Comparative genomics of 16 *Microbacterium* spp. that tolerate multiple heavy metals and antibiotics

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Sixteen different strains of *Microbacterium* spp. were isolated from contaminated soil and enriched on the carcinogen, hexavalent chromium [Cr(VI)]. The majority of the isolates (11 of the 16) were able to tolerate concentrations (0.1 mM) of cobalt, cadmium, and nickel, in addition to Cr(VI) (0.5-20 mM). Interestingly, these bacteria were also able to tolerate 3 different antibiotics (ranges: ampicillin 0-16 μg ml^{-1}, chloramphenicol 0-24 μg ml^{-1}, and vancomycin 0-24 μg ml^{-1}). To gain genetic insight into these tolerance pathways, the genomes of these isolates were assembled and annotated. The genomes of these isolates have some shared genes (core genome) but also have a large amount of variability. The genomes also contained an annotated Cr(VI) reductase (*chrR*) that could be related to Cr(VI) reduction. Further, various heavy metal tolerance (e.g. Co/Zn/Cd efflux system) and antibiotic resistance genes were identified, which provide insight into the isolates’ ability to tolerate metals and antibiotics. Overall, these isolates showed a wide range of tolerances to heavy metals and antibiotics and genetic diversity, which was likely required of this population to thrive in a contaminated environment.
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Abstract

Sixteen different strains of Microbacterium spp. were isolated from contaminated soil and enriched on the carcinogen, hexavalent chromium [Cr(VI)]. The majority of the isolates (11 of the 16) were able to tolerate concentrations (0.1 mM) of cobalt, cadmium, and nickel, in addition to Cr(VI) (0.5-20 mM). Interestingly, these bacteria were also able to tolerate 3 different antibiotics (ranges: ampicillin 0-16 $\mu$g ml$^{-1}$, chloramphenicol 0-24 $\mu$g ml$^{-1}$, and vancomycin 0-24 $\mu$g ml$^{-1}$). To gain genetic insight into these tolerance pathways, the genomes of these isolates were assembled and annotated. The genomes of these isolates have some shared genes (core genome) but also have a large amount of variability. The genomes also contained an annotated Cr(VI) reductase (chrR) that could be related to Cr(VI) reduction. Further, various heavy metal tolerance (e.g. Co/Zn/Cd efflux system) and antibiotic resistance genes were identified, which provide insight into the isolates’ ability to tolerate metals and antibiotics. Overall, these isolates showed a wide range of tolerances to heavy metals and antibiotics and genetic diversity, which was likely required of this population to thrive in a contaminated environment.

Introduction

Heavy metals, while naturally occurring, cause harm to both human and ecosystem health. Specifically, chromium is a mutagen and carcinogen and its presence in the environment can be natural or due to anthropogenic activities, such as industrial manufacturing (e.g. metal plating and tanneries) and mining (chromite ore) (Barak et al., 2006; Brose and James, 2010; Cheng et al., 2012). In the environment, chromium can exist as hexavalent chromium [Cr(VI)], which is soluble and more toxic, or as insoluble trivalent chromium [Cr(III)] (Bartlett, 1991; Cheng et al., 2012). Thus, the redox cycling of chromium is critical to understanding its impacts on the environment.
In natural systems, Cr(III) can be oxidized by manganese oxides and hydrogen peroxide, while Cr(VI) can be reduced by ferrous iron and hydrogen sulfides (Brose and James, 2010; Oze et al., 2004; Viti et al., 2013).

Many bacteria are able to tolerate Cr(VI) stress based on their ability to transport it outside the cell or enzymatically reduce it to the less toxic form Cr(III). The efflux pump, chrA, has been associated with providing Cr(VI) resistance by transporting it outside the cell (Cervantes and Ohtake, 1988; Cervantes et al., 1990; Nies et al., 1990). A 2008 study found 135 chrA orthologs of this efflux pump (Ramirez-Diaz et al., 2008), however, the presence of chrA is not always a sole predictor of the amount of Cr(VI) that bacteria can resist (Henne et al., 2009a). Bacteria can also have additional efflux pumps that provide resistance to other metals (Nies, 2003; Silver, 1996), which is advantageous as anthropogenically impacted sites are often contaminated with multiple stressors. Further, there is a well-known association between metal and antibiotic tolerance (Baker-Austin et al., 2006; Seiler and Berendonk, 2012; Wright et al., 2006). For example, Staphylococcus species use multiple efflux pumps to tolerate chromium, lead, and penicillin (Ghosh et al., 2000) and Salmonella abortus equi strains were able to tolerate chromium, cadmium, mercury and ampicillin (Ug and Ceylan, 2003). Thus, efflux pumps can play a vital role in how bacteria thrive in contaminated ecosystems.

Bacteria are also known to catalyze the reduction of Cr(VI) aerobically. Specifically, certain bacteria use a soluble NADH/NADPH dependent oxidoreductase to reduce Cr(VI) (Ackerley et al., 2004a; Barak et al., 2006; Cheung and Gu, 2007; Gonzalez et al., 2005; Park et al., 2000). Two well-studied Cr(VI) reductases are chrR and yieF. In Pseudomonas putida, chrR reduces Cr(VI) via the transfer of one electron, generating Cr(V), a reactive intermediate, which then requires a second electron transfer to generate the more stable insoluble Cr(III) (Park et al., 2000).
Conversely, *E. coli* uses *yieF* to catalyze the transfer of four electrons to reduce Cr(VI) (Ackerley et al., 2004b; Barak et al., 2006; Ramirez-Diaz et al., 2008).

The objective of this study was to examine the genomes of 16 *Microbacterium* spp. strains isolated from metal contaminated soil to identify their genetic potential and physiological ability to reduce Cr(VI) and to tolerate both heavy metals and antibiotics. A recent genomic study of four Cr(VI) reducing *Microbacterium* spp. identified two putative reductases (Henson et al., 2015), one genetically similar to *chrR* in *Thermus scotoductus* (Opperman et al., 2008) and the other to *yieF* of *Arthrobacter* sp. RUE61a (Niewerth et al., 2012), however, these putative reductases had low sequence similarity to the well-studied reductases found in *P. putida* and *E. coli*. While other studies have shown *Microbacterium* isolates are able to reduce Cr(VI) (e.g. Humphries et al., 2005; Liu et al., 2012; Pattanapipitpaisal et al., 2001; Soni et al., 2014), there are still unknowns related to the genetics of Cr(VI) reduction and resistance. Further, the study by Henson et al. (2015) documented high levels of genomic variability between the isolates, which might be related to ecotypes. However, the conclusions of this study were limited based on the low number of genomes examined. Comparative genomics has been used in various environments and microorganisms to examine inter-strain variation and ecotypes (e.g. Briand et al., 2009; Coleman and Chisholm, 2010; Denef et al., 2010; Frangeul et al., 2008; Humbert et al., 2013; Martiny et al., 2006; Meyer and Huber, 2014; Meyer et al., 2017). Thus, this study examined the genomic and physiological variability of heavy metal and antibiotic resistance of 16 *Microbacterium* strains from the same contaminated soil environment to elucidate the potential genomic flexibility of closely related strains.

**Methods**
Bacterial Isolation

Isolation of Microbacterium spp. from soil is described in Kourtev et al. (Kourtev et al., 2009) and Henson et al. (Henson et al., 2015). Bill Jervis from the Indiana Department of Transport provided site access (no permit was required) and the project did not involve endangered or protected species. Briefly, contaminated soil samples were collected from a Department of Transportation site in Seymour, IN. Previous studies have documented the site was contaminated with Pb (1,156 μg\textsuperscript{-1} soil), Cr (5,868 μg\textsuperscript{-1} soil), and hydrocarbons (toluene and xylenes: >200 μg g\textsuperscript{-1} soil) (Joynt et al., 2006; Kourtev et al., 2006; Nakatsu et al., 2005). Bacteria were initially isolated from the soil on 50% tryptic soy agar (TSA) amended with 0.25 mM Cr(VI) (K\textsubscript{2}CrO\textsubscript{4}). From this, sixteen isolates were further selected based on their ability to grow on at least 0.5 mM Cr(VI). The isolates were stored in glycerol stocks at -80°C until further used.

Cr(VI) Reduction

For Cr(VI) reduction experiments, isolates were grown in 25% tryptic soy broth (TSB) with 0.5 mM or 1 mM Cr(VI) at 30°C and 225 rpm for 72 hours. Two concentrations were used as certain isolates had relatively lower growth at 1mM compared to other isolates. The cultures were pelleted and Cr(VI) reduction was determined using a colorimetric assay (Henson et al., 2015; Urone, 1955).

DNA Sequencing and bioinformatics

Isolates were grown in TSB with 0.5 mM or 2 mM Cr(VI) at 30°C and 225 rpm for 24-72 hours. The cultures were pelleted via centrifugation and stored at -20°C until used in DNA extractions. FastDNA Spin Kits (MP Biomedical) were used to extract genomic DNA from the
112 thawed pellets. The resulting DNA was stored at -20°C. The DNA from the 16 bacterial isolates
113 were sent to Cincinnati Children’s Hospital Medical Center’s Genetic Variation and Gene
114 Discovery Core facility for whole genome shotgun sequencing using one lane of an Illumina HiSeq
115 2000 (100bp PE). The resulting raw genomic reads can be found in the National Center for
116 Biotechnology Information’s (NCBI) Short Read Archives (SRA), accession number:
117 SRP120551.
118
119 Raw reads were evaluated for quality with FastQC
120 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and trimmed and quality filtered
121 with Trimmomatic (v 0.33) (Bolger et al., 2014). Sequence quality scores were low on R2
122 (assessed by FastQC), so the trimmed reads were cut (length of 70bp) and passed through another
123 quality filter (-Q33 -q 30 -p 50) with FastX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/).
124 Reads for each sample were subsampled to 4 million reads (multiple subsampling depths were
125 tested) with seqtk (https://github.com/lh3/seqtk). Using multiple different read depths, MEGAhit
126 (Li et al., 2016) was used to assemble the data, which were then assessed for quality with QUAST
127 (Gurevich et al., 2013). CheckM (Parks et al., 2015) was used to examine genome completeness
128 and contamination (taxon marker Microbacterium). The resultant genomes were annotated by the
129 Department of Energy’s Joint Genome Institute Integrated Microbial Genomes (IMG) system
130 (Markowitz et al., 2012) and are publicly available (see Table S1). A pangenomic analysis of 20
131 genomes (from this study and Henson et al. 2015) was conducted using the An’vio program
132 (version 3) (Eren et al., 2015) following the pangenomics workflow from Delmont and Eren 2018
133 (Delmont and Eren, 2018). The pangenomic analysis within An’vio also utilized other programs
134 like HMMER (Eddy, 2011), Prodigal (Hyatt et al., 2010), and NCBI’s blastp (Altschul et al.,
134 1997). An’vio was also used to define clusters of orthologous groups (COGs) for the pangenomic analysis using the command anvi-run-ncbi-cogs.

136 All 16, 16S rRNA gene sequences from the Cr(VI) isolates were downloaded from IMG. These genes were used to generate a maximum likelihood phylogenetic tree in MEGA7 (Kumar et al., 2016) and evolutionary history was inferred using the maximum likelihood Tamura-Nei model (Tamura and Nei, 1993). Within IMG, annotations were searched for genes related to Cr(VI) resistance and Cr(VI) reduction via keywords (e.g. efflux, reductase, Cr(VI)). Annotated Cr(VI) reductase genes were compared via BLASTP (one-way comparison on NCBI’s website) to a known Cr(VI) reducing chrR from Pseudomonas putida KT2440 (NP_746257) (Ackerley et al., 2004b). Genes related to heavy metal resistance and antibiotic resistance were searched using functional categories. Beta-lactam, chloramphenicol, and vancomycin resistance genes were also identified using KEGG pathway KO identifiers. The COG category “Inorganic ion transport and metabolism” was used to find annotated heavy metal efflux genes and metal resistance genes.

148 Metal and antibiotic tolerance assays

149 Bacterial isolates were grown on agar plates (10% TSB, 1.5% agar, and 0.5 mM Cr(VI)) at 30°C until colonies were visible. Colonies were then streaked onto fresh agar plates amended with different concentrations of heavy metals (Cd 0-5 mM, Co 0-5 mM, Cr 0-20 mM, Cu 0-1 mM, Ni 0-15 mM, and Zn 0-1 mM). Duplicate plates were incubated at 30°C for 14 days and growth was confirmed by the presence of visible colonies. All bacterial isolates were plated on each metal concentration in duplicate.

155 The Minimum Inhibitory Concentration (MIC) for ampicillin, chloramphenicol, and vancomycin was tested on each isolate using MIC test strips (Liofilchem®). To do this, each of
the sixteen *Microbacterium* spp. isolates was streaked onto agar plates (50% TSB and 1.5% agar) with 0.5 mM Cr and incubated at 30° C for five days. Next, each isolate was suspended in a 0.85% sterile saline solution until it reached a density that approximated the 0.5 McFarland Turbidity Standard. The isolates were then spread uniformly on a Mueller-Hinton agar plate. A MIC test strip was placed in the center of each plate. The plates were incubated at 30° C for 24 hours. After the 24 hours, the plates were removed and the MIC of each isolate to each antibiotic was determined by visually documenting the zones of inhibition.

Results and Discussion

Isolation of Cr(VI) reducing bacteria

Though originally isolated and studied for their ability to reduce Cr(VI) (Henson et al., 2015; Kourtev et al., 2009), it was speculated that these isolates may also be tolerant to other contaminants, because the soils were contaminated with other heavy metals and organic solvents (Joynt et al., 2006; Kourtev et al., 2006; Nakatsu et al., 2005). Among the 16 isolates studied here, a wide range of Cr(VI) tolerance and reducing ability was found (Table 1). The majority (13) of the isolates could tolerate 2mM Cr(VI), while three of the isolates (K2B2, K36, and K40) could only tolerate 0.5mM Cr(VI). Interestingly, five of the 13 isolates that were able to tolerate 2mM Cr(VI) (A20, K24, K30, K33, and PF3) had observable colonies on plates containing up to 20mM Cr(VI). Further, the 16 isolates also showed a wide range in Cr(VI) reduction (0-88.8%) over a 72-hour incubation (Table 1). Only two isolates, K27 and K33, reduced over 80% of the Cr(VI) in the experiment. Interestingly, K27 reduced 83% of the Cr(VI) in liquid medium but was only able to tolerate 0.5mM Cr(VI) when grown on solid medium, while K33 tolerated up to 20mM Cr(IV) and reduced 88% of the Cr(IV) present. Two additional isolates, K31 and PF3, reduced
over 50% of the Cr(VI) in the experiment. There was no statistical relationship between the ability of isolates to tolerate and their ability to reduce Cr(VI).

Genomic assembly and pangenomic analysis

Previous research has shown Cr(VI) tolerance to be related to a resistance gene \((chrA)\) (Cervantes and Ohtake, 1988; Cervantes et al., 1990) and/or Cr(VI) reductases \((chrR\) and \(yieF\);(Ackerley et al., 2004b; Barak et al., 2006; Park et al., 2000; Ramirez-Diaz et al., 2008). To gain a better understanding of the Cr(VI) tolerance of these isolates, their genomes were assembled and annotated. The isolate genomes ranged in total length from 3.31 to 4.23 Mb and all had GC content ranging from 68-70% (Table 2). A genomic study of 10 Macrobacterium sp. documented similar GC ranges and genome length (Corretto et al., 2015). The assembled genomes were generally over 96.7% complete and all genomes had under 4% contamination (Table 2).

Phylogenetic analysis of the 16S rRNA gene indicated that all of the isolates belong to the genus \(Microbacterium\) (Figure S1 and Table S2). Specifically, the isolates were closely related to \(M.\) oxydans, \(M.\) maritypicum, or \(M.\) paraoxydans. \(Microbacterium\) spp. have been isolated from sites contaminated with metals (Avramov et al., 2016; Bollmann et al., 2010; Corretto et al., 2015), radionuclides (Nedelkova et al., 2007), and petroleum (Avramov et al., 2016; Chauhan et al., 2013; Wang et al., 2014). Moreover, some environmental isolates, such as \(M.\) laevaniformans strain OR221, have shown to tolerate multiple metals (Ni, Co, and Cd) (Bollmann et al., 2010). A genomic analysis of the latter strain provided evidence of genes (e.g. transporter and detoxification genes) that could aid the strain’s ability to tolerate metals (Brown et al., 2012). Other \(Microbacterium\) spp. have been known to reduce Cr(VI): \(Microbacterium\) sp. SUCR140 (Soni et
The pan-genome size of the 20 isolates was 7,902 protein clusters with the core genome encompassing 2,073 protein clusters (26%) (Figure 1). The majority of the genes in the core genome of the 20 isolates (36%) were placed in the “Metabolism” COG (Figure 2). Genes in the COG categories “Cellular processes and signaling” and “Information storage and processing” comprised 21 and 17%, respectively of the core genome (Figure 2). The flexible pangenome was dominated by genes in the COG categories “Metabolism” and “Poorly characterized/No assigned COG” (36% for both) (Figure 2). Relative to the core pangenome, the flexible pangenome had few genes in the COG categories “Cellular processes and signaling (21%)” and “Information storage and processing 17%” (Figure 2). The percentage of core genes in a genome can vary from 3 to 84% (McInerney et al., 2017). For example, the Bacillus cereus core genome is 27% of the pangenome, whereas the core genome of Bacillus anthracis is 65% (McInerney et al., 2017). An analysis of 20 Microcystis sp. showed the genome was comprised of 34-49% core genes and 51%-66% flexible genes (Meyer et al., 2017). A study on the genomes of 12 Prochlorococcus isolates found a core genome ranging from 40-67% (Kettler et al., 2007). The relatively large genomic variability seen with the Microbacterium spp. examined here could be due to selective pressures that drove to gene loss or horizontal gene transfer, which in the end enhanced the ability of the isolates to survive in a contaminated sediment environment.

The COG categories “Inorganic ion transport and metabolism (P)” and “Self-defense mechanism (V)” were examined due to their relation to heavy metal and antibiotic transport. The flexible genome has relatively higher percentages of genes in both the self-defense (1% of the core genome and 3% of the flexible genome) and inorganic ion transport and metabolism (5% of the
core genome and 6% of the flexible genome) COG category. Each of these COG categories includes predicted gene functions for heavy metal transport and antimicrobial resistance and antibiotic efflux/transporters (Figure 1). Interestingly, the core and flexible genomes contain different Co/Zn/Cd efflux system component genes and multidrug efflux pump genes, which suggest tolerance to heavy metals and antibiotics. The variability found here may be related to the ability of each isolate to tolerate different types and concentration of heavy metals and antibiotics.

**Genomic insights of Cr(VI) reduction**

The annotated genomes from this study provided evidence that these isolates could reduce Cr(VI). All possess annotated Cr(VI) reductase, *chrR* (Table S3). In addition, *M. sp* K24 and K30 contained two annotated *chrR* genes. The Cr(VI) reductases were compared with a known Cr(VI) reductase from *Pseudomonas putida* KT2440 (Park et al., 2000) and BLASTP results showed that all the *Microbacterium* spp. *chrR* genes shared a high degree of homology (ranging from 40-46% identity) (Table S3). Thus, it is likely that these *chrR* genes are responsible for the Cr(VI) reduction ability of these isolates. Interestingly, other *Microbacterium* spp. isolated from this site did not have annotated Cr(VI) reductases but BLAST searches were able to identify genes homologous to *chrR* and *yeiF* (Henson et al., 2015), further suggesting high interspecies genetic diversity in the putative *Microbacterium* spp. Cr(VI) reductases found in the same soil.

Cr(VI) tolerance might not be related to efflux pumps and Cr(VI) reductases. While the genomes did contain Cr(VI) specific reductases, they did not contain a Cr(VI) efflux pump (*chrA*). The lack of an assembled and annotated *chrA* was interesting since the ability of the isolates to reduce and resist Cr(VI) did not correlate. While the lack of an assembled *chrA* does not confirm its absence from the genome, tolerance could be related to other genes. Cr(IV) tolerance has been shown to
be related genes that involve oxidative stress response (Ackerley et al., 2004a; Cheng et al., 2009), DNA repair (Hu et al., 2005; Miranda et al., 2005), and metabolism (Brown et al., 2006; Decorosi et al., 2009; Henne et al., 2009b).

**Tolerance to multiple heavy metals and antibiotics**

The isolates’ genomes all contained numerous genes that suggest these bacteria can tolerate multiple heavy metals (e.g. Co, Zn, and Cd) and antibiotics. When examining KEGG pathways that related to transport, all of the isolates had genes that were annotated to be Co/Zn/Cd efflux system components (Table S4). In addition, some isolates had a putative cadmium resistance protein (Table S4). KEGG pathways also documented genes that could provide tolerance to three different antibiotics (ampicillin, chloramphenicol, and vancomycin) (Table S4).

The isolates’ physiological ability to tolerate heavy metals were examined to determine whether the inferred resistance based on the putative metal transport genes could be confirmed. All the isolates were streaked onto plates with various concentrations of Cd, Co, Cr, Cu, Ni, and Zn. Overall, 11 of the 16 isolates tolerated between 0.1 to 1.0 mM of all six tested metals (Table 1). All 16 isolates were able to tolerate Cr, Ni, Zn, and Cu (Table 1). As the isolates were enriched with Cr, it was expected that they would be able to tolerate higher concentrations of Cr. Overall, the bacteria isolated in this study were able to tolerate many heavy metals, a finding seen in other studies. *Microbacterium* spp. enriched from Ni-rich serpentine soils have been previously documented to tolerate multiple heavy metals including Cd (0.5-2.5 mM), Co (0.1-5 mM), Cr (1-5 mM), Cu (0.25-10 mM), Ni (5-15 mM) and Zn (5-10 mM) (Abou-Shanab et al., 2007). Another study found that multiple isolates closely related to *M. oxydans* were able to tolerate Cu (0.25-16 mM), Cr (0.5-16 mM), and Ni (0.25-16 mM) (Nedelkova et al., 2007).
have been isolated from contaminated environments, it is not surprising to find they can tolerate heavy metal contamination. While this study did not attempt to confirm the function of the Co/Zn/Cd efflux system components found in these genomes (Table S4), the presence of these genes could be related to the ability of these isolates to tolerate multiple heavy metals. Whether in culture (e.g. *Pseudomonas putida* or *Cupriavidus necator*, formerly *Alcaligenes eutrophus*) (Manara et al., 2012; Nies, 1995) or in soil microcosms (Cabral et al., 2016), cobalt-zinc-cadmium efflux system proteins have been shown to provide tolerance to heavy metals. Thus, is it possible that the presence of these genes plays the same role in the isolates documented in this study.

There is a long history of research linking metal tolerance and antibiotic resistance (e.g. Baker-Austin et al., 2006; Calomiris et al., 1984; Henriques et al., 2016; Seiler and Berendonk, 2012). In this study, thirteen of the sixteen isolates showed tolerance to ampicillin, chloramphenicol, and vancomycin (Table 3). Specifically, 13 out of 16 isolates were able to grow in the presence of ampicillin with tolerances ranging from 1.5 to 16 μg ml⁻¹ (Table 3). Chloramphenicol tolerance was found in 15 out of 16 isolates, of which 4 isolates, K24, K35, K36 and K5D, tolerated 24 μg ml⁻¹ of the antibiotic (Table 3). *Microbacterium* isolates have been previously documented to tolerate various antibiotics. *Microbacterium* isolates from fish mucus have shown high antibiotic resistance to both ampicillin (>1600 μg ml⁻¹) and chloramphenicol (>960 μg ml⁻¹) (Ozaktas et al., 2012). Human isolated *Microbacterium* spp. have been reported to grow in the presence of vancomycin in concentrations ranging from 0.25 to 15 μg ml⁻¹ (Gneiding et al., 2008). Other clinical *Microbacterium* isolates had MIC for ampicillin from 1 to 1.5 μg ml⁻¹ and vancomycin from 3 to 4 μg ml⁻¹ (Laffîneur et al., 2003). A 2015 study found 26% of *Microbacterium* isolates were resistant to vancomycin (Bernard and Pacheco, 2015).
Metal and antibiotic co-tolerance has been reported in a number of environmentally isolated bacteria. Bacteria isolated from drinking water with noted antibiotic resistance were also tolerant to high levels of Cu\(^{2+}\), Pb\(^{2+}\), and Zn\(^{2+}\) (Calomiris et al., 1984). Environmental isolates from wastewater treatment plants have been shown to tolerate various heavy metals and antibiotics (Shafique et al., 2016; 2017). Bacillus spp. isolated from river water tolerated multiple heavy metals and antibiotics (Shammi and Ahmed, 2016). Co-tolerance to heavy metals, antibiotics, and polychlorinated biphenyls was also observed in isolates from Antarctic sediments (Giudice et al., 2013). Further, evidence of shared genetic tolerance mechanisms for metals and antibiotics was found in Staphylococcus aureus isolated from a polluted riverbank. S. aureus contained a novel emrAB operon encoding efflux pumps that were inducible by the heavy metals Cr(VI) and manganese and the antibiotics ampicillin and chloramphenicol (Zhang et al., 2016).

While this study does not prove function, the data suggests the antibiotic tolerances found in these isolates could be related to the antibiotic resistance genes documented in the genomes. For example, 15 out of 16 isolates were able to tolerate chloramphenicol matching the 15 genomes that contained an annotated chloramphenicol resistance gene (cmlR, cmx) (Table S4), a gene previously shown to provide Corynebacterium striatum with chloramphenicol tolerance (Schwarz et al., 2004; Tauch et al., 1998). It is possible cmx could therefore provide tolerance to the Microbacterium isolates. Similarly, all the Microbacterium isolates had an annotated class A beta-lactamase gene (penP), a gene that provides ampicillin tolerance to numerous bacteria (Bush and Jacoby, 2010), though not all isolates were resistant to ampicillin (Table 3).

**Implications to community functions**
All *Microbacterium* spp. were isolated from the same contaminated soil and have highly similar 16S rRNA genes (99-100% BLAST identity). Yet, these isolates displayed both genomic variation and varying abilities to tolerate multiple heavy metals and antibiotics. Other studies have shown that closely related isolates can exhibit variable ranges of genomic and physiological differences (Coleman and Chisholm, 2010; Henson et al., 2015; Hunt et al., 2008; Martiny et al., 2006; Meyer and Huber, 2014; Qamar et al., 2017; Rocap et al., 2003; Simmons et al., 2008; Welch et al., 2002). A study of 78 *Myxococcus xanthus* isolates from a small soil plot found 21 different genotypes (Vos and Velicer, 2006). Thus, it is speculated that genomic and physiological diversity would allow populations to differentiate, potentially increasing resistance and/or resilience that would aid the community to thrive during metals and other contaminant stress. Further support for this could be found when examining the Cr(VI) reduction and resistance data. While none of the isolates had an annotated Cr(VI) resistance efflux pump (*chrA*), they all contained a Cr(VI) reductase (*chrR*), yet there was variability found in their ability to reduce and resist Cr(VI). As the isolates from this study were from a soil with multiple contaminants, specific isolates that more efficiently reduce Cr(VI) (making it less bioavailable) may allow other community members (without resistance genes) to thrive and possibly degrade other contaminants. Thus, the variation observed in these isolates could be a reflection of how the environmental stressors (e.g. heavy metals) can promote genomic diversity and a more stable community.

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References

Abou-Shanab RAI, Van Berkum P, Angle JS 2007. Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of *Alyssum murale*. Chemosphere 68:360–367. DOI: 10.1016/j.chemosphere.2006.12.051.

Ackerley DF, Gonzalez CF, Keyhan M, Blake R, Matin A 2004a. Mechanism of chromate reduction by the *Escherichia coli* protein, NfsA, and the role of different chromate reductases in minimizing oxidative stress during chromate reduction. *Environ Microbiol* 6:851–860. DOI: 10.1111/j.1462-2920.2004.00639.x.

Ackerley DF, Gonzalez CF, Park CH, Blake R, Keyhan A, Matin A 2004b. Chromate-reducing properties of soluble Flavoproteins from *Pseudomonas putida* and *Escherichia coli*. *Applied and Environmental Microbiology* 70:873–882. DOI: 10.1128/Aem.70.2.873-882.2004.

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic acids research* 25:3389–3402. DOI: 10.1093/nar/25.17.3389.

Avramov AP, Couger MB, Hartley EL, Land C, Wellendorf R, Hanafy RA, Budd C, French DP, Hoff WD, Youssef N 2016. Draft genome sequence of *Microbacterium oleivorans* strain Wellendorf implicates heterotrophic versatility and bioremediation potential. *Genomics Data* 10:54–60. DOI: 10.1016/j.gdata.2016.09.005.

Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV 2006. Co-selection of antibiotic and metal resistance. *Trends Microbiol* 14:176–182. DOI: 10.1016/j.tim.2006.02.006.
Barak Y, Ackerley DF, Dodge CJ, Banwari L, Alex C, Francis AJ, Matin A 2006. Analysis of novel soluble chromate and uranyl reductases and generation of an improved enzyme by directed evolution. *Applied and Environmental Microbiology* 72:7074–7082. DOI: 10.1128/AEM.01334-06.

Bartlett RJ 1991. Chromium Cycling in Soils and Water - Links, Gaps, and Methods. *Environmental Health Perspectives* 92:17–24.

Bernard K, Pacheco AL 2015. In Vitro Activity of 22 Antimicrobial Agents against *Corynebacterium* and *Microbacterium* Species Referred to the Canadian National Microbiology Laboratory. *Clinical Microbiology Newsletter* 37:187–198. DOI: 10.1016/j.clinmicnews.2015.11.003.

Bolger AM, Lohse M, Usadel B 2014. Trimmomatic: a flexible trimer for Illumina sequence data. *Bioinformatics* 30:2114–2120.

Bollmann A, Palumbo AV, Lewis K, Epstein SS 2010. Isolation and Physiology of Bacteria from Contaminated Subsurface Sediments. *Applied and Environmental Microbiology* 76:7413–7419. DOI: 10.1128/Aem.00376-10.

Briand E, Escoffier N, Straub C, Sabart M, Quiblier C, Humbert J-F 2009. Spatiotemporal changes in the genetic diversity of a bloom-forming *Microcystis aeruginosa* (cyanobacteria) population. *The ISME Journal* 3:419–429. DOI: 10.1038/ismej.2008.121.

Brose DA, James BR 2010. Oxidation-Reduction Transformations of Chromium in Aerobic Soils and the Role of Electron-Shuttling Quinones. *Environ Sci Technol* 44:9438–9444.

Brown SD, Palumbo AV, Panikov N, Arlyawansa T, Klingeman DM, Johnson CM, Land ML, Utturkar SM, Epstein SS 2012. Draft Genome Sequence for *Microbacterium laevaniformans*
Strain OR221, a Bacterium Tolerant to Metals, Nitrate, and Low pH. *J Bacteriol* 194:3279–3280. DOI: 10.1128/JB.00474-12.

Brown SD, Thompson MR, Verberkmoes NC, Chourey K, Shah M, Zhou J, Hettich RL, Thompson DK 2006. Molecular dynamics of the *Shewanella oneidensis* response to chromate stress. *Mol Cell Proteomics* 5:1054–1071. DOI: 10.1074/mcp.M500394-MCP200.

Bush K, Jacoby GA 2010. Updated functional classification of beta-lactamases. *Antimicrobial agents and chemotherapy* 54:969–976. DOI: 10.1128/AAC.01009-09.

Cabral L, Júnior GVL, Pereira de Sousa ST, Dias ACF, Lira Cadete L, Andreote FD, Hess M, de Oliveira VM 2016. Anthropogenic impact on mangrove sediments triggers differential responses in the heavy metals and antibiotic resistomes of microbial communities. *Environmental Pollution* 216:460–469. DOI: 10.1016/j.envpol.2016.05.078.

Calomiris JJ, Armstrong JL, Seidler RJ 1984. Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. *Applied and Environmental Microbiology* 47:1238–1242.

Cervantes C, Ohtake H 1988. Plasmid-determined resistance to chromate in *Pseudomonas aeruginosa*. *FEMS Microbiology Letters* 56:173–176.

Cervantes C, Ohtake H, Chu L, Misra TK, Silver S 1990. Cloning, nucleotide sequence, and expression of the chromate resistance determinant of *Pseudomonas aeruginosa* plasmid pUM505. *Journal of Bacteriology* 172:287–291.

Chauhan A, Green S, Pathak A, Thomas J, Venkatramanan R 2013. Whole-Genome Sequences of Five Oyster-Associated Bacteria Show Potential for Crude Oil Hydrocarbon Degradation. *Genome Announcements* 1:e00802–13–e00802–13. DOI: 10.1128/genomeA.00802-13.
Cheng Y, Holman H-Y, Lin Z 2012. Remediation of Chromium and Uranium Contamination by Microbial Activity. *Elements* 8:107–112.

Cheng Y, XIE Y, ZHENG J, WU Z, CHEN Z, MA X, LI B, Lin Z 2009. Identification and characterization of the chromium(VI) responding protein from a newly isolated *Ochrobactrum anthropi* CTS-325. *Journal of Environmental Sciences-China* 21:1673–1678. DOI: 10.1016/S1001-0742(08)62472-9.

Cheung KH, Gu JD 2007. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: A review. *International Biodeterioration & Biodegradation* 59:8–15. DOI: 10.1016/j.ibiod.2006.05.002.

Coleman ML, Chisholm SW 2010. Ecosystem-specific selection pressures revealed through comparative population genomics. *Proceedings of the National Academy of Sciences of the United States of America* 107:18634–18639. DOI: 10.1073/pnas.1009480107.

Corretto E, Antonielli L, Sessitsch A, Kidd P, Weyens N, Brader G 2015. Draft Genome Sequences of 10 *Microbacterium* spp., with Emphasis on Heavy Metal-Contaminated Environments. *Genome Announcements* 3:e00432–15. DOI: 10.1128/genomeA.00432-15.

Decorosi F, Tatti E, Mini A, Giovannetti L, Viti C 2009. Characterization of two genes involved in chromate resistance in a Cr(VI)-hyper-resistant bacterium. *Extremophiles: life under extreme conditions* 13:917–923. DOI: 10.1007/S00792-009-0279-6.

Delmont TO, Eren AM 2018. Linking pangenomes and metagenomes: the *Prochlorococcus* metapangenome. *Peerj* 6:e4320. DOI: 10.7717/peerj.4320.

Denef VJ, Kalnejais LH, Mueller RS, Wilmes P, Baker BJ, Thomas BC, VerBerkmoes NC, Hettich RL, Banfield JF 2010. Proteogenomic basis for ecological divergence of closely related bacteria in natural acidophilic microbial communities. *Proceedings of the National Academy of Sciences of the United States of America* 107:18634–18639. DOI: 10.1073/pnas.1009480107.
Eddy SR 2011. Accelerated Profile HMM Searches. 7:e1002195. DOI: 10.1371/journal.pcbi.1002195.

Eren AM, Esen OC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO 2015. Anvi'o: an advanced analysis and visualization platform for "omics data. PeerJ 3:e1319. DOI: 10.7717/peerj.1319.

Focardi S, Pepi M, Focardi SE 2014. Microbial Reduction of Hexavalent Chromium as a Mechanism of Detoxification and Possible Bioremediation Applications. In: Chamy R, Rosenkranz F eds. Biodegradation - Life of Science. InTech. DOI: 10.5772/56365.

Frangeul L, Quillardet P, Castets A-M, Humbert J-F, Mattheis HC, Cortez D, Tolonen A, Zhang C-C, Gribaldo S, Kehr J-C, Zilliges Y, Ziemert N, Becker S, Talla E, Latifi A, Billault A, Lepelletier A, Dittmann E, Bouchier C, de Marsac NT 2008. Highly plastic genome of Microcystis aeruginosa PCC 7806, a ubiquitous toxic freshwater cyanobacterium. BMC Genomics 9:274. DOI: 10.1186/1471-2164-9-274.

Ghosh A, Singh A, Ramteke PW, Singh VP 2000. Characterization of Large Plasmids Encoding Resistance to Toxic Heavy Metals in Salmonella abortus equi. Biochemical and Biophysical Research Communications 272:6–11. DOI: 10.1006/bbrc.2000.2727.

Giudice Lo A, Casella P, Bruni V, Michaud L 2013. Response of bacterial isolates from Antarctic shallow sediments towards heavy metals, antibiotics and polychlorinated biphenyls. Ecotoxicology 22:240–250. DOI: 10.1007/S10646-012-1020-2.
Gneiding K, Frodl R, Funke G 2008. Identities of Microbacterium spp. encountered in human clinical specimens. *Journal of Clinical Microbiology* 46:3646–3652. DOI: 10.1128/JCM.01202-08.

Gonzalez CF, Ackerley DF, Lynch SV, Matin A 2005. ChrR, a soluble quinone reductase of *Pseudomonas putida* that defends against H2O2. *Journal of Biological Chemistry* 280:22590–22595. DOI: 10.1074/jbc.M501654200.

Gurevich A, Saveliev V, Vyahhi N, Tesler G 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075.

Henne KL, Nakatsu CH, Thompson DK, Konopka AE 2009a. High-level chromate resistance in *Arthrobacter* sp. strain FB24 requires previously uncharacterized accessory genes. *BMC Microbiology* 9:199. DOI: 10.1186/1471-2180-9-199.

Henne KL, Turse JE, Nicora CD, Lipton MS, Tollaksen SL, Lindberg C, Babnigg G, Giometti CS, Nakatsu CH, Thompson DK, Konopka AE 2009b. Global Proteomic Analysis of the Chromate Response in *Arthrobacter* sp. Strain FB24. *Journal of Proteome Research* 8:1704–1716. DOI: 10.1021/pr800705f.

Henriques I, Tacão M, Leite L, Fidalgo C, Araújo S, Oliveira C, Alves A 2016. Co-selection of antibiotic and metal(loid) resistance in gram-negative epiphytic bacteria from contaminated salt marshes. *Marine pollution bulletin* 109:427–434. DOI: 10.1016/j.marpolbul.2016.05.031.

Henson MW, Santo Domingo JW, Kourtev PS, Jensen RV, Dunn JA, Learman DR 2015. Metabolic and genomic analysis elucidates strain-level variation in *Microbacterium* spp. isolated from chromate contaminated sediment. *PeerJ* 3:e1395. DOI: 10.7717/peerj.1395.
469 Hu P, Brodie EL, Suzuki Y, McAdams HH, Andersen GL 2005. Whole-Genome Transcriptional Analysis of Heavy Metal Stresses in Caulobacter crescentus. J Bacteriol 187:8437–8449. DOI: 10.1128/JB.187.24.8437-8449.2005.

471 Humbert J-F, Barbe V, Latifi A, Gugger M, Calteau A, Coursin T, Lajus A, Castelli V, Oztas S, Samson G, Longin C, Medigue C, de Marsac NT 2013. A Tribute to Disorder in the Genome of the Bloom-Forming Freshwater Cyanobacterium Microcystis aeruginosa. Plos One 8:e70747. DOI: 10.1371/journal.pone.0070747.

476 Humphries AC, Nott KP, Hall LD, Macaskie LE 2005. Reduction of Cr(VI) by immobilized cells of Desulfovibrio vulgaris NCIMB 8303 and Microbacterium sp. NCIMB 13776. Biotechnology and Bioengineering 90:589–596. DOI: 10.1002/bit.20450.

479 Hunt DE, David LA, Gevers D, Preheim SP, Alm EJ, Polz MF 2008. Resource partitioning and sympatric differentiation among closely related bacterioplankton. Science 320:1081–1085. DOI: 10.1126/Science.1157890.

482 Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC bioinformatics 11:119. DOI: 10.1186/1471-2105-11-119.

485 Joynt J, Bischoff M, Turco R, Konopka A, Nakatsu CH 2006. Microbial community analysis of soils contaminated with lead, chromium and petroleum hydrocarbons. Microbial Ecology 51:209–219. DOI: 10.1007/s00248-005-0205-0.

488 Kettler GC, Martiny AC, Huang K, Zucker J, Coleman ML, Rodrigue S, Chen F, Lapidus A, Ferreira S, Johnson J, Steglich C, Church GM, Richardson P, Chisholm SW 2007. Patterns and Implications of Gene Gain and Loss in the Evolution of Prochlorococcus. PLOS Genetics 3:e231. DOI: 10.1371/journal.pgen.0030231.
Kourtev PS, Nakatsu CH, Konopka A 2006. Responses of the anaerobic bacterial community to addition of organic C in chromium(VI) - and iron(III)-amended microcosms. Applied and Environmental Microbiology 72:628–637. DOI: 10.1128/Aem.72.1.628-637.2006.

Kourtev PS, Nakatsu CH, Konopka A 2009. Inhibition of nitrate reduction by chromium(VI) in anaerobic soil microcosms. Applied and Environmental Microbiology.

Kumar S, Stecher G, Tamura K 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular biology and evolution 33:1870–1874. DOI: 10.1093/molbev/msw054.

Laffineur K, Avesani V, Cornu G, Charlier J, Janssens M, Wauters G, Delmée M 2003. Bacteremia due to a novel Microbacterium species in a patient with leukemia and description of Microbacterium paraoxydans sp. nov. Journal of Clinical Microbiology 41:2242–2246. DOI: 10.1128/JCM.41.5.2242-2246.2003.

Li DH, Luo RB, Liu CM, Leung CM, Ting HF, Sadakane K, Yamashita H, Lam TW 2016. MEGAHIT v1.0: A fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102:3–11.

Liu ZM, Wu Y, Lei CF, Liu PM, Gao MY 2012. Chromate reduction by a chromate-resistant bacterium, Microbacterium sp. World Journal of Microbiology & Biotechnology 28:1585–1592. DOI: 10.1007/s11274-011-0962-5.

Manara A, DalCorso G, Bialiardini C, Farinati S, Cecconi D, Furini A 2012. Pseudomonas putida Response to Cadmium: Changes in Membrane and Cytosolic Proteomes. Journal of Proteome Research 11:4169–4179. DOI: 10.1021/pr300281f.

Markowitz VM, Chen IMA, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang JH, Williams P, Huntemann M, Anderson I, Mavromatis K, Ivanova NN, Kyrpides NC
2012. IMG: the integrated microbial genomes database and comparative analysis system. *Nucleic acids research* 40:D115–D122. DOI: 10.1093/Nar/Gkr1044.

Martiny AC, Coleman ML, Chisholm SW 2006. Phosphate acquisition genes in *Prochlorococcus* ecotypes: Evidence for genome-wide adaptation. *Proceedings of the National Academy of Sciences of the United States of America* 103:12552–12557. DOI: 10.1073/pnas.0601301103.

McInerney JO, McNally A, O’Connell MJ 2017. Why prokaryotes have pangenomes. *Nature Microbiology* 2:17040. DOI: 10.1038/nmicrobiol.2017.40.

Meyer JL, Huber JA 2014. Strain-level genomic variation in natural populations of *Lebetimonas* from an erupting deep-sea volcano. *The ISME journal* 8:867–880. DOI: 10.1038/ismej.2013.206.

Meyer KA, Davis TW, Watson SB, Denef VJ, Berry MA, Dick GJ 2017. Genome sequences of lower Great Lakes *Microcystis* sp. reveal strain-specific genes that are present and expressed in western Lake Erie blooms. *Plos One* 12:e0183859. DOI: 10.1371/journal.pone.0183859.

Miranda AT, González MV, González G, Vargas E, Campos-Garcia J, Cervantes C 2005. Involvement of DNA helicases in chromate resistance by *Pseudomonas aeruginosa* PAO1. *Mutation research* 578:202–209. DOI: 10.1016/j.mrfmmm.2005.05.018.

Nakatsu CH, Carmosini N, Baldwin B, Beasley F, Kourtev P, Konopka A 2005. Soil microbial community responses to additions of organic carbon substrates and heavy metals (Pb and Cr). *Applied and Environmental Microbiology* 71:7679–7689. DOI: 10.1128/Aem.71.12.7679-7689.2005.

Nedelkova M, Merroun ML, Rossberg A, Hennig C, Pobell SS 2007. *Microbacterium* isolates from the vicinity of a radioactive waste depository and their interactions with uranium. *Fems Microbiology Ecology* 59:694–705. DOI: 10.1111/j.1574-6941.2006.00261.x.
Nies A, Nies DH, Silver S 1990. Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from *Alcaligenes eutrophus*. *Journal of Biological Chemistry* 265:5648–5653.

Nies DH 1995. The cobalt, zinc, and cadmium efflux system CzcABC from *Alcaligenes eutrophus* functions as a cation-proton antiporter in *Escherichia coli*. *J Bacteriol* 177:2707–2712. DOI: 10.1128/jb.177.10.2707-2712.1995.

Nies DH 2003. Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiology Reviews* 27:313–339. DOI: 10.1016/S0168-6445(03)00048-2.

Niewerth H, Schuldes J, Parschat K, Kiefer P, Vorholt JA, Daniel R, Fetzner S 2012. Complete genome sequence and metabolic potential of the quinaldine-degrading bacterium *Arthrobacter* sp. Rue61a. *BMC Genomics* 13. DOI: 10.1186/1471-2164-13-534.

Opperman DJ, Piater LA, van Heerden E 2008. A novel chromate reductase from *Thermus scotoductus* SA-01 related to old yellow enzyme. *J Bacteriol* 190:3076–3082. DOI: 10.1128/Jb.01766-07.

Ozaktas T, Taskin B, Gozen AG 2012. High level multiple antibiotic resistance among fish surface associated bacterial populations in non-aquaculture freshwater environment. *Water Research* 46:6382–6390. DOI: 10.1016/j.watres.2012.09.010.

Oze C, Fendorf S, Bird DK, Coleman RG 2004. Chromium geochemistry in serpentinized ultramafic rocks and serpentine soils from the Franciscan Complex of California. *American Journal of Science* 304:67–101.

Park CH, Keyhan M, Wielinga B, Fendorf S, Matin A 2000. Purification to Homogeneity and Characterization of a Novel *Pseudomonas putida* Chromate Reductase. *Applied and Environmental Microbiology* 66:1788–1795. DOI: 10.1128/aem.66.5.1788-1795.2000.
Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. DOI: 10.1101/gr.186072.114.

Pattanapipitpaisal P, Brown NL, Macaskie LE 2001. Chromate reduction by *Microbacterium liquefaciens* immobilised in polyvinyl alcohol. *Biotechnology Letters* 23:61–65. DOI: 10.1023/A:1026750810580.

Qamar N, Rehman Y, Hasnain S 2017. Arsenic-resistant and plant growth-promoting Firmicutes and γ-Proteobacteria species from industrially polluted irrigation water and corresponding cropland. *Journal of Applied Microbiology* 123:748–758. DOI: 10.1111/jam.13535.

Ramirez-Diaz MI, Diaz-Perez C, Vargas E, Riveros-Rosas H, Campos-Garcia J, Cervantes C 2008. Mechanisms of bacterial resistance to chromium compounds. *Biometals* 21:321–332. DOI: 10.1007/s10534-007-9121-8.

Rocap G, Larimer FW, Lamerdin J, Malfatti S, Chain P, Ahlgren NA, Arellano A, Coleman M, Hauser L, Hess WR, Johnson ZI, Land M, Lindell D, Post AF, Regala W, Shah M, Shaw SL, Steglich C, Sullivan MB, Ting CS, Tolonen A, Webb EA, Zinser ER, Chisholm SW 2003. Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature* 424:1042–1047. DOI: 10.1038/Nature01947.

Schwarz S, Kehrenberg C, Doublet B, Cloeckaert A 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews* 28:519–542. DOI: 10.1016/j.femsre.2004.04.001.

Seiler C, Berendonk TU 2012. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Frontiers in Microbiology* 3:399. DOI: 10.3389/fmicb.2012.00399.
Shafique M, Jawaid A, Rehman Y 2016. As(V) Reduction, As(III) Oxidation, and Cr(VI) Reduction by Multi-metal-resistant Bacillus subtilis, Bacillus safensis, and Bacillus cereus Species Isolated from Wastewater Treatment Plant. Geomicrobiology Journal 34:687–694. DOI: 10.1080/01490451.2016.1240265.

Shafique M, Jawaid A, Rehman Y 2017. Redox biotransformation of arsenic along with plant growth promotion by multi-metal resistance Pseudomonas sp. MX6. Comptes Rendus Biologies 340:330–338. DOI: 10.1016/j.crvi.2017.05.002.

Shammi T, Ahmed S 2016. Heavy Metal Tolerance and Antibiotic Resistance of Bacillus spp. Isolated from Two Major Rivers in Bangladesh. Bangladesh Journal of Microbiology 30:17–22. DOI: 10.3329/bjm.v30i1-2.28448.

Silver S 1996. Bacterial resistances to toxic metal ions - A review. Gene 179:9–19. DOI: 10.1016/S0378-1119(96)00323-X.

Simmons SL, DiBartolo G, Denef VJ, Goltsman DSA, Thelen MP, Banfield JF 2008. Population Genomic Analysis of Strain Variation in Leptospirillum Group II Bacteria Involved in Acid Mine Drainage Formation. PLoS Biol 6:e177. DOI: 10.1371/journal.pbio.0060177.

Soni SK, Singh R, Awasthi A, Kalra A 2014. A Cr(VI)-reducing Microbacterium sp. strain SUCR140 enhances growth and yield of Zea mays in Cr(VI) amended soil through reduced chromium toxicity and improves colonization of arbuscular mycorrhizal fungi. Environmental Science and Pollution Research 21:1971–1979. DOI: 10.1007/s11356-013-2098-7.

Tamura K, Nei M 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular biology and evolution 10:512–526. DOI: 10.1093/oxfordjournals.molbev.a040023.
Tauch A, Zheng Z, Pühler A, Kalinowski J 1998. *Corynebacterium* striatum Chloramphenicol Resistance Transposon Tn5564: Genetic Organization and Transposition in *Corynebacterium glutamicum*. *Plasmid* 40:126–139. DOI: 10.1006/plas.1998.1362.

Ug A, Ceylan Ö 2003. Occurrence of Resistance to Antibiotics, Metals, and Plasmids in Clinical Strains of Staphylococcus spp. *Archives of Medical Research* 34:130–136. DOI: 10.1016/S0188-4409(03)00006-7.

Urone PF 1955. Stability of colorimetric reagent for chromium, s-Diphenylcarbazide, in various solvents. *Division of Industrial Hygiene* 27.

Viti C, Marchi E, Decorosi F, Giovannetti L 2013. Molecular mechanisms of Cr(VI) resistance in bacteria and fungi. *FEMS Microbiology Reviews* 38:633–659. DOI: 10.1111/1574-6976.12051.

Vos M, Velicer GJ 2006. Genetic population structure of the soil bacterium *Myxococcus xanthus* at the centimeter scale. *Applied and Environmental Microbiology* 72:3615–3625. DOI: 10.1128/AEM.72.5.3615-3625.2006.

Wang H, Xiang T, Wang Y, Song J, Zhai Y, Chen X, Li Y, Zhao B, Bin Zhao, Ruan Z 2014. *Microbacterium petrolearium* sp. nov., isolated from an oil-contaminated water sample. *International Journal of Systematic and Evolutionary Microbiology* 64:4168–4172. DOI: 10.1099/ijs.0.061119-0.

Welch RA, Burland V, Plunkett G, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, Stroud D, Mayhew GF, Rose DJ, Zhou S, Schwartz DC, Perna NT, Mobley HLT, Donnenberg MS, Blattner FR 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* 99:17020–17024. DOI: 10.1073/pnas.252529799.
Wright MS, Peltier GL, Stepanauskas R, McArthur JV 2006. Bacterial tolerances to metals and antibiotics in metal-contaminated and reference streams. *Fems Microbiology Ecology* 58:293–302. DOI: 10.1111/j.1574-6941.2006.00154.x.

Zhang H, Ma Y, Liu P, Li X 2016. Multidrug resistance operon *emrAB* contributes for chromate and ampicillin co-resistance in a *Staphylococcus* strain isolated from refinery polluted river bank. *SpringerPlus* 5:1648. DOI: 10.1186/s40064-016-3253-7.

**Figure Legends**

Figure 1. Pangenomic analysis of heavy metal tolerant *Microbacterium* spp. The figure was made in An’vio with the items order in presence/absence (D: Euclidean; L: Ward) and the samples ordered by PC frequency. The outer most complete ring (labeled Num cont.) represents the number of genomes that a gene occurs in, with the core genome highlighted with an additional black bar. The internal rings represent an isolate’s genome with the black color representing the presence of genes in a genome (gray is the absence of a gene). The bar charts shows quality control measurements (total genome length [base pairs], GC content, and percent completion) and also the abundance of genes that were annotated as Co, Zn, Cd efflux system genes and antibiotic tolerance genes (beta lactam, vancomycin and chloramphenicol resistance).

Figure 2. COG distribution of genes found in the core and flexible genomes of 20 *Microbacterium* isolates isolated from heavy metal contaminated soil.

Figure S1. Molecular Phylogenetic analysis by Maximum Likelihood method. The tree with the highest log likelihood is shown. Initial tree(s) for the heuristic search were obtained automatically.
by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using
the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with
superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the
number of substitutions per site. All positions containing gaps and missing data were eliminated.
There were a total of 1181 positions in the final dataset.
**Table 1** (on next page)

Heavy metal tolerance and chromate reduction.

Heavy metal (cadmium, chromium, cobalt, copper, nickel, and zinc) tolerance (in mM) of each isolate and chromate reduction (%) data.
## Table 1. Heavy metal tolerance and chromate reduction

| Isolate               | Cr  | Ni  | Co  | Cd  | Zn  | Cu  | Cr reduction (%)* |
|-----------------------|-----|-----|-----|-----|-----|-----|-------------------|
| Microbacterium sp. A20| 20.0| 1.0 | 0.1 | 0.1 | 1.0 | 1.0 | 41.6              |
| Microbacterium sp. K19| 2.0 | 1.0 | 0.1 | 0.1 | 1.0 | 1.0 | 34.0              |
| Microbacterium sp. K21| 2.0 | 1.0 | 0.1 | 0.1 | 1.0 | 1.0 | 39.3              |
| Microbacterium sp. K22| 2.0 | 1.0 | 0.1 | 0.1 | 1.0 | 1.0 | 42.9              |
| Microbacterium sp. K24| 20.0| 1.0 | 1.0 | 0.0 | 1.0 | 1.0 | 41.6              |
| Microbacterium sp. K27| 2.0 | 1.0 | 1.0 | 0.1 | 1.0 | 1.0 | 83.0              |
| Microbacterium sp. K2B2| 0.5 | 0.1 | 0.0 | 0.1 | 0.1 | 0.1 | 36.0              |
| Microbacterium sp. K30| 20.0| 1.0 | 1.0 | 0.1 | 1.0 | 1.0 | 41.6              |
| Microbacterium sp. K31| 2.0 | 0.1 | 0.1 | 0.1 | 1.0 | 1.0 | 55.3              |
| Microbacterium sp. K33| 20.0| 1.0 | 0.1 | 0.1 | 1.0 | 1.0 | 88.8              |
| Microbacterium sp. K35| 2.0 | 0.1 | 0.1 | 0.1 | 1.0 | 1.0 | 3.6               |
| Microbacterium sp. K36| 0.5 | 1.0 | 0.1 | 0.1 | 1.0 | 1.0 | 10.7              |
| Microbacterium sp. K40| 0.5 | 1.0 | 0.1 | 0.1 | 1.0 | 1.0 | 30.3              |
| Microbacterium sp. K41| 2.0 | 1.0 | 0.1 | 0.0 | 0.1 | 1.0 | 28.1              |
| Microbacterium sp. K5D| 2.0 | 1.0 | 0.1 | 0.0 | 0.1 | 1.0 | 0.0               |
| Microbacterium sp. PF3| 20.0| 1.0 | 0.1 | 0.0 | 1.0 | 1.0 | 50.6              |

*Reduction % after 72 hours with 1 mM Cr except for K19, 31, 27 which was 0.5mM. Data shown in percentages since two chromate concentrations were used.
Table 2 (on next page)

Assembly statistics and quality assurance data

Genome assembly statistics (total length, GC%, and number of contigs) and quality assurance data (completeness, contamination, and strain heterogeneity).
### Table 2. Assembly statistics and quality assurance data

| Isolate               | Total length (Mb) | GC (%) | Number of contigs | Largest contig (bp) | N50 (bp) | Est. Sequencing Coverage | Comp. (%) | Cont. (%) | Strain heterogeneity (%) |
|-----------------------|-------------------|--------|-------------------|---------------------|----------|--------------------------|-----------|-----------|--------------------------|
| Microbacterium sp. A20| 3.94              | 68.53  | 75                | 436954              | 166569   | 142                      | 99.06     | 0.54      | 0                        |
| Microbacterium sp. K19| 3.89              | 68.69  | 54                | 479964              | 187157   | 144                      | 99.44     | 1.01      | 20                       |
| Microbacterium sp. K21| 3.85              | 68.33  | 34                | 537739              | 330631   | 146                      | 99.06     | 0.18      | 0                        |
| Microbacterium sp. K22| 3.94              | 68.52  | 96                | 258133              | 83317    | 142                      | 99.06     | 0.71      | 33.33                    |
| Microbacterium sp. K24| 4.23              | 68.90  | 142               | 248507              | 56118    | 132                      | 99.35     | 3.93      | 9.09                     |
| Microbacterium sp. K27| 3.75              | 68.51  | 37                | 342064              | 187856   | 149                      | 98.66     | 0.18      | 0                        |
| Microbacterium sp. K2B2| 3.94              | 68.52  | 76                | 436909              | 120725   | 142                      | 99.06     | 0.54      | 0                        |
| Microbacterium sp. K30| 4.06              | 69.16  | 96                | 295140              | 88745    | 138                      | 98.39     | 2.61      | 0                        |
| Microbacterium sp. K31| 3.77              | 68.43  | 39                | 443452              | 183886   | 148                      | 98.66     | 0.18      | 0                        |
| Microbacterium sp. K33| 3.89              | 68.68  | 110               | 311089              | 66684    | 144                      | 99.44     | 1.49      | 0                        |
| Microbacterium sp. K35| 3.62              | 70.91  | 379               | 168068              | 17080    | 155                      | 96.70     | 1.82      | 12.5                     |
| Microbacterium sp. K36| 3.31              | 70.53  | 91                | 183861              | 73738    | 169                      | 98.10     | 0.60      | 0                        |
| Microbacterium sp. K40| 3.77              | 68.40  | 45                | 442332              | 195355   | 148                      | 99.06     | 0.09      | 0                        |
| Microbacterium sp. K41| 3.62              | 70.75  | 454               | 89969               | 15114    | 155                      | 97.62     | 1.37      | 0                        |
| Microbacterium sp. K5D| 3.80              | 68.35  | 49                | 442284              | 195368   | 147                      | 99.06     | 0.09      | 0                        |
| Microbacterium sp. PF5| 3.74              | 70.80  | 144               | 125065              | 47267    | 150                      | 97.23     | 1.82      | 0                        |
Table 3 (on next page)

Antibiotic Minimum Inhibitory concentrations

Minimum inhibitory concentrations (µg/ml) of ampicillin, chloramphenicol, and vancomycin for each isolate.
# Table 3. Minimum Inhibitory concentrations (µg ml⁻¹)

| Isolates               | Amp* | Cam* | Van* |
|------------------------|------|------|------|
| Microbacterium sp. A20 | 0.0  | 8.0  | 0.5  |
| Microbacterium sp. K19 | 3.0  | 12.0 | 3.0  |
| Microbacterium sp. K21 | 3.0  | 12.0 | 4.0  |
| Microbacterium sp. K22 | 1.5  | 1.5  | 1.0  |
| Microbacterium sp. K24 | 3.0  | 24.0 | 6.0  |
| Microbacterium sp. K27 | 0.0  | 12.0 | 4.0  |
| Microbacterium sp. K2B2| 0.0  | 0.0  | 4.0  |
| Microbacterium sp. K30 | 3.0  | 16.0 | 3.0  |
| Microbacterium sp. K31 | 2.0  | 8.0  | 4.0  |
| Microbacterium sp. K33 | 2.0  | 16.0 | 3.0  |
| Microbacterium sp. K35 | 2.0  | 24.0 | 1.5  |
| Microbacterium sp. K36 | 3.0  | 24.0 | 1.5  |
| Microbacterium sp. K40 | 3.0  | 12.0 | 3.0  |
| Microbacterium sp. K41 | 1.0  | 1.5  | 1.5  |
| Microbacterium sp. K5D | 1.0  | 24.0 | 4.0  |
| Microbacterium sp. PF3 | 16.0 | 6.0  | 0.1  |

*ampicillin (Amp), chloramphenicol (Cam), and vancomycin (Van)
Figure 1

Pangenomic analysis of heavy metal tolerant Microbacterium spp.

The figure was made in An’vio with the items order in presence/absence (D: Euclidean; L: Ward) and the samples ordered by PC frequency. The outer most complete ring (labeled Num cont.) represents the number of genomes that a gene occurs in, with the core genome highlighted with an additional black bar. The internal rings represent an isolate’s genome with the black color representing the presence of genes in a genome (gray is the absence of a gene). The bar charts shows quality control measurements (total genome length [base pairs], GC content, and percent completion) and also the abundance of genes that were annotated as Co, Zn, Cd efflux system genes and antibiotic tolerance genes (beta lactam, vancomycin and chloramphenicol resistance).
Manuscript to be reviewed
Figure 2

COGs of share genes

COG distribution of genes found in the core and flexible genomes of 20 Microbacterium isolates isolated from heavy metal contaminated soil.