Chapter 5

Evaluation of bacterial strains isolated from subsurface sediment samples at Rayka and Rampura for heavy metal tolerance and Cr(VI) removal ability

Karmanye Vadhikaraste, Ma phaleshou kada chana, Ma Karma Phala Hetur Bhurmatey Sangostva Akarmani

- Bhagavad Gita, Chapter 2, Verse 47

5.1 Introduction

River sediment is ecologically important and harbors a large pool of heavy metals such as Cu, Cr, Hg, Ni (Sin et al. 2001; Mora et al. 2019). These heavy metals find their presence into river sediment by natural processes such as weathering of minerals, erosion, diagenesis etc. and/or due to various anthropogenic activities during the transport and deposition of the sediment (Tchounwou et al. 2012; Sin et al. 2001). Land uses and management practices also lead to heavy metal contamination of river sediment (Kumar et al. 2017). A comprehensive understanding of metal tolerance capacity and metal removal ability of subsurface microbes needs special attention to prevent and counter the contamination of groundwater. However, most studies have focused on metal tolerance and metal removal ability of microbes isolated from surface soils or upper sediment systems (Bao et al. 2006; Nithya et al. 2011; Tamindžija et al. 2019).

Vertical sediment profiles spanning long term depositional periods can preserve a historical sequence of pollution and provide a record of the level of contamination over time (Liaghati et al. 2004). The evaluation of microbial metal tolerance ability of vertical sediment profiles gives information on the long-term ecotoxicological impact of pollutants. Additionally, investigation of physiological capacities such as metal tolerance and metal immobilization abilities of culturable microbes is also necessary to understand their functioning in the environment (Fry 2000). Many metal tolerant microbes can remove or immobilize toxic heavy metals from their surrounding environments through various mechanisms such as sorption, accumulation, transformation (oxidation-reduction process), and leaching (Gadd 2004, 2010). Metal-microbe interaction in soils or sediments is interesting since microbes also directly interact with soil particles (Chenu and Stotzky 2002) and these interactions can influence metal-microbe interactions. For instance, clay particles can provide a surface for the adhesion of bacterial cells (Ruan et al. 2020) and the clay-microbe interactions can have distinct effects on microbe-metal interactions (Wang et al. 2020). Besides, adherence to soil mineral components such as goethite has also been shown to influence bacterial metal reduction ability (Li et al. 2020). However, the metal tolerance ability of microbes in subsurface samples and its influence on metal removal is not well-studied. Especially in the case of subsurface sediments having multiple lithological horizons derived from different depositional sources and consisting of

distinct grain size fractions as well as distinct geological composition, it would be interesting to study the role of microbial flora with respect to their metal tolerance and metal removal ability.

Among all heavy metals, chromium (Cr) is the fifth most important element in economic importance (Katz and Salem 1994) and the 17th most abundant element in the earth's mantle (Pratush et al. 2018). Chromium can be readily transported through soils/sediments into groundwater (Puls et al. 1994). Moreover, among the two most stable oxidation states, i.e., Cr(III) and Cr(VI) the latter is highly mobile, toxic, and carcinogenic as compared with Cr(III) (Fendorf 1995; Pratush et al. 2018). Leaching of Cr(VI) into groundwater is influenced by various abiotic factors such as grain size of sediment materials, pH (Puls et al. 1994). Several studies reported elevated concentration of Cr(VI) in groundwater, higher than the World Health Organization's limit for drinking water (i.e., 50 μg Cr(VI) per liter) either due to natural factors (e.g., Cr(III) rich ultramafic and serpentinite derived soils/sediments released higher Cr(VI) into groundwater due to natural geochemical and oxidation processes) or due to anthropogenic activities (Robles-Camacho and Armientac 2000; Oze et al. 2007; Stanin 2005). Thus Cr(VI) removal capability of subsurface microbes is an important aspect that needs to be understood.

The tolerance of subsurface microbes towards various toxic chemicals such as heavy metals, polycyclic aromatic hydrocarbons is considered an indicator of pollution of soil and groundwater (Blakely et al. 2002; Hinojosa et al. 2004; Niemeyer et al. 2012). In this study, we determined the heavy metal tolerance capability of various bacterial strains obtained from ~28 m deep and ~25 m deep sediment cores recovered from Rayka (RYD) and Rampura (RMD) locations of the Mahi river basin. Rayka and Rampura cores resemble each other geologically with respect to their depositional source, age, and stratigraphic sequences (refer section 4.2.1; Fig. 4.2). However the RMD core is located near Mini River (which is heavily polluted with industrial effluents) at Nandesari Industrial Estate of Vadodara (Gujarat) while to the best of our knowledge Rayka core location is not disturbed by any anthropogenic activities (refer section 4.2.1; Fig. 4.1). Hence, this study was carried out in order to understand discrimination in metal tolerance ability of bacterial isolates obtained from both cores, role of Cr(VI) tolerant bacteria obtained from both cores for Cr(VI) removal in liquid media as well as in sediment fractions of different consistency.

5.2 Results

5.2.1 Taxonomic identity of bacterial strains obtained from RYD and RMD sediment profiles and their heavy metal tolerance capacity

A total of 59 bacterial strains were isolated based on the abundance of isolates [i.e., colony-forming unit (CFU) counts] and their morphological characteristics. Identification of obtained isolates by 16S rRNA gene sequence revealed that bacterial strains belonged to 27 different genera totally. Among these, members of 9 genera were commonly recovered from both the sediment profiles while 11 genera were unique to RYD and 7 genera to RMD sediment profile (Fig. 5.1a; Table 5.1, 5.2). Genus level identities (with >96% sequence similarity) along with the GenBank accession numbers of the 16S rRNA gene sequences of the abundant morphotypes of heterotrophic bacteria obtained from sediment samples of the RYD and RMD are shown in Table 5.1 and 5.2, respectively. Phylogenetic relatedness of heterotrophic isolates obtained from RYD and RMD sediment profiles is depicted in Fig. A-V and A-VI respectively (Appendix). Phylum level distribution of the bacterial isolates shown in Fig. 5.1b, reveals that the culturable bacterial communities of both sediment profiles were dominated by *Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes* (Fig. 5.1b; Table 5.1, 5.2). One bacterial isolate belonging to the *Deinococcus-Thermus* phylum was observed in the RMD sediment profile. Likewise, one bacterial isolate affiliated with phylum β-*Proteobacteria* was observed in the RYD sediment profile [Fig. 5.1; Table 5.1, 5.2; Fig. A-V, A-VI (Appendix)].

The metal tolerance ability of all the bacterial strains is shown in (Fig. 5.2). As seen, the bacteria exhibited certain heterogeneity in their susceptibility towards the five heavy metals tested (Fig. 5.2), irrespective of them belonging to the same genera (refer Table 5.1 and Table 5.2 for genus level identity). For instance, *Nocardioides* sp. RYD12.5E (RY-G), *Nocardioides* sp. RYD17.5E (RY-J), *Nocardioides* sp. RYD18.3C (RY-K), *Nocardioides* sp. RYD19.1A (RY-L), *Nocardioides* sp. RMD 11.1A (RM-D), *Nocardioides* sp. RMD 15.1A (RM-E) showed assorted tolerance pattern for five heavy metals. The maximum number of strains (25%) showed high tolerance (i.e., >100 ppm) towards Cr(VI) followed by 13% of total bacterial isolates which showed tolerance towards >50 ppm Cd(II) while ~12% of bacterial strains showed tolerance towards 200-300 ppm Ni(II). Four bacterial strains obtained from RYD showed the

Table 5.1: Genus level identification of bacterial isolates obtained from Rayka (RYD) sediment profile.

Isolate code	Depth (m)	Total count (CFU)*	CFU of identified isolate	Genus level identity of the bacterial isolate (GenBank accession number)	Phylum
RY-A	0 -0.2	3.18E+05	8.50E+04	*Sphingomonas* sp. RYD1.4D (MK077721)	α-Proteobacteria
RY-B[1]	1.4-1.6	2.18E+05	7.14E+04	*Microbacterium* sp. RYD3.1A (MK077722)	Actinobacteria
RY-B[2]			8.95E+03	*Arthrobacter* sp. RYD3.5E (MK077723)	Actinobacteria
RY-C	4.4-4.5	3.55E+05	4.23E+04	*Novosphingobium* sp. RYD7.4D (MK077724)	α-Proteobacteria
RY-D[1]	5-5.1	8.85E+05	3.11E+04	*Roseomonas* sp. RYD9.5E (MK077725)	α-Proteobacteria
RY-D[2]			2.31E+04	*Gordonia* sp. RYD9.6F (MK123357)	Actinobacteria
RY-D[3]			3.40E+03	*Lysobacter* sp. RYD9.8H (MK077726)	γ-Proteobacteria
RY-E[1]	5.3-5.5	8.40E+05	3.41E+05	*Microbacterium* sp. RYD10.1A (MK077727)	Actinobacteria
RY-E[2]			5.50E+04	*Rhodococcus* sp. RYD10.5E (MK123358)	Actinobacteria
RY-F	6.4-7	4.93E+05	2.13E+05	*Methylobacterium* sp. RYD11.2B (MK077728)	α-Proteobacteria
RY-G	7-7.6	9.03E+05	6.05E+04	*Nocardioides* sp. RYD12.5E (MK077729)	Actinobacteria
RY-H	9.5-10.3	2.12E+05	9.82E+03	*Brevundimonas* sp. RYD14.3C (MK077730)	α-Proteobacteria
RY-I[1]	10.8-10.9	5.39E+05	2.49E+05	*Bacillus* sp. RYD15.1A (MK077731)	Firmicutes
RY-I[2]			3.50E+04	*Oharaeibacter* sp. RYD15.3C (MK077732)	α-Proteobacteria
RY-J	11.3-11.4	5.07E+05	7.33E+03	*Nocardioides* sp. RYD17.5E (MK077733)	Actinobacteria
RY-K	12.3-12.5	1.00E+05	1.12E+04	*Nocardioides* sp. RYD18.3C (MK077734)	Actinobacteria
RY-L	12.7-13	1.01E+05	3.75E+04	*Nocardioides* sp. RYD19.1A (MK077735)	Actinobacteria
RY-M	13.3-13.5	5.20E+05	3.75E+05	*Herbaspirillum* sp. RYD20.1A (MK163506)	β-Proteobacteria
RY-N	13.6-13.7	2.44E+05	9.13E+03	*Paracoccus* sp. RYD21.3C (MK077736)	α-Proteobacteria
RY-O	14-14.2	1.26E+05	3.81E+04	*Bacillus* sp. RYD 23.2B (MK077737)	Firmicutes
RY-P	14.9-15.03	3.12E+05	2.41E+05	*Paenibacillus* sp. RYD25.1A (MK077738)	Firmicutes
RY-Q	16.8-17	1.05E+06	4.93E+05	*Pseudomonas* sp. RYD29.1A (MK077739)	γ-Proteobacteria
RY-R[1]	21.8-22	7.75E+04	4.20E+04	*Pedobacter* sp. RYD32.1A (MK077740)	Bacteroidetes
RY-R[2]			9.75E+03	*Gordonia* sp. RYD32.3C (MK163507)	Actinobacteria
RY-R[3]			7.30E+03	*Pseudomonas* sp. RYD32.5E (MK077741)	γ-Proteobacteria
RY-S[1]	22.3-22.5	6.54E+05	3.31E+05	*Rhodococcus* sp. RYD33.1A (MK077742)	Actinobacteria
RY-S[2]			1.56E+05	*Pseudomonas* sp. RYD33.2B (MK123359)	γ-Proteobacteria
RY-T	23-23.2	1.37E+05	4.32E+04	*Rhodococcus* sp. RYD34.2B (MK077743)	Actinobacteria
RY-U	23.7-24	3.87E+05	3.61E+05	*Brevundimonas* sp. RYD36.1A (MK077744)	α-Proteobacteria
RY-V	24-24.7	3.10E+05	2.90E+05	*Rhodococcus* sp. RYD37.1A (MK077745)	Actinobacteria
RY-W	25.8-26	4.58E+05	9.40E+04	*Rhodococcus* sp. RYD38.2B (MK077746)	Actinobacteria
RY-X[1]	27.7-28	4.67E+05	2.59E+05	*Pontibacter* sp. RYD40.1A (MK077747)	Bacteroidetes
RY-X[2]			1.70E+05	*Yonghaparkia* sp. RYD40.2B (MK077748)	Actinobacteria

*Colony-forming units (CFU) expressed as bacterial counts per g dry weight of sediment.

Table 5.2: Genus level identification of bacterial isolates obtained from Rampura (RMD) sediment profile.

Isolate code	Depth (m)	Total count (CFU)*	CFU of identified isolate	Genus level identity of the bacterial isolate (GenBank accession number)	Phylum
RM-A	0-0.6	3.00E+04	2.5E+04	*Micrococcus* sp. RMD 1.1A (MK027328)	Actinobacteria
RM-B[1]	1.4-1.7	3.27E+04	9.8E+03	*Sinorhizobium* sp. RMD 4.1A (MK027329)	α-proteobacteria
RM-B[2]			8.7E+03	*Bacillus* sp. RMD 4.3C (MK027330)	Firmicutes
RM-C[1]	3.4-3.6	1.28E+05	7.8E+04	*Saccharothrix* sp. RMD 8.1A (MK027331)	Actinobacteria
RM-C[2]			2.3E+04	*Pseudomonas* sp. RMD 8.2B (MK027332)	γ-Proteobacteria
RM-D	4.2-4.4	1.02E+05	7.50E+04	*Nocardioides* sp. RMD 11.1A (MK027333)	Actinobacteria
RM-E	6.4-6.6	5.37E+04	5.19E+04	*Nocardioides* sp. RMD 15.1A (MK027334)	Actinobacteria
RM-F	7.8-8	1.31E+05	7.79E+04	*Lysobacter* sp. RMD 18.1A (MK027335)	γ-Proteobacteria
RM-G	8.5-8.7	1.87E+05	1.58E+05	*Kocuria* sp. RMD 20.1A (MK027336)	Actinobacteria
RM-H	9.6-9.8	2.43E+05	1.05E+05	*Kocuria* sp. RMD 22.1A (MK027337)	Actinobacteria
RM-I	11.4-11.6	1.95E+04	9.50E+03	*Kocuria* sp. RMD 27.1A (MK027338)	Actinobacteria
RM-J[1]	13.4-14	1.93E+04	5.30E+03	*Gordonia* sp. RMD 30.2B (MK027339)	Actinobacteria
RM-J[2]			5.00E+02	*Microbacterium* sp. RMD 30.4D (MK027340)	Actinobacteria
RM-K	14-14.8	2.22E+04	1.37E+04	*Brevibacillus* sp. RMD 31.1A (MK027341)	Firmicutes
RM-L	16.7-17	1.89E+04	8.70E+03	*Microbacterium* sp. RMD 35.1A (MK027342)	Actinobacteria
RM-M	18-18.2	3.5E+04	2.01E+04	*Lysobacter* sp. RMD 38.1A (MK027343)	γ-Proteobacteria
RM-N	18.4-18.6	3.02E+04	9.20E+03	*Paracoccus* sp. RMD 40.1A (MK027344)	α-Proteobacteria
RM-O	18.8-19	1.54E+04	1.31E+04	*Streptomyces* sp. RMD42.2B (MK027345)	Actinobacteria
RM-P	19.4-19.6	1.14E+04	6.75E+03	*Pseudomonas* sp. RMD 43.1A (MK027346)	γ-Proteobacteria
RM-Q[1]	21.2-21.6	7.17E+04	3.80E+04	*Sphingomonas* sp. RMD 46.1A (MK027347)	α-Proteobacteria
RM-Q[2]			8.73E+03	*Microbacterium* sp. RMD 46.2B (MK027348)	Actinobacteria
RM-Q[3]			8.51E+03	*Brevibacillus* sp. RMD 46.4D (MK027349)	Firmicutes
RM-R	22.2-22.4	4.67E+04	4.70E+03	*Paracoccus* sp. RMD 48.3C (MK027350)	α-Proteobacteria
RM-S	23.4-23.7	1.28E+04	1.04E+04	*Deinococcus* sp. RMD 51.1A (MK027351)	Deinococcus-Thermus
RM-T	23.7-24	1.42E+04	1.01E+04	*Pontibacter* sp. RMD 52.1A (MK027352)	Bacteroidetes
RM-U	24.3-24.8	1.31E+04	1.03E+04	*Lysobacter* sp. RMD 53.1A (MK027353)	γ-Proteobacteria

*Colony-forming units (CFU) expressed as bacterial counts per g dry weight of sediment.

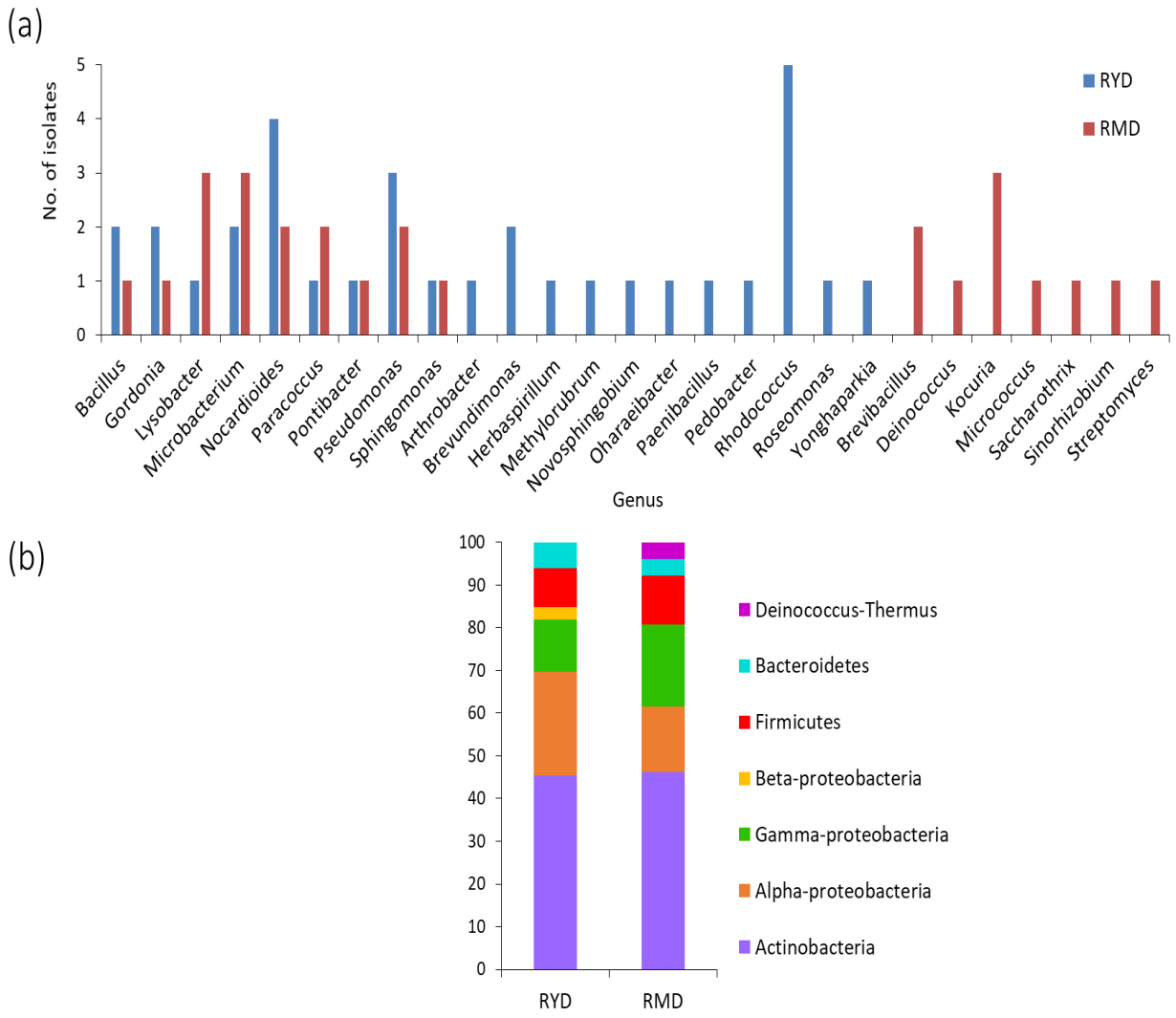

Figure 5.1: Genus level and phyla/class level distribution of bacterial strains obtained from Rayka (RYD) and Rampura (RMD). (a) The number of bacterial strains (isolates) belonging to a particular genus as identified by NCBI-BLASTn and Sequence Match tool of RDP (b) Stacked bar graph showing phyla/class level abundance of isolated bacterial strains.

highest tolerance i.e. 10-20 ppm for Hg(II) while within the RMD sediment profile the highest tolerance was observed between 5 and 10 ppm of Hg(II). Among all bacterial strains, only 5% of strains had the highest tolerance capacity of Cu(II) which is between 300 and 400 ppm (Fig. 5.2).

(a) **RYD**

Isolate code	Lowest metal concentration (ppm) at which growth inhibition was observed				
	Hg	Cd	Cr	Cu	Ni
RY-A	3	20	100	200	200
RY-B[1]	3	50	100	100	100
RY-B[2]	10	20	100	200	100
RY-C	10	20	100	100	100
RY-D[1]	20	50	100	100	100
RY-D[2]	5	70	100	300	300
RY-D[3]	20	50	100	400	100
RY-E[1]	3	50	100	100	100
RY-E[2]	10	100	200	200	200
RY-F	10	50	100	100	100
RY-G	3	20	100	300	200
RY-H	10	>100	100	100	200
RY-I[1]	5	50	>400	100	100
RY-I[2]	10	20	100	100	100
RY-J	3	20	100	200	200
RY-K	3	20	100	100	100
RY-L	3	50	100	200	100
RY-M	5	20	100	100	100
RY-N	5	20	100	100	100
RY-O	3	20	100	100	100
RY-P	3	20	100	100	100
RY-Q	10	50	200	200	100
RY-R[1]	3	20	100	100	200
RY-R[2]	20	20	400	200	200
RY-R[3]	3	50	100	300	100
RY-S[1]	3	50	200	200	300
RY-S[2]	3	20	100	100	100
RY-T	5	50	300	300	300
RY-U	3	100	100	200	100
RY-V	10	100	100	200	200
RY-W	20	20	100	200	100
RY-X[1]	5	20	100	100	100
RY-X[2]	10	100	100	100	300

(b) **RMD**

Lowest metal concentration (ppm) at which growth inhibition was observed					
Hg	Cd	Cr	Cu	Ni	Isolate code
3	50	100	300	100	RM-A
3	20	100	100	100	RM-B[1]
3	50	200	200	100	RM-B[2]
3	20	100	100	100	RM-C[1]
10	50	100	400	100	RM-C[2]
3	20	100	200	100	RM-D
5	20	100	100	100	RM-E
3	20	100	200	100	RM-F
3	50	100	200	200	RM-G
3	20	100	100	100	RM-H
3	20	100	200	100	RM-I
3	20	200	200	100	RM-J[1]
3	20	200	100	100	RM-J[2]
5	50	300	200	100	RM-K
3	20	100	200	100	RM-L
10	20	100	200	300	RM-M
5	20	200	100	100	RM-N
5	100	>400	400	100	RM-O
10	20	100	300	100	RM-P
10	50	200	300	300	RM-Q[1]
5	50	100	200	300	RM-Q[2]
3	20	200	100	200	RM-Q[3]
5	50	100	200	100	RM-R
3	>100	200	200	200	RM-S
3	20	100	100	100	RM-T
3	50	100	200	100	RM-U

Color Key

3	5	10	20	Hg(II)
20	50	70	100	>100 Cd(II)
100	200	300	400	>400 Cr(VI)
100	200	300	400	Cu(II)
100	200	300		Ni(II)

Figure 5.2: Heavy metal [Hg(II), Cd(II), Cr(VI), Cu(II), and Ni(II)] tolerance of bacterial strains obtained from RYD and RMD cores (a) Metal tolerance of bacterial strains obtained from sediment samples of RYD (b) Metal tolerance of bacterial strains obtained from sediment samples of RMD. Values in the figure represent the lowest concentration (in ppm) of metal at which growth inhibition was observed. The isolate code given in the figure represents specific bacterial isolate as described in Table 5.1 for RYD and Table 5.2 for RMD. A color key represents a shade of different colors corresponding to metal concentration (ppm).

5.2.2 MIC of Cr(VI) exhibited by selected bacterial strains obtained from RYD and RMD sediment profiles

The 15 strains that showed tolerance capacity >100 ppm for Cr(VI) included six bacterial strains that were obtained from the RYD core and nine bacterial strains that were obtained from the RMD core (Table 5.3). All 15 strains displayed a MIC of Cr(VI) within the range of <20 ppm to 100 ppm, indicating that tolerance obtained by the broth dilution method was lower than the tolerance obtained by the plate diffusion method. Among these, five bacterial strains that exhibited MIC >40 ppm were *Bacillus* sp. RYD 15.1A (RY-I[1]), *Gordonia* sp. RYD 32.3C (RY-R[2]), *Rhodococcus* sp. RYD 34.2B (RY-T), *Brevibacillus* sp. RMD 31.1A (RM-K) and *Streptomyces* sp. RMD 42.2B (RM-O) (Table 5.3). These five isolates were further used to determine their efficiency of chromate removal.

Table 5.3: MIC of bacterial strains obtained from RYD and RMD cores for Cr(VI). Bacterial strains showing MIC >40 ppm are represented in bold.

RYD		**RMD**	
Isolate code	**MIC (ppm)**	**Isolate code**	**MIC (ppm)**
RY-E[2]	20-40	RM-B[2]	<20
RY-I[1]	**80-100**	RM-J[1]	<20
RY-Q	20-40	RM-J[2]	<20
RY-R[2]	**60-80**	**RM-K**	**40-60**
RY-S[1]	20-40	RM-N	20-40
RY-T	**40-60**	**RM-O**	**80-100**
		RM-Q[1]	<20
		RM-Q[3]	<20
		RM-S	20-40

5.2.3 Removal of chromate in R2A and SE + peptone broth by selected bacterial strains obtained from RYD and RMD sediment profiles

Bacillus sp. RYD 15.1A showed nearly 54% survival at 20 ppm Cr (VI) while *Gordonia* sp. RYD 32.3C and *Rhodococcus* sp. RYD 34.2B showed 50% and 38% survival at 20 ppm Cr (VI) respectively in R2A broth (Fig. 5.3). At an initial concentration of 2 ppm Cr (VI), *Bacillus* sp. RYD 15.1A was able to remove 53%, *Gordonia* sp. RYD 32.3C showed 20% removal while *Rhodococcus* sp. RYD 34.2B was not able to remove Cr(VI) at all in R2A broth. *Brevibacillus* sp. RMD 31.1A was able to exhibit only 29% survival at 20 ppm and able to give around 40% Cr(VI) removal at 2 ppm and 12% Cr(VI) removal at 5 ppm, while *Streptomyces* sp. RMD

42.2B showed almost 94% survival up to 20 ppm and able to remove 15% Cr(VI) at 2 ppm in R2A broth (Fig. 5.3).

In the SE + peptone broth, *Bacillus* sp. RYD 15.1A showed 78% survival at 20 ppm Cr(VI) and was able to remove 100% chromate at 2 ppm Cr(VI) and different lower values for higher Cr(VI) concentrations (Fig. 5.3). *Gordonia* sp. RYD 32.3C showed 60% survival at 20 ppm and was able to remove chromate 35% only at 2 ppm. *Rhodococcus* RYD 34.2B exhibited 52% survival at 20 ppm but was not able to remove Cr(VI) even at 2 ppm initial concentration (Fig. 5.3). At 20 ppm Cr(VI), 60% and 96.5% survival of *Brevibacillus* sp. RMD 31.1A and *Streptomyces* sp. RMD 42.2B was observed in SE + peptone broth. *Brevibacillus* sp. RMD 31.1A exhibited 99.5%, 70.6%, 31% and *Streptomyces* sp. RMD 42.2B exhibited 100%, 98%, 90% chromate removal at 2, 5, 10 ppm Cr(VI) respectively in this medium (Fig. 5.3).

These findings suggested that all isolates showed significantly (paired t-test, $p<0.05$) high survival in SE + peptone broth as compared with R2A broth at higher Cr(VI) concentration, except *Streptomyces* sp. RMD 42.2B which grew equally efficiently in both media but exhibited a significant high Cr(VI) removal efficiency in SE + peptone broth as compared with R2A broth (paired t-test, $p<0.01$) (Fig. 5.3). Among all five isolates, *Bacillus* sp. RYD 15.1A exhibited higher Cr(VI) removal efficiency in R2A as well as in SE + peptone broth while *Streptomyces* sp. RMD 42.2B exhibited the highest Cr(VI) removal efficiency among all isolates in SE+ peptone broth (Fig. 5.3). Hence these two bacterial strains were used further for microcosm studies. Their morphologies are shown in Fig. 5.4.

5.2.4 Microcosm study to assess the influence of Bacillus sp. RYD 15.1A and Streptomyces sp. RMD 42.2B on chromate leaching from sediments of different consistency

Bacillus sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B was used to study their effect on the release of chromate in a microcosm study using packed-bed columns containing sediments of different consistency. Figure 5.5 shows the total amount of chromate leached out (i.e., the cumulative concentration of Cr(VI) that is observed in all fractions). It can be seen that the Cr(VI) leached out varied significantly ($p<0.05$) for each type of column material, the highest amount of (i.e., ~44 μg) of Cr(VI) was observed in the leachate of the control column of MS while lowest amount (i.e., ~21 μg) of Cr(VI) was observed in the leachate of SC control column (Fig. 5.5).

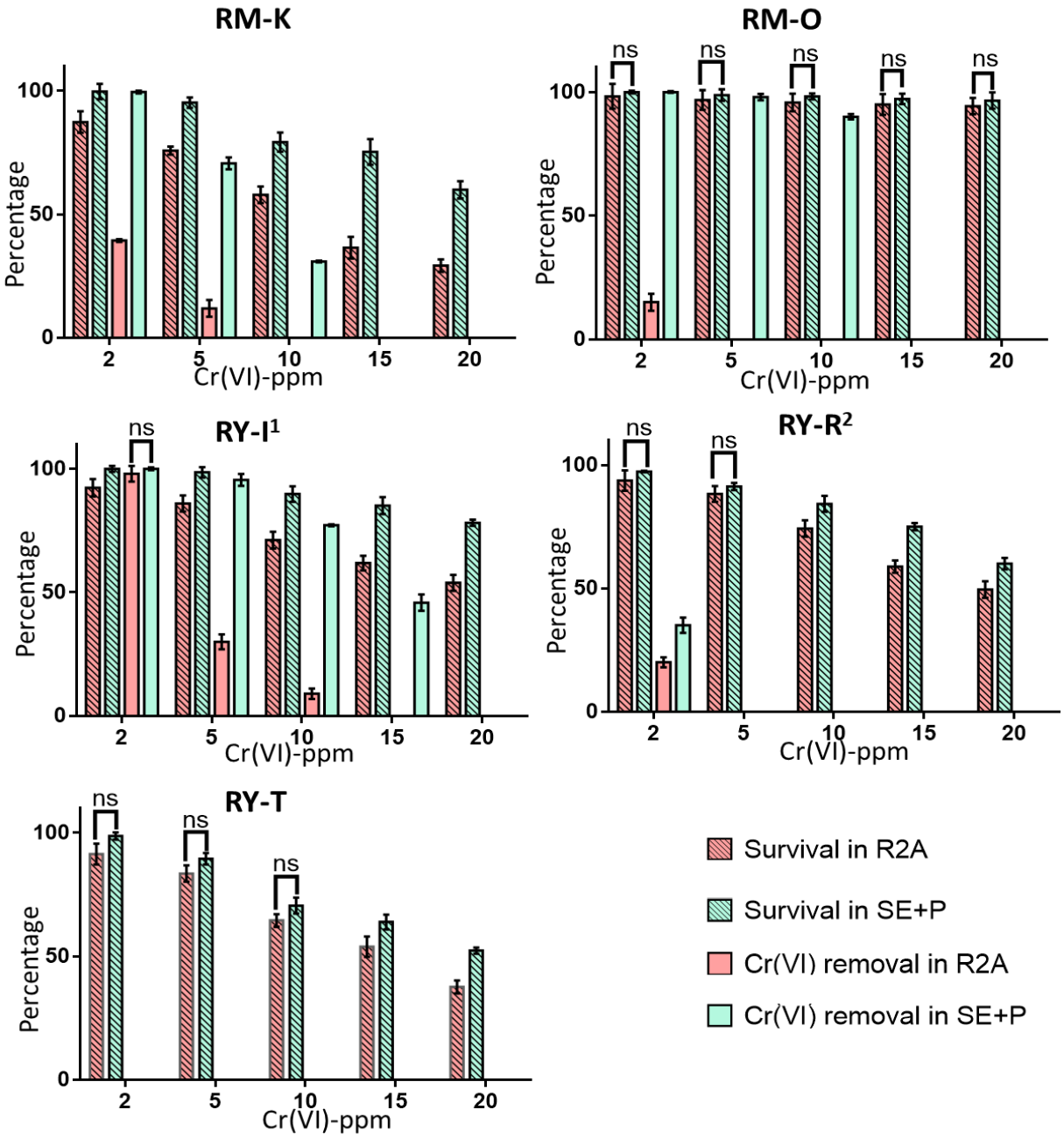

Figure 5.3: Percent survival and removal of chromate by selected bacterial strains obtained from RYD and RMD cores in R2A and SE + peptone broth amended with 2, 5, 10, 15, and 20 ppm Cr(VI). Percent survival and removal of Cr(VI) exhibited by isolates in R2A and SE + peptone broth differ significantly (Paired t-test, two-tailed, $p<0.05$) except those marked with ns (not significant). RY-I[1]: *Bacillus* sp. RYD 15.1A; RY-R[2]: *Gordonia* sp. RYD 32.3C; RY-T: *Rhodococcus* sp. RYD 34.2B; RM-K: *Brevibacillus* sp. RMD 31.1A; RM-O: *Streptomyces* sp. RMD 42.2B.

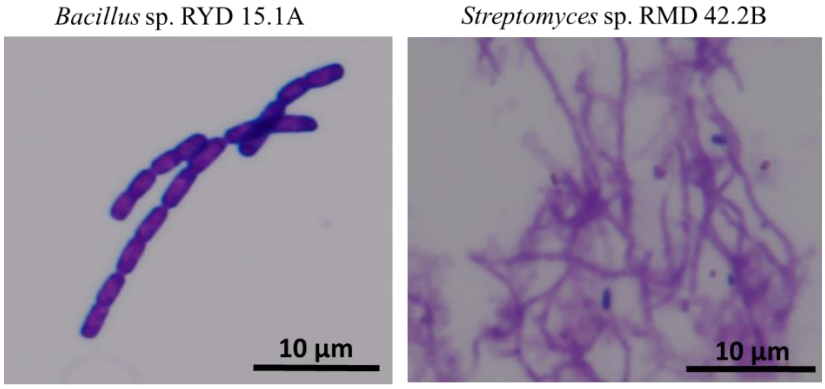

Figure 5.4: Morphology of *Bacillus* sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B.

When the bacterial strains were applied to the columns, the chromate level in the leachate was further reduced as compared to control columns without any microbe. In the case of *Bacillus* sp. RYD 15.1A, Cr (VI) in leachate was seen to be decreased by 86% in MS, by 89% in FS, and by 48% in SC with respect to the corresponding control columns and overall Cr(VI) was decreased by 89%, 94%, and 78% respectively as compared with the applied concentration of 5 ppm (Fig. 5.5). Likewise, in the leachate of experimental columns treated with *Streptomyces* sp. RMD 42.2B, amount of Cr(VI) decreased up to 92% in MS, 94% in FS, and 69% in SC with respect to the control columns and an overall decrease by 92% in MS, 96% in FS, and 87% in SC was observed as compared with the applied concentration of 5 ppm (Fig. 5.5). This suggests that both strains prevented chromate leaching efficiently from MS and FS columns as compared with the SC column (Fig. 5.5).

However, SC columns could retain a large fraction of Cr(VI) even in absence of the bacteria. The significant reduction ($p<0.05$) in the amount of Cr(VI) in the leachate of all experimental columns in comparison with their respective control columns indicated that both bacterial strains were capable of reducing the mobility of Cr(VI) from different types of sediment materials into the water percolating through the sediment bed (Fig. 5.5). The *Streptomyces* sp. RMD 42.2B was somewhat more efficient than the *Bacillus* sp. RYD 15.1A in retaining Cr(VI). There was no significant difference observed in the pH of the first fraction of the experimental column leachate with respect to that of the control column leachates. However, a significant ($p<0.05$) lower EC was observed in the first fraction of the experimental columns leachates with respect to the control columns leachates indicating changes in ion mobility and

reduction in heavy metal outflow due to *Bacillus* sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B treatment (Table 5.4).

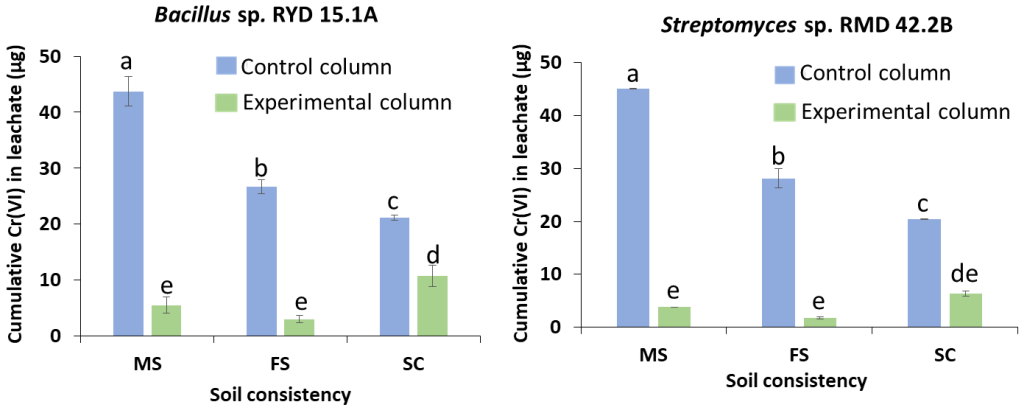

Figure 5.5: Effect of *Bacillus* sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B on chromate removal in the leachate of packed bed columns filled with the soil of different consistency. Cumulative amount of Cr(VI) (i.e. addition of Cr(VI) concentration obtained in each fraction) obtained within leachate of control and experimental columns treated with *Bacillus* sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B is shown here. A significant difference between the amount of Cr(VI) observed in the leachate of each column was determined using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test ($p<0.05$). Different letters indicate significant differences. MS: medium sand, FS: fine sand, SC: silt and clay.

Table 5.4: pH and EC of leachate fraction-1 obtained from control and experimental columns treated with *Bacillus* sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B.

Bacterial strains	Parameters	Column	Control column leachate F1	Experimental column leachate F1
RYD 15.1A	pH	MS	8.35(0.05)	8.25(0.07)
		FS	8.22 (0.11)	8.15(0.12)
		SC	8.24(0.08)	8.26(0.04)
	EC(µS)	MS	120.13 (5.53)	101.13 (3.67)*
		FS	110.83 (3.45)	79.76(4.06)**
		SC	198.1 (8.3)	171(2.58)*
RMD 42.2B	pH	MS	8.37(0.05)	8.23(0.08)*
		FS	8.28(0.04)	8.18(0.05)
		SC	8.02(0.17)	7.9(0.07)
	EC(µS)	MS	116.4(4.37)	90.41(3.24)**
		FS	95.31(4.39)	78.3(3.46)**
		SC	195.86(3.6)	160.23(5.98)***

* $P \leq 0.05$, >0.01; ** $P \leq 0.01$,>0.001; ***P<0.001 (paired t-test, 2-tailed). MS: medium sand, FS: fine sand, SC: silt and clay.

5.3 Discussion

Soil pollution by heavy metals has become of great concern worldwide which could arise due to geogenic and/or anthropogenic sources (Hernandez et al. 2003; Frohne et al. 2014; Liu et al. 2018). Heavy metal tolerance and metal removal ability of soil indigenous microbes provide insights into ecotoxicology and bioavailability of heavy metals and can pave the way for the application of soil microorganisms in bioremediation of soils/sediments (Giller et al. 2009; Beelen and Doelman 1997). Here, we investigated heavy metal tolerance for Cu(II), Ni(II), Cd(II), Cr(VI), and Hg(II) as well as Cr(VI) removal ability of bacterial isolates obtained from laterally extended, geologically resembled, Late Quaternary sediment sequences of Rayka (RYD) and Rampura (RMD). Bacterial isolates belong to phyla *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* were observed in both cores which have been among the most abundantly sampled organisms due to the ease of their cultivation (Hugenholtz 2002). Many of the bacteria recovered here are facultative anaerobic bacteria (e.g. *Bacillus* sp., *Lysobacter* sp., *Micrococcus* sp., *Nocardioides* sp., *Paracoccus* sp., *Pseudomonas* sp.) or spore forming bacteria (e.g. *Bacillus* sp., *Brevibacillus* sp., *Paenibacillus* sp., *Streptomyces* sp.) that might be present in dormant spore form in the deep core samples. Further, Escudero et al. (2018) reported that bacteria belonging to these phyla are most commonly detected within the continental subsurface environment. *Bacillus* sp., *Lysobacter* sp., *Microbacterium* sp., *Nocardioides* sp., *Paracoccus* sp., and *Pseudomonas* sp. were recovered from all three cores (including CRD core; refer section 3.3.5, Fig. 3.8) of the Mahi River basin. While, *Algoriphagus* sp., *Arenimonas* sp., *Dietzia* sp., *Lysinimonas* sp., *Pseudarthrobacter* sp., and *Rhizobium* sp. observed only within CRD core sediment samples and *Arthrobacter* sp. *Rhodococcus* sp., *Yonghaparkia* sp. were observed within CRD and RYD core samples but not within the RMD core samples (refer section 3.3.5, Fig. 3.8; Fig. 5.1). Bacterial isolates obtained from RYD and RMD cores showed tolerance between 3 to 20 ppm for Hg(II), between 20 to 100 ppm for Cd(II) (except one isolate), between 100 to 400 ppm for Cr(VI) (except two isolate), between 100 to 400 ppm for Cu(II), and between 100 to 300 ppm for Ni(II). This result demonstrated no remarkable discrimination in metal tolerance ability of bacteria isolates obtained from both RYD and RMD cores. The following order of metal tolerance was observed in terms of preponderance of tolerant isolates recovered: Hg(II)<Cd(II)< Ni(II)<Cu(II)=Cr(VI).

All bacterial strains (obtained from RYD and RMD core) that were selected for MIC determination by broth culture method for Cr(VI) exhibited lower MIC than tolerance value observed by the plate diffusion method e.g. *Bacillus* sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B exhibited MIC of Cr(VI) between 80 and 100 ppm which is a lower concentration than that obtained by the plate diffusion method (>400 ppm). The higher tolerance of Cr(VI) observed in the plate diffusion assays as compared with the broth dilution method might be due to diffusion limitation of the metal salt solution in agar media due to complexation with media components (Silva et al. 1996; Hassen et al. 1998). However, relative tolerance levels of different bacteria for all the metals tested remained the same regardless of the method of determination. A significant difference in Cr(VI) removal ability of bacterial strains in two different liquid media was also observed (Fig. 5.3). Likewise, Pal and Paul (2004) demonstrated the effect of growth medium and the presence of electron donors on Cr(VI) reduction carried out by the *B. sphaericus* isolate AND303 obtained from serpentine soil. Goyal et al. (2003) also demonstrated the effect of growth media composition on the biosorption of Cr(VI) carried out by *Saccharomyces cerevisiae.* Moreover, results of Cr (VI) removal ability in liquid media indicate that bacterial strains that have high Cr(VI) resistance/tolerance ability need not necessarily be proficient at Cr(VI) removal even at lower Cr(VI) concentration (Fig. 5.3) reinstating that bacterial Cr(VI) resistance/tolerance and Cr(VI) removal ability are not dependent on each other (Dhal et al. 2013; Narayani and Shetty 2013). *Bacillus* sp. RYD 15.1A showed 77% removal while the *Streptomyces* sp. RMD 42.2B exhibited 90% removal of Cr(VI) in SE + peptone broth at 10 ppm Cr(VI) indicating the potential Cr(VI) removal ability of these two strains in liquid media. In general, the mechanisms by which bacterial strains are known to tolerate high levels of Cr(VI) are efflux, biosorption, bioaccumulation, and/or enzymatic reduction to the less toxic Cr(III) form (Srinath et al. 2002; Thatoi et al. 2014). Among these resistance mechanisms, biosorption, and enzymatic reduction processes are also responsible for the removal of Cr(VI) from the growth medium (Thacker et al. 2006; Polti et al. 2007; Morales et al. 2007). In the present study, the mechanism of Cr(VI) tolerance has not been ascertained; however, the efficient Cr(VI) removal ability exhibited by the two strains indicates that the strains may carry out biosorption and/or enzymatic reduction.

A packed bed column study revealed that both bacterial strains were capable of reducing Cr(VI) flux into leachate from sediments of different consistency amended with 5 ppm Cr (VI)

(Fig. 5.5). Together with this, the packed bed column experiments also revealed a lower Cr(VI) leaching from finer particles which might be due to higher Cr(VI) absorbance or retention capacity of fine particles (Puls et al. 1994) because of high surface area or because of their surface charge and surface geochemistry (Fendorf 1995). A greater amount of Cr(VI) leaching in medium sand (MS) and fine sand (FS) as compared with silt and clay (SC) indicated an increased probability of percolation of Cr(VI) into groundwater by sandy material. Experimental columns treated with *Bacillus* sp. RYD 15.1A and *Streptomyces* sp. RMD 42.2B exhibited a 94% to 86% reduction in Cr (VI) leaching from 5 ppm Cr(VI) containing MS and FS columns. These findings emphasize that subsurface microbes have the potential of influencing the percolation of Cr(VI) from soils or sediments of different consistencies. On the whole, the present work indicates that at least some of the metal tolerant isolates found in the subsurface vadose zone have the potential role in preventing the leaching of the metal ions and thus reducing groundwater contamination.

5.4 Conclusion

Evaluation of metal tolerance and Cr(VI) removal capability of bacterial strains obtained from ~25 m (Rampura) and ~28 m (Rayka) deep subsurface sediment profiles of the Mahi river basin was carried out in this study. Bacterial strains affiliated with phyla *Actinobacteria*, *Proteobacteria, Firmicutes,* and *Bacteroidetes* were the predominantly recovered culturable members from both sediment profiles. Metal tolerance capacity of bacterial strains obtained from these sediment profiles revealed that subsurface microbes have the capability for tolerating heavy metals to varied extents. Chromate removal studies in liquid media and a packed bed column experiment revealed that bacterial strains obtained from the subsurface have the ability to remove Cr(VI) from liquid media as well as prevent its leaching through sediments of different consistency. Moreover, this finding indicates that subsurface microbes have the potential for Cr(VI) removal from soils/sediments comprising different textural characteristics.