Emergence of Highly Mercury Tolerant Plant Growth promoting Bacteria in Tea Plantation Soil of Darjeeling Hills

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Abstract

This study characterizes three strains of Gram-negative bacteria MTD10B, MTD10C and MTD10D isolated from soil collected from tea plantations of Darjeeling hills, exhibiting extreme tolerance towards mercury. The minimum inhibitory concentration of mercury against these strains sits at a high level of 0.2 mg/mL of $HgCl_2$. The isolates also display an expansive pattern of resistance to known clinically relevant synthetic antibiotics and a host of other potent heavy metals such as lead, cadmium, arsenic, chromium, silver, nickel etc. Biochemical and molecular characterization via 16S rRNA sequencing identified MTD10B and MTD10C as strains of Brevundimonas diminuta and MTD10D as Alcaligenes faecalis respectively.

This study also explores the plant growth promoting abilities of these strains and their respective growth trends under normal conditions in comparison to when they are under mercury stress. This work attempts to cultivate an understanding of their potential for use as candidates for the bioremediation of mercury contamination in diverse environments.

Keywords: *Alcaligenes faecalis*, antibiotics, *Brevundimonas diminuta*, heavy metals, mercury.

Introduction

Heavy metal contamination of our environment has been an ever-present danger since the dawn of civilization. Among these toxic metals, mercury holds a top tier position due to the extreme consequences that acute or chronic exposure may cause to practically all forms of life. It poses a real, tangible threat, most notably to human life when present at excess concentrations in the environment. This may be a direct result of anthropogenic activities like mining, over reliance on fossil fuel, poor management and disposal of industrial and hospital waste, excessive use of pesticides etc.[4] or it may even be of geo-genic origin[30].

The notoriety of mercury as a major environmental pollutant is irrefutable. This status can be attributed to its ability to undergo bio-transformations to various noxious forms with the help of microbes as a natural part of its bio-geochemical cycle as well as its troubling ability to bio-accumulate and bio-magnify at various trophic levels[16]. A classic example of the ramifications of unchecked mercury contamination is the Minamata Bay disaster of Japan that not only took lives but also irreversibly crippled generations to come[8]. Mercury affects organisms at the cellular level as it has a particular affinity for binding to thiol groups of proteins, thereby rendering them non-functional within the cells[9]. It is also known to cause oxidative damage to cells via binding to selenohydryl and sulfhydryl groups and to cause disruption of cellular membranes[26].

Like other heavy metals, mercury also proves difficult to get rid of due to its chemically persistent nature. There have been various attempts made to combat this predicament. Natural biological processes of eradication are available. These are extremely versatile and can be tweaked easily to our advantage. Although most microorganisms are unsurprisingly very susceptible to mercury toxicity, when faced with such stress, some populations develop a tolerance towards the contaminant on account of their propensity to adapt to varied environments. Biological methods built around such tolerant populations not only surpass the limitations of conventional methods such as reverse osmosis, ion exchange, membrane filtration, chemical precipitation etc. but are easier to handle and subsequently manipulate towards our target objectives[19].

The design of remediation tactics featuring bacteria against pollutants such as mercury is rapidly gaining a promising body of work with a growing number of microbes being reported as tolerant towards elevated levels of mercury. The study and utilization of such bacterial strains provides a great opportunity for the definitive reclamation of mercury contaminated areas. With this study we intend to explore the soil microflora of Darjeeling tea plantations for such mercury tolerant strains of bacteria as soil is an important reservoir for mercury[22]. We aim to evaluate their abilities for the prospect of application as future candidates for remediation solutions.

Material and Methods

Sample collection, bacterial isolation and determination of minimum inhibitory concentration: Soil samples were collected from tea plantations over the Darjeeling hills. Nutrient broth inoculated with the samples for growth of microfloral consortium was tested for tolerance against mercuric chloride ($HgCl_2$) at concentrations 2.5 mg/mL and 5 mg/mL[6]. Pure culture isolation was followed for tolerant populations. Tolerance ability of the isolates was re-evaluated by growth on nutrient agar plates supplemented

with $HgCl_2$. The three pure culture isolates thus obtained were designated MTD10B, MTD10C and MTD10D. $HgCl_2$ discs of varying concentrations (0.1-1 mg/mL) placed on agar plates inoculated with the isolates elucidated the minimum inhibitory concentration (MIC) of mercury against these bacteria and their maximum tolerance concentration (MTC) towards the same.

Biochemical characterization: Biochemical characterization was conducted starting with Gram staining followed by tests for citrate utilization, nitrate reduction, gelatin and starch hydrolysis, methyl red (MR), Voges–Proskauer (VP), triple sugar iron test (TSI), test for motility (SIM) and for coagulase, catalase and urease activities[5].

Plant growth promotion (PGP): The isolates were qualitatively tested for production of indole acetic acid (IAA)[18], hydrogen cyanide (HCN) and ammonia[12]. Their phosphate solubilizing ability was evaluated by streaking on Pikovskayas agar and observing for a halo. Detection of siderophores was conducted following the SD-CASA plate assay[24]. Positive tests for PGP were repeated with added $HgCl_2$ (0.025mg/mL and 0.05mg/mL) in media to assess their ability to produce the same results under mercury stress.

Heavy metal cross tolerance: Tolerance towards other heavy metals was studied using salts of twelve prevalent heavy metals to determine their susceptibility towards them[6]. The salts were used at concentrations of 2.5 and 5 mg/mL as previously used to select for the tolerant microbes against mercury.

Growth Study: The growth pattern of the isolates under mercury stress was monitored by growing them in nutrient broth supplemented with $HgCl_2$ (0.05 mg/mL and 0.1 mg/mL). Broth devoid of mercury (Hg-) was used for comparison. Broths containing 0.05 and 0.1 mg/mL of mercury were tagged as Hg5+ and Hg 10+ respectively. The broths were inoculated with log phase MTD10B, MTD10C and MTD10D and incubated at 30ºC. The optical density (OD) was measured at 600 nm using Spectrophotometer (Agilent Cary 60 UV–Vis) at 24h intervals.

16S rRNA sequencing: Genomic DNA was isolated. 16S rDNA region of isolated DNA was amplified and purified via column purification and bi-directional cycle sequencing was carried out on ABI 3730 genetic analyzer. GeneTool was used to generate a consensus sequence and Blast analysis was done against sequences available in the NCBI GenBank database.

Antibiotic susceptibility study: The isolates were tested for susceptibility to commonly used, synthetic, semi-synthetic and natural antibiotics using antibiotic discs purchased from HiMedia, India. Assessment of inhibition zones was as per interpretive criteria provided by the antibiotic disc manufacturer (HiMedia). Previously reported data on cases of resistance were also used. Accordingly, they were classified as susceptible (S), intermediate (I) and resistant (R). Wherever clinical susceptibility breakpoints were unavailable, inhibition zones measuring above 13mm were marked susceptible, less than or equal to 5mm were marked resistant and zones measuring between 6mm and 12mm were considered intermediate in their susceptibility to the given antibiotic for that isolate.

Pot culture: Plastic pots filled with soil were sown with seeds of the common green bean (*Phaseolus vulgaris*) previously treated with each of the three bacterial isolate cultures. The experiment was carried out in triplicate sets for each isolate. The pots were kept at room temperature and irrigated with distilled water as required on alternative days. Initial inoculum of 100µl was added to the pots after one day. Inoculum was thereafter added on days 4, 8 and 12 at the rate of 100µl to each pot. A triplicate set of pots with untreated seeds sown was taken as control and no addition of inoculum was done for this set. Various parameters like initiation of shooting, increase in shoot length, leaf length and number were recorded over a period of 15 days. On the 15th day, the plants were uprooted and their root length and dry weight were also recorded for consideration.

Results and Discussion

The diverse microflora obtained from the soil of Darjeeling tea plantations tested for tolerance against mercury yielded three particularly high tolerance isolates. These were designated as MTD10B, MTD10C and MTD10D, are the subject of this study. The sequences obtained for MTD10B and MTD10C demonstrated 98.2% and 98.12% homology respectively with the bacterium *Brevundimonas diminuta* while MTD10D produced 99.18% homology with *Alcaligenes faecalis*. Sequence data of all three isolates have been deposited in GenBank with accession numbers MZ068202 (MTD10B), MZ068206 (MTD10C) and MZ068207 (MTD10D) respectively.

Morphologically, MTD10B and MTD10C formed circular, convex, opaque colonies without any distinct pigmentation on nutrient agar plates. MTD10D colonies were whitish grey, flat, with irregular edges. Interestingly, the same isolates when grown on plates supplemented with mercury showed significant differences in their colony morphology. MTD10B and MTD10C formed irregularly shaped colonies with visibly different dark yellow pigmentation. The pigmentation turned amber over extended periods of time. MTD10D also formed low convex dark yellow colonies with smoother edges. This change in colony morphology of the isolates can be ascribed to their dealing with extreme mercury stress.

Mercury has been known to cause significant morphological distortions of bacterial cells, previously reported for strains of *Brevundimonas diminuta* as well where it was shown to be an indication of mercury accumulation within cells[15]. Gram staining revealed Gram negative rods for all three

isolates, consistent with the molecular identification results. Biochemical tests carried out characterized the isolates further (Table 1). The isolates were found to be motile and catalase positive. The MR test yielded strong positive results for MTD10B but weak positive results for MTD10C and MTD10D. TSI results in case of MTD10B implied the fermentation of dextrose, lactose and/or sucrose.

In *Brevundimonas dimunuta*, lactose/sucrose utilization is generally absent[10]. As such MTD10B differs from the consensus but MTD10C is consistent with expected results. MTD10B demonstrated no ability to utilize citrate while MTD10C and MTD10D gave positive results for the same. *Brevundimonas diminuta* is typically unable to utilize citrate[17] which contradicts the positive results obtained for MTD10C. Nitrate reduction was only found in case of MTD10C as is sometimes known to be present in strains of *Brevundimonas diminuta*[17].

The MIC of mercury against the three isolates was found to be at a tremendous 0.2mg/mL of $HgCl_2$ which is considerably higher than reported for any mercury tolerant strains. The only other strain with a higher MIC for mercury is of MTD10A at 0.3mg/mL, also previously characterized by us and interestingly, from the same region of Darjeeling hills[3]. The isolates grew easily at their MTC of 0.1mg/mL $HgCl_2$ and thrived at 0.05mg/mL on both solid and liquid media. Mercury tolerance in *Brevundimonas diminuta* has been reported before[3], one of the cases being of the strain IITISM22 with a tolerance of 0.05mg/mL of $HgCl_2$ and a biosorption capacity of 666.6 mg/g at 298 K[27].

The level of tolerance being reported in this study is double that exhibited by IITISM22. *Alcaligenes faecalis* has few more reports of tolerance towards mercury. Especially noteworthy is a study on *Alcaligenes faecalis* isolated from industrial sludge capable of growth in the presence of mercury at concentrations as high as 5mg/g[11]. Tolerance is also reported in another study on marine *Alcaligenes sp.* to grow in the presence of 0.05mg/mL of mercury[7]. The tolerance ability of MTD10D in comparison to both studies is significantly higher.

PGP activity tests recorded the production of ammonia and absence of phosphate solubilization as well as IAA and HCN production in the isolates. In MTD10D, the ammonia production was much more pronounced. *Alcaligenes faecalis* has been characterized as being capable of degrading urea, thereby creating ammonia, consistent with the results obtained in this study. Ammonia production under mercury stressed condition was shown to be hindered for all three isolates. This suggests possible reallocation of resources towards survival resulting in the down regulation of the ability of these isolates, most notably MTD10D, to produce ammonia. The three isolates produced siderophores. Continued production of siderophores under mercury stressed condition may be a positive indication towards better ability of the cells to sustain themselves in limiting conditions.

Siderophore detection performed in this study under mercury stress highlighted MTD10B as a sturdier isolate as it gave positive results under both concentrations of mercury used. MTD10C and MTD10D only tested positive for siderophores at the reduced stress of 0.025mg/mL of mercury (Table 2). This is again a demonstration of diminished abilities of these isolates under stressed conditions.

Table 1
Morphological and biochemical characteristics

TEST	MTD10B	MTD10C	MTD10D
Colony Morphology under normal condition	Smooth, glistening, circular, convex, non-pigmented	Smooth, circular, glistening, convex, non-pigmented	Whitish grey, flat, frayed edges
Colony Morphology under mercury stress	Undulated margin, dull, dark yellow to amber, convex	Undulated margin, dull, dark yellow to amber, convex	Low convex, yellow, smoother edges
Transparency	Opaque	Opaque	Opaque
Shape	Rods	Rods	Rods
Gram stain	-	-	-
Methyl Red test	+	+	+
Triple Sugar Iron test	+	-	-
Citrate utilization	-	+	+
Coagulase test	-	-	-
Gelatin Hydrolysis	-	-	-
Nitrate reduction	-	+	-
Urease test	-	-	-
VP	-	-	-
SIM	+	+	+
Starch hydrolysis	-	-	-
Catalase	+	+	+

"-" = negative, "+" = positive

Significant differences were recorded in the tolerance pattern of the isolates towards the heavy metals used to test for cross tolerance (Table 3). It should be noted that the concentrations of heavy metals being used for this experiment are quite high with regard to standard expectations.

MTD10B exhibited extensive tolerance towards most of heavy metals tested for both 2.5 and 5mg/mL, only yielding to copper, silver and tin at 5mg/mL. MTD10C performed comparatively poorly only showing tolerance towards zinc, iron, cobalt, nickel and chromium at 2.5mg/mL and additionally lead at both 2.5 and 5mg/mL.

MTD10D demonstrated tolerance towards most of the heavy metals at the lower concentration of 2.5mg/mL but this tolerance was hindered at the higher concentration of 5mg/mL. When comparing all three isolates, copper, silver and tin proved most successful in inhibiting their growth.

Brevundimonas diminuta has detailed reports on tolerance towards arsenic and copper and has even been successfully utilized in conjunction with *Oryza sativa L.* and *Helianthus annuus L.* respectively for alleviation of the said heavy metals by enhanced phytoremediation[25,28].

It has also been used in the production of zinc nanoparticles owing to its good tolerance towards the metal[21]. While MTD10B and MTD10C have shown arsenic and zinc resistance at prominently elevated levels of 5mg/mL, these strains do not possess the tools necessary for survival under copper stress. *Alcaligenes faecalis* has various reports of being highly tolerant towards heavy metals like silver, lead, cadmium, arsenic, zinc, copper etc.

The results depicted by MTD10D corroborate this fact. The evaluation of multi-tolerance ability conducted of bacterial strains already characterized as being tolerant/resistant to a target heavy metal is important as the goal is to establish these strains as candidates for remediation of the said target metal. In all likelihood, these bacteria have to survive varied contaminants acting against them in a noxious mix in order to be viable remediation candidates outside of Petri dishes. As such, cross tolerance studies are an important first step to fully understand their potential in dealing with and eliminating stresses and informing our designs for utilization of the bacterial strains.

Table 2
Plant growth promoting properties

TEST	MTD10B		MTD10C		MTD10D	
IAA production	-		-		-	
HCN production	-		-		-	
Ammonia production	+		+		++	
Ammonia production under mercury (Hg) stress (mg/mL)	**0.025**	**0.05**	**0.025**	**0.05**	**0.025**	**0.05**
	-	-	-	-	-	-
Siderophore production	+		+		+	
Siderophores production under mercury (Hg) stress (mg/mL)	**0.025**	**0.05**	**0.025**	**0.05**	**0.025**	**0.5**
	+	+	+	-	+	-
Phosphate solubilization	-		-		-	

"-" = negative, "+" = positive, "++" = strongly positive

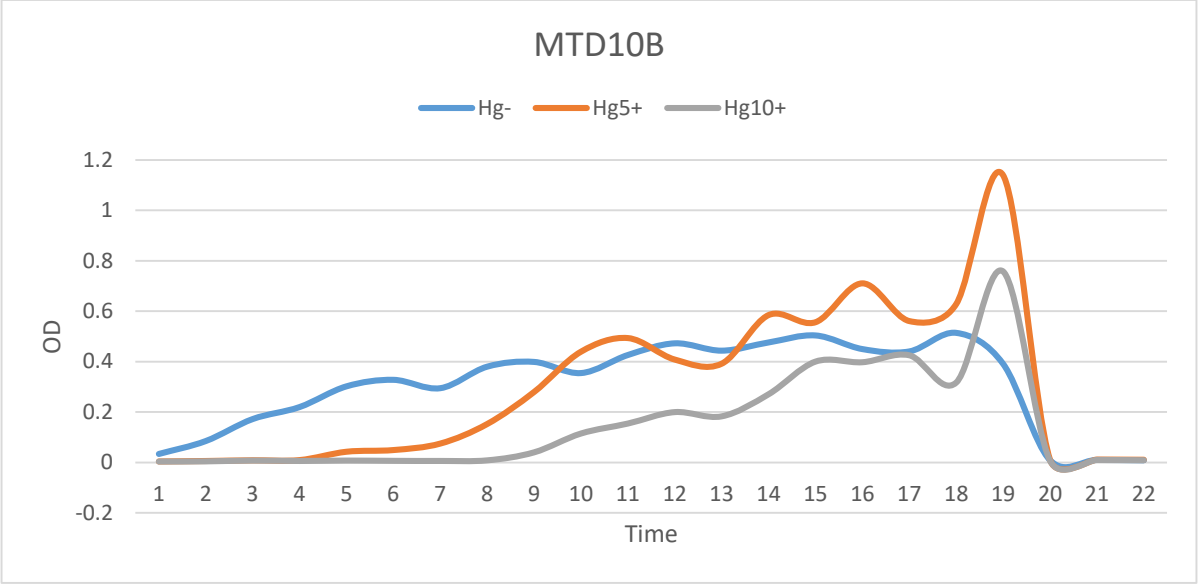
Table 3
Heavy metal cross tolerance

HEAVY METAL	MTD10B (mg/mL)		MTD10C (mg/mL)		MTD10D (mg/mL)	
	2.5	**5**	**2.5**	**5**	**2.5**	**5**
Pb	-	-	-	+	+	+
Sn	-	+	+	+	-	+
Cu	-	+	+	+	+	+
Mn	-	-	+	+	-	+
As	-	-	+	+	-	+
Zn	-	-	-	-	-	+
Cd	-	-	+	+	-	+
Fe	-	-	-	-	-	+
Ag	-	+	+	+	+	+
Co	-	-	-	-	-	-
Ni	-	-	-	-	-	-
Cr	-	-	-	-	-	-

"-" = negative, "+" = positive

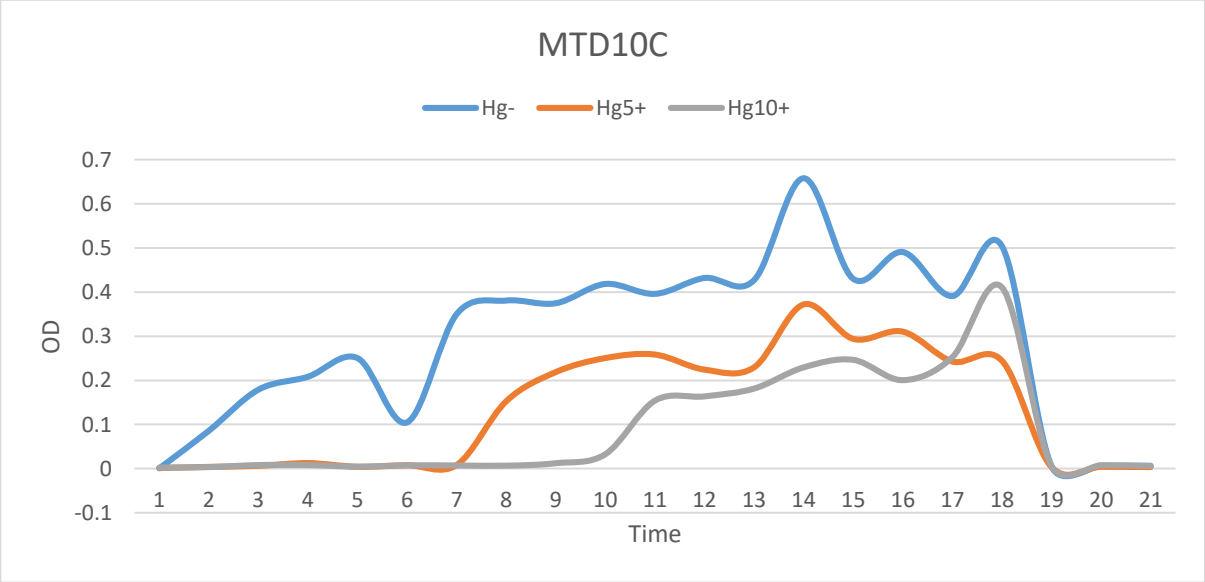
Growth pattern of the three isolates proved to be quite distinct. It is to be noted that this study on all three isolates was carried out at the same time and incubated under the same conditions. In the case of MTD10B, broth Hg- followed quite a different trend than Hg5+ and Hg10+ respectively. These in turn exhibited very similar growth to each other, albeit slightly delayed in onset in the case of Hg10+ (Figure 1). In case of MTD10C, growth pattern of all three broths was very similar in trend (Figure 2). The time of initiation of growth in the mercury stressed broths was the main difference shown. The Hg- broth expectedly reached the maximum OD. For MTD10D, the Hg5+ and Hg10+ broths varied significantly in growth. The Hg10+ broth visibly struggled with the mercury stress (Figure 3). Overall if we compare growth patterns of all three isolates in their respective broths with 0.05mg/mL of $HgCl_2$ (Figure 4), we can conclude that MTD10B is able to deal with the stress most effectively based on relative OD attained with and without stress. If we also take into consideration the point of initiation of growth and duration, they are able to withstand the mercury stress. MTD10D shows the better results. This comparison is arbitrary as the three isolates may have quite varying attributes with regards to growth rate, generation time etc. but does provide a baseline perspective on the overall performance of the isolates in optimum conditions versus under stress in relation to each other.

The antibiotic susceptibility pattern of the three isolates was analyzed based on their identification results (Table 4). Predictably the resistance profiles of MTD10B and MTD10C were almost interchangeable.



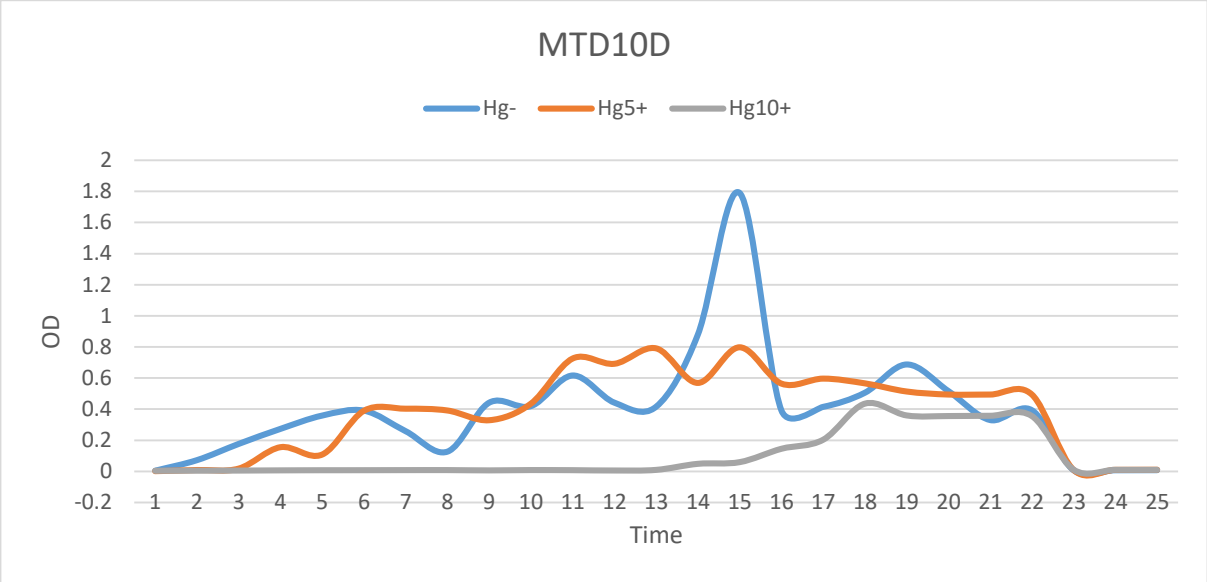
Hg- = broth devoid of $HgCl_2$, Hg5+ = broth with 0.05 mg/mL $HgCl_2$, Hg10+ = broth with 0.1 mg/mL $HgCl_2$

Figure 1: Comparative growth pattern of MTD10B



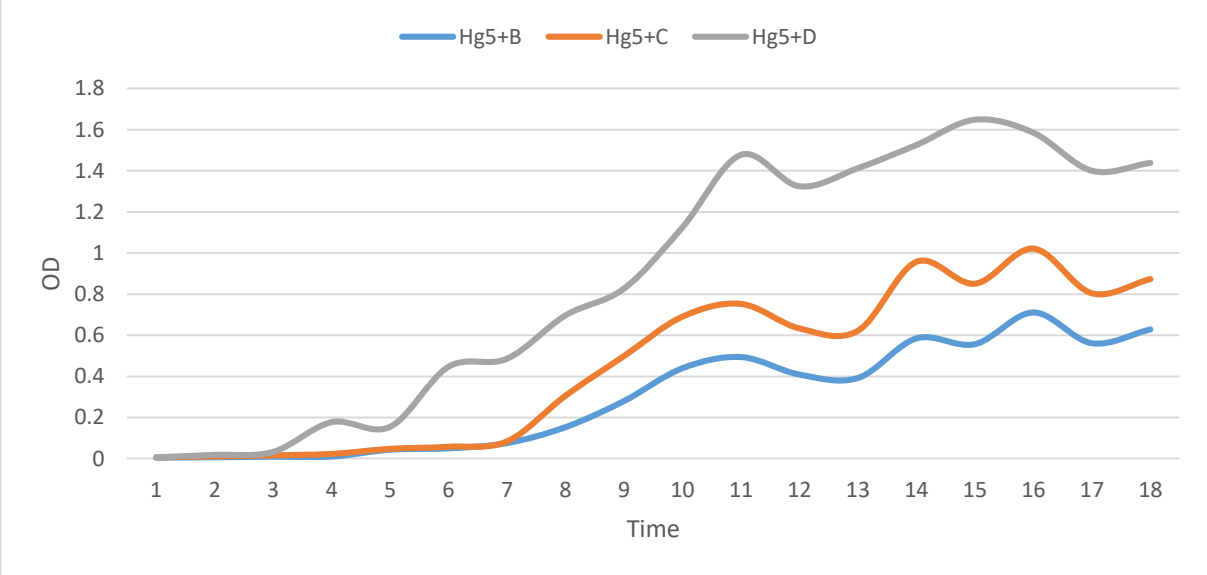
Hg- = broth devoid of $HgCl_2$, Hg5+ = broth with 0.05 mg/mL $HgCl_2$, Hg10+ = broth with 0.1 mg/mL $HgCl_2$

Figure 2: Comparative growth pattern of MTD10C



Hg- = broth devoid of $HgCl_2$, Hg5+ = broth with 0.05 mg/mL $HgCl_2$, Hg10+ = broth with 0.1 mg/mL $HgCl_2$

Figure 3: Comparative growth pattern of MTD10D



Hg5+B = MTD10B broth with 0.05 mg/mL $HgCl_2$, Hg5+C = MTD10C broth with 0.05 mg/mL $HgCl_2$, Hg5+D = MTD10D broth with 0.05 mg/mL $HgCl_2$

Figure 4: Comparison in growth pattern between MTD10B, MTD10C and MTD10D

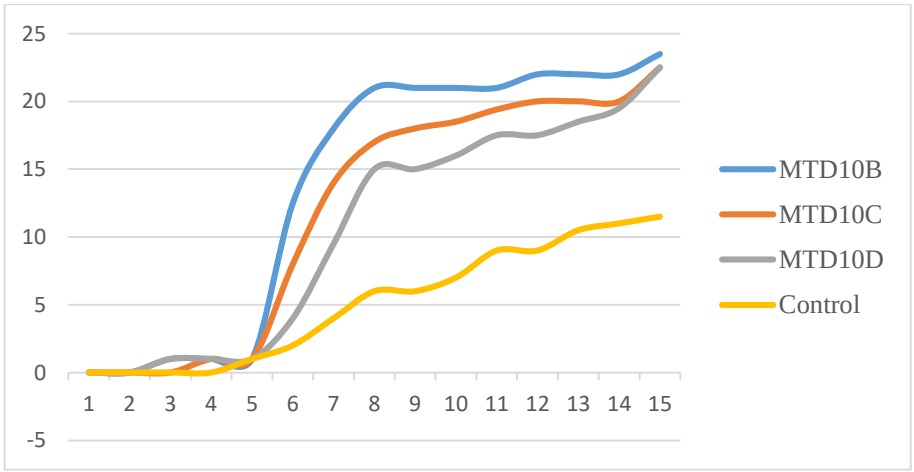


Figure 5: Comparative increase in shoot length in pot culture

Differences in susceptibility towards a few were still evident with MTD10B showing susceptibility towards streptomycin, tigecycline and chloramphenicol and resistance towards colistin. A colistin resistant strain of *Brevundimonas diminuta* has been studied as an opportunistic pathogen in a patient with cystic fibrosis[20]. Tigeclycline, a glycylcycline (minocycline derivative), is often shown to have an effect on organisms that are resistant to other tetracyclines.

MTD10C was in turn resistant to streptomycin and tigecycline and showed intermediate susceptibility to colistin and chloramphenicol. MTD10C was also resistant to erythromycin for which MTD10B gave intermediate susceptibility. Both isolates were additionally susceptible to amoxicillin, kanamycin, azithromycin, spectinomycin, pristinamycin and bacitracin.

The results suggest more susceptibility towards natural antibiotics than the synthetic antibiotics used. The susceptibility pattern of MTD10D displayed widespread resistance to most of the antibiotics used which is in congruence with the extensive reports of antibiotic resistance in *Alcaligenes faecalis*[13].

Table 4
Antibiotic susceptibility of MTD10B, MTD10C and MTD10D

Drug Class	Antibiotics	mcg/units	MTD10B	MTD10C	MTD10D
Cephalosporin/Beta-lactam	Cephalothin	30	R	R	R
	Penicillin	10	I	I	R
	Ampicillin	10	R	R	R
	Cefoxitin	30	R	R	I
	Cefuroxime	30	R	R	R
	Methicillin	5	R	R	R
	Amoxicillin	30	S	S	R
	Ceftazidime	30	R	R	R
Carbapenem	Imipenem	10	R	R	I
Sulfonamide	Cotrimoxazole	25	R	R	R
Non-sulfonamide	Trimethoprim	5	R	R	R
Fluoroquinolones	Ofloxacin	5	R	R	R
	Ciprofloxacin	5	R	R	R
	Enoxacin	10	R	R	R
	Moxifloxacin	5	I	I	R
	Gemifloxacin	5	R	R	R
Aminocoumarin	Novobiocin	5	R	R	R
	Novobiocin	30	R	R	R
Antimycobacterial	Rifampin	5	R	R	R
Aminoglycoside	Gentamicin	10	R	R	R
	Streptomycin	10	S	R	S
	Kanamycin	30	S	S	S
Glycyclcycline	Tigecycline	15	S	R	R
Macrolide	Erythromycin	15	I	R	R
	Azithromycin	15	S	S	I
Chloramphenicol	Chloramphenicol	30	S	I	R
Fosfomycin	Fosfomycin	200	R	R	R
Glycopeptide	Vancomycin	30	R	R	R
	Teicoplanin	30	R	R	R
Lincomycin	Clindamycin	2	R	R	R
Tetracycline	Tetracycline	30	I	I	R
Oxazolidinones	Linezolid	30	I	I	R
Nitrofurantoin	Nitrofurantoin	300	R	R	R
Fusidane	Fusidic Acid	10	R	R	R
Aminocyclitol	Spectinomycin	100	S	S	S
Polymyxin	Polymyxin B	300	I	I	S
	Colistin	10	R	I	S
Streptogramin	Pristinamycin	15	S	S	S
Polypeptide	Bacitracin	8	S	S	S

S = susceptible, I = Intermediate, R = Resistant

Table 5
Pot culture assessment

Parameters	MTD10B	MTD10C	MTD10D	Control
Average shoot length (cm)	23.5	22.5	22.5	11.5
Average root length (cm)	14.4	13.5	13.07	12
Average leaf length (cm)	5	3.7	5.7	3.5
Dry weight (g)	0.76	0.39	0.54	0.3

MTD10D was only susceptible to bacitracin, pristinamycin, colistin, polymixin B, spectinomycin, streptomycin and kanamycin, peculiarly, all natural antibiotics used. The susceptibility of *Alcalignes faecalis* to kanamycin is recorded in published literature as a consistent trait[23]. MTD10D also exhibited intermediate susceptibility for imipenem and azithromycin. Cefoxitin resistance is usually a sign of *mec*A mediated resitance in bacterial strains.

Resistance to it was demonstrated by MTD10B and MTD10C but not MTD10D which had intermediate susceptibility towards it. This suggests presence of *mec*A mediated resistance to beta-lactam antibiotics in the former two isolates. This correlates with the results obtained for the other beta-lactam antibiotics used in the study except for penicillin to which they were marked intermediate. *BlaVIM-2* and *BlaVIM-13*, mediators of resistance to almost all β-lactams, have previously been reported in both environmental and clinical isolates of *Brevundimonas diminuta*[1]. All three isolates were resistant to most fluoroquinolones except moxifloxacin, a fourth-generation fluoroquinolone.

Moxifloxacin showed intermediate activity against MTD10B and MTD10C. The presence of intrinsic quinolone resistance has been reported for *Bevundimonas diminuta* isolated from clinical samples due to the presence of QRDR or quinolone resistance determining region hosted by the bacterium[14]. There is mounting evidence for a positive correlation between heavy metal and antibiotic resistance within microbial communities. It has been suggested that the genes involved in heavy metal resistance and antibiotic resistance may be co-selected, especially when present on chromosomes rather than plasmids.

In case of plasmids, a negative correlation has also been suggested in metagenomic studies[29]. The undue stress of heavy metals in the environment may even directly create selective pressure for the picking or retention of antibiotic resistance genes. The resistance pattern of the three isolates in this study is quite expansive and advocates for this hypothesis. These strains were isolated from soil collected from tea plantations.

Hence there is no conceivable potential for exposure to such antibiotics to cultivate a resistance towards them. The skewed susceptibility towards natural antibiotics is also odd considering their location suggesting a more naturally innocuous microbial community in these areas. The pot culture experiment carried out clearly depicts an improvement in the growth rate of the common bean plant when compared to the control. The 15day experiment recorded faster initiation of shooting, increased average shoot length, increased average leaf length and average root length for the plants in comparison to control.

The average dry weight recorded also showed an increase against the control. Among the three isolates, MTD10B produced the best overall results followed by MTD10D and then MTD10C (Table 5). The graph (Figure 5) illustrates clearly the increased growth rate of the plants in presence of these isolates and strongly suggests the potential benefit of integrating such bacterial isolates into a holistic approach to reclamation and rejuvenation of degraded soil.

Conclusion

Often when looking to study heavy metal tolerant microflora, scientists logically search deeply contaminated sites. In these environments, microbes, especially tolerant bacterial populations diversify and devise numerous innovative ways to alleviate the contaminant from within themselves and the environment around them. In this study we considered the Darjeeling tea plantations as the area of study. The preliminary positive outcome of this study affirms the potential of the unique geological signature of this region as a part of the Himalayas, a young fold mountain and highlights the need to explore the diverse and distinctive microflora harbored by this relatively unexplored region.

Evidently, strains isolated from this region have exhibited not only a much higher capacity of tolerance towards highly toxic heavy metals but also unusually extensive resistance towards antibiotics commenting again on the rampant spread of antibiotic resistance in the world today. Tea plantation soil provides an exclusive niche for the exploration of relevant microbes with important qualities such as high mercury tolerance that needs further investigation. This may be due to the distinctive geological and climactic profile of the region or the unchecked abuse of biocides that has unfortunately ensued for too long in the numerous small and even larger, world-renowned tea plantations encompassing this area.

The problem with mercury has been its extremely high toxicity in comparison to most other pollutants that make it especially uncooperative and therefore, persistent. Bacterial isolates with higher tolerance abilities, able to face the challenges of mercury toxicity with a stronger arsenal may be a way forward to circumnavigate part of the problem. Thus, it is of imperative importance that high tolerance

bacterial strains such as MTD10B, MTD10C and MTD10D be studied thoroughly in order to properly comprehend and evaluate their potential for effective use in bioremediation.

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