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Potential of Mercury-Resistant Marine Bacteria for Detoxification of Chemicals of Environmental Concern

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The hypothesis that mercury-resistant bacteria exposed to polluted environments such as coastal areas can tolerate, detoxify, or biotransform a variety of other toxicants was examined. Several mercury-resistant marine bacteria from the coastal waters of India were evaluated for their ability to biotransform the heavy metals mercury, cadmium and lead as well as xenobiotics like polychlorinated biphenyls and tributyltin. These salt-tolerant bacteria removed mercury by means of volatilization and were successfully used to detoxify mercury-amended growth medium for the culturing of mercury-sensitive *Phormidium* sp. Over 70% cadmium and 95% of the lead from the growth medium were either cell-bound (cadmium) or precipitated (lead) by some of these bacteria. A pseudomonad strain, CH07, aerobically degraded fourteen toxic polychlorinated biphenyls including congeners with five or more chlorine atoms on the biphenyl ring and was also equally efficient in degrading more than 54% of the tributyltin. These bacteria offer great biotechnological opportunities in the bioremediation of toxic chemicals.

Key words: mercury-resistant marine bacteria, detoxification, heavy metals, xenobiotics, bioremediation

Bioremediation encompasses technologies that accelerate natural processes for degrading harmful chemicals and thereby provide a good cleanup strategy for many, if not all, types of pollution. Toxic metals such as mercury, cadmium and lead etc. are not biodegradable in the same sense as carbon-based molecules, posing hindrances to bioremediation efforts. Thus, unless removed completely from a system, heavy metals will persist indefinitely[36]. Heavy metals like cadmium, copper, lead, mercury, nickel, and zinc are included as the most hazardous in the US Environmental Protection Agency's (USEPA) list of priority pollutants[8]. In general, areas polluted by organic compounds, i.e. fossil fuels or their derivatives, pesticides, polychlorinated biphenyl (PCB)s, tributyltin (TBT) etc., are often also contaminated by some heavy metals.

Mercury (Hg) is the most toxic of the heavy metals with a widespread use in industry[28]. Worldwide, many areas are polluted with mercury and present a threat to people and the environment[17]. Syndromes due to mercury poisoning at different trophic levels are many, but the worst affecting human has been Minamata disease[21]. Cadmium (Cd) is another toxic heavy metal causing several environmental problems including the very painful *itai itai* disease[25]. Lead (Pb) is well known to inhibit the biosynthesis of heme, and consequently hemoglobin, and to decrease the life span of circulating red blood cells[33]. Once thought to be safe, even at low concentrations Pb results in a decreased Intelligence Quotient, slow growth, hearing problems and kidney damage. The PCBs are among the most persistent organic pollutants (POPs) and thus their usage has been banned[47]. Since they persist, get dispersed over vast areas, and have

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an estimated half-life in the environment of several months, concerted efforts must be made to cleanup PCB contamination. It has been shown that TBT may be responsible for the thickening of oyster and mussel shells as well as retardation of growth in aquatic snails[1,22]. Keeping the deleterious effects in the fore, the International Maritime Organization (IMO) has already passed a resolution to ban the use of TBT-based antifouling compounds[11]. However, TBT is also a long persisting toxicant and, ships, recreational boats and other vessels painted with TBT amalgamations will continue to leach this toxicant into the marine environment. Thus, any attempt made to realize a potential remedy is indeed important.

Resistance to mercury is quite well understood in a variety of bacteria. This extensively studied resistance system based on clustered genes in the *mer* operon, allowing bacteria to detoxify Hg^{2+} into volatile mercury by enzymatic reduction, has been thoroughly investigated[4,20,26,30,43,45]. Several studies[2,3,14,18] have examined mercury-resistant bacteria (MRB) and their potential to catabolize toxic xenobiotics. The ability of bacteria to detoxify mercury can be utilized to bioremediate mercury-contaminated wastewaters and sites[7,10,13,40,46] as well as other toxic chemicals[14]. Pain *et al.*[32] reported that most TBT-resistant bacteria are also resistant to six heavy metals (Hg, Cd, Zn, Sn, Cu, and Pb), which suggest that resistance to many types of toxicants may be present in the same organism. In addition, many moieties of chromosomal DNA have been shown to be important in resistance to heavy metals. For example, Cánovas *et al.*[9] reported that the genome sequence of *Pseudomonas putida* KT2440 has 61 open reading frames likely to be involved in metal tolerance/resistance. The present investigation was carried out to address such multiple resistances and the potential of mercury-resistant marine bacteria in bioremediation of mixed wastes containing heavy metals and xenobiotics.

Materials and Methods

Isolation and identification of MRB

Mercury-resistant marine bacteria were isolated from seawater and sediment on seawater nutrient agar medium (SWNA: 5.0 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 500 ml aged seawater, 500 ml deionised water and 15 g agar) amended with 10 mg/l Hg (as $HgCl_2$). These MRB were isolated from Mormugao (15°24"35' N, 73°48"2' E; Hg concentration 152–456 ng/l in water and 53–194 ng/g dry sediment), Gopalpur (19°18"12' N, 84°57"55' E; Hg concentration 2–117 ng/l in water and 72–128 ng/g dry sediment) and Chennai (13°6"40' N, 80°18"3' E; Hg concentration 100–2100 ng/l in water and 237–338 ng/g dry sediment). Several single colonies were picked and streaked onto SWNA plates containing 25 mg/l mercury for further purification. These isolates showed an obligate requirement of sodium for their growth indicating their marine origin[5]. The isolates were characterized biochemically[23] and a select set of MRB were identified by 16S rDNA sequencing[39].

Detoxification and removal of heavy metals by MRB

1. Mercury (Hg). Seven MRB were grown in SWNB (SWNB: 5.0 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 500 ml aged seawater, 500 ml deionised water) amended with Hg concentrations of 10 and 50 mg/l and growth was monitored by measuring optical density at 660 nm (OD_{660}). Eleven MRB isolates viz. GP15 (*Alcaligenes faecalis*), CM10 (*Bacillus* sp.), CH07 (*Pseudomonas aeruginosa*), GP08 (*Bacillus pumilus*), GP13 (*Brevibacterium iodinium*), GO02 (*A. faecalis*), GP16 (*A. faecalis*), GP17 (*A. faecalis*), GP14 (*B. pumilus*), GP06 (*A. faecalis*), CH13 (*B. pumilus*), 3C (*B. pumilus*; a contaminant), one mercury-sensitive isolate (unidentified), and *P. putida* KT2442::mer73 (positive control) were grown in marine broth for 24 h and the cells from the broth culture were pelleted by centrifugation at 10000 rpm. The cells were washed with phosphate buffer and placed in wells of microtitre plates. Mercurated phosphate buffer (10 mg/l Hg as the final concentration) was added and the cells were incubated at 30°C in the dark for 4 h. After this incubation, the XAR film was removed and developed to check whether Hg was volatilized[27] by these MRB. A pseudomonad CH07 (*P. aeruginosa*) was grown in M9 medium amended with different concentrations of Hg and kinetics of Hg removal was measured in terms of mercury volatilization as detected by cold vapor atomic absorption spectrometry.

Axenic cultures of *Phormidium* sp. (a marine cyanobacterium) were grown in artificial seawater nutrient medium (ASN-III[34]). The minimal inhibitory concentration of mercury (as $HgCl_2$) for this strain was determined by inoculating exponentially growing cultures in ASN-III medium amended with various concentrations of Hg ranging from 10 to 200 μg/l. Growth in terms of chlorophyll *a* was estimated by the acetone extraction method[19]. Two MRB, namely CH07 and S3 (*B. pumilus*), were used to detoxify ASN-III medium amended with 10 mg/l mercury ($HgCl_2$). After 7 days, the medium was filtered through 0.22 μm membrane filter to exclude the bacterial cells. The filtrate after being supplemented with mineral salts was inoculated

with an exponentially growing culture of *Phormidium* sp. Once the algal growth became visible, chlorophyll *a* was measured on the 7th day after inoculation.

2. Cadmium (Cd) and lead (Pb). Two isolates (CH07 and GP06) were grown in seawater nutrient broth (SWNB) amended with Cd ($CdCl_2$). Three isolates (CH07, GP13, and S3) were grown in medium amended with $(CH_3COO)_2Pb$ to final concentrations of 10, 50, and 100 mg/l. The flasks were incubated on a rotary shaker (200 rpm) at room temperature (ca. 28±2°C) for 120 h and the OD_{660} of each culture was measured to monitor growth. A sensitive strain, CH05 (*Proteus* sp.) and heat-killed bacterial cells were included as negative controls. The removal of the metal was calculated by analyzing the metal content of the medium and in the cells following suitable methods[37] of extraction. Once in every 24 h, a 1 ml sample was withdrawn aseptically into a 1.5-ml sterile microcentrifugation tube. The tubes were centrifuged at 13,000 rpm for 15 min at 24°C. The supernatant was filtered through preweighed membrane filters with a pore size of 0.22 μm and the filtrate was digested with 10% HNO_3 for estimation of the content of heavy metal (either Cd or Pb) removed from the medium. The pellets were treated overnight using 1 M HCl and treated further including a sonification step twice for 45 sec followed by centrifugation at 10,000 rpm for 5 min. The supernatant was collected and digested with 10% HNO_3 for estimation of the amount of heavy metal (either Cd or Pb) accumulated by the cells. The cell pellets were dried for 48 h at 70°C and weighed for determining bacterial biomass. The Cd concentrations were determined by inductively coupled plasma-atomic emission spectrometry and Pb was measured using an atomic absorption spectrophotometer following the manufacturer's instructions. Their concentrations were calculated using proper blanks and several standards ranging from 5 to 20 mg/l were used for calibration. The bacterial cells were studied using scanning electron microscopy (SEM) and energy dispersive X-ray spectrometry (EDS) to investigate the possible mechanism(s) involved in the transformation of the heavy metals.

Degradation of xenobiotics

1. PCBs. The marine pseudomonad strain CH07 was checked for its potential to degrade different congeners of PCBs from the technical mixture Clophen A-50 in a final concentration of 100 mg/l (w/v in distilled *n*-hexane) in SWNB. The technical mixture of PCBs (Clophen A-50) was obtained from Bayer (Leverkusen, Germany) and the PCB standards were from Promochem (Wesel, Germany). A 24 h old broth culture of strain CH07 was added to two replicates of test medium (SWNB+Clophen A-50) and normal SWNB (without any addition of Clophen A-50). Controls in duplicate were also maintained without addition of the organism in one set and with heat-killed bacterial cells in another set at room temperature (28±2°C). Samples were taken out aseptically and prepared for gas chromatographic analysis. The comparison of degradation of PCBs was done with the control without added bacteria and test condition with the live bacterial cells. The PCBs were extracted following standardized methods[41] and were analyzed by gas chromatography (Varian GC-3380; Middelburg, Netherlands) coupled with an ECD and an autosampler 8200. A capillary column VA-5 (30 m×0.25 mm) was employed with the ECD for peak detection using argon with 5% methane as the carrier gas. The injector temperature was fixed at 250°C and the analysis of PCBs was calibrated using the standards for individual congeners of PCBs obtained from Promochem, Germany.

2. Tributyltin. Strains CH07 and GP15 were grown in M3 mineral salt medium[24] supplemented with 5 mg/l TBT (concentration in terms of Sn) as the sole carbon source. Heat-killed bacterial cells were inoculated in one flask as a control. Samples were collected from each dark brown flask at "0", 48 and 312 h for analysis of TBT and its breakdown products. TBT was extracted, derivatized using tripropyltin as an internal standard following standard procedures[6]) and analyzed by gas chromatography (Agilent HP 6890; Wilmington, Delaware, US). In brief, 500 μl of sample was extracted with double-distilled dichloromethane in presence of sodium borohydrate, sodium sulfate after adding appropriate amounts of tripropyltin. The sample was concentrated to 500 μl with nitrogen gas, dissolved in double-distilled hexane, concentrated again finally to around 500 μl and stored in the freezer prior to analysis. Standards were prepared with tributyltin, dibutyltin and tripropyltin. In a separate experiment, the growth of strains CH07 and GP15 was examined by providing one-fourth strength SWNB and 10 mg/l of TBT to check if bacteria can grow at rates as fast as they do in normal strength SWNB as a result of cometabolism. An isolate CH08, (unidentified), served as the control.

Results

Bacterial isolates

Three isolates (GP08, CH13, and S3) were identified as *Bacillus pumilus*, seven isolates (GO01, GO02, GP06, GP14, GP15, GP16, and GP17) as *Alcaligenes faecalis*, and one each as *Brevibacterium iodinium* (GP13), *Pseudomonas aeruginosa* (CH07) and *Bacillus* sp. (CM10) from 16S

Table 1. Growth response of bacteria highly resistant to mercury in the presence of heavy metals

Isolates groups		PA	AF						BI	BP
Strain		CH07	GO02	GP06	GP14	GP15	GP16	GP17	GP13	GP08
Heavy metals	Conc[a]									
Mercury	25	+	+	+	+	+	+	+	+	+
Mercury	50	+	−	−	+	+	+	+	+	−
Mercury	55	−	−	−	−	−	−	−	−	−
Mercury	75[b]	+	NT	NT	+	+	NT	NT	+	−
Cadmium	100	+	+	+	+	+	+	+	+	−
Copper	100	+	+	+	+	+	+	+	+	+
Zinc	100	+	+	+	+	+	+	+	+	+
Lead	100	+	+	+	+	+	+	+	+	+

[a] parts per million (mg/l) spiked concentrations; +, positive growth; −, no growth; NT, not tested; PA, *Pseudomonas aeruginosa*; AF, *Alcaligenes faecalis*; BI, *Brevibacterium iodinium*; BP, *Bacillus pumilus.*; [b] in SWNA; in all other cases it was in SWNB.

Table 2. Growth response of bacteria highly resistant to mercury in the presence of xenobiotics

Isolate groups		PA	AF						BI	BP
Strain		CH07	GO02	GP06	GP14	GP15	GP16	GP17	GP13	GP08
Xenobiotics	Conc[a]									
DDT[b]	100	+	−	−	+	+	+	+	−	−
Penconazole[b]	93	−	−	−	+	+	−	+	+	−
Propiconazole[b]	95	+	+	+	+	+	+	+	+	+
Metolachlor[b]	95	+	+	+	+	+	+	+	+	+
Pretilachlor[b]	96	+	+	+	+	+	+	+	+	+
Profenofos[b]	91	+	+	+	+	+	+	+	+	+
Phenol	50	+	+	+	+	+	+	+	+	+
Phenol	1000	NT	NT	+	NT	NT	-	NT	+	NT
TCE	10% (v/v)	NT	NT	NT	+	NT	+	NT	NT	NT
TBT	10	+	−	−	−	+	−	−	−	−
PCBs[b]	100	+	NT	−	NT	−	NT	−	−	−

[a] mg/l spiked concentrations; [b] stock solutions prepared using hexane; +, positive growth; −, no growth; NT, not tested; DDT, dichlorodiphenyltrichloroethane; TCE, trichloroethylene; PA, *Pseudomonas aeruginosa*; AF, *Alcaligenes faecalis*; BI, *Brevibacterium iodinium*; BP, *Bacillus pumilus.*

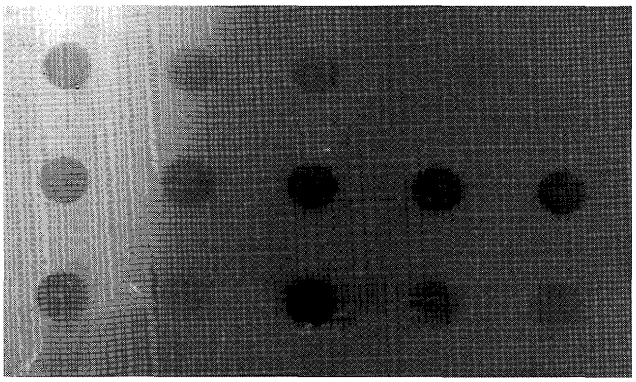
rDNA sequencing (accession numbers; DQ377441–DQ377468). An overview of the resistance potential of these isolates is shown in Tables 1 and 2.

Hg detoxification

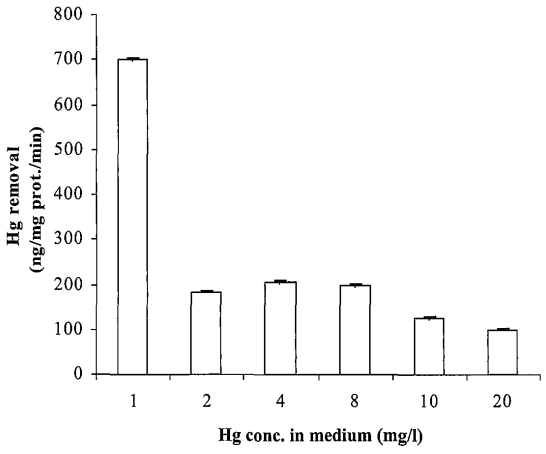
The toxic effect of Hg prolonged the lag phase of the MRB but growth was normal once the cells adapted to the toxic Hg by means of detoxification. All the MRB isolates volatilized (Fig. 1a) mercury from the assay medium. The highest Hg removal rate was observed at an Hg concentration of 1 mg/l though the rate was quite good at 8 mg/l Hg in the medium (Fig. 1b). The fact that the *Phormidium* sp. whose growth was affected at 50 μg/l Hg (Fig. 1c), could grow later in bioremediated growth medium which initially contained 10 mg/l Hg (approximately 200 times) further shows the efficient detoxification of Hg performed by the MRB (Table 3).

Cd and Pb removal

The toxicity of Cd or Pb had hardly any effect on the growth of MRB isolates. In medium amended with 100 mg/l Cd, the concentration of Cd dropped to 17.4 mg/l of Cd in strain CH07 (Fig. 2a) and 19.2 mg/l in GP06 by 72 h, and the accumulation of Cd in the biomass reached the maxi-



a



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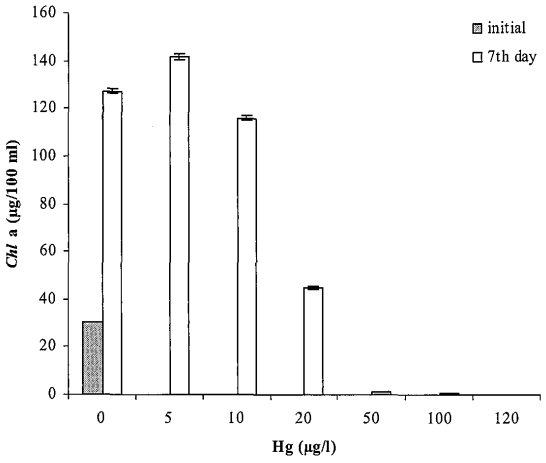


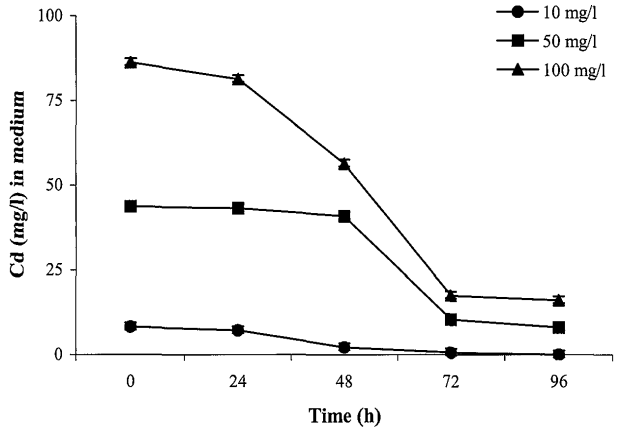
c

Fig. 1 a) Volatilization of mercury by MRB as visualized on Kodak XAR film. Upper row: CH13 (*B. pumilus*), GP06 (*A. faecalis*), 3C (*B. pumilus*), non MRB isolate (negative control), mercurated PBS used in the experiment (no bacteria added); Middle row: GP14 (*A. faecalis*), GP17 (*A. faecalis*), GP16 (*A. faecalis*), GO02 (*A. faecalis*), GP13 (*B. iodinium*); Lower row (from left to right): positive control (*P. putida* KT2442::mer73), GP08 (*B. pumilus*), CH07 (*P. aeruginosa*), CM10 (*Bacillus* sp.), GP15 (*A. faecalis*). b) Removal of Hg (ng/mg protein/min) by strain CH07. c) Growth response of *Phormidium* sp. for detection of Minimum inhibitory concentration of Hg.

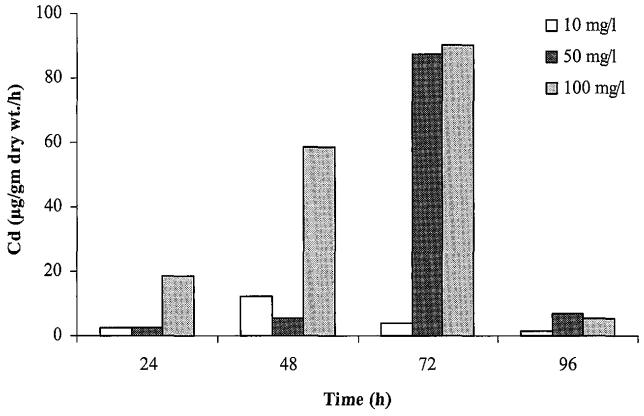
Table 3. Chlorophyll *a* concentration (µg/100 ml) in flask cultures of *Phormidium* sp. after Hg was removed through bioremediation using CH07 and a combination of CH07 and S3

Sample	*Chl* a (µg/100 ml)
Initial[a]	1.93±0.09
Control[b]	127.29±1.1
CH07[b]	58.81±0.8
CH07 & S3[b]	17.46±0.3

[a] Concentration of chlorophyll *a* at the start of the experiment.
[b] Concentration of chlorophyll *a* on day 7.



a



b

Fig. 2 a) Kinetics of Cd removal by strain CH07 from SWNB amended with 10 mg/l Cd (circles), 50 mg/l Cd (squares) and 100 mg/l Cd (triangles). b) Cell biomass-associated quantities of Cd (µg/g dry wt./h) with strain CH07 in media containing different concentrations of this toxic metal.

mum by 72 hours (Fig. 2b). Thus, both CH07 and GP06 were capable of removing >70% Cd from the growth medium. Further, all three strains of MRB removed Pb from the growth medium. In case of CH07, the concentration of Pb in medium amended with 100 mg/l Pb reached as low as

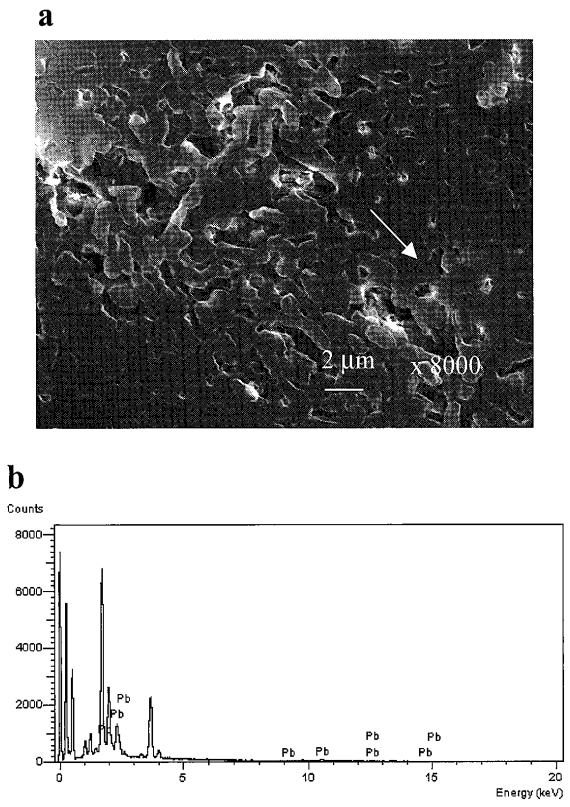


Fig. 3. Removal of Pb from SWNB amended with 50 mg/l Pb. a) SEM pictures of the EPS-entrapped Pb (white arrow). b) The signal reflected from Pb as revealed by EDS. Results shown here are for the mercury-resistant marine pseudomonad strain CH07.

1.8 mg/l (>98% removal) in 96 h and Pb was found to be entrapped in the extracellular polymeric substances (EPS), as revealed by the SEM and EDS (Fig. 3). This could be due to the efflux commonly seen in Gram-negative bacteria as a detoxification measure[29]. Removal of the metals in the controls was negligible. Strains GP13 and S3 removed >87% of the Pb in the same period and precipitated it as lead sulfide. It is clear that the MRB have cellular mechanisms to either immobilize, as in the case of Cd, or precipitate (Pb) toxic heavy metals.

Degradation of PCBs

Among the different congeners of PCBs present in Clophen A-50, fourteen chlorobiphenyls were degraded by MRB to varying degrees. Of the three most toxic coplanar PCBs, CB-126 (3,3',4,4',5-pentachlorobiphenyl) was degraded completely by CH07 in about 40 h. Another coplanar congener, CB-77 (3,3',4,4'-tetrachlorobiphenyl) was degraded by over 24% within a period of 40 h. One heptachlorobiphenyl, CB-181 (2,2',3,4,4',5,6) was degraded completely within 40 h (Fig. 4). Two asymmetric di-ortho chlorinated biphenyls, 2,2',4,5,5'-pentachlorobiphenyl and

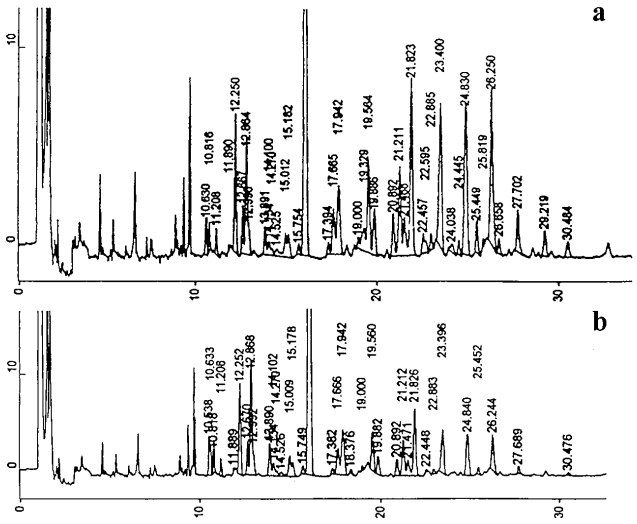


Fig. 4. Degradation of PCBs by the marine pseudomonad CH07. a) Initial peaks of different congeners. b) The peaks of congeners after 40 h of bacterial degradation.

2,3',4,4',6 pentachlorobiphenyl, were degraded by 20.19% and 19.66% respectively (Table 4). The control with the dead cells did not show any remarkable decrease of PCBs from the growth medium indicating that the PCBs were biodegraded by the actions of MRB.

Degradation of TBT

The pseudomonad strain CH07 degraded the TBT faster than strain GP15 (*A. faecalis*). At the end of the experiment i.e. after 312 h, CH07 degraded nearly 54% of the initial TBT concentration (approximately 3564.4 ng/ml) in comparison to a 34% degradation by GP15 (Fig. 5a and b). The concentration of DBT in the media also increased with time and at the end of 312 h, was 320 and 83.2 ng/ml in the case of CH07 and GP15, respectively. The appearance of DBT in varying amounts implies that these marine MRB strains were able to degrade TBT quite effectively. The control with the dead cells showed hardly any decrease of TBT indicating that TBT were degraded by bacterial action. With organic enrichment, the amounts of TBT degraded were similar for both strains but the degradation rate was faster.

Discussion

Lower costs and higher efficiency at low metal concentrations make biotechnological processes very attractive in comparison with physicochemical methods for heavy metal removal[16]. Among the principal processes, microbial degradation/biotransformation may be the most efficient way of removing chemical pollutants and their toxicity from the

Table 4. Degradation (percent) of different congeners of PCBs in Clophen A-50 by CH07

Chlorobiphenyls	Molecular Formula	Retention time (Min)	PCBs at 0 hr (ng/ml)	PCBs at 40 hrs. (ng/ml)	Degradation of PCBs (%)
CB-101 (2,2',4,5,5')	$C_{12}H_5Cl_5$	19.564	18.17	14.50	20.19
CB-119 (2,3',4,4',6)	$C_{12}H_5Cl_5$	19.886	8.07	6.48	19.66
CB-97 (2,2',3',4,5)	$C_{12}H_5Cl_5$	20.892	8.17	6.57	19.69
CB-116 (2,3,4,5,6)	$C_{12}H_5Cl_5$	21.211	10.09	8.06	20.04
CB-77 (3,3',4,4')	$C_{12}H_6Cl_4$	21.823	53.37	40.42	24.25
CB-151 2,2',3,5,5',6)	$C_{12}H_4Cl_6$	22.595	2.04	1.28	37.32
CB-118 (2,3',4,4',5)	$C_{12}H_5Cl_5$	23.400	1.31	0.77	40.72
CB-105 (2,3,3',4,4')	$C_{12}H_5Cl_5$	24.830	17.54	9.29	46.69
CB-141 (2,2',3,4,5,5')	$C_{12}H_4Cl_6$	25.449	3.57	1.59	55.38
CB-138 (2,2',3,4,4',5')	$C_{12}H_4Cl_6$	25.819	1.62	0.71	55.97
CB-126 (3,3',4,4',5)	$C_{12}H_5Cl_5$	26.658	2.75	00.00	100
CB-128 (2,2',3,3',4,4')	$C_{12}H_4Cl_6$	27.702	5.02	1.79	64.33
CB-181 (2,2',3,4,4',5,6)	$C_{12}H_3Cl_7$	29.219	2.87	00.00	100
CB-180 (2,2',3,4,4',5,5')	$C_{12}H_3Cl_7$	30.484	1.64	0.63	61.33

environment. The principal goal of bioremediation is to enhance the natural biological-chemical transformations that render pollutants harmless as minerals and thus to provide a means to deal with the problems of contaminated environments. Serious attention began to be paid to the bioremediation of metal contamination only in the 1990s[45].

It is quite likely that multi-metal resistant strains such as CH07, GP14, GP15, and S3 possess genetic components for dealing with many toxic metal ions. Though there is a potential threat of contamination of unaffected areas by Hg due to its dispersal over time and space, the efficient removal of this most toxic heavy metal from the environment is of prime importance. As they release relatively less toxic gaseous mercury into the atmosphere, MRB thus hold the key to the successful detoxification of mercury at least at a local level. Bioremediation of the mercury-containing ASN-III medium to promote the growth of the mercury-sensitive *Phormidium* sp. was a successful demonstration of the detoxifying efficiency of the MRB. Common methods to remove Hg^{2+} from contaminated waters are mostly based on sorption to materials such as ion exchange resins[31,35]. One of the initial efforts to retain mercury in bacterial bioreactors was made by[7] Canstein *et al.*[10], who demonstrated the removal of mercury from chloralkali electrolysis wastewater by a mercury resistant *Pseudomonas putida* strain. A genetically engineered *E. coli* strain with a Hg^{2+} transport system and metallothionein has been used to bioaccumulate mercury from wastewater[15]. There was a clear correlation between the amount of Cd taken up by the MRB and the amount of Cd removed from the medium. This phenomenon may be explained by the role of microbial metabolism into bioabsorption[29]. Although no detailed analysis, either at the enzymatic or molecular genetic level, of Cd resistance mechanisms was attempted during this study, it is quite likely that at least one of the following mechanisms reported in the literature operate in the marine MRB examined during this study. Sulfide precipitation of Pb implies to sulfur-rich (such as cysteine) enzymatic detoxification, which could also detoxify other metals such as mercury. Roane *et al.*[38] reported that *R. eutropha* JMP134, a 2,4-D degrader which was sensitive to Cd, could degrade 2,4-dichlorophenoxyacetic acid even in the presence of Cd when it was grown in a consortium with Cd-detoxifying bacteria. Zeroual *et al.*[48] observed that a strain of *Klebsiella pneumoniae* could tolerate 2400 μM of mercury and 1000 μM of cadmium. The resting cells of *P. aeruginosa* PU21 (Rip64) have been reported to take up upto 110 mg Pb/g dry cell mass whereas, the inactivated cells could absorb 70 mg Pb/g dry cell[12]. Henceforth, the biotransformed metals can be treated suitably either to recover the toxic metals or buried away from conditions that might cause them to leach back to the environment. The extent of degradation of different congeners of PCBs in the presence of other chlorobiphenyls and with varying degrees of polarity and stereochemical asymmetry is a clear indication that bacterial strains such as CH07, isolated from marine environments can be used effectively for their detoxification. Most importantly, highly chlorinated congeners, CB-180 and CB-181 were found to be degraded sufficiently. Thus, the conclusive demonstration of an aerobic microbial process involv-

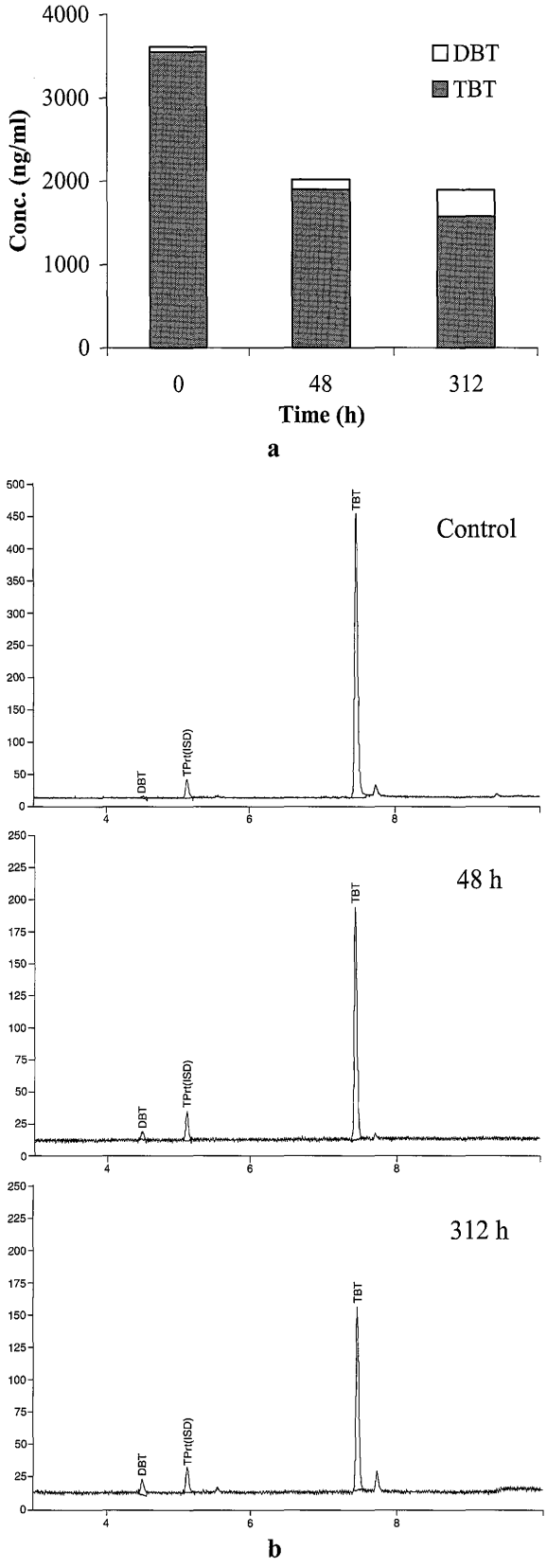


Fig. 5. a) Degradation of TBT from minimal medium into DBT by the marine pseudomonad CH07. b) Gas Chromatograms of TBT degradation by CH07 at different time points.

ing the marine bacterium CH07 warrants further research to understand the degradation mechanism. From the fact that the MRB strain degraded 54% of the initial TBT concentration within a week, potential of such environmental strains needs to be more thoroughly established. This can be substantiated by the appearance and increase of DBT in the media with time. Though no attempt was made to check whether DBT was further degraded to monobutyltin or elemental tin, it was clear from the decrease of TBT and, as a consequence, appearance of DBT, in varying amounts, that these marine MRB were able to degrade TBT quite effectively. With organic enrichment, amounts of TBT degraded were similar by both strains but the degradation rate was faster. Results from such experiments are useful to show that TBT is usually worked upon by the native microflora with the wherewithal to break it down. The use of indigenous microflora in biotreatment has been successfully employed for hydrocarbon remediation[42]. The marine isolates used in this study were able to grow in a salinity ranging from 15 to 35‰. As the experiments with different chemicals were carried out at quite a high NaCl concentration, it is possible to suggest that these marine MRB strains are effective in dealing with these chemicals in truly marine and estuarine saline environment.

In principle, if a single strain can perform several metabolic activities, the efficiency and predictability of the process may be significantly enhanced. The successful application of mercury-resistant marine bacteria like CH07 in detoxification/degradation of several heavy metals or xenobiotics adds to bioremediation technology where mixed waste containing heavy metals and xenobiotics can be dealt naturally with the same organism. It can be surmised that despite the alarming present scenario of chemical pollution, there is hope is provided by these MRB possessing an array of armory for alleviating health hazards.

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