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Screening and Evaluation of Multi-metal Tolerance of Chromate Resistant Marine Bacteria Isolated from Water and Sediment Samples of Paradip Port, Odisha Coast

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Abstract: Contamination with various toxic metal ions has been detected in sediment and water samples of Paradip Port environment caused by various metal handling operations. Presence of chromium in the sediment and water samples has led to isolate Cr(VI) resistant marine bacteria from these environments. In total 44 chromate resistant bacteria were isolated from surface water, bottom water and sediment samples using nutrient agar medium supplemented with 50 mg/l Cr(VI) which were screened for their tolerance to increasing Cr concentrations in nutrient agar medium. The screening test has resulted in selection of 11 bacterial isolates tolerating Cr(VI) concentrations as high as 1500 mg/l. These 11 strains were then subjected to purification by two subsequent quadrant streak in nutrient agar plates and evaluated for determination of their minimum inhibitory concentration (MIC) towards Cr(VI) in nutrient broth by broth dilution method with further increasing concentrations from 1500 to 2500 mg/l of Cr(VI). Based on MIC test, a bacterium, CrRPSD40 isolated from marine sediment was selected because of very high tolerance towards Cr(VI) with MIC value of 2100 mg/l. This strain was found to exhibit multiple metal tolerance capacity when screened for tolerance towards other 6 toxic heavy metals such as Cd(II), Cu(II), As(III), Zn, Pb(II) and Ni in nutrient broth medium. Based on morphological and biochemical characterisation the bacterium was identified as a *Brevibacillus* sp. Identification of *Brevibacillus* sp as a high chromium resistant bacteria with multi-metal tolerance ability could be a potential candidate for bioremediation of chromium along with other metals from saline industrial waste effluents.

Key words: Chromate resistance, marine bacteria, water and sediment, metal toxicity, bioremediation.

Introduction

Paradip Port is situated on the east coast of India along the Bay of Bengal, a deep water sea Port of Odisha that acts as a principal hub for national and international cargo exchange. The water and sediment of the semi-closed Port harbour are affected mainly by loading and unloading operations of large quantities of various materials including ores like iron, manganese, chromites and aluminium. It is, therefore, possible to get metal resistant marine bacteria from the water and sediment samples of Port harbour which could be the potential candidates for bioremediation of heavy metals from metal contaminated saline environments such as industrial effluents. The marine bacteria are as such potential because of their adaptation to most adverse marine conditions such as varying temperature, pH, salinity, currents, precipitation regimes and wind patterns. [9]. Organisms from saline habitat have been shown

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to be resistant to many toxic metals as they survive in highly stress conditions [11].

Among the various metal ores those are transported from Pradip Port, chromium is one of the important ores with high export demand. Chromite ores produced from chromite mines located in the Sukinda Valley of Odisha are transported to foreign countries through Paradip Port on regular basis. As a result, the Port environment is highly contaminated with chromite ores. Hence, the present study, is aimed to isolate novel Cr(VI) resistant marine bacteria from the Port harbour which is frequently contaminated by various toxic metals including chromium by ore handling activity. After isolation the bacteria were screened for their tolerance towards increasing concentrations of chromium as well as other heavy metals to select a high Cr(VI) tolerant bacterium with mutimetal tolerance potential. Further, the high Cr(VI) resistant and multimetal tolerant bacterium was characterised and identified as a potential candidate to be used for bioremediation of metal contaminated saline environments..

Materials and methods

Sampling location and sample collection

Water and Sediment samples were collected from Paradip Port harbour of Odisha coast during May 2014. Port harbour was frequently contaminated with different metal ore handling activity and the locations were selected in the basis of probability of finding more metal resistant marine bacteria. Inside Port harbour ten major sampling points such as, Approach channel (AC), Oil jetty (OJ), Iron ore handling berth (IOHB), Coal handling berth (CHB), North quay (NQ), General cargo berth (GCB), South quay (SQ), East quay (EQ), Fertiliser berth (FB) and Marine site (MS) were selected (Figure 1). Surface water, bottom water and sediment samples were collected from each sampling points inside harbour. Surface and bottom water (10 m depth) were collected by the help of a bucket and Niskin water sampler respectively. Sediment samples were collected by using a Van Veen Grab sampler.

Estimation of heavy metal and bacterial load in Port water and sediment samples

Concentration of various heavy metals like Cr, Cd, Pb, Zn, Ni, Cu, and Co present in collected surface water, bottom water and sediment sample were analysed. In case of water sample, sea water was extracted by APDC-MIBK method [4]. Sediment samples were dried and digested with acid mixture of concentrated HF, $HClO_4$ and HNO_3 and remaining digested residues were brought into solution by dissolving in HCl [16]. Heavy metals of extracted water and digested sediment samples

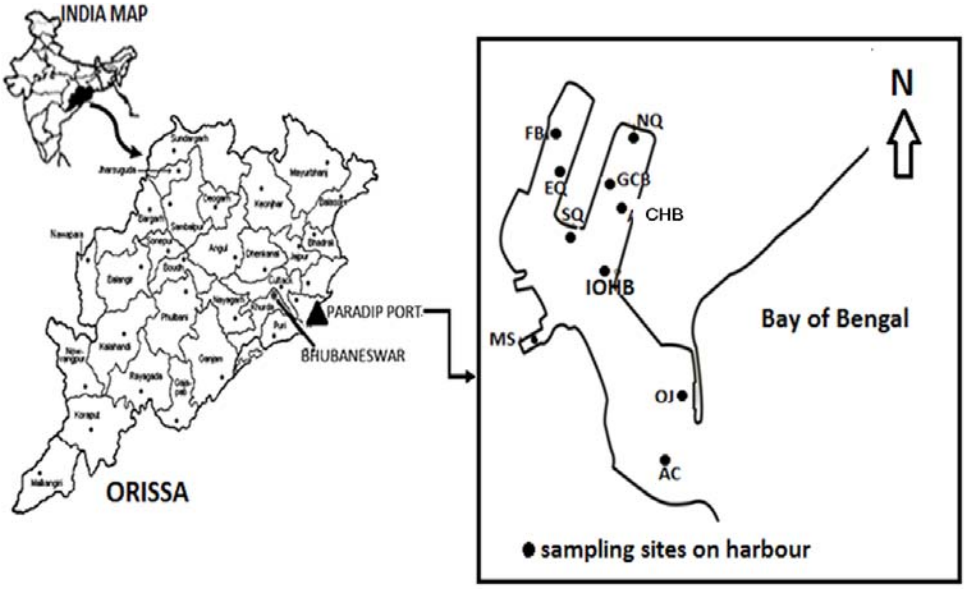

Figure 1. Ten sampling locations inside Paradip Port harbour

were analysed by atomic absorption spectrophotometer (AAS Shimadzu, AA7000).

For microbial analysis, water samples were collected in the pre-sterilized 100 ml glass bottles and brought under ice pack to laboratory. Bacterial load in water and sediment samples were estimated by using pour plate method with proper dilution [2]. Bacterial population such as total viable bacteria (TVB) and total coliform bacteria (TCB) were estimated by counting the colony forming unit (CFU) on the nutrient agar (NA) plate and Eosine-methylene blue (EMB) agar plate, respectively.

Isolation and Screening of Cr(VI) resistant bacteria

Isolation of Cr(VI) resistant bacteria were carried out in nutrient agar plate containing 50 mg/l Cr(VI). This procedure eliminates non-resistant bacteria's presence in the sample and allows the growth of only the Cr(VI) resistant bacteria. Sediment samples were diluted up to 10^{-3} times in sterile distilled water by serial dilution method before use. 100 µl from both water and diluted sediment samples were spread on nutrient agar plate supplemented with 50 mg/l Cr(VI) and incubated at 37°C for 24 hours. Isolated strains that were grown in the 50 mg/l Cr(VI) were screened against higher Cr(VI) concentration such as 200, 500, 700, 1000 and 1500 mg/l by subsequent streaking nutrient agar plate containing Cr(VI) from lower to higher concentration step by step.

Purification and MIC study of the selected Cr(VI) resistant bacteria

Strains those resistant and able to grow in 1500 mg/l Cr(VI) were subjected to purification by two subsequent quadrant streak in nutrient agar plate to obtained a single type of strain. After that minimum inhibitory concentration (MIC) of Cr(VI) was determined for the selected strains in nutrient broth by broth dilution method [13]. For that, test tubes having 10 ml of nutrient broth with increasing concentrations from 1500 to 2500 mg/l of Cr(VI) are prepared and inoculated by 0.1 ml fresh bacterial culture of each strain separately. The growth of the strains were confirmed by both naked eye observation and taking O.D. at 600 nm after 24 to 48 hrs of incubation.

Multi-metal resistant study

The strain showing maximum MIC value was subjected to multimetal resistant test for ascertaining whether the strain can show resistance to additional toxic metals other than Cr(VI). For this study, test tubes having 10 ml of nutrient broth with different heavy metals like Zn, As, Cu, Cd, Pb and Ni in varied concentration ranges from 10-1200 mg/l were prepared and inoculated with the strain. After 48 h incubation, the bacterial resistance in the form of its growth was measured by taking OD at 600 nm in a UV-Vis spectrophotometer.

Morphological and biochemical characterisation

The selected Cr(VI) resistant bacterial strain (CrRPSD40) was subjected to various morphological and biochemical characters by following Bergy's manual of systemic bacteriology [12]. Grahm's staining of the bacterium was performed and colony morphology like size, shape, surface, opacity, texture, elevation and pigmentation were recorded by visual observation under a light microscope. Biochemical characterisation, such as indole production, citrate utilisation, methyl red test, Voges Prokauer's test and production of enzymes by the strain such as oxidase, catalase, urease, alkaline phosphatase, casein hydrolase, gelatine hydrolase, amylase and lipase were carried out as per standard procedures [8,12]. Carbohydrate utilisation tests for 35 sugars were performed by using Himedia Carohydrate test kit KB009A, KB009B1 and KB009C.

Antibiotic resistance study was carried out for the selected Cr(VI) resistant strain against sixteen antibiotics on antibiotic assay agar medium (Himedia) by following disc diffusion antibiotic sensitivity test (Kirby-Bauer's disc diffusion method). In this method, the broth culture of the test organism was swabbed by sterile cotton swab on the antibiotic assay agar plate and then the antibiotic discs were placed aseptically on it. After 24 h incubation at 37°C, inhibition zone diameter was measured in millimetres. The test result was obtained based on the antimicrobial zone size

interpretative chart provided by Himedia Laboratories.

Results and discussion

Assessment of heavy metal load

Results of analysis of different heavy metals (Cr, Pb, Cd, Ni, Zn, Co and Cu) of water and sediment samples of Paradip Port harbour of Odisha were presented in Table 1. In surface water, the range and average concentrations (mg/l) were 0.003-0.011 (0.0059) for Cr, 0.004-0.011(0.007) for Pb, 0.0003-0.0006 (0.00046) for Cd, 0.001-0.002 (0.0012) for Ni, 0.013-0.032(0.0232) for Zn, 0.0003-0.0013 (0.00066) for Co, 0.002-0.007 (0.0047) for Cu. In case of bottom water, the range and average concentrations (mg/l) were 0.004-0.012 (0.0077) for Cr, 0.004-0.014(0.0083) for Pb, 0.0003-0.0007 (0.00052) for Cd, 0.001-0.003 (0.0014) for Ni, 0.010-0.056(0.0301) for Zn, 0.0004-0.0026 (0.00086) for Co, 0.003-0.009 (0.0063) for Cu. Metal contamination in both surface and bottom layer of the Port harbour water samples were within BIS and CCME [5,6] prescribed limits. In water samples, the average values of metal concentrations of ten locations was in the order of Zn>Pb>Cr>Cu>Ni>Co>Cd. In comparision to metal contamination in Port Elizabeth harbour water as reported by Fatoki and Mathabatha [10], the metal contamination in Paradip Port harbour water is very less and well within the standard limit.

In sediment samples, the range and average concentrations (mg/kg) of metal ions were 167.25-238.59 (197.77) for Cr, 90.34-126.29 (109.17) for Pb, 0.41-0.71(0.56) for Cd, 46.27-71.52 (57.42) for Ni, 47.56-84.27 (70.99) for Zn, 31.55-56.08 (42.89) for Co and 41.08-63.57 (50.61) for Cu. In comparison with interim marine sediment quality guideline (ISQG) and probable effect levels (PELs) of Canadian Council of Ministers of the Environment [7]; Cr, Cu and Pb values were exceeded the toxic limit. Average value of Cr in the Port sediment was also crossed the PELs limit. Metal concentration in Port harbour was found more in sediment than water due to deposition of metal ore dust through water column in the sediment. Among all the analysed metals Cr showed the highest contamination level as seen from the sequence (Cr>Pb>Zn>Ni>Cu>Co>Cd) which may be due to major handling of chromites ore from Paradip Port. The highest Cr concentration was observed at South quay (SQ) and the isolated Cr(VI) resistant strain CrRPSD40 from this point showed more tolerance capacity towards Cr(VI). Heavy metal contamination in marine and Port's surface sediments was studied by various authors. In the present study, the average value of metals like Cr, Ni, Co and Pb of Paradip Port sediment were found higher than the marine sediment of Bay of Bengal coast as reported earlier by Raj *et al.*[21]. Similarly, contamination level of Cr, Cu, Co, Ni and Pb values of Paradip Port sediment observed to be higher than the sediment of Aden Port, Yemen [19], suface sediment of Naples City Port [1] and Port Klang, Selanger, Malaysia [25]. Hence, from the present study it is concluded that sediment environment of the Paradip Port is more contaminated with various toxic metals than the above reported ports.

Enumeration of bacterial population

Enumeration of bacterial population in the form of total viable bacterial count and total coliform count was presented in the Table 2. From the table it was found that in suface water, total viable heterotrophic bacteria (TVB) (CFU/ml) were detected in the range and average of 60-550 (170). Total coliform bacteria (TCB) (CFU/100 ml) were present in the range and average of 400-5400 (1700). In case of bottom water, TVB were found in the range and average of 80-230 (128) CFU/ml. TCB were enumerated in the range and average of 500-3600(1530) CFU/100ml. Similarly, in sediment samples TVB and TCB (CFU/gm) were enumerated in the range and average of 135514-55608 (315375), and 16340-66850(39669) respectively. Overall, bacterial population was found more in the sampling locations like FB, IOHB, EQ and MS. These points are important due availability of more nutrients from fertilizer spillage from IFFCO fertilizer plant, cargo handling and human faecal contamination during shipping activity.

The number of viable and coliform bacteria counts detected in the water samples were matching with the data range of Paradip coastal region and Mahanadi transect with little variation as reported by Banoo *et al.* [3]. The main reason for in-

Table 1. Heavy metal content (concentration) of water and sediment samples of Paradip Port harbour, Odisha Coast.

Sampling station code	[Cr] Mgl^{-1} /*$mgkg^{-1}$			[Pb] Mgl^{-1} /*$mgkg^{-1}$			[Cd] Mgl^{-1} /*$mgkg^{-1}$			[Ni] Mgl^{-1} /*$mgkg^{-1}$			[Zn] Mgl^{-1} /*$mgkg^{-1}$			[Co] Mgl^{-1} /*$mgkg^{-1}$			[Cu] Mgl^{-1} /*$mgkg^{-1}$		
	SW	BW	SED*	SW	BW	SED*	SW	BW	SED*	SW	BW	SED*	SW	BW	SED*	SW	BW	SED*	SW	BW	SED*
AC	0.008	0.010	183.54	0.006	0.007	111.25	0.0005	0.0006	0.47	0.002	0.002	46.27	0.032	0.056	47.56	0.0008	0.0011	35.27	0.006	0.007	43.27
OJ	0.007	0.008	167.25	0.006	0.008	120.54	0.0005	0.0007	0.51	0.001	0.002	57.54	0.017	0.024	61.28	0.0007	0.0008	46.58	0.004	0.006	49.36
IOHB	0.007	0.010	203.57	0.011	0.014	105.93	0.0004	0.0004	0.43	0.002	0.003	71.52	0.026	0.030	76.26	0.0008	0.0010	41.36	0.007	0.009	58.62
CHB	0.006	0.009	198.37	0.009	0.010	124.37	0.0005	0.0007	0.58	0.001	0.001	56.24	0.026	0.040	81.67	0.0007	0.0007	51.62	0.006	0.009	63.57
NQ	0.003	0.006	211.63	0.004	0.006	109.27	0.0004	0.0006	0.61	0.001	0.001	68.24	0.014	0.021	64.37	0.0004	0.0005	56.08	0.007	0.008	41.08
GCB	0.003	0.004	235.57	0.006	0.007	117.63	0.0006	0.0004	0.66	0.001	0.001	61.57	0.026	0.037	71.38	0.0003	0.0005	49.37	0.002	0.003	53.66
SQ	0.004	0.006	238.59	0.004	0.007	98.53	0.0006	0.0004	0.71	0.001	0.001	46.34	0.026	0.031	70.39	0.0004	0.0004	31.55	0.003	0.005	46.75
EQ	0.004	0.006	191.32	0.006	0.004	87.63	0.0003	0.0005	0.53	0.001	0.001	49.62	0.021	0.026	72.48	0.0005	0.0005	30.11	0.006	0.007	48.64
FB	0.011	0.012	171.64	0.010	0.011	90.34	0.0004	0.0006	0.41	0.001	0.001	61.48	0.031	0.026	84.27	0.0013	0.0026	40.68	0.004	0.006	51.63
MS	0.006	0.006	176.25	0.008	0.009	126.29	0.0004	0.0003	0.66	0.001	0.001	55.37	0.013	0.010	80.31	0.0007	0.0005	46.27	0.002	0.003	49.57
Minimum	0.003	0.004	167.25	0.004	0.004	90.34	0.0003	0.0003	0.41	0.001	0.001	46.27	0.013	0.010	47.56	0.0003	0.0004	31.55	0.002	0.003	41.08
Maximum	0.011	0.012	238.59	0.011	0.014	126.29	0.0006	0.0007	0.71	0.002	0.003	71.52	0.032	0.056	84.27	0.0013	0.0026	56.08	0.007	0.009	63.57
Average	0.006	0.008	197.77	0.007	0.008	109.17	0.0005	0.0005	0.56	0.001	0.0014	57.42	0.023	0.030	70.99	0.0007	0.0009	42.89	0.005	0.006	50.61

SW-Surface water
BW-Bottom water
SED-Sediment

Table 2. Bacterial analysis of Port harbour water and sediment samples

Sampling station code	Total viable bacterial count			Total coliform bacteria count		
	SW CFU/ 1 ml	BW CFU/ 1 ml	SED CFU/ 1 gm	SW CFU/ 100 ml	BW CFU/ 100 ml	SED CFU/ 1 gm
AC	60	150	173010	600	500	16340
OJ	80	90	135514	700	800	26199
IOHB	70	230	558608	400	1400	66850
CHB	70	140	268398	600	1100	38961
NQ	100	80	378274	2000	1000	52376
GCB	70	110	384246	1400	1500	59558
SQ	230	80	368217	1800	2200	41667
EQ	340	160	325655	5400	2300	31771
FB	530	90	281642	1500	900	36699
MS	150	150	280186	2600	3600	26267
Minimum	60	80	135514	400	500	16340
Maximum	530	230	558608	5400	3600	66850
Average	170	128	315375	1700	1530	39669

SW-Surface water
BW-Bottom water
SED-Sediment
CFU- Colony forming unit

crease in bacterial population in Port harbour is due to human induced activities during Port's operation.

Bacterial isolation, screening and MIC test

In total 44 numbers of bacteria were isolated from the water and sediment samples using Nutrient agar supplemented with 50 mg/l Cr(VI). These include 13 bacteria (CrRPSW01-13) from surface water, 16 bacteria (CrRPSW14-29) from bottom water and 15 bacteria (CrRPSD30-44) from sediment samples (Table 3). After subsequent screening for higher Cr(VI) concentrations (i.e. 200, 500, 700, 1000, 1500 mg/l), only 11 bacteria such as CrRPSW08, CrRPSW11, CrRPBW14, CrRPBW18, CrRPBW21,

Table 3. Isolation and Screening of Cr(VI) resistant bacteria from Paradip Port harbour

Sample type	Location names	Location codes	Strain code	Cr(VI) mg/l					
				50	200	500	700	1000	1500
Surface water	Approach Channel	AC	CrR PSW01	++	++	+	-	-	-
	Oil Jetty	OJ	CrR PSW02	++	++	++	++	+	-
	Iron Ore Handling Berth	IOHB	CrR PSW03	++	+	-	-	-	-
	Coal Handling Berth	CHB	CrR PSW04	++	+	-	-	-	-
	North Quay	NQ	CrR PSW05	++	++	++	+	+	-
	North Quay	NQ	CrR PSW06	++	+	-	-	-	-
	General Cargo Berth	GCB	CrR PSW07	++	+	-	-	-	-
	South Quay	SQ	CrR PSW08	++	++	++	++	++	+
	East Quay	EQ	CrR PSW09	++	+	-	-	-	-

table 3. (continued).

Sample type	Location names	Location codes	Strain code	Cr(VI) mg/l 50	200	500	700	1000	1500
	Fertilizer Berth	FB	CrR PSW10	++	+	-	-	-	-
	Marine Site	MS	CrR PSW11	++	++	++	++	++	+
	Marine Site	MS	CrR PSW12	++	++	++	++	+	-
	Marine Site	MS	CrR PSW13	++	++	+	-	-	-
Bottom water	Approach channel	AC	CrR PBW14	++	++	++	++	++	+
	Approach channel	AC	CrR PBW15	++	++	+	-	-	-
	Oil Jetty	OJ	CrR PBW16	++	++	-	-	-	-
	Oil Jetty	OJ	CrR PBW17	++	++	+	-	-	-
	Oil Jetty	OJ	CrR PBW18	++	++	++	++	++	+
	Iron Ore Handling Berth	IOHB	CrR PBW19	++	+	-	-	-	-
	Iron Ore Handling Berth	IOHB	CrR PBW20	++	+	-	-	-	-
	Iron Ore Handling Berth	IOHB	CrR PBW21	++	++	++	++	++	+
	Coal Handling Berth	CHB	CrRPBW22	++	++	+	-	-	-
	North Quay	NQ	CrRPBW23	++	++	++	++	++	+
	General Cargo Berth	GCB	CrRPBW24	++	+	-	-	-	-
	South Quay	SQ	CrRPBW25	++	+	-	-	-	-
	East Quay	EQ	CrRPBW26	++	+	-	-	-	-
	Fertilizer Berth	FB	CrRPBW27	++	+	-	-	-	-
	Fertilizer Berth	FB	CrRPBW28	++	++	++	+	-	-
	Marine Site	MS	CrRPBW29	++	+	-	-	-	-
Sediment	Approach channel	AC	CrRPSD30	+	-	-	-	-	-
	Oil Jetty	OJ	CrRPSD31	+	-	-	-	-	-
	Iron Ore Handling Berth	IOHB	CrRPSD32	++	++	++	++	+	-
	Iron Ore Handling Berth	IOHB	CrRPSD33	++	++	+	-	-	-
	Coal Handling Berth	CHB	CrRPSD34	+	-	-	-	-	-
	North Quay	NQ	CrRPSD35	++	++	++	++	+	-
	North Quay	NQ	CrRPSD36	++	++	++	++	+	+
	General Cargo Berth	GCB	CrRPSD37	++	++	+	+	-	-
	General Cargo Berth	GCB	CrRPSD38	++	++	++	++	+	+
	General Cargo Berth	GCB	CrRPSD39	++	++	++	++	+	+
	South Quay	SQ	CrRPSD40	++	++	++	++	++	++
	East Quay	EQ	CrRPSD41	++	++	++	++	+	-
	Fertilizer Berth	FB	CrRPSD42	++	++	++	++	+	-
	Fertilizer Berth	FB	CrRPSD43	++	++	-	-	-	-
	Marine Site	MS	CrRPSD44	++	++	++	++	++	+

++: Indicates extensive growth of bacteria
+: Indicates less growth of bacteria
- : Indicates no growth of bacteria

Table 4. Minimum Inhibitory Concentration (MIC) test of selected Cr(VI) resistant bacteria

Strain Code	Concentration of Cr(VI) in mg/l										
	1500	1600	1700	1800	1900	2000	2100	2200	2300	2400	2500
CrRPSW08	+	+	+	-M	-	-	-	-	-	-	-
CrRPSW11	+	+	-M	-	-	-	-	-	-	-	-
CrRPBW14	+	-M	-	-	-	-	-		-	-	-
CrRPBW18	+	+	-M	-	-	-	-		-	-	-
CrRPBW21	+	+	+	-M	-	-	-	-	-		-
CrRPBW23	+	+	-M	-	-	-	-	-	-	-	-
CrRPSD36	+	+	+	-M	-	-	-	-	-	-	-
CrRPSD38	+	+	+	+	-M	-	-	-	-	-	-
CrRPSD39	+	+	+	+	-M	-	-	-	-	-	-
CrRPSD40	++	++	++	++	++	+	-M	-	-	-	-
CrRPSD44	+	+	-M	-	-	-	-	-	-	-	-

++: Indicates extensive growth of bacteria
+: Indicates less growth of bacteria
- : Indicates no growth of bacteria
M: MIC

CrRPBW23, CrRPSD36, CrRPSD38, CrRPSD39, CrRPSD40 and CrRPSD44 out of 44 bacteria were found to exhibit tolerance as high as 1500 mg/l Cr(VI) (Table 3). Based on MIC test (Table 4), the bacterium CrRPSD40 isolated from SQ sediment was found to exhibit high Cr(VI) resistance having highest MIC value (2100 mg/l) among the selected 11 Cr(VI) resistant bacteria. Hence this bacterium was selected for further studies.

Evaluation of multi-metal tolerance of the bacterium

The highest Cr(VI) resistant bacteria (CrRPSD40) is evaluated for its tolerance towards a variety of toxic heavy metals. The maximum tolerance concentration toward various metals in different concentration ranges are shown in figure 2. It was found that the strain can tolerate Cd, Pb, Zn, Ni, As(III) and Cu up to 150, 1000, 60, 50, 50 and 100 mg/l respectively. Bacteria isolated from metal polluted environments adapt to toxic heavy metals present in the environment and became metal resistant, which is a natural phenomenon [22]. As Paradip Port harbour is frequently contaminated with multiple metals during ore handling activity, bacteria grow in this environment can develop tolerance to multiple metal toxicity. Hence, the isolated strain shows multiple metal tolerance towards seven tested toxic metals including Cr(VI).

Bacterial identification

Morphological and biochemical characterisation

Based on morphological and biochemical test results (Table 5 a, b, c & d), the strain CrRPSD40 was found to be Gram positive, spore forming, rod shape and motile bacteria. The strain showed TSI alakaline, non fermentative and oxidative reaction. It was not utilizing Indole and also showed negative result for MR-VP test (Table 5a). The strain has positive results against hydrolysis of enzymes like casein hydrolase, gelatine hydrolase, alakaline phosphatase, oxidase and catalase (Table 5b). This strain had also utilized Glycerol and Salicin as a carbohydrate source for its growth (Table 5c). The strain also showed resistance against antibiotics like Ampicillin, Cefixime, Erytromycin, Rifampicin and Penicillin (Table 5d). The above phenotypic characters of strain CrRPSD40 were almost matched to the strain of

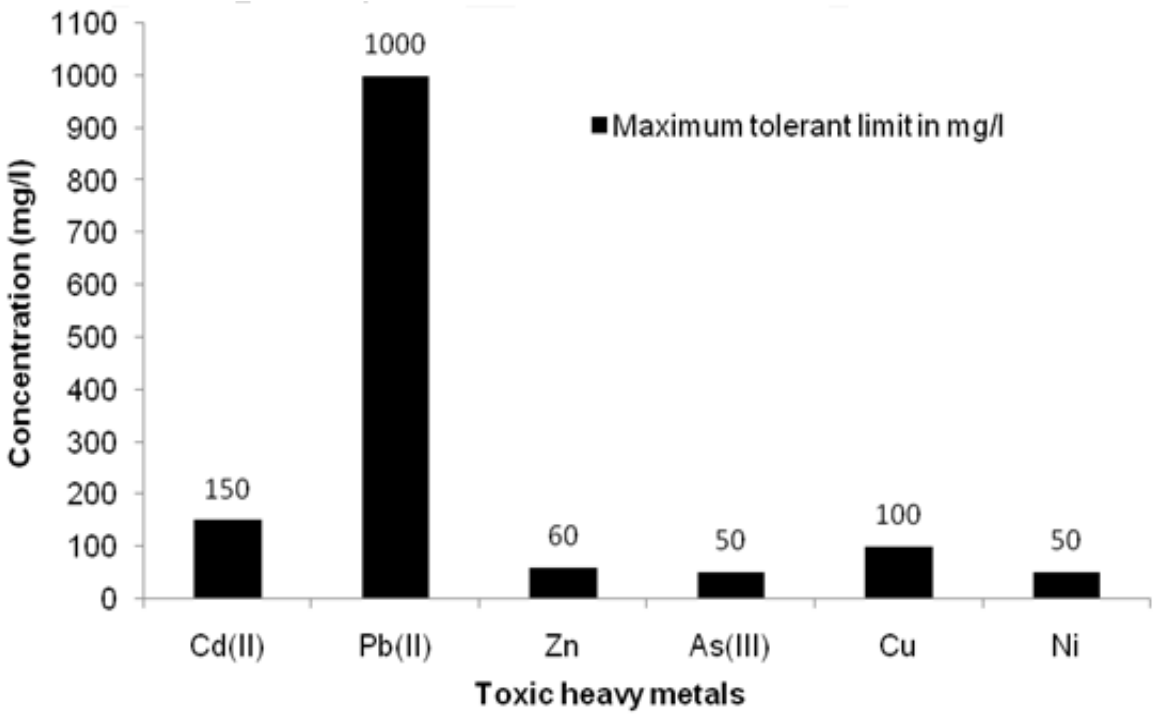

Figure 2. Multi-metal resistance of the Cr(VI) resistant strain CrRPSD40 against six different metals other than Cr(VI)

Table 5a. Morphological and biochemical characterisation of CrRPS40

No.	Test	Result and observation
1	Gram's Staining	Gram-Positive, Rod, Spore forming
2	Mannitol Motility	Positive, fully motile
3	Oxidation Fermentation	oxidation
4	Triple Sugar Iron Agar	Alkaline
5	Indole	Negative
6	Methyl Red	Negative
7	Voges-Proskauer	Negative
8	Citrate Utilization	Negative

Table 5b. Enzyme production by CrRPSD40

No.	Enzyme Test	Result and Observation
1	Amylase	Negative
2	Casein Hydrolase	Positive (14 mm)
3	Gelatin Hydrolase	Positive (12 mm)
4	Lipase	Negative
5	Alkaline Phosphatase	Positive
6	Urease	Negative
7	Oxidase	Positive
8	Catalase	Positive

Table 5c. Carbohydrate utilisation by CrRPSD40

No.	Carbohydrate Test	Result and Observation
1	Lactose	Negative
2	Xylose	Negative
3	Maltose	Negative
4	Fructose	Negative
5	Dextrose	Negative
6	Galactose	Negative
7	Raffinose	Negative
8	Trehalose	Negative
9	Melibiose	Negative
10	Sucrose	Negative
11	L-Arabinose	Negative
12	Mannose	Negative
13	Inulin	Negative
14	Sodium gluconate	Negative
15	Glycerol	Positive
16	Salicin	Positive
17	Dulcitol	Negative
18	Inositol	Negative
19	Sorbitol	Negative
20	Mannitol	Negative
21	Adonitol	Negative
22	Arabitol	Negative
23	Erythritol	Negative
24	á-Methyl-D-glucoside	Negative
25	Rhamnose	Negative
26	Cellobiose	Negative
27	Melezitose	Negative
28	á-Methyl-D-mannoside	Negative
29	Xylitol	Negative
30	ONPG	Negative
31	Esculin hydrolysis	Negative
32	D-Arabinose	Negative
33	Citrate utilization	Negative
34	Malonate utilization	Negative
35	Sorbose	Negative

Brevibacillus genus by Song *et al*. [26] and Holt *et al*. [12]. According to Ruiu [23], *Brevibacillus* genus is a new genus comes within *Brevibacillus brevis* cluster. According to Joshi *et al*. [25], the genus *Brevibacillus* was recognized within the family *Paenibacillaceae* and members of this genus rod-shaped, Gram-positive or Gram-variable, motile by means of peritrichous flagella, and strictly aerobic. Few members of this genus have agricultural importance and used as bio-control and denitrifying agents. *Brevibacillus* sp. an As (III) metabolizing bacteria contains an arsenate reductase enzyme which has reduction potential of the toxic arsenate to arsenite has been reported by Sanyal *et al*. [25]. An As resistant *Brevibacillus* sp. which could resist 17 mM As(III) and having ar-

Table 5d. Antibiotic resistance/sensitivity of CrRPSD40

No.	Antibiotic	Code with conc. in mcg	Result
1	Ampicillin	AMP^{10}	Resistant
2	Azithromycin	AZM^{30}	Intermediate
3	Cefixime	CFM^{5}	Resistant
4	Chloramphenicol	C^{30}	Sensitive
5	Ciprofloxacin	CIP^{5}	Sensitive
6	Erythromycin	E^{15}	Resistant
7	Gatifloxacin	GAT^{5}	Sensitive
8	Gentamicin	GEN^{10}	Intermediate
9	Kanamycin	K^{30}	Sensitive
10	Nalidixic Acid	NA^{30}	Intermediate
11	Neomycin	N^{30}	Intermediate
12	Norfloxacin	NX^{10}	Sensitive
13	Penicillin-G	P^{10}	Resistant
14	Rifampicin	RIF^{5}	Resistant
15	Streptomycin	S^{10}	Intermediate
16	Tetracycline	TE^{30}	Sensitive

senic removal capacity under aerobic culture conditions was reported by Mallick *et al.*[17]. Jirasripongpun *et al.* [14] has also reported the decolourization of an azo dye, navy blue 3G by *Brevibacillus laterosporus* MTCC2298 and found 80 % dye removal of 50 mg/l concentration within 48 hour under static condition . Hence, in the present study isolation of Cr(VI) resistant *Brevibacillus* sp. and its evaluation of multimetal tolerance would reveal the potentiality of the bacterium for bioremediation of the hexavalent chromium is from metal contaminated marine environment.Remediation of Cr(VI) from polluted environment by biologically transformation of Cr(VI) to relatively non toxic and insoluble form Cr(III) by chromium resistant bacteria is emerging as a safe and cost-effective technology as an alternative to the expensive traditional physicochemical methods [18,20]. However, availability of effective Cr(VI) resistance and reducing bacteria is an essential requirements for the bio-reduction based remediation of Cr(VI) contaminated water and soil [18]. Hence, in the present study, metal resistant bacterium, *Brevibacillus* sp. isolated from metal contaminated marine sediment might prove useful applications in bioremediation of the metal-contaminated saline environments.

Conclusion

In conclusion, analysis of heavy metals of environmental samples from the Port harbour revealed that, the surface water, bottom water and sediments are contaminated with different metals mostly by ongoing ore handling activities of the Port. The bacteria isolated from the water and sediment samples were found to be Cr(VI) resistant which could be more suitable candidates for bioremediation of metal pollutants from saline environment. In the present study, a potent Cr(VI) resistant marine bacterium, *Brevibacillus* sp, isolated and identified from sediment sample of Paradip Port has also showed resistance to various other toxic metals like Cd, Pb, Zn, Ni, As(III) and Cu in different concentration ranges. Hence, this bacterium can be exploited for bioremediation of saline environments containing multiple toxic metals with development of suitable bioremediation process.

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