ACE estimated the number and richness of species. The Chao1 and ACE values of sample MRS5 were highest (1158 and 1183, respectively) indicating a higher number of species and richness in the sample, followed by MRS6 while MRS3, followed by MRS1, had the least values and thus with lower species richness. On the other hand, the values of Shannon and Simpson diversity indexes which are measures of species diversity, revealed MRS2 to be most diverse and MRS6 least diverse. Figure 1 shows the alpha diversity pairwise analysis of variance between groups, which indices indicate non-tailing sites to have more number and richness of species while the tailings to have more diversity of bacterial species. The samples from non-tailing areas were shown to be richer in the sense of the number of species, while the samples from tailing areas were more diverse. Various and unique microbial assemblies with potentially biotechnologically significant genes for biosynthesis and biodegradation are sheltered in mine tailings (Sibanda *et al.*, 2019). Any possible colonization of the region by vegetation is impaired by high concentrations of toxic metals in tailing soils and the input of organic matter content in soils is inversely affected, which would otherwise serve as a source of nutrients for soil microbiota (Sibanda *et al.*, 2019).

Structure of the bacterial community in the rhizosphere soil of *M. pudica* in an ex-tin mining soil

The OTUs from all six samples were placed to 23 different bacterial phyla, 72 classes, 165 orders, 248 families and 357 genera. The most represented and dominant phylum among all the rhizosphere soils was

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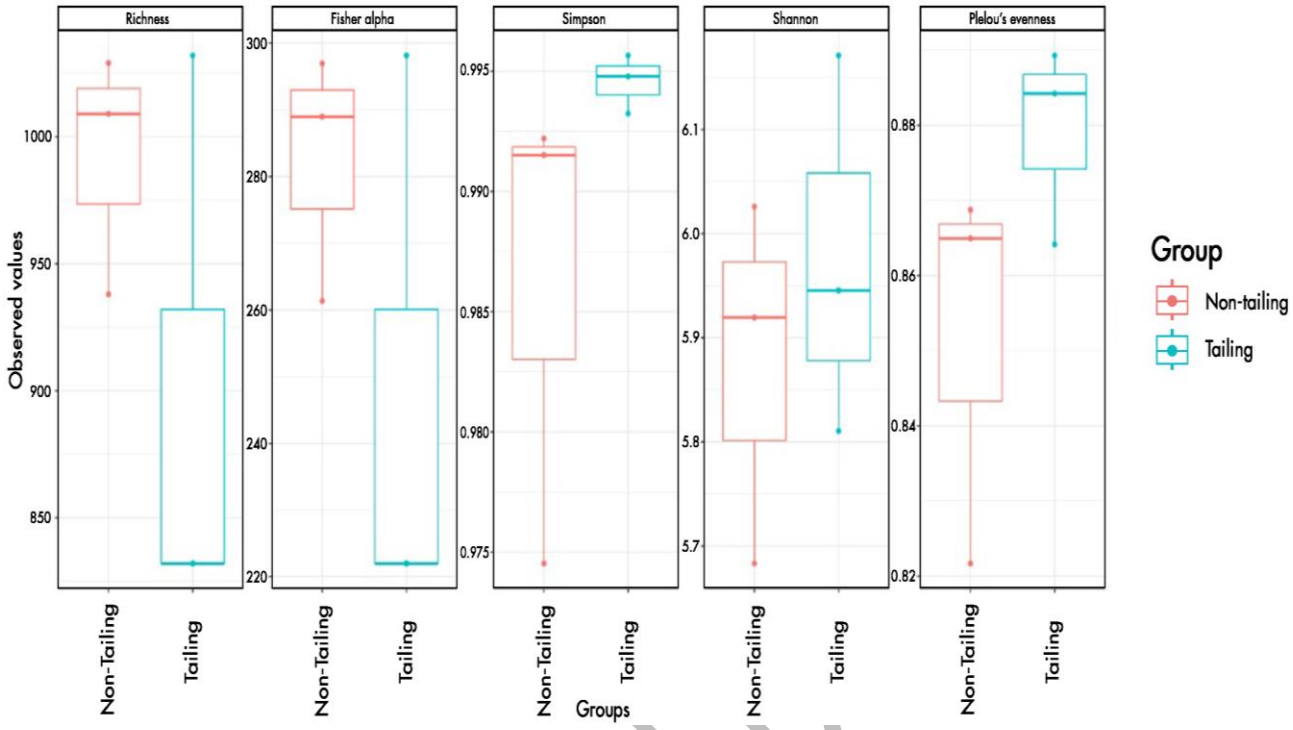

Figure 1: Alpha diversity with ANOVA showing pairwise analysis of variance between groups and its significance annotated on the plots. The *p*-value threshold for significance set at 0.05.

Proteobacteria (41.2%), followed by Acidobacteria (17.1%) and Actinobacteria (14.4%). The sample MRS6 had the highest percentage of Proteobacteria (49.2%), while MRS3 had the highest of Acidobacteria and Actinobacteria (20.2% and 17.0%, respectively). Firmicutes were found at the highest abundance in MRS1. Several studies on bacterial composition in heavy metals polluted soils showed Proteobacteria as the most dominant phylum (Costa *et al.*, 2015; Ghosh and Das, 2018; Lopez *et al.*, 2019) as in the case of this study while few others indicated the dominance of other phyla such as Actinobacteria (Sibanda *et al.*, 2019). The occurrence of bacterial taxa in preferred locations is a clear indication that the environmental factors/soil conditions and heavy metals pollution greatly affect the bacterial distribution. The bacteria, therefore, have a good adaptation to the system. The top ten bacterial phyla are presented in Figure 2. At the genus level, 357 genera were recovered from the 6 rhizosphere soils. Figure 3 showed the top ten abundant genera detected. The genera *Bacillus* and *Acidibacter* were more abundant in MRS1 and MRS2. *Nitrospira* were much more in MRS3 compared to other samples with very low representation in MRS2. MRS3, MRS4 and MRS5 had a reasonable number of *Acidothermus* while MRS1 had very low of such. The genus *Paraburkholderia* was interestingly in high abundance in sample MRS6 compared to other genera in the sample and compared to the same genus in other samples.

Bacterial community differences between tailing and non-tailing sites

Venn diagrams (Figure 4a–c) illustrated the relationships in unique and shared OTUs between and among all samples and groups of tailing and non-tailing origin. In general, out of the total 1,790 OTUs, 168 were available only in tailing samples, 309 only in non-tailing samples and then 1313 found in both the 2 sample groups. The results showed that more OTUs were present in the non-tailing site compared to the tailing site. The phyla Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes, Gemmatimonadetes and Rokubacteria (18.56, 15.13, 3.54, 3.06, 2.14 and 5.24%, respectively) were more abundant in the tailing area; while Bacteroidetes, Planctomycetes, Proteobacteria and Verrucomicrobia (5.37, 4.55, 44.93 and 3.53%, respectively) were more in the non-tailing area (Figure 5). By far, members of Proteobacteria (e.g. *Paraburkolderia* – most represented) are most abundant among all the bacteria revealed in the study and are more in the non-tailing sites. This may make the non-tailing sites to have higher number of bacteria. Also, higher concentrations of heavy metals in the tailings may lead to great selective pressure on the assemblage of bacterial communities. The tailing area had more of the genera *Bacillus* and *Nitrospira* in comparison to the non-tailing area, which, on the other hand, had more of *Acidothermus*, *Bradyrhizobium*, *Burkholderia* and *Candidatus solibacter* genera. Among

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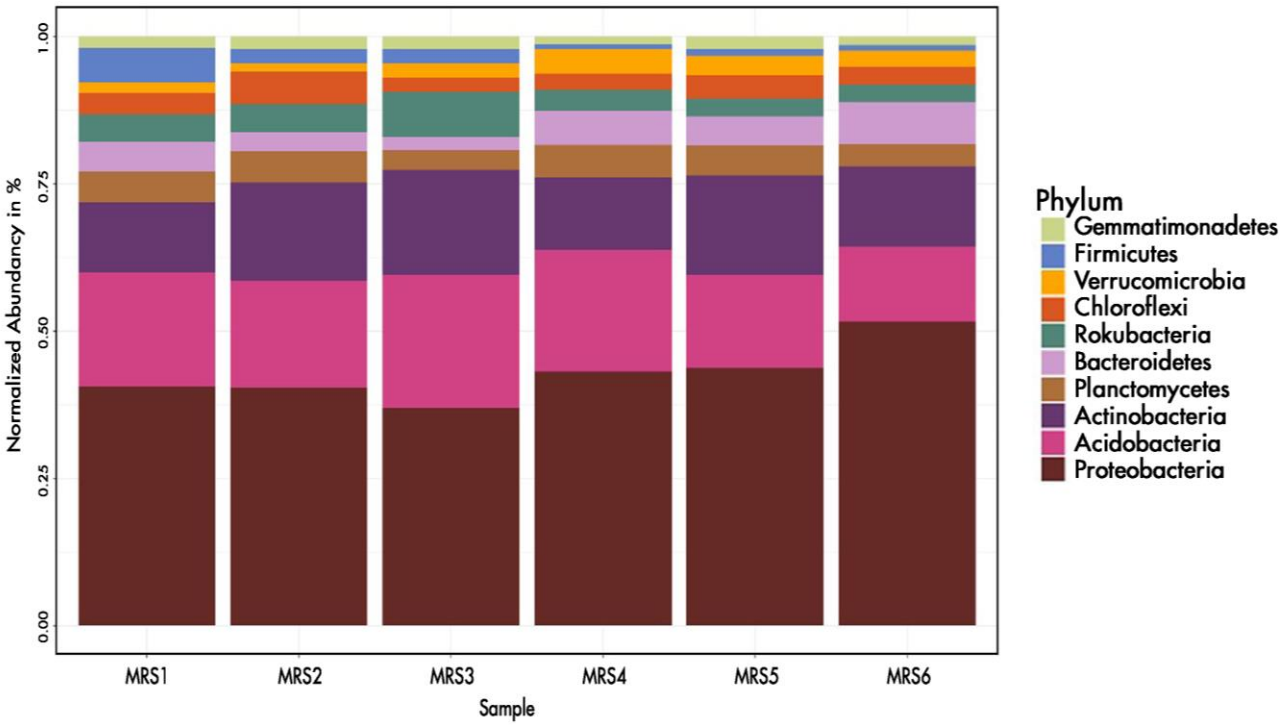

Figure 2: Relative abundance of bacteria (top ten) at phylum level identified from the rhizosphere soil of *M. pudica* in an ex-tin mining soil.

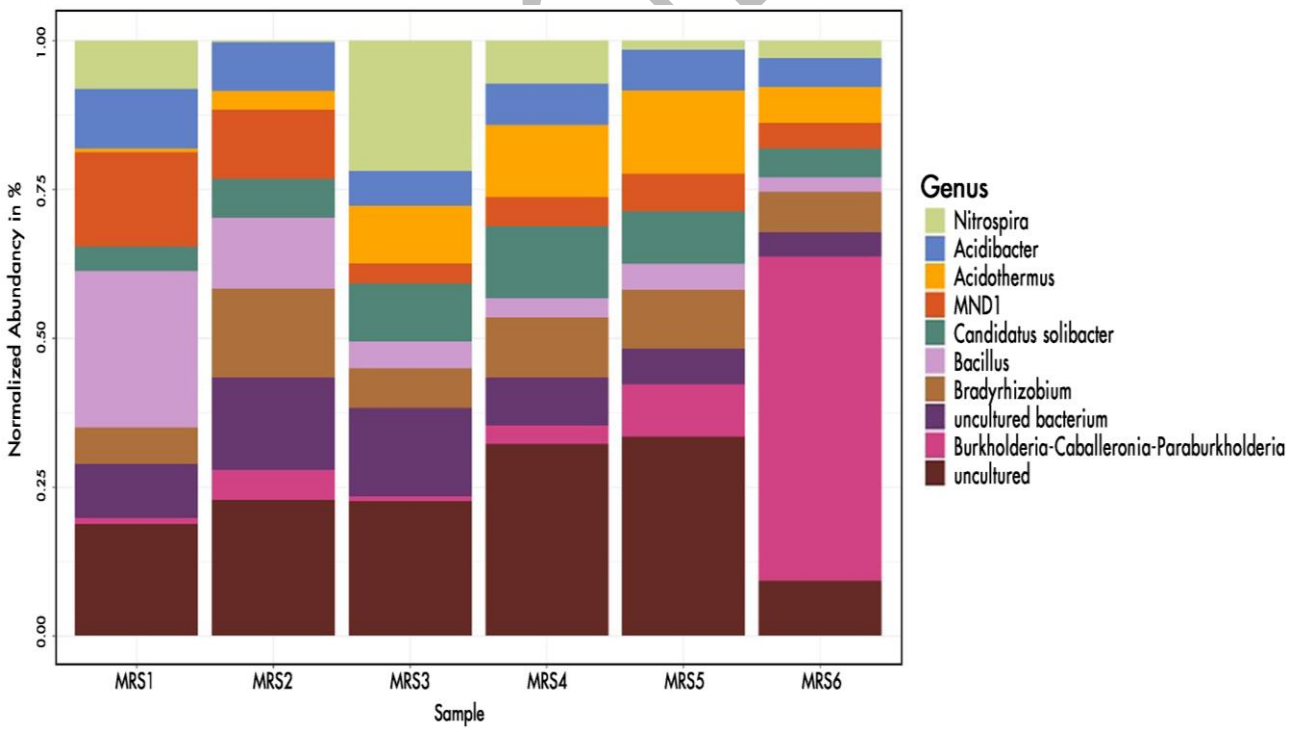

Figure 3: Relative abundance of bacteria (top ten) at genus level identified from the rhizosphere soil of *M. pudica* in an ex-tin mining soil.

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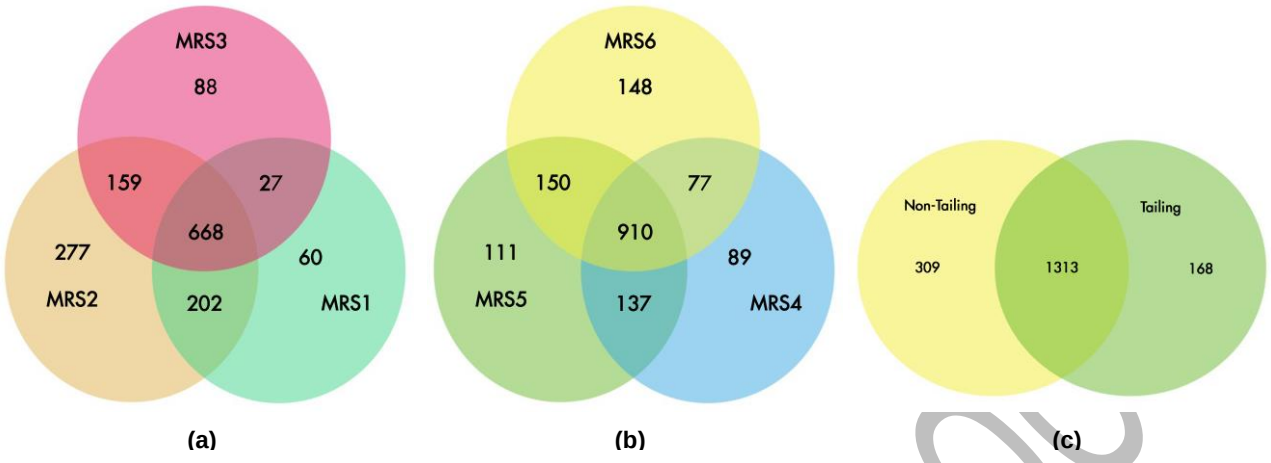

Figure 4: Venn diagrams showing the unique and shared OTUs of (a) tailing samples, (b) non-tailing samples and (c) tailing vs non-tailing.

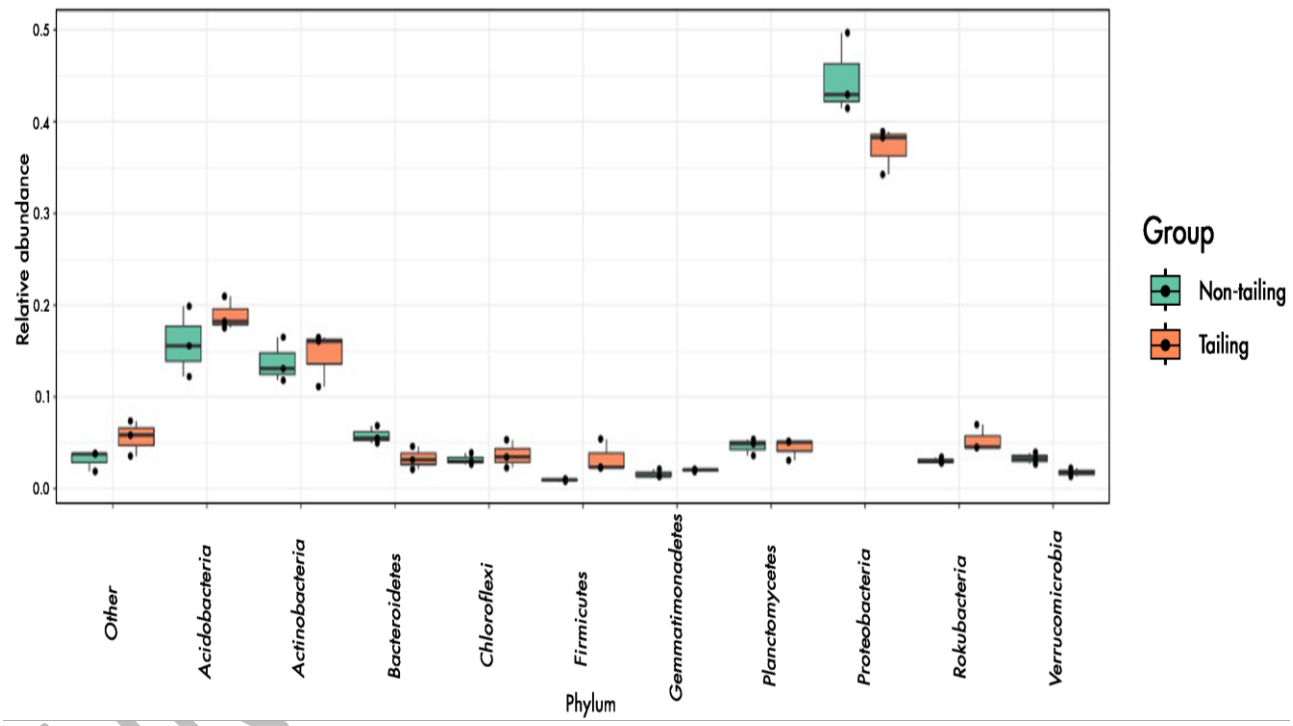

Figure 5: Relative abundance of most represented bacterial phyla in the tailing and non-tailing sites of the ex-mining area.

the identifiable genera in this study, some were present in both the tailing and non-tailing areas, while others are specifically present in only one of the two areas. One of the main causes of soil pollution by heavy metals is mining. Still, the mining sites are a rich source of microbial diversity, which has produced many metal-tolerant mechanisms and metabolic processes that can greatly help to remediate metal-polluted sites (Ghosh and Das, 2018). Many bacteria have evolved different immune mechanisms to adapt and withstand metal toxicity (Costa *et al*., 2014; Ojuederie and Babalola, 2017; Nayak *et al*., 2019). Furthermore, in especially heavy metal polluted soils such as mine tailing, bacteria that grow have metal toxicity resistant genes for their survival (Sibanda *et al.*, 2019). Detection of such metal tolerating genes in the studied sites explain their likely source of resistance.

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Table 2: Heavy metals tolerance genes predicted from the metagenome of *M. pudica* rhizosphere soil.

Function	Genes
Arsenic resistance	*ars*C, *ars*A, *ars*R, *ars*B, *ars*H
Chromium resistance	*chr*A, *chr*R
Cobalt resistance	*cbi*D, *cbi*E, *cbi*G, *cbi*K, *cbi*T, *cob*K, *cob*M, *cob*H, *cob*N, *cob*S, *cob*T
Copper resistance	*pco*D, *cop*A, *cop*B, *cop*C, *cue*O, *cue*R, *cus*R, *cut*C, *cut*F, *ctp*V
Iron resistance	*afu*C, *afu*B, *afu*A, *feo*A, *feo*B, *feo*C, *efe*O, *efe*B, *suf*C, *suf*B, *suf*D, *isc*A, *znu*A, *znu*B, *znu*C, *fhu*F
Mercury resistance	*mer*R, *mer*A, *mer*T, *mer*P, *mer*C
Nickel resistance	*sod*N, *hyp*A, *hyp*B, *nix*A, *nik*A, *nik*B, *nik*D
Zinc resistance	*zup*T, *pqq*L, *znu*A, *znu*B, *znu*C, *zur*R, *znt*B, *znt*R

Predicted functional genes involved in heavy metals tolerance, degradation and detoxification

Predicted genes for heavy metal tolerance were detected and presented in Table 2. For example, arsenic resistant genes of the *ars* operon (*ars*C, *ars*A, *ars*R, *ars*B and *ars*H) involved in arsenic tolerance, reduction and transport were detected in the metagenome. The *ars* operons are the most popular systems for arsenic resistance in microbes, and numerous *ars* operons with a wide range of genes and multiple combinations are found in prokaryotic genomes (Ben Fekih *et al*., 2018). ArsR is a transacting transcriptional repressor protein that binds to the promoter region of *ars* operons. Its interaction with arsenite will dissociate the repressor protein from the DNA that can, therefore, allow transcription of the operon (Ben Fekih *et al*., 2018). Alleviation of arsenic contamination in the environment is considered a high-priority issue, and studies were carried out that revealed different bacteria and genes in an association that can detoxify the toxic effects of As (Costa *et al*., 2014; Luo *et al*., 2014; Gu *et al*., 2017; 2018).

Two chromium resistant genes *chr*A (chromate transporter) and *chr*R (chromate reductase) were found as well as several cobalt resistant genes of the *cbi* cluster (*cbi*D, *cbi*E, *cbi*G, *cbi*K and *cbi*T) and *cob* cluster (*cob*K, *cob*M, *cob*H, *cob*N, *cob*S and *cob*T). Determinants of chromate resistance found in archaea, bacteria and eukarya consist of genes from the superfamily of chromate ion transport (CHR) that typically include the *chr*A gene (Viti *et al.*, 2014). Reduction of Cr(VI) to Cr(III) by microbes can be regarded as a chromate detoxification mechanism (Viti *et al.*, 2014). The best chromate reductase studied so far is the *chr*R, which is a soluble flavin mononucleotide-binding enzyme involved in catalyzing the reduction of Cr(VI) to Cr(III) (Viti *et al.*, 2014).

Also detected were genes for cobalt-zinc-cadmium resistance predominantly of the *czc* operon (*czc*C, *czc*A, *czc*B and *czc*D). Czc is said to be a chemiosmotic efflux pump for Cd^{2+}, Zn^{2+} and Co^{2+}, that forms essential internal (CzcA), external (CzcC) and membrane (CzcB) proteins involved in transport of cation from the cytoplasm (Xavier *et al.*, 2019). The czc operon was found in several other studies such as one detected to comprise three structural genes, *czc*A, *czc*B and *czc*C, and two regulatory ones, *czc*D and *czc*R (Asaf *et al.*, 2018). This operon is known to confer resistance to the three heavy metals, namely zinc, cobalt and cadmium. In a study on the microbial community of metal-rich tropical stream sediment (Costa *et al.*, 2015), the bacterial metagenome revealed a number of genes related to tolerance of different heavy metals with the cobalt-zinc-cadmium being the most abundant (47%).

Genes for copper resistance (*pco*D, *cop*A, *cop*B, *cop*C, *cue*R, *cue*O, *cus*R, *cut*C, *cut*F and *ctp*V) and for iron resistance and transport (*afu*C, *afu*B, *afu*A, *feo*A, *feo*B, *feo*C, *efe*O, *efe*B, *hug*Z, *suf*C, *suf*B, *suf*D, *isc*A, *erp*A, *znu*A, *znu*B, *znu*C and *fhu*F) were also found. Other heavy metals resistance genes revealed in this study were those involved in mercury resistance (*mer*R, *mer*A, *mer*T, *mer*P and *mer*C), nickel resistance (*sod*N, *hyp*A, *hyp*B, *nix*A, *nik*A, *nik*B and *nik*D) and zinc resistance (*zup*T, *pqq*L, *znu*A, *znu*B, *znu*C, *zur*R, *znt*B and *znt*R). Two genes, *cop*A and *cue*O are regulated by the gene *cue*R, where *cop*A is known to be a P-type ATPase and *cue*O said to encode a multicopper oxidase involve in oxidizing Cu^{+} [Cu(I) to a less toxic Cu(II)] and reducing dioxygen to water (Das *et al.*, 2016). Two mercury resistant genes, *mer*A and *mer*R were detected in this study. The narrow-spectrum *mer* operon is made up of many genes that are induced by inorganic mercury (Hg^{2+}) and solely provide resistance to inorganic mercury salts, with *mer*R serving as the operon's positive transcriptional regulator (Das *et al.*, 2016). *mer*A gene codes for mercuric ion reductase that function in reducing Hg^{2+} in the cell to form volatile Hg^{0}, that can easily be released from the cell (Das *et al.*, 2016).

Predicted functional genes linked to plant growth-promoting (PGP) traits

Also detected are various predicted genes associated with plant growth-promoting traits (Table 3). Genes associated with the production of indole acetic acid (IAA) revealed in this study were *iaa*M, *ipd*C and *ald*A. Besides these, genes involved in tryptophan production which is the precursor of the IAA were also detected and included *trp*C, *trp*A, *trp*B, *trp*E, *trp*F, *trp*G, *trp*GD, *trp*CF and *trp*R. Production of IAA by bacteria may be tryptophan-

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Table 3: Plant growth-promoting genes predicted from the metagenome of *M. pudica* rhizosphere soil.

Function	Genes
IAA production	*iaa*M, *ipd*C, *ald*A, *trp*C, *trp*A, *trp*B, *trp*E, *trp*F, *trp*G, *trp*GD, *trp*CF, *trp*R
ACC deaminase	*rim*, *dcy*D, *acd*, *acd*H, *acd*A
Siderophore production	*ent*A, *ent*B, *ent*C, *ent*D, *ent*F, *ent*S, *fiu*
Nitrogen fixation	*nif*A, *nif*Z, *nif*B, *nif*D, *nif*E, *nif*H, *nif*K, *nif*N, *nif*T, *nif*V, *nif*W, *nif*X, *nif*Z, *gln*B, *gln*K, *nac*
Phosphate solubilization	*pqq*A, *pqq*B, *pqq*C, *pqq*D, *pqq*E, *pst*B, *pst*C, *pst*A, *pst*S, *pho*U, *pho*D, *pho*R, *pho*Q, *pho*B, *phn*C, *phn*E, *phn*F, *phn*D, *ids*A, *gpm*I, *gpm*B, *bcr*C, *yfb*T, *yni*C
Potassium solubilization	*trk*H, *trk*G, *ktr*B, *trk*A, *ktr*A, *kdp*A, *kdp*B, *kdp*C, *kdp*F, *kef*C, *kef*F, *kef*V, *kef*G

dependent or tryptophan-independent (Zhang *et al.*, 2019). The indole-3-acetamide (IAM) pathway is the best-studied IAA pathway in bacteria (Spaepen *et al.*, 2007). Here, the tryptophan-2-monooxygenase enzyme (IaaM), encoded by the *iaa*M gene, converts tryptophan to IAM, which is subsequently converted to IAA in the second step by an IAM hydrolase (IaaH), expressed by the *iaa*H gene. Another pathway is the indole-3-pyruvate (IPyA) pathway. The conversion of tryptophan to IPyA is the initial step, followed by IPyA being decarboxylated to indole-3-acetaldehyde (IAAld) by indole-3-pyruvate decarboxylase (IPDC) and IAAld being oxidized to IAA (Spaepen *et al.*, 2007). The enzyme IPDC is encoded by *ipd*C. IAA is the most crucial and prevalent natural auxin, that plays a very vital role in cell division, cell elongation, root and fruit development and senescence (Duca *et al.*, 2014; Upadhyay *et al.*, 2018). In a huge-scale genetic analysis study to determine the distribution of tryptophan-dependent IAA synthesis routes and associated genes, 82.2% of the analyzed genomes showed the potential to synthesize IAA from the tryptophan (Trp) or its intermediates (Zhang *et al.*, 2019).

Genes associated with the production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (*rim*M, *dcy*D, *acd*, *acd*H and *acd*A) were also found, likewise those for siderophore production (*ent*A, *ent*B, *ent*C, *ent*D, *ent*F, *ent*S and *fiu*). ACC deaminase activities by some plant-associated microbes help host plants to survive well under different environmental stresses by decreasing the level of ethylene that is known to inhibit the growth of such plants (Singh *et al.*, 2015). Most of the plant growth inhibition related to environmental stress is caused by higher ethylene levels resulting from the stress (Kong and Glick, 2017). The ACC deaminase is said to break down ACC (ethylene precursor) into ammonia and -ketobutyrate, both of which bacteria can use for growth and metabolism (Singh *et al.*, 2015). In a study that reported ACC deaminase from *Pseudomonas stutzeri* A1501 to aid rice growth in the presence of heavy metals or salt, the *P. stutzeri* A1501 was reported to have a single gene *acd*S encoding ACC deaminase (Han *et al.*, 2015). One of the most abundant elements on the planet is iron, but it is not always available for direct use by living organisms (Kong and Glick, 2017). In the process of obtaining iron, several bacteria make siderophores that bind Fe and act as solubilizing agents, which promote iron uptake by plants (Kong and Glick, 2017). The siderophores can as well create stable complexes with other forms of metals, in addition to iron such as Cd, Al, Ga, In, Cu, Pb and Zn, which can make the beneficial siderophore-synthesizing bacteria useful in the heavy metals phytoremediation processes (Kong and Glick, 2017).

The genes for nitrogen fixation, regulation and assimilation revealed included the *nif* cluster (*nif*A, *nif*Z, *nif*B, *nif*D, *nif*E, *nif*H, *nif*K, *nif*N, *nif*T, *nif*V, *nif*W, *nif*X and *nif*Z), the *gln* cluster (*gln*B and *gln*K) and *nac*. The molybdenum-dependent nitrogenase subtype encoded by *nif*H, *nif*D and *nif*K is the most well-studied (Dos Santos *et al.*, 2012). Like this study, a previous study on the draft genome sequence of *Rhizobium sullae* strain IS123^T focused on the genes for symbiosis with its host *Hedysarum coronarium* L. (Sablok *et al.*, 2017) identified the structural nitrogenase units *nif*H, *nif*D and *nif*K, as well as other regulatory or accessory determinants such as *nif*A, *nif*B, *nif*E, *nif*N, *nif*S, *nif*T, *nif*U, *nif*W and *nif*Z. In addition to the *nif* genes for the nitrogenase activity and biosynthesis. In nitrogen fixation, non-*nif* genes encoding transporters for Mo, Fe and S play an important role (Shi *et al.*, 2016). Biological nitrogen fixation is a critical component of the global nitrogen cycle and the primary pathway for converting atmospheric N_2 to NH_3 (Dos Santos *et al.*, 2012; Shi *et al.*, 2016).

Another important and essential macronutrient needed by living organisms including plants is phosphorus. Most of the phosphorus in the soil is in an insoluble form and therefore not always available for plant consumption as plants can utilize only free orthophosphate (PO_4^{3-}) (Andrés-Barrao *et al.*, 2017). Some plant growth-promoting bacteria could provide inorganic phosphorus available to plants through phosphate solubilization. Several clusters of genes involved in phosphate solubilization, transport and assimilation were found. These were the *pqq* cluster (*pqq*A, *pqq*B, *pqq*C, *pqq*D and *pqq*E), *pst* cluster (*pst*B, *pst*C, *pst*A and *pst*S), *pho* cluster (*pho*U, *pho*D, *pho*R, *pho*Q and *pho*B), *phn* cluster (*phn*C, *phn*E, *phn*F and *phn*D), *gpm* cluster (*gpm*I and *gpm*B) and then *ids*A, *bcr*C and *yfb*T/*yni*C. These were also detected in other studies of plant growth-promoting bacteria (Bruto *et al.*, 2014; Gupta *et al.*, 2014; Asaf *et al.*, 2018; Luziatelli *et al.*, 2020). Lastly, the potassium solubilization genes were also detected in the study and these were *trk*H, *trk*G, *ktr*B, *trk*A, *ktr*A, *kdp*A, *kdp*B, *kdp*C, *kdp*F, *kef*C, *kef*F, *kef*V and *kef*G. Potassium, together

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with nitrogen and phosphorus, form part of the most crucial nutrients needed by plants for growth and development (Andrés-Barrao *et al.*, 2017).

Several bacteria on exposure to different toxic heavy metals have developed metal resistant genes that serve as means of survival and adaptation in the harsh environment (Das *et al.*, 2016). The bacterial community present in the studied ex-tin mining soil could, therefore, be one of the most important elements affecting development and survival of *M. pudica* and other metalliferous plants in such heavy metal contaminated conditions. These bacteria and genes can further be exploited for the purpose of bioremediation of the metal-contaminated environment.

CONCLUSION

The study revealed complex and diverse bacterial taxa with Proteobacteria as the most dominant phylum (41.2%), followed by Acidobacteria (17.1%) and Actinobacteria (14.4%). Commonly found genera detected were *Paraburkholderia*, *Bradyrhizobium*, *Bacillus*, *Candidatus solibacter*, *Acidothermus*, *Acidibacer* and *Nitrospira*. The study also showed bacterial community in the non-tailing sites had a higher number and richness of species and those in the tailing sites were more diverse. Predicted genes for both heavy metal tolerance (As, Cr, Co, Ni, Zn, Cd, Cu, Fe and Hg) and plant-growth promotion (synthesis of IAA, ACC deaminase and siderophore; solubilization of phosphate and potassium and nitrogen fixation) were detected in the samples. These provided holistic information for potential plant growth-promoting rhizobacteria that are heavy metal tolerant, that can cope with heavy metal polluted soils which can be employed for bioremediation or phytoremediation of heavy metal contaminated soils. It also explained why *M. pudica* is endemic in the present study area even though it is highly contaminated with heavy metals.

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