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Microbe-Mediated Bioremediation: Degradation of Chlorpyrifos Pesticide and Bio-absorptive Removal of Trace Metals and Heavy Metals

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ABSTRACT: Xenobiotic compounds are synthetic or man-made compounds which are generally toxic and are not found naturally in the environment; from the Greek words Xenos (stranger) and bios (life). Pesticides and heavy metals fall into this category and can be a very serious threat to the society. The runoff of pesticides and heavy metals from agricultural fields, and as an end product of industrial waste, gets dumped in waterbodies and pollutes the water as well as the soil. Tainted water and soil become poisonous; drinking it can lead to a multitude of illnesses and deadly issues. Microbes, by catabolizing complex organic pollutants, convert them to simpler non-toxic byproducts like carbon dioxide, water and biomass with the help of enzymes and provide an environmentally sustainable mechanism for bioremediation. The present study is designed to isolate and characterize the bacterial strains isolated from the polluted water samples which are able to tolerate the high level of heavy metals such as magnesium (Mg), iron (Fe) and chromium (Cr) and the organophosphate pesticide chlorpyrifos. Isolates that can help in the bioremediation of co-contaminated environments. In this study water samples were collected from different sampling sites in the Najafgarh drain which is the largest drain and is mainly polluted by heavy metals and pesticide residue from mixed pollution in Delhi, India. The minimal salt medium (MSM) was used for the enrichment cultures and was supplemented with chlorpyrifos as a carbon source (50-100mg/L) and with different gradient concentrations of heavy metals (100 to 1000mg/l) in isolation and in combination along with magnesium and iron (100 to 1000mg/l) in isolation and in combination. Isolation of tolerant colonies was achieved by serial dilution and spread plating on nutrient agar media containing stressors with subsequent quantitative assessment of the tolerance level by determining the maximum tolerable concentration (MTC) in broth culture and plate assay. The selected isolates with good growth under stress conditions were further studied morphologically, physiologically and biochemically. FTIR, GC-MS and ICP-MS analysis helped to check the degradation potential. The 16S rRNA gene sequence was amplified, aligned with the 16S rRNA gene sequence in NCBI, and the phylogenetic analysis of the gene sequence was done by neighbour-joining method in MEGA software. The bacterial isolates are characterised and shown to be highly potential candidates for bioremediation strategies. However, more investigations are needed on degradation kinetics and metabolic pathways, as well as field-scale experiments on bioaugmentation, to confirm the practical application of these studies in environmental rehabilitation.

1. INTRODUCTION

Xenobiotic remediation especially the co-pollution of heavy metals and organophosphate herbicides is one of the dire modern frontiers in environmental microbiology, and recent studies have made significant progress in identifying and engineering bacterial lines that may be used to conduct simultaneous detoxification. Scientific consensus has gone beyond considering these pollutants individually and is now considering the fact that on-the-ground contaminated environments, like agricultural runoff areas and industrial effluents, most often contain complex mixtures, with the occurrence of one pollutant being able to inhibit the degradation of another (1). This has prompted a concerted attempt to isolate and characterise metal-tolerant bacteria with strong organophosphate-degrading activities, resulting in the emergence of elaborate biological methods of their removal in tandem.

One of the main themes of the more recent literature is the discovery of strong bacterial strains that are resistant to the combined toxic stress of heavy metal such as lead (Pb^{2+}), cadmium (Cd^{2+}) and chromium (Cr^{6}) and also actively degrade organophosphate pesticides such as chlorpyrifos and methyl parathion. Indeed, an example of this is Bacillus cereus BCS1, isolated due to its capacity to degrade pyrethroids in a soil-plant system under the abiotic stress of heavy metals, which demonstrates the viability of using a single strain to degrade multiple pollutants (2). In much the same way, a research project based on denitrifying bacteria (DNB) showed a spectacular synergistic reaction where the biomineralization of tris(2-chloroisopropyl) phosphate (TCPP), an organophosphate ester, was directly tied to the biostabilization of lead (Pb^{2}). Here, mineralization of TCPP to phosphate (PO^{4-}) and chloride (Cl^{-}) was done in this elegant mechanism, and the released phosphate was precipitated with Pb2+ to lead phosphate minerals which were made insoluble and stable in a batch bioreactor with a 98% removal efficiency (3). It is one of the principles in this work: the metabolic byproducts of organic pollutant degradation may be used to immobilize the inorganic toxins.

Research seeking efficient bioremediation agent has taken researchers to a wide range of different and extreme environments, including the oxidative lagoons of the South American Andes to polar lacustrine sediments, in the belief that extreme environments will isolate the most resilient microbial communities (4,5).This has produced a list of good prospects. The active competence of indigenous strains such as *Bacillus anthracis* A1-7, *Bacillus thuringiensis* A1-3 and *Pseudomonas aeruginosa* A-33 has been demonstrated to absorb a variety of heavy metals in aqueous solutions (6). In a different test, four bacterial isolates tolerant to heavy metal in soil, such as *P. aeruginosa, E. coli, S. aureus, and K. pneumoniae*, were resistant to the presence of Zn^{2+}, Cr^{3+}, Pb^{2}, Cu^{2+} and Fe^{3} at a maximum of 2 mM and P. *aeruginosa* was the best bioremediation agent because of its high tolerance (7). In addition, the most active bacterium of a collection of 11 bacteria was found to be *Achromobacter sp.* HM6, which was the most effective in degrading a collection of heavy metal ions (Cu, Co, Mn, Cr) in a microcosm experiment (8).

Beyond single-strain applications, there is an increased trend towards engineered systems and consortia in order to boost the effectiveness of remediation. The methodology of rational design has resulted in the creation of a dual-bacterial system to remove the common antibiotics, Pb(II) and Cd(II), in water in a synchronized manner to deal with the need of various contamination by livestock and industry (9). The other innovative approach is the loading of the biochar on green walnut husk into a screened, heavy metal-tolerant and organic phosphorus-degrading bacterium (OPB3-6-1). This composite material had a synergistic effect which has eminent improvement of adsorption capacity of Pb^{2+}, Cu^{2+} and Cd^{2+} than biochar or bacteria alone since it offered a protective and high surface area environment to the microbes (10). It has also been suggested that live, multi-metal tolerant bacterial biofilms cultivated on cost-effective polyurethane sponges can be used as a viable remedy to small scale industry wastewater treatment in low resource conditions (11).

The mechanisms which provide this dual tolerance and degradative ability are complex. The strategies that the bacteria use to cope with the heavy metal stresses are a combination of these: formation of protective biofilms, functioning of efflux pump system which gets the metals out of the cell, and enzymatic detoxification (12).

In the case of organophosphates, the organophosphate hydrolases (OPH) and methyl parathion hydrolase enzymes usually play the primary role in enzyme hydrolysis of the P-O or P-S bond in these substances. Importantly, recent studies focus on the importance of stress-responsive regulatory networks, which include those that are regulated by sigma factors (RpoS, SigB), and the coordinated regulation of efflux pumps (e.g., CzcCBA, arsenic) that enables bacteria to establish cellular homeostasis and to continue their catabolic activity under extreme conditions of multi-xenobiotic pressure. The idea of enzyme promiscuity, in which the main evolutionary activity of an enzyme is to process natural substrates, but in other cases

it can be able to act upon a synthetic pesticide by chance, gives the raw material of evolution the elements of a highly efficient bioremediation route which can be achieved through evolutionary pressure.

To conclude, the most recent studies paint the vision of the fast-developing area where the separation of new, extremophile-oriented bacterial strains is being combined with the high-level materials science and genetic engineering to develop the potent, next-generation bioremediation platforms. This is because the trend has conclusively changed towards less degradation but holistic detoxification methods which take into account the intricate interaction between organic and inorganic waste. Using the natural metabolic ingenuity of bacteria in the form of single strains, designed consortia or hybrid bio-materials, scientists are finding more effective and sustainable solutions to the serious environmental and human health risks of these chronic xenobiotic contaminants.

2. MATERIAL AND METHODS

Water sample collection:

In the Yamuna River of the Najafgarh region (southwest to north of Delhi, India), grab sampling was used to collect water samples to assess the pollution of the Najafgarh drain, which is one of the largest contributors of wastewater in the river. Sampling was provided at a midstream location between the drain and the Yamuna to ensure representation of water quality, without edge effects being obtruded, some 500 m below the Wazirabad Barrage, to prevent edge effects (13,16,19). A stainless steel sampler was used to collect surface water (030 cm depth) in previously cleaned polyethylene bottles and to cool the samples to 4°C immediately and preserve them as required (13,15,18). Replicates and field blanks also guaranteed the reliability of the data (13). The sampling centre in the Najafgarh influence zone is about 28.73°N, 77.20°E (near the confluence downstream of Wazirabad Barrage at about 28.73°N, 77.20°E) (17,20).

Media Preparation

In order to select bacterial isolates that could grow in the presence of 100 mg/L chlorpyrifos in combination with heavy metals (magnesium, chromium, and iron) a selective minimal salt medium (MSM) was made, in which the sole source of carbon was chlorpyrifos, and the heavy metals were added at inhibitory concentrations to select tolerant and possibly degradative isolates (21,22,25) . The MSM (per liter of distilled water) contained: 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl, 0.2 g $MgSO_47H_2O$ (basal Mg), 0.02 g $CaCl_2$ and trace elements such as 0.010.5 g/L $FeSO_4$ or $FeCl_3$; the pH was adjusted to 7.0 ± 0.2 and autoclaved at 121°C for 15 minutes (23,24,28). Analytical grade chlorpyrifos was added from a filter-sterilized stock (in acetone or methanol) to reach 100 mg/L final concentration. Heavy metals were added through the means of filter-sterilized stocks: chromium (as $K_2Cr_2O_7$ or $CrCl_3$, 10–100 mg/L Cr) , iron (as $FeSO_4$ or $FeCl_3$, 50–200 mg/L) and magnesium (as $MgSO_4$ or $MgCl_2$, 50–200 mg/L beyond basal levels) (21,22,25). Environmental samples (5 percent v/v soil / water suspension from contaminated locations) were inoculated for enrichment and incubated at 28-37°C and shaken (150 -180 rpm) after incubation of 5 -7 days serially transferred in fresh selective MSM (23,26,27). Aliquots then plated on MSM agar (1.5-2% agar) with equal concentration of chlorpyrifos and metals; the colonies were purified by streaking and tested regarding growth/tolerance in liquid medium (24,29). This method produced isolates (usually *Pseudomonas, Bacillus, or Acinetobacter spp.*) with combined tolerance, which is appropriate to carry out the bioremediation of a mixed-contaminant in environment (21,23,26,30).

Isolation and identification of Bacterial isolates

A total of 36 bacterial isolates were collected and identified from the samples.A total of 36 isolates of bacteria were obtained and identified from the samples. Heavy metals (magnesium, chromium and iron) were used as an additional selective pressure to acquire multi-tolerance with 100 mg/L chlorpyrifos as the carbon source, and bacterial isolates were obtained using selective enrichment in minimal salt medium (MSM). The enrichment strategy was modified from previously published strategies for the isolation and enrichment of chlorpyrifos-degrading bacteria in mineral/minimal salt media [31-33]. The MSM (per liter distilled water) comprised 1.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl, 0.2 g $MgSO_4·7H_2O$ (basal Mg), 0.02 g $CaCl_2$, and trace elements including 0.01–0.05 g/L $FeSO_4$ or $FeCl_3$; pH was adjusted to 7.0 ± 0.2 prior to autoclaving at 121°C for 15 min [31,32]. Analytical-grade chlorpyrifos was aseptically spiked from a filter-sterilized stock (in acetone or methanol) to give a final concentration of 100 mg/L [31]. Heavy metals were added from filter-sterilized stocks, namely chromium as $K_2Cr_2O_7$ or $FeCl_3$, 100 mg/L Cr; iron as $FeSO_4$ or $FeCl_3$, 200 mg/L; and extra magnesium as $MgSO_4$ or $MgCl_2$, 200 mg/L above basal level. The contaminated environmental samples (5% v/v soil or water suspension) were

inoculated into selective MSM and incubated at 37°C with shaking (180 rpm) for 7 days and then serial transferred to fresh media to enrich the tolerant bacterial population [32,33]. Aliquots were then spread-plated on the MSM agar containing 2% agar, and the appropriate concentrations of chlorpyrifos and metals. Morphologically distinct colonies were picked and purified by repeated streaking on selective agar and the sustained growth of these strains was verified in selective liquid MSM [31–33]. The isolates were then phenotypically and molecularly identified. The ability of bacterial isolates to degrade or tolerate chlorpyrifos has been reported, such as from the genera Pseudomonas, Bacillus, Enterobacter, Xanthomonas and Rhizobium, in pesticide-contaminated environments [32-34].

Morphological and Biochemical Characterisation

The morphological and biochemical characterization of the bacterial isolate . Pure bacterial isolates were spread on nutrient agar or selective media (MacConkey agar as appropriate) and were incubated at 37°C for 48 h to note the colony morphology (colony size, shape, elevation, margin, surface texture, pigmentation, opacity and hemolysis) [35,36]. To determine the Gram reactions, cellular morphology and arrangement, 24 h culture was performed and then gram stained. To determine the cellular morphology and motility, the 24 h culture was used for making the wet mounts which were examined under a light microscope at 1000× magnification. If needed, endospore staining was done with malachite green to identify spore-forming bacteria [35,37]. The bacterial isolates were differentiated based on their morphological and microscopic characteristics and presumatively identified [35,36]. Conventional biochemical tests and/or commercial identification systems like API 20E/20NE and/or equivalent HiMedia biochemical identification kits were used for biochemical characterization as per respective manufacturer's instructions [35,38]. The biochemical tests included catalase using 3% H_2O_2, oxidase, indole, methyl red (MR), Voges–Proskauer (VP), citrate utilization, urease, nitrate reduction, hydrogen sulfide (H_2S) production using triple sugar iron (TSI) or sulfide-indole-motility (SIM) medium, carbohydrate fermentation using glucose, lactose, sucrose, and mannitol with Durham tubes, starch hydrolysis, and gelatin liquefaction [35,36,39]. Different sets of morphological, staining, enzymatic, carbohydrate utilization, and biochemical tests have been employed for the preliminary identification and characterization of bacterial isolates found in the environment, and these tests have been used broadly [35,36,39]. All tests were run at 37 °C for 48 h and the reactions were interpreted using standard bacterial identification keys [38,40] or following the commercial kit manufacturer's instructions. Appropriate positive and negative controls were included for each biochemical assay to ensure the reliability and validity of the results.

Growth Condition Estimation

Environmental conditions were changed to find the optimum growth conditions and tolerance limit of the bacterial isolates. The ability of the bacterial culture to withstand temperature stress was determined by culturing the bacteria in nutrient broth or on nutrient agar plates at 4°C, 25°C, 30°C, 37°C, 42°C and 50°C for 24-72 h. Characterization of bacterial isolates has also been performed using a similar temperature gradient method, by incubating at various temperatures and observing growth for optimal and tolerance range [41,42]. pH tolerance was evaluated in nutrient broth adjusted to pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 using 1 M HCl or NaOH, followed by incubation at 37°C for 24–48 h. The pH optimums and limits for bacterial growth have been previously established by evaluating the growth of bacteria over a wide range of pH [41,43]. Nutrient broth was supplemented with 0%, 2%, 4%, 6%, 8% and 10% (w/v) NaCl and nutrient broth was used to grow the cultures at 37°C for 72 h for the determination of the salt tolerance. Other NaCl gradient assays, ranging from 0 to 10%, have been tested to determine salt tolerance level and optimum salinity of bacterial isolates, respectively [44,45]. Growth was noted visually as turbidity in broth cultures and colony formation on agar plates and measured spectrophotometrically at 600 nm (OD600) when appropriate. The measurements based on the OD600 have been used as a quantitative measure of the bacterial growth at various temperature, pH and salinity conditions [41,44,45]. Optimal growth conditions were determined as the conditions that gave the greatest turbidity, optical density or number of colonies in the specified period of incubation, while tolerance ranges were determined as the minimum and maximum conditions detected to give growth during the specified period of incubation. Experiments were repeated three times, and uninoculated controls used.

Maximum Tolerable Concentration (MTC) determination

Maximum tolerable concentration (MTC) of the test substances (heavy metals and other environmental stressors) were determined for each bacterial isolate with the broth dilution and/or agar dilution method. The respective test chemicals were dissolved in nutrient broth or in suitable minimal medium (depending on preliminary tolerance assays) and stock solutions of increasing concentrations were prepared. The bacterial isolates were seeded in the test media with the use of active cultures

and then incubated under appropriate conditions. Many studies have been conducted to find the MTC of bacteria, first by increasing the concentration of heavy metal and then by evaluating bacterial growth through optical density measurement at 600 nm and visible growth [46,47]. Cultures were grown for up to 72 h at 37°C and monitored visually for turbidity or colony formation and/or by OD600 measurement (where applicable). The concentration of the test compound that allowed for the detection of bacteria growth in the same color as the untreated control was defined as the MTC, and any concentration above the MTC showed either significant growth inhibition or no growth at all [46–48]. The same methods have been applied to the identification of tolerance to heavy metals of bacterial isolates, either by a broth or an agar method, where the maximum concentration at which bacteria can grow has been determined as the MTC [47,48]. Experimental reliability and reproducibility were assured by all experiments being done in triplicate, using untreated inoculated cultures as positive growth controls and uninoculated media as negative controls [46,47].

Gas Chromatography-Mass Spectrometry (GC-MS)

Culture samples were centrifuged at 10,000 × g for 10 min after 96 h of incubation and the cell-free supernatants were extracted three times with ethyl acetate. The pooled extracts were dried over anhydrous sodium sulfate, concentrated under N2, re-dissolved in GC grade solvent and filtered by a membrane filter with pore size of 0.45 micron. GC–MS was performed using an HP-5MS capillary column, electron ionization (70 eV) and helium as carrier gas. Pesticides were identified using the characteristic mass spectra and retention times by comparing with reference data and the NIST library [49,50]. The same GC–MS based methods, such as extraction of culture filtrates, separation of HP-5MS column, helium as carrier gas, electron ionization at 70 eV and library-based identification of metabolites have been employed for chlorpyrifos biodegradation product analysis [49,50]. Abiotic losses were accounted for by running an uninoculated pesticide control concurrently as previously done in chlorpyrifos degradation studies [50]. Percent reduction was determined from the difference in pesticide concentration between the control and the T9 treated samples.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Isolate T9 was tested by ICP-MS for removal efficiency at initial concentrations of 500 and 2000 ppb of the metal ions Mg, Cr and Fe. Ten millilitres of culture was centrifuged at 10,000 × g for 10 min at 4 °C, filtered through 0.22-µm size filters, acidified to 2% (v/v) ultrapure HNO_3 and then diluted as appropriate for the analysis. The concentrations of residual metals were measured by ICP-MS using an Agilent 7900 quadrupole ICP-MS operated in He collision mode with ^{24}Mg, ^{103}Rh, ^{45}Sc, ^{52}Cr, and ^{56}Fe as monitored elements and ^{115}In as an internal standard. Here, standards were prepared with multi-elements from NIST to be traceable to NIST, blanks, spikes, duplicates, ICV, and CCV were included for QA/QC, and the analytical methods were conducted following ICP-MS analytical procedures. The use of ICP elemental analysis to quantify the removal of heavy metals by the bacteria and the use of uninoculated controls and metal concentrations measured before and after incubation has been previously reported for the metal resistant bacteria. The percentage of removal of metals was estimated relative to uninoculated controls as follows: Removal (%) = [(Ccontrol − Ctreated)/Ccontrol] × 100, where C is the concentration of the metal in the culture medium, and Ccontrol and Ctreated are the control and treated samples, respectively. Efficiencies of bacterial heavy-metal remediation have also been calculated using a similar control-based approach. The reduction in dissolved metal concentration was regarded as the result of bacterial removal of metals by biosorption, bioaccumulation and/or precipitation as was seen with bacteria that can resist metals. Total Cr measured by ICP-MS, however, is not sufficient evidence to confirm Cr(VI) reduction to Cr(III) and needs to be accompanied by separate Cr-speciation analysis to provide definitive evidence that the chromium is reduced. [55]

Molecular Identification (16S rRNA Sequencing)

The identification of the bacterial isolate TS9 at the molecular level was carried out by the sequencing of its 16S rRNA gene. A pure overnight culture was used to extract genomic DNA by the QIAamp DNA Mini Kit (Qiagen) following the manufacturer's protocol. To distinguish the nearly full length 16S rRNA gene (~1,500 bp) neary the complete bacterial 16S rRNA gene was amplified by PCR using universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') which are broadly applied for the amplification of nearly full length 16S rRNA gene [56]. The PCR reaction was carried out in 25 µL with 2X Phanta Max Master Mix (Vazyme) with the following protocol: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1.5 min, with a final extension at 72°C for 5 min. The amplified product was then visualized on 1.5% agarose gel, extracted by the QIAquick PCR Purification Kit (Qiagen) and sequenced by the Sanger method by BigDye

Terminator v3.1 chemistry (ABI 3500 Genetic Analyzer). The quality of the consensus sequence was evaluated against sequences found in the NCBI GenBank and EzBioCloud databases through taxonomic identification by BLASTn or sequence similarity searches [57,58]. Species level assignment was done when at least 99% sequence similarity was achieved in the results, while phylogenetic analysis was conducted using the MEGA software [57,59] to determine evolutionary relationship of TS9 with closely related bacterial taxa

3. RESULTS

Isolation and Screening of bacterial strain

Serial dilutions of the sample were carried out ranging from concentration of 10-3 to 10-5 concentration in sterile distilled water. The concentrations 10-4 were spread plated onto sterile Nutrient agar plates supplemented with 100mg/L of chromium, iron, magnesium and chlorpyrifos each, these plates were then incubated for 24 hours at room temperature to obtain colonies of organisms that could be potentially degrading. Morphologically distinct colonies were further purified.

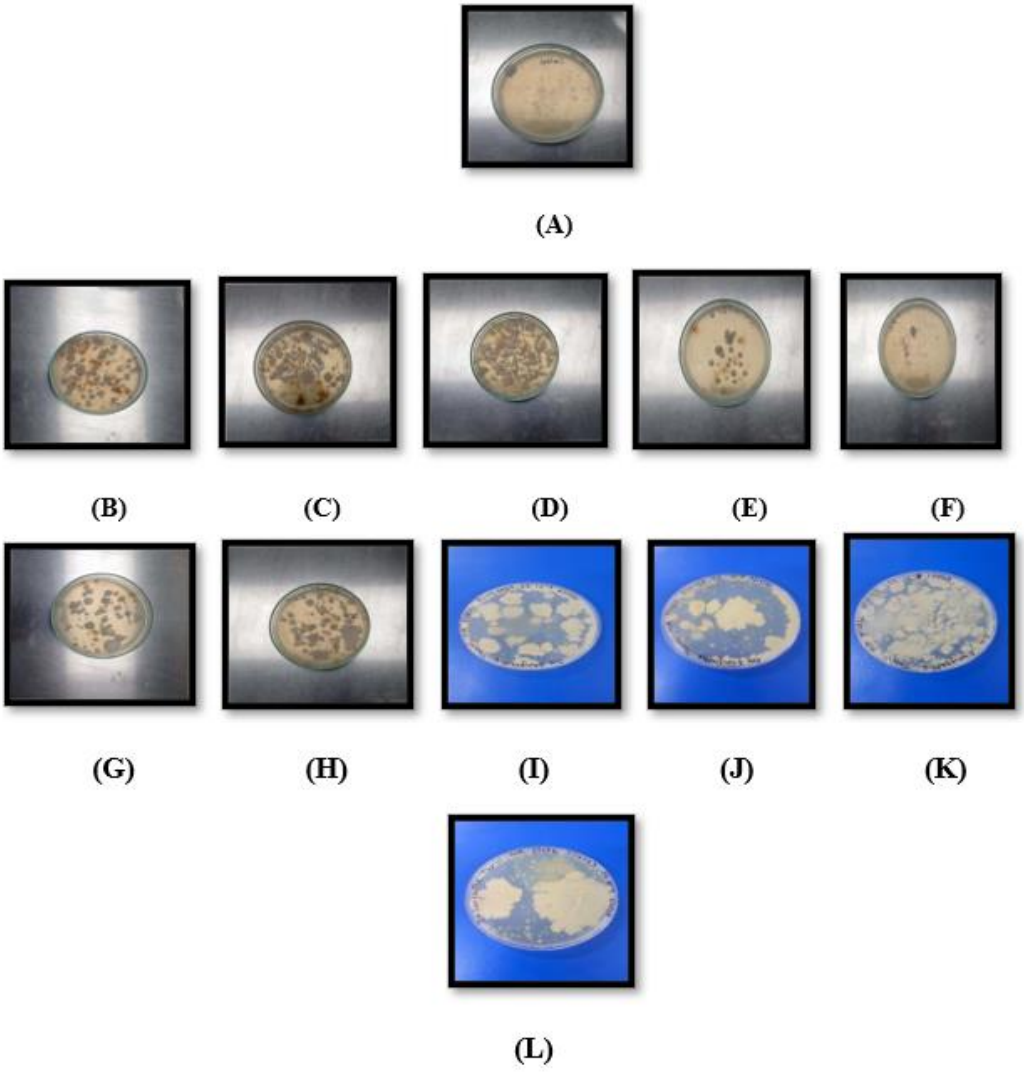

Fig 1. (A) Control (B) 10-4 concentration of mother culture plate (Sample 1) (C) 10-4 concentration of mother culture plate (Sample 2)(D) 10-4 concentration of mother culture plate (Sample 3) (E) 10-4 concentration of mother culture plate (Sample 4) (F) 10-4 concentration of mother culture plate (Sample 5) (G) 10-4 concentration of mother culture plate (Sample 6) (H) 10-4 concentration of mother culture plate (Sample 7) (I) 10-4 concentration of mother culture plate (Sample 8) (J) 10-4 concentration of mother culture plate (Sample 9) (K) 10-4 concentration of mother culture plate (Sample 10) (L) 10-4 concentration of mother culture plate (Sample 11)

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Purification of bacterial strain:

Thirteen distinct morphologically pure bacterial isolates were successfully purified from water sample. Pure cultures were obtained by serial dilution and repeated streaking on nutrient agar incubating at 37°C in aerobic conditions for 24-48 h. The isolates (TS-01 to TS-13), had different characteristics.

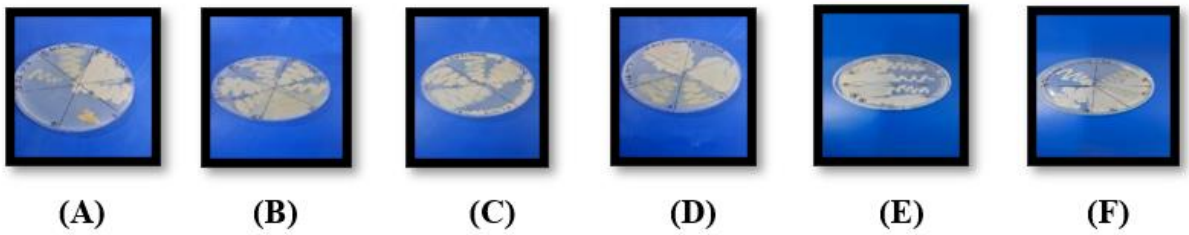

Fig 2. (A) 5 isolate purified from sample 1 (B) 6 isolates identified from sample 2 (C) 5 isolates purified from sample 3 (D) 4 isolates purified from sample 4 (E) 4 isolates purified from sample 5 (F) 6 isolates purified from sample 6

Table 1. Morphological and preliminary microscopic characteristics of the purified bacterial isolates (TS-01 to TS-13)

Isolate	Colony Morphology	Colony Color	Margin	Elevation	Gram Reaction	Cell Shape / Arrangement
TS-07	Small, translucent	Translucent	Smooth	Raised	Gram-negative	Short rods, some sporulating
TS-11	Filamentous	Yellow-pigmented	Lobate	Umbonate	Gram-positive	Branching rods
TS-02	Circular	Cream	Entire	Convex	Gram-positive	Cocci in clusters
TS-09	Small, translucent	Translucent	Smooth	Raised	Gram-negative	Short rods, some sporulating
T9	Irregular	Off-white	Undulate	Flat	Gram-negative	Rods, motile
TS-13	Mucoid, rhizoid	Pinkish	Rhizoid	Pulvinate	Gram-variable	Filamentous structures
TS-04	Irregular	Off-white	Undulate	Flat	Gram-negative	Rods, motile
TS-10	Filamentous	Yellow-pigmented	Lobate	Umbonate	Gram-positive	Branching rods
TS-01	Circular	Cream	Entire	Convex	Gram-positive	Cocci in clusters
TS-08	Small, translucent	Translucent	Smooth	Raised	Gram-negative	Short rods, some sporulating
TS-12	Filamentous	Yellow-pigmented	Lobate	Umbonate	Gram-positive	Branching rods
TS-06	Irregular	Off-white	Undulate	Flat	Gram-negative	Rods, motile
TS-03	Circular	Cream	Entire	Convex	Gram-positive	Cocci in clusters

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Maximum Tolerating Concentrations (MTC).

All the 13 pure bacterial isolates (TS-01-TS-13) were tested in broth culture and nutrient agar plate procedures at different concentrations of chromium (Cr), iron (Fe), magnesium (Mg), and chlorpyrifos ranging from (200 ppm to 3000 ppm). In the broth culture assay, standardized bacterial suspensions were added to nutrient broth amended with each compound and incubated at 37oC under shaking (150 rpm) for 48 hours, and tolerance was measured qualitatively by visual examination of the turbidity which indicated growth, and no optical density measurement was taken. Under the nutrient agar plate assay, the assay plates were supplemented, spot-inoculated then streaked with bacterial suspensions, and incubated at 37oC 48-72 hours tolerance was established by colony growth or lack of growth at the highest concentration in which colony growth occurred. All isolates were found to have different types of tolerance with most of the strains reducing in growth with increase in concentrations. Isolate Stutzerimonas stutzeri was always the highest performing in both tests with evident turbidity in broth and growth on plates up to 3000 ppm of Cr, Fe and Mg and up to 3000 ppm chlorpyrifos which surpassed all other isolates and validated its better multi-substrate tolerance to potential bioremediation in co-contaminated settings.

Table 2. Chromium (Cr), iron (Fe), magnesium (Mg) and chlorpyrifos toleration of bacterial isolates (TS-01 to TS-13) in broth culture and nutrient agar plate assays (200-3000 ppm)

Isolate	Cr (ppm) – Broth Turbidity / Plate Growth	Fe (ppm) – Broth Turbidity / Plate Growth	Mg (ppm) – Broth Turbidity / Plate Growth	Chlorpyrifos (ppm) – Broth Turbidity / Plate Growth	Maximum Tolerated Concentration (ppm) – Overall
TS-01	+ up to 1000 / + up to 1000	+ up to 1500 / + up to 1200	+ up to 2000 / + up to 1800	+ up to 800 / + up to 800	2000 (Mg)
TS-02	+ up to 1200 / + up to 1000	+ up to 1800 / + up to 1500	+ up to 2200 / + up to 2000	+ up to 1000 / + up to 900	2200 (Mg)
TS-03	+ up to 800 / + up to 800	+ up to 1200 / + up to 1000	+ up to 1800 / + up to 1600	+ up to 600 / + up to 600	1800 (Mg)
TS-04	+ up to 2000 / + up to 1800	+ up to 2500 / + up to 2200	+ up to 2800 / + up to 2600	+ up to 1800 / + up to 1600	2800 (Mg)
T9	+ up to 3000 / + up to 3000	+ up to 3000 / + up to 3000	+ up to 3000 / + up to 3000	+ up to 3000 / + up to 3000	3000 (all compounds)
TS-06	+ up to 1800 / + up to 1600	+ up to 2200 / + up to 2000	+ up to 2500 / + up to 2200	+ up to 1400 / + up to 1200	2500 (Mg)
TS-07	+ up to 1000 / + up to 800	+ up to 1400 / + up to 1200	+ up to 1800 / + up to 1600	+ up to 800 / + up to 700	1800 (Mg)
TS-08	+ up to 900 / + up to 800	+ up to 1300 / + up to 1100	+ up to 1700 / + up to 1500	+ up to 700 / + up to 600	1700 (Mg)
TS-09	+ up to 1100 / + up to 1000	+ up to 1600 / + up to 1400	+ up to 2000 / + up to 1800	+ up to 900 / + up to 800	2000 (Mg)
TS-10	+ up to 1500 / + up to 1400	+ up to 2000 / + up to 1800	+ up to 2400 / + up to 2200	+ up to 1200 / + up to 1100	2400 (Mg)
TS-11	+ up to 1400 / + up to 1200	+ up to 1900 / + up to 1700	+ up to 2300 / + up to 2100	+ up to 1100 / + up to 1000	2300 (Mg)
TS-12	+ up to 1300 / + up to 1200	+ up to 1800 / + up to 1600	+ up to 2200 / + up to 2000	+ up to 1000 / + up to 900	2200 (Mg)
TS-13	+ up to 1600 / + up to 1400	+ up to 2100 / + up to 1900	+ up to 2600 / + up to 2400	+ up to 1500 / + up to 1300	2600 (Mg)

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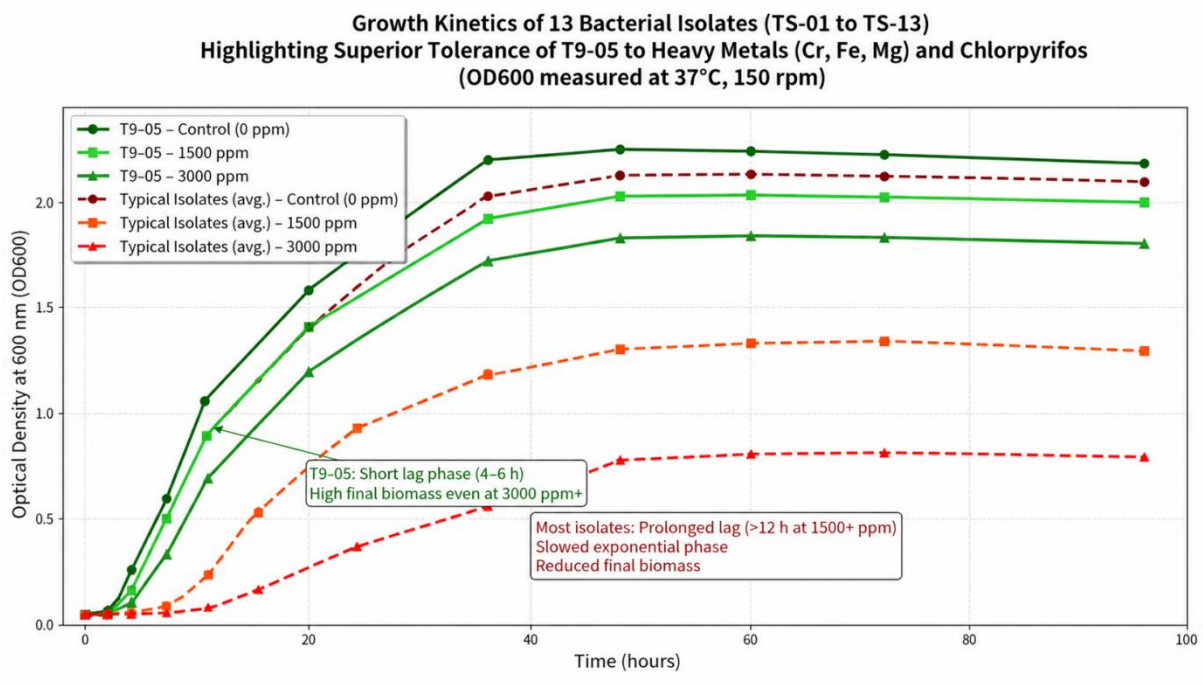
Growth condition estimation

Growth kinetic of all the 13 bacterial isolates (TS-01 to TS-13) was determined in nutrient broth with incremental addition of chromium (Cr), iron (Fe), magnesium (Mg) and chlorpyrifos in nutrient broth with shaking condition (150 rpm) at 37 0 C. The protocol was repeated in triplicate with uninoculated controls and involved blank nutrient broth (0 baseline) with the spectrophotometer, 100 mL flasks containing the prepared amended broth and 1 mL aliquots of the sample taken every 1 hour and OD600 recorded in quartz cuvettes (1 cm path length); the sample was returned to the flasks to minimize loss of sample volume. Most isolates showed progressive growth inhibition across the concentration gradient with prolonged lag phases (>12 hours at 1500 ppm or over), slowed exponential growth rate and reduced final biomass, although isolate Stutzerimonas stutzeri always had the lowest amount of inhibition, giving high final OD600 values (>12 hours) at all concentrations (3000 ppm or above) after 9tationary phases.6 hours, with short lag phases (4 to 6 hours) and s These findings highlight the high tolerance and vigor of growth of Stutzermonas stutzeri to increasing levels of heavy metal and pesticides, which makes it the best candidate to be used in bioremediation research.



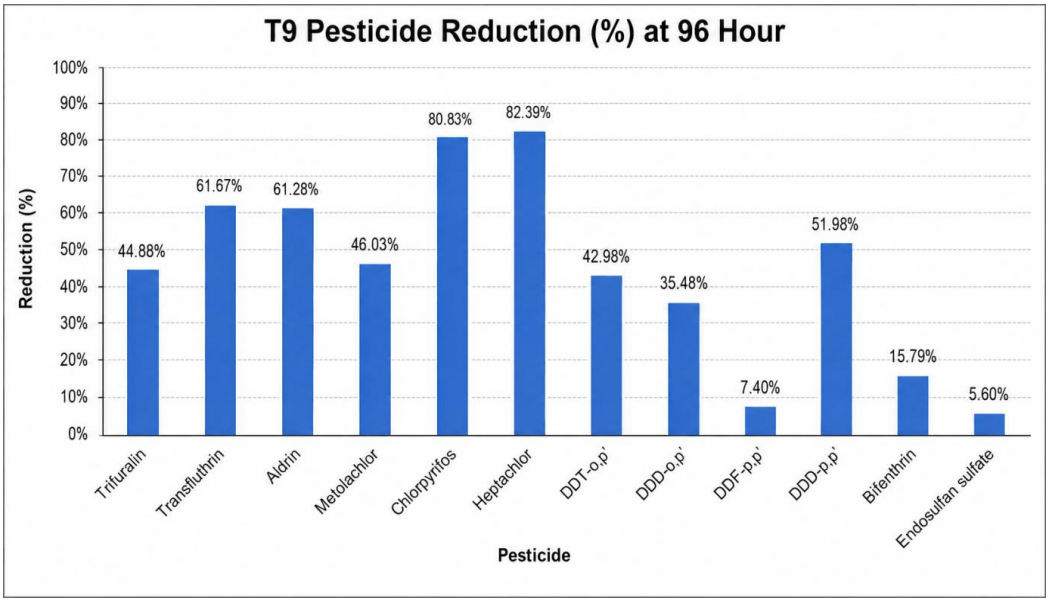
Graph 1. Growth kinetics of 13 bacterial isolates highligting superior tolereance of T9

GC-MS pesticide degradation profile

After 96 h of incubation, the bacterial isolate T9 was subjected to GC–MS analysis to check the ability to degrade a series of pesticides. The chromatographic quantification showed a significant decrease of the pesticides detected in the T9-treated samples in relation to the untreated controls, suggesting that the isolate has the ability to degrade and/or transform the pesticides. Heptachlor showed the maximum reduction (82.39%) followed by chlorpyrifos (80.83%). T9 also exhibited considerable degradation of transfluthrin (61.67%), aldrin (61.28%), DDD-p,p' (51.98%), and metolachlor (46.03%). Moderate reductions were recorded for trifluralin (44.88%), DDT-o,p' (42.98%), and DDD-o,p' (35.48%). Whereas, comparatively lesser reduction was seen with DDT-p,p' (7.40%), endosulfan sulfate (5.60%) and bifenthrin (15.75%) indicating the differential susceptibility of these compounds to microbial transformation. The reduction for the compounds tested were from 9.7754 to 1.874, or 80.83% for chlorpyrifos, and from 6.8584 to 1.208, or 82.39% for heptachlor. Overall, the results demonstrate that Stutzerimonas sp. T9 has a wide spectrum of pesticide transformation activity, especially against heptachlor and chlorpyrifos. Microbial-mediated degradation is suggested by the significant reduction of parent compounds detected by GC–MS after 96 h; the identification of the specific degradation pathways would require the identification of the corresponding transformation products, which would be done by comparing retention time and mass spectral fragmentation patterns.

Table 3. GC-MS Pesticide Biodegradation at 96 hour reduction (%) by Stutzerimonas stutzeri at for T9 Test 1, Test 2 and Test 3.

Pesticide	Control	T9-treated	Reduction (%) / status
Trifluralin	28.0001	15.435	44.88%
Transfluthrin	37.8442	14.507	61.67%
Aldrin	18.3615	7.1090	61.28%
Metolachlor	39.4073	21.267	46.03%
Chlorpyrifos	9.7754	1.874	80.83%
Heptachlor	6.8584	1.208	82.39%
DDT-o,p'	14.6280	8.341	42.98%
DDD-o,p'	17.0236	10.983	35.48%
DDT-p,p'	2.1026	1.947	7.40%
DDD-p,p'	6.0264	2.894	51.98%
Bifenthrin	13.8023	11.629	15.75%
Endosulfan sulfate	31.6741	29.9009	5.60%



Graph 2. GC-MS Pesticide Biodegradation at 96 hour reduction (%) by Stutzerimonas stutzeri at for T9 Test 1, Test 2 and Test 3.

ICP-MS heavy metal biotransformation

The results of ICP–MS suggested that the bacteria identified as Stutzerimonas sp. At 96 h incubation, T9 showed metal removal that depended on the element, at both 500 and 2000 ppb. The highest bioremoval was consistently observed for Cr (73.96–74.32%), followed byFe (63.05–63.89%),Pb (63.07–64.35%), and Ni (62.16–65.91%). Moderate removal was recorded for Zn (38.85–40.79%), V (37.77–38.63%), Be (33.33–34.16%), Cd (27.51–35.63%), and Mn (29.88–30.83%), whereas B, Al, Co and Cu showed comparatively lower reductions. Minimal removal was observed for Sr, Ba, Tl and U suggesting a differential interaction of T9 with the individual metal ions. The similarity of the reduction values for the three experimental

replicates indicates good repeatability of the response observed. In general, the ICP–MS data suggest that Stutzerimonas sp. T9 is very effective at removing/biotransforming some metals, such as Cr, Fe, Ni and Pb, and could be used in the bio-remediation of metal polluted environments.

Table 4. ICP-MS Metal Biotransformation at 96 Hours Comparative reduction (%) by Stutzerimonas stutzeri at 500 ppb for T5 Test 1, Test 2 and Test 3.

Metal/ element	**Control 500 ppb concentration (96 hour)**	***Stutzerimonas stutzeri* (T9 test 1) treated (96 hour)**	**Bio-removal Reduction (%)**	***Stutzerimonas stutzeri* (T9 test 2) treated (96 hour)**	**Bio-removal Reduction (%)**	**Stutzerimonas stutzeri (T9 test 3) treated (96 hour)**	**Bio-removal Reduction (%)**
Be	0.252	0.168	33.33%	0.168	33.33%	0.168	33.33%
B	15.579	12.122	22.19%	12.122	22.19%	12.122	22.19%
Al	2.833	2.222	21.57%	2.202	22.28%	2.202	22.28%
Ti	1.182	1.018	13.87%	1.018	13.87%	1.018	13.87%
V	0.233	0.143	38.63%	0.143	38.63%	0.143	38.63%
Cr	4.739	1.234	73.96%	1.230	74.04%	1.230	74.04%
Mn	3.837	2.654	30.83%	2.654	30.83%	2.654	30.83%
Fe	8.250	2.979	63.89%	2.979	63.89%	2.979	63.89%
Co	0.974	0.767	21.25%	0.767	21.25%	0.767	21.25%
Ni	1.493	0.565	62.16%	0.564	62.22%	0.565	62.16%
Cu	7.580	5.675	25.13%	5.675	25.13%	5.676	25.13%
Zn	30.878	18.882	38.85%	18.882	38.85%	18.882	30.85%
Sr	5.535	5.489	0.83%	5.489	0.83%	5.489	0.83%
Mo	0.073	0.062	15.07%	0.062	15.07%	0.062	15.07%
Cd	0.338	0.245	27.51%	0.245	27.51%	0.245	27.51%
Ba	0.759	0.759	0.00%	0.759	0.00%	0.759	0.00%
Tl	0.396	0.375	5.30%	0.377	4.80%	0.375	5.30%
Pb	0.807	0.298	63.07%	0.296	63.32%	0.296	63.32%
U	0.006	0.006	0.00%	0.006	0.00%	0.00	0.00%

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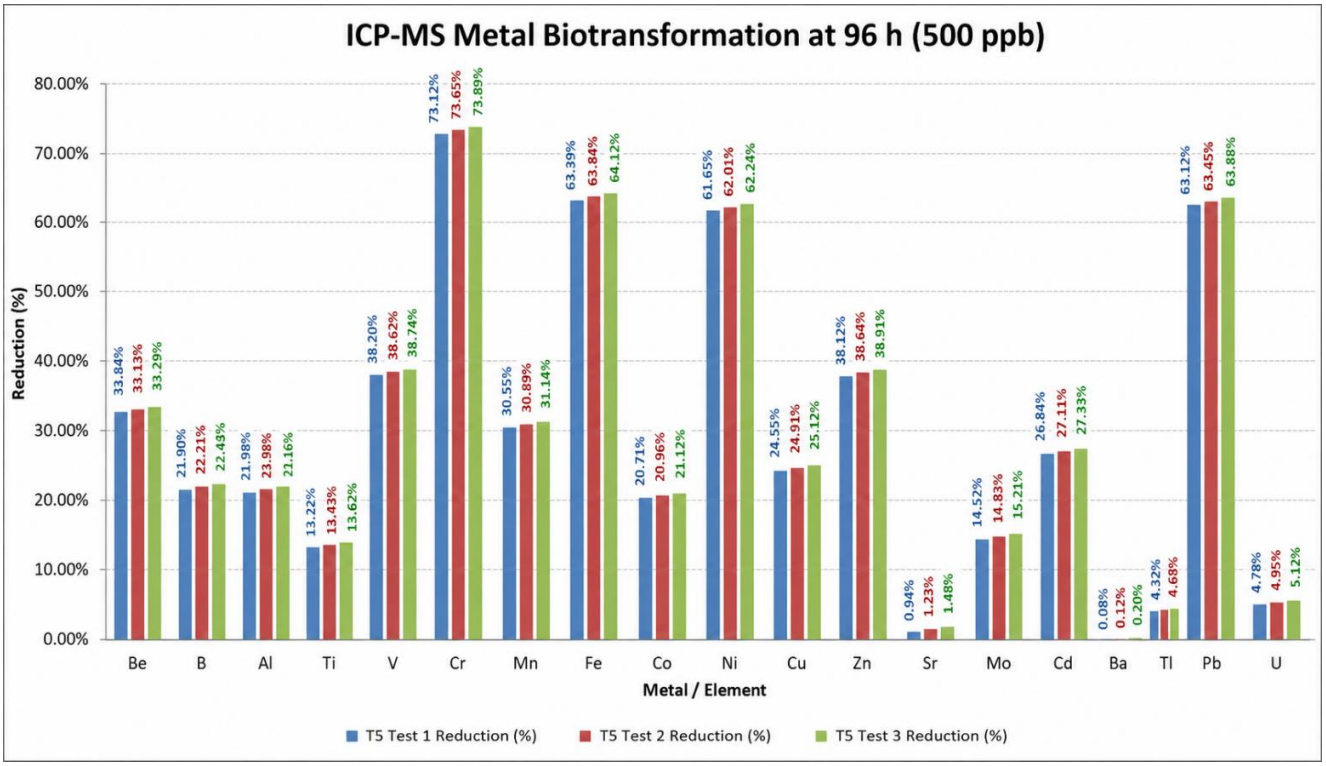
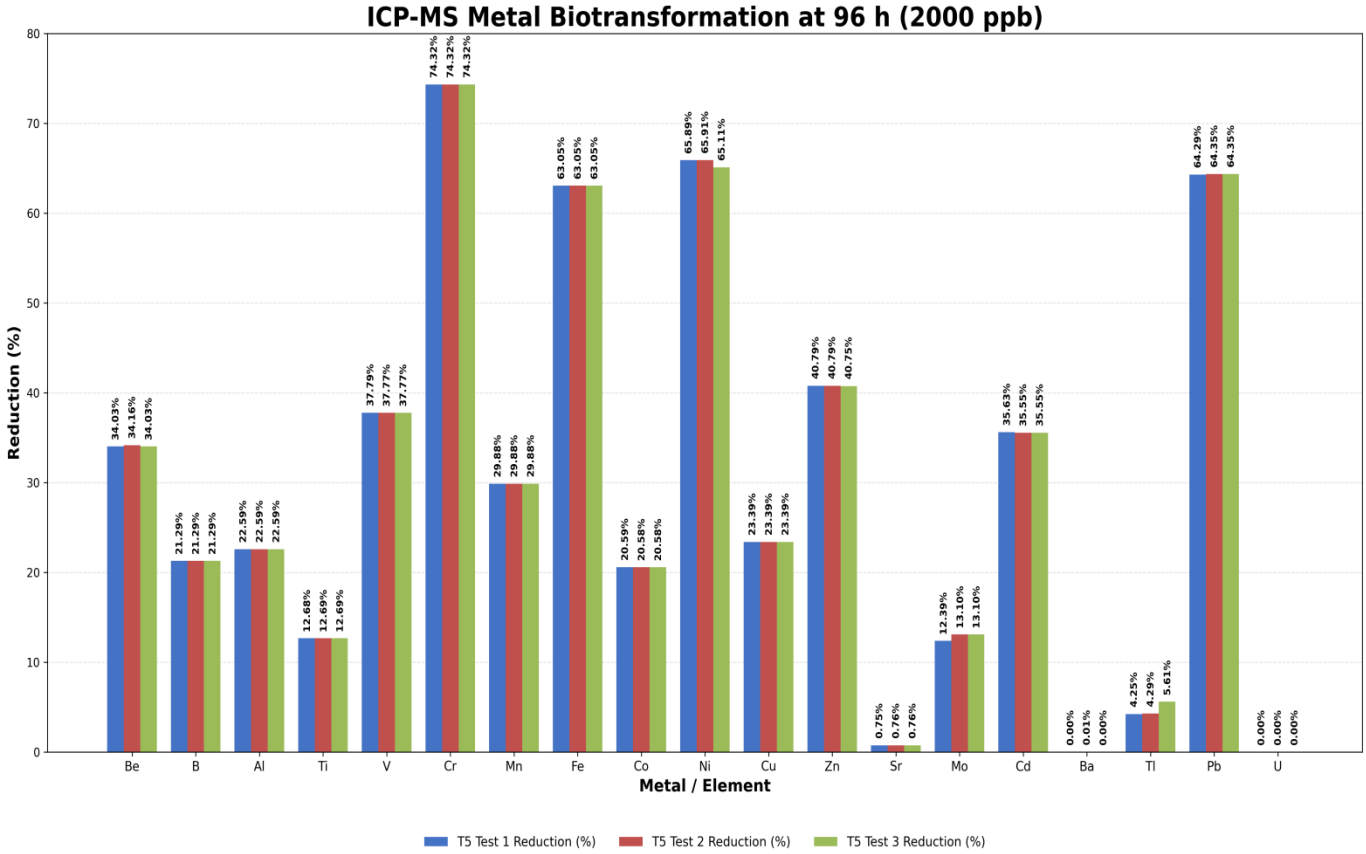


Graph 3 ICP-MS Metal Biotransformation at 96 Hours Comparative reduction (%) by Stutzerimonas stutzeri at 500 ppb for T5 Test 1, Test 2 and Test 3.

Table 5 ICP-MS Metal Biotransformation at 96 Hours Comparative reduction (%) by Stutzerimonas stutzeri at 2000 ppb for T5 Test 1, Test 2 and Test 3.

Metal/element	**Control 2000 ppb concentration (96 hour)**	***Stutzerimonas stutzeri* (T9 test 1) treated (96 hour)**	**Bio-removal Reduction (%)**	***Stutzerimonas stutzeri* (T9 test 2) treated (96 hour)**	**Bio-removal Reduction (%)**	**Stutzerimonas stutzeri (T9 test 3) treated (96 hour)**	**Bio-removal Reduction (%)**
Be	0.521	0.344	34.03%	0.325	34.16%	0.343	34.03%
B	24.561	19.332	21.29%	19.297	21.29%	19.299	21.29%
Al	8.231	6.372	22.59%	5.987	22.59%	6.372	22.59%
Ti	1.284	1.121	12.68%	1.087	12.69%	1.085	12.69%
V	0.323	0.201	37.79%	0.259	37.77%	0.255	37.77%
Cr	6.316	1.622	74.32%	1.509	74.32%	1.594	74.32%
Mn	4.742	3.325	29.88%	3.325	29.88%	3.312	29.88%
Fe	14.035	5.1859	63.05%	5.2569	63.05%	5.2590	63.05%
Co	1.521	1.208	20.59%	1.211	20.58%	1.208	20.58%
Ni	1.596	0.544	65.89%	0.495	65.91%	0.557	65.11%
Cu	7.152	5.479	23.39%	5.479	23.39%	5.479	23.39%

Zn	36.660	21.706	40.79%	21.789	40.79%	21.715	40.75%
Sr	5.810	5.766	0.75%	5.734	0.76%	5.734	0.76%
Mo	0.084	0.073	12.39%	0.71	13.10%	0.073	13.10%
Cd	0.436	0.281	35.63%	0.285	35.55%	0.285	35.55%
Ba	0.964	0.9639	0.001%	0.964	0.01%	0.964	0.00%
Tl	0.606	0.580	4.25%	0.573	4.29%	0.572	5.61
Pb	0.589	0.210	64.29%	0.215	64.35%	0.210	64.35
U	0.014	0.014	0.00%	0.00	0.00%	0.00	0.00



Graph 6 ICP-MS Metal Biotransformation at 96 Hours Comparative reduction (%) by Stutzerimonas stutzeri at 2000 ppb for T5 Test 1, Test 2 and Test 3.

Molecular Identification (16S rRNA Sequencing)

The isolate, tested T9 Stutzerimonas stutzeri, was molecularly identified as Stutzerimonas stutzeri by 16S rRNA gene sequencing, which resulted in sequence identities of 99–100% with multiple reference strains (including SM13, J15, yjy10, and 24a43). The phylogenetic analysis by the NJ method showed that the isolate was grouped into the γ-proteobacteria group, with extremely short branch length (scale bar = 0.0007 substitutions per site), and collapsed clades as a result of the negligible genetic distances (Fig. X). It is a species that has been well documented for its metabolism of a wide range of pesticides and is considered highly promising for bioremediation purposes with isolate T9 showing good potential for this.

Fig 3. Phylogenetic Tree of Stutzerimonas stutzeri by 16 S rRNA Sequencing

DISCUSSION

The present study showed that the indigenous bacterial strains isolated from contaminated water can be used as bioreactor for the bioremediation of pesticide and metal-contaminated environment. The collected water samples yielded a total of 13 morphologically different bacterial isolates, suggesting the presence of a variety of bacterial species that are able to survive under polluted environment. The combination of selective media with chlorpyrifos, Cr, Fe and Mg proved to be an effective method for enrichment of multiple stress tolerant microorganisms. Previous studies have used enrichment-based strategies for the recovery of chlorpyrifos-degrading bacteria from pesticide-contaminated soil, water, plant, and animal habitats, with bacteria from Acinetobacter, Pseudomonas, Bacillus, Enterobacter and Stenotrophomonas species exhibiting high tolerance and capability for degrading chlorpyrifos [60–64]. Pailan et al. reported that a heavy metal tolerant Acinetobacter strain could

use chlorpyrifos as a carbon source and could degrade around 98% of the pesticide in 144 h, emphasizing the selection of microorganisms from contaminated environment which could be used for effective bioremediation of chlorpyrifos [60].

Variations among the 13 strains in their tolerance to the environmental contaminants, Cr, Fe, Mg and chlorpyrifos, suggest that the reactions of bacteria to environmental contaminants are strain dependent. In the present investigation, most of the isolates had growth reduction with the increasing concentration of the contaminants while isolate T9 maintained growth up to 3000 ppm of all the contaminants tested at both broth and agar growth media for Cr, Fe, Mg and chlorpyrifos. The tolerance of T9 to several contaminants at a relatively high concentration can be significant as the contaminated aquatic environment is usually a mixture of organic and inorganic contaminants, not a single contaminant. This has also been described for bacteria that have been collected from polluted environments, where the bacteria may have acquired various resistance and detoxification genes when exposed to chronic exposure of persistent pollutants [61,65]. Under combined chlorpyrifos and metal stress, accumulating of heavy metals in engineering microorganisms was found to enhance the ability to degrade chlorpyrifos, suggesting that microorganisms tolerant to multiple pollutant stress would be useful in the future [64].

The fact that T9 grows best under higher concentrations of the contaminants tested further illustrate its physiological adaptability. T9 adapted relatively fast and seemed to grow at a relatively high rate at higher contaminant levels, but several isolates had extended lag times and decreased biomass. This type of behavior may relate to the organism's capacity to regulate intracellular pH and cellular homeostasis via metal efflux, sequestration of metals, production of extracellular polymeric substances, enzymatic detoxification and other stress-related processes [65]. The resistance of bacteria to heavy metals is typically not just a single mechanism; rather, multiple mechanisms can be involved, such as biosorption onto cell surface functional groups, extracellular precipitation, active efflux and intracellular accumulation [65,71]. In addition, EPS can bind metal ions by functional groups, like carboxyl, hydroxyl and phosphate groups, which reduces the concentration of free metal in the surrounding environment and shields the bacteria from the toxic effects of metal ions [72]. Hence, the tolerance of T9 in the present study could be due to a combination of several physiological and biochemical mechanisms.

The results of GC–MS further support the ability of T9 for wide xenobiotic transformation. The percentage reduction of chlorpyrifos and heptachlor was the lowest (80.83%) and the highest (82.39%), respectively, after 96 h of incubation. Significant transformation of transfluthrin, aldrin, and metolachlor was also seen, and there were relatively low reductions for DDT-p,p' and endosulfan sulfate. The differential degradation may be due to differences in their chemical structure, hydrophobicity, molecular stability and/or their susceptibility to bacterial enzymes. Microbial degradation of chlorpyrifos usually begins with hydrolysis of the phosphoester bond by organophosphate-hydrolyzing enzymes to produce intermediates like 3,5,6-trichloro-2-pyridinol (TCP) and diethyl thiophosphoric acid (DETP) [61,62,66]. A considerable degradation of chlorpyrifos under laboratory conditions has been reported with bacterial isolates, such as Bacillus thuringiensis MB497 (degradation of ~99%) and Acinetobacter sp. (degradation of ~98%). MemCl4 [60,63].

The 80.83% degradation of chlorpyrifos found in the present study is in good agreement with the previous reports showing the transformation capacity of bacteria for organophosphate pesticides. But the degradation of parent compound is not a definitive sign of mineralization, as chlorpyrifos degradation may create TCP as an initial degradation product that is also environmentally persistent. Previous studies have shown that microorganisms able to degrade chlorpyrifos can either accumulate TCP as an intermediate or further transform TCP to less persistent products [63,67]. GC–MS analyses have showed the formation of TCP and phosphorylation metabolites during bacterial degradation of chlorpyrifos [60,63] in particular. Thus, the reduction seen in the present study suggests significant amounts of chlorpyrifos were transformed by T9 and additional characterization and quantification of metabolites would be necessary to definitively establish complete degradation pathway and complete detoxification.

The ICP–MS analysis confirmed that T9 was also able to remove a wide spectrum of dissolved metal elements from the culture media. The highest reduction was observed for Cr, reaching approximately 73.96–74.32%, followed by Fe (63.05–63.89%), Pb (63.07–64.35%) and Ni (62.16–65.91%) at the tested concentrations. Moderate removal was observed for Zn, V, Be, Cd and Mn while very limited removal was observed for Sr, Ba, Tl and U. This element-dependent pattern suggests that the removal of metal by T9 is not non-specific, and that it could be related to physicochemical properties like ionic radius, charge, affinity for functional groups of bacteria, and affinity for bacterial cell membrane. This differential uptake of metal has also been noted for indigenous metal-tolerant bacteria, where individual strains were found to have varying affinities for Cr, Fe, Cu, Zn, Cd, Pb and other metals [68,71]. Also, studies on Pseudomonas/Stutzerimonas stutzeri have shown its potential

for interaction and removal of metals, further indicating the suitability of this bacterial group for environmental metal remediation.

The relatively high elimination of chromium noted during the current study is especially notable because of the high mobility and toxicity of hexavalent chromium and the fact that chromium pollution is a significant environmental concern. The ability of Pseudomonas stutzeri to interact with chromium in contaminated wastewater has been investigated earlier for its biosorption/reduction properties of Cr(VI) and it has been observed that the members of this bacterial group can interact with Cr(VI) in contaminated wastewater effectively [73]. However, the current ICP–MS data does not provide an independent measure of conversion of the Cr(VI) to Cr(III). Speciation analysis for chromium would need to be used with an appropriate method for the analysis to confirm the biological reduction of Cr(VI) to Cr(III), which is the less harmful oxidation state. It is important to note this distinction when interpreting the present results as the reduction observed may be accompanied by biosorption, bioaccumulation, precipitation and/or redox transformation of the Cr species.

A similar situation may occur with the removal of Fe and other metals. The extracellular polymeric substances can be metal binding matrices and can either immobilize or precipitate metal ions; the surfaces of bacterial cells have functional groups that bind the metal ions, which are negatively charged [71,72]. Moreover, metal stress can trigger bacterial defence mechanisms such as enhanced EPS production, efflux and enzymatic detoxification [65,72]. The observed relatively consistent values for the three replicates of the experiments in the present ICP–MS analysis further indicate good reproducibility of the observed pattern of metal removal. The relative stability of the interaction between T9 and individual metals can be deduced from the similarity in the major removed elements at both 500 and 2000 ppb.

The molecular identification of the selected isolate was a further confirmation of its bioremediation potential. The isolate T9 was identical (99–100%) in 16S rRNA gene sequences to Stutzerimonas stutzeri and was closely related to other strains of S. stutzeri in the phylogenetic tree based on 16S rRNA gene sequences. The identification is in line with recent studies demonstrating that Stutzerimonas stutzeri is able to degrade chlorpyrifos. Recently, Hoda and Aggarwal isolated S. stutzeri from the phyllosphere of Eichhornia crassipes and indicated that this species helped in the degradation of chlorpyrifos, where the bacterial consortium degraded nearly 98% of the chlorpyrifos in seven days [74]. The proteomic analysis also indicated involvement of phosphodiesterases, metallo-beta-lactamases and oxidoreductases in pesticide transformation, suggesting the possibility of enzymatic mechanisms in the ability of S. stutzeri to transform xenobiotics

Overall, the results suggest that T9/Stutzerimonas stutzeri has a unique set of pesticide transformation and metal removal properties. The present isolate was tolerant to both organic pesticides and inorganic metals and was able to reduce several concentrations of both groups, as opposed to conventional treatment methods that typically reduce either group of contaminant alone. This is especially important for mixed contaminated sites, such as agricultural drainage or industrially affected water bodies, where pesticides and metals can co-occur and can potentially interact with one another to affect microbial activity. Previous studies also highlighted the need for microbial systems to be developed that can handle multiple contaminants instead of just single organic or inorganic contaminants [64,69,70].

In summary, the present study showed that the indigenous bacteria (Stutzerimonas stutzeri) is a worthy candidate for microbial bioremediation due to its high tolerance towards Cr, Fe, Mg, high chlorpyrifos transformation capacity, wide spectrum pesticide activity and efficient removal of several dissolved metals. The lab results, however, should be viewed as merely a preliminary indicator of potential and not as actual field-scale remediation. Future research should include studies on degradation kinetics, identification and quantification of transformation products, identification of genes and enzymes involved in pesticide degradation, elucidation of the exact mechanisms for metal uptake, and, controlled microcosm and field-scale experiments. Specifically, the speciation of chromium will need to be determined to determine if any chromium was removed as Cr(VI) and reduced to Cr(III). This type of research would give better mechanistic justification for using T9 as a bioaugmentation tool for the treatment of environments contaminated with mixtures of pesticides and metals.

CONCLUSION

Overall, the present study revealed the tolerance and bioremediation potential of the bacterial isolate T9 which was identified as Stutzerimonas stutzeri against chlorpyrifos and various heavy metals. The T9 was found to have a broad ability to transform pesticides, with the highest reduction of chlorpyrifos (80.83%) and heptachlor (82.39%) after 96 h; ICP-Ms analysis revealed high removal of Cr, Fe, Pb and Ni. The results demonstrate that S. stutzeri T9 is able to withstand and degrade various pollutants and can thus be considered as a potential bacterial strain for bioremediation of contamination affected

areas. Further research is needed to verify degradation pathways, transformation products and degradation mechanisms under field conditions.

DECLARATIONS

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Conflict of interest

The authors declare no conflicts of interest.

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DATA AVAILABILITY

All raw data, including laboratory assay reports from the contracted testing facility are available from the corresponding author on reasonable request.

Authors' Contributions Tanvi Singh: Conceptualization, Methodology, Investigation, Writing – original draft.

Anjali Phogat: Data curation, Formal analysis.

Rajendra Prasad: Validation, Visualization.

Atanu Banerjee: Review & editing.

Sangeeta Gohel: Resources and review.

Sangeeta Kumari: Supervision, Project administration, Writing – review & editing.

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Consent to Participate: Not applicable.

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