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Indigenous Bacillus *paramycoides* spp. and *Alcaligenes faecalis*: sustainable solution for bioremediation of hospital wastewater

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Indigenous *Bacillus paramycoides* spp. and *Alcaligenes faecalis*: sustainable solution for the bioremediation of hospital wastewater**Aneeba Rashid[a,d], Safdar A. Mirza[a], Ciara Keating[b], Sikander Ali[c], Luiza C. Campos[d*]**

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Abstract

Farmers near towns and cities are using a wide range of highly polluted wastewaters for crop irrigation in Pakistan due to severe freshwater shortage. The present study aimed to promote indigenous bacterial strains isolated from domestic, hospital, textile, pharmaceutical and mixed wastewaters to remove contaminants and colour and render these wastewaters safer for irrigation. Thirty seven bacterial strains were isolated from five wastewater samples collected from different sites in Lahore, Pakistan. Under optimal growth conditions, three isolates (D6, D7 and P1) showed >93% decolourisation potential in the treatment of hospital wastewater. 16S rDNA sequencing identified two of these isolates (D6 and D7) as showing 100 and 99.86 % homology to *Bacillus paramycoides* spp. - novel strains from *B. cereus* group. Isolate P1 showed 97.47 % homology to *Alcaligenes faecalis*. GCMS analysis of the untreated hospital wastewater revealed the presence of pharmaceutic pollutants i.e. Phenol (876 µg/L), Salicylic acid (48 µg/L), Caffeine (7 µg/L), Naproxen (23 µg/L), Octadecene (185 µg/L) and Diazepam (14 µg/L). The analysis of treated hospital wastewaters showed percentage degradation of pharmaceutic pollutants (100 – 43 %) and significant reduction in the BOD_5 (91 – 68 %), COD (89 – 52 %) and heavy metals concentrations. These strains therefore can represent a low-cost and low-tech alternative to bioremediate complex matrices of hospital wastewater prior to crop irrigation to support the achievement of clean re-usable water in developing countries like Pakistan.

Keywords: *Bacillus paramycoides* spp., *Alcaligenes faecalis*, wastewaters, biotreatment, irrigation.

1. Introduction

The Planet Earth contains less than 1 % of freshwater [1]. The increasing population, urbanization, human activities and excessive usage of freshwater are the main contributors to water shortages [2]. The South Asian region, mainly Pakistan, has the worst condition in this scenario [3, 4]. Pakistan's per capita annual fresh water availability is just 1017 m^3 [5] which is on the precipice of the scarcity threshold level (1000 m^3). Being an agricultural country, this scarcity of freshwater resources has driven local farmers in Pakistan to reuse untreated wastewaters for irrigation of crops [6]. These wastewaters contain many harmful chemicals and heavy metals which accumulate in plant crops [7-10] and accumulate further up the food chain making them hazardous for animal and human consumption. It is estimated that the total wastewater production in Pakistan is ~96 M gal per annum [11]. These wastewater effluents are generally discharged to sewerage drains or fresh-water bodies directly without receiving any prior treatment [12].

The domestic, industrial, pharmaceutical and hospital wastewater effluents in Pakistan contain extensive quantities of dyes, suspended solids, heavy metals, additives, soaps, washing powders, surface-active agents and cancer-causing amines. Additionally, formaldehyde [13], organic and inorganic compounds, macro-solids, gases, emulsions, toxins, microplastics [14], pharmaceuticals like endocrine disrupting compounds, hormones, antibiotics, anesthetics, perfluorinated compounds [15], siloxanes [16], drugs of abuse [17] and various biological pathogens [18] are also reported to be found in the untreated wastewaters. These untreated wastewaters also contain higher metal concentration which leads to the inhibition of the microbial populations and pose a serious public health and environmental threat [19]. The complex nature of these effluents and lack of centralized wastewater treatment infrastructure make sufficient treatment difficult in Pakistan. One area, that is a considerable challenge is the removal of colour contamination. The higher coloured contamination depicts the presence of higher unused concentrations of dyes and chemicals in these wastewaters [20]. These dyes, impurities and chemicals released from the textile industries impart colour to wastewater drains and cause colour contamination, thus diminishing the water quality [21].

Various physicochemical methods have been used worldwide to remove colour and impurities from wastewater, for example, adsorption [22], ion exchange [23], membrane

filtration [24], ozonation [25], photooxidation [26] and reverse osmosis [27]. Pakistan being a developing economy has not adopted any of these methods on a large scale as these methods are prohibitively expensive and require large complex infrastructure [28]. With the exception of two metropolitan cities, Islamabad and Karachi, centralised treatment facilities are rare [29]. The plant in Islamabad, Pakistan was set up for treating domestic wastewaters by conventional activated-sludge process, but the biological degradation efficacy for this treatment plant was lower than the anticipated levels for COD (73 %) and BOD_5 (65 %). Moreover, this process is inflexible and has a high operational cost [30]. Karachi also has sewage treatment infrastructure but none of them are currently operational, owing to maintenance issues [31]. Thus, for these cities, even with centralised infrastructure, it is estimated that less than eight percent of wastewater receives treatment because of high operational and maintenance costs [31, 32]. Decentralised biological treatment methods could offer a potential low-cost and low-tech solution for communities in developing countries such as Pakistan.

The bioremediation potential of specific bacterial isolates is a financially practical and ecofriendly method. These may offer a simple substitute to more complex engineered and expensive methods such as the activated sludge process [30]. One strategy – to use native or indigenous isolates from wastewater to degrade, detoxify and decolour specific compounds has been the source of intensive research e.g. *Bacillus cereus* isolated from domestic wastewater for degrading acrylamide [33], *Acinetobacter tandoii* RTE1.4 [34, 35] and *Rhodococcus* sp. CS1 from chemical industry and tannery wastewater for degrading phenol [34], *Pseudomonas aeruginosa* from synthetic wastewater for heavy metal removal [35], *Achromobacter* sp. (AIEB-7), *Pseudomonas* sp. (AIEB-4), *Enterobacter* sp. (AIEB-3), and *Acinetobacter* sp. (AIEB-2) from oil refinery wastewater for degrading phenol and benzene [36], *Aeromonas sp. TXBc10* from tannery wastewater for degrading octylphenol polyethoxylated [37], *B. licheniformis* for degrading phenol [38] or *Marinobacter hydrocarbonoclasticus* and *Nitratireductor kimnyeongensis* for the bioremediation of tannery wastewater [39]. However, in these biotreatments, the bacterial strains are isolated and tested for the bioremediation of specific lab-derived compounds and thus, the potential to degrade a complex matrix of compounds in raw wastewaters is largely unknown. This, therefore, is not sufficient for the real-world situation in countries like Pakistan particularly where wastewaters from household, hospitals and a wide range of industries are combined

in drainage systems.

The present research aimed to i) isolate and identify specific decolourising bacteria from raw wastewaters (domestic, hospital, textile and pharmaceutical sources), ii) characterise the physicochemical components present in these wastewaters, and iii) determine the efficiency of these specific isolates to decolour and degrade the constituents in hospital wastewater. This work is designed to investigate the feasibility of a low-cost and low-tech mono-culture system alternative to bioremediate complex matrices of hospital wastewater to support the achievement of clean water and sanitation in developing countries like Pakistan.

2. Materials and methods

2.1 Collection of wastewaters

Four wastewaters (domestic, hospital, textile and pharmaceutical) samples (50 L each) were collected in sterile bottles according to the standard protocols from the points of discharge of drainage sites in Lahore, Pakistan [40]. The geographical coordinates of Lahore city are 31 ° 34 ’ 55.36 ” north and 74 ° 19 ’ 45.75 ” east at an altitude of 217 m (712 ft). Mixed wastewater (50 L) was also collected from a collective drainage site of the different wastewaters. All five samples were collected in March, 2019. The temperature of the wastewaters and environment were noted down on-site by using digital thermometer (HUBDIC).

2.2 Characterisation of the wastewaters

The wastewaters were analysed immediately after the collection for the characterisation to ensure the bacterial viability and to avoid any self-degradation of organic compounds. Following standard protocols [40, 41], the following physicochemical parameters were investigated, *i.e.* colour, smell, temperature, pH, electrical conductivity (EC), total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), biological oxygen demand (BOD_5), salinity (ppt) and turbidity (NTU). The concentrations of the following heavy metals were estimated through Atomic Absorption Spectrophotometer (AA 7000 F with Autosampler and Hydride Vapour Generator, Shimadzu, Japan): Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb) and Nickel (Ni).

2.3 Isolation and screening of bacteria

The isolation of bacterial strains from each of the five types of wastewaters was performed using serial dilution [42]. The isolates from each wastewater’s inocula were

incubated on sterile nutrient agar medium plates in static incubator at 37 ℃ for 24 hours and were then purified by streaking on nutrient agar medium plates. The purified colonies were transferred to prepared slants of Luria-Bertani medium (LB) with agar in test tubes and were preserved in a refrigerator (4 ℃). The bacterial slants were maintained every two weeks on freshly prepared agar slants [43]. For initial screening, the isolated screened isolates were inoculated in domestic wastewater (100 mL) incubated at 37 ℃ for 24 hours. The percentage decolourisation was estimated by UV/VIS (AE-S80) spectrophotometer at 545 nm [44]. The bacterial isolates showing more than 90 % decolourisation were further selected for testing optimal conditions (see Supplementary Material, *S1.1.1*) for colour contamination removal in complex wastewaters.

2.4 Identification of the screened bacterial isolates

The three bacterial isolates showing maximum decolourisation potential (> 90 %) were selected for identification using 16S rDNA sequencing [45]. Polymerase chain reaction (PCR) was carried out on the three isolates using the following forward and reverse primer set (see Supplementary Material, *S1.2*): 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (TAC GGY TAC CTT GTT ACG ACT T) [46]. The DNA samples with the extension products were then added to Hi-Di formamide (Applied Biosystems, Foster City, CA). This mixture was incubated for 5 min at 95 ℃, placed on ice for 5 min and subsequently the sequencing reaction was carried out in an ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). The forward and reverse sequence chromatograms (abi files) were initially viewed in FinchTV version 1.5.0 and then interrogated using MacVector version 17.5.4. The forward and reverse reads were imported into BioEdiT version 7.2. A consensus sequence per strain was subsequently assembled using the contig assembler program (CAP) [47] using the forward read and reverse complement of the reverse read. The full sequence information and raw chromatogram details are presented in the Supplementary Material (S1.2, Figure S4 – S25). Basic Local Alignment Search Tool (BLAST) analysis was carried out on the assembled sequences. The sequences of the three isolates were deposited in GenBank with accession numbers [GenBank: MT477810], [GenBank: MT477812] and [GenBank: MT477813].

2.5 Testing decolourisation potential of bacteria

After the optimization of bacterial growth conditions (see Supplementary Material, *S1.1.1*), the three selected bacterial isolates (*B. paramycoides* D6, *B. paramycoides* D7 and *A. faecalis* P1) were inoculated separately in five sources of wastewater (domestic, hospital,

textile, pharmaceutical and mixed wastewater) to find out the decolourisation percentages. Approximately 100 mL of each type of autoclaved wastewater was inoculated (10 %) with each of the three bacterial isolates separately in conical flasks (250 mL) for 48 hours at 37 and 51 ℃ [48]. The experiments were performed in triplicates. The percentage decolourisation was calculated using decolourisation percentage equation [49]. The physicochemical parameters that were investigated for the characterisation of the untreated wastewaters were carried out after the decolourisation tests and were compared with untreated wastewaters (Section 2.2). Biodegradability index (BI) was also calculated for finding the extent of biodegradability of the wastewaters before and after biotreatment [50].

2.5.1 Organic compounds degradation

For the organic compounds' degradation potential, the wastewater sample showing maximum decolourisation percentual and biodegradability index, i.e. hospital wastewater, (Section 2.2 and 2.5) was further analysed by gas chromatography mass spectrometry (GCMS) technique with Agilent Gas Chromatograph (GC, AgiTech-7260) and Mass Spectrometer (MS, Maspec-6595). In total, four samples (10 mL each) were prepared for the analysis, *i.e.* one raw hospital wastewater sample (as uninoculated control) and three inoculated (i.e. decolourised) hospital wastewater samples. All GC separations were achieved using 20 m × 0.3 mm fused-silica capillary column with a 0.45 µm coated 6 % phenylmethyl silicone film in the instrument.

The sample aliquot (5 µL) was injected in split-less mode (0.5 min) at 290°C. The temperature of oven was set as follows: initial temperature (45 °C), raised to 58-92 °C / min and then 12-210 °C/min, 10-285 °C/min and 6-320 °C/min with a hold time of 5 min. Mass Spectrometer was adjusted as follows: 120 °C analyzer, 210 °C source, 280 °C interface and electron ionization at 80 eV. The data was collected from 50-450 atomic mass unit (amu). The retention time (±0.1 min), quantification ions, confirmation ions (156.18 and 184.25 m/z) and internal standards (Acenaphthene and Phenanthrene) of each sample were set at optimal levels (Spiking level = 0.05 µg/g; recovery = 98.9 and 93.47 %; coefficient of variation (CV) = 4.22 and 7.39 %) and run in accordance with the system sequence. The base-peak ion was employed for quantitation and two qualifier ions were used for confirmation. The compound concentrations were compared with internal standard quantitation (LoQ = 0.05 mg/kg) and calibration curves. For identifying the degraded compounds as a result of biotreatment, the mass spectra were compared with NIST (National Institute of Standards and Technology)

database library software present in the instrument. The retention times were also compared with the authentic compounds available in the database.

2.6 Metal tolerance limits

100 mg/L solutions of the following ten metal salts were prepared in deionized water, *i.e.* lead nitrate ($PbNO_3$), cobalt chloride ($CoCl_2$), calcium chloride ($CaCl_2$), zinc sulphate ($ZnSO_4$), manganese sulphate ($MnSO_4$), magnesium sulphate ($MgSO_4$), iron sulphate ($FeSO_4$), Potassium dichromate ($K_2Cr_2O_7$), sodium molybdate (Na_2MoO_4) and copper sulphate ($CuSO_4$) (Sigma Aldrich, Uk). The three bacterial isolates (*B. paramycoides* D6, *B. paramycoides* D7 and *A. faecalis* P1) were streaked on the prepared metal salt-nutrient agar plates and incubated at 37℃ for 24 h in static conditions. Salt-nutrient agar plates without the addition of metal salts were used as controls. At 100 mg/L concentration, the metal salt plates with more than 65% bacterial growth were selected for further investigation with the bacterial isolates. The solutions of these metal salts specifically $CaCl_2$, $MgSO_4$, $K_2Cr_2O_7$, Na_2MoO_4 and $PbNO_3$ were then prepared in 50, 100, 150, 200, 250 and 300 mg/L concentrations with each of the three isolates (*B. paramycoides* D6, *B. paramycoides* D7 and *A. faecalis* P1).

2.7 Statistical analysis

The results were presented as means ± S.D. (standard deviation). The data from each of the different parameters of which there were three replicate values (pH, EC, TSS, TDS, COD, BOD_5, Salinity, Turbidity and Heavy metals) before and after biotreatment were compared using a t test with Welch's correction and two-tailed p-value calculation using Graphpad Prism software. For all comparisons, differences were considered significant when the probability level was < 0.05.

3. Results and discussion

3.1 Characterisation of the wastewaters

Following biotreatment, the results showed that the concentrations of the physicochemical parameters (pH, EC, TSS, TDS, COD, BOD_5, Salinity, Turbidity and Heavy metals) were within the levels of National Environment Quality Standards in comparison with the untreated wastewaters [51] (See Table 1).

Table 1. Physiochemical characterisation of untreated and treated wastewaters (Domestic wastewater – DWW; Hospital wastewater – HWW; Textile wastewater – TWW; Pharmaceutical wastewater – PWW and Mixed wastewater – MWW) in comparison to National Environment Quality Standards (NEQS). Nd = not detectable. Significance (Welch's test) is indicated by P < 0.05*, P < 0.01**, P < 0.001***, P < 0.0001****

Parameters	NEQS	Wastewaters									
		DWW		HWW		TWW		PWW		MWW	
		Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
Colour (PCU)	-	Light grey	Colourless	Light yellow	Colourless	Greenish grey	Colourless	Light brown	Colourless	Blackish	Colourless
	-	101	28	188	55	221	61	103	38	311	64
Smell	Acceptable / Bearable	Pungent	No smell	Fishy	No smell	Pungent	No smell	Fishy	No smell	Pungent	No smell
Temperature (°C)	=<3°C -	25	4	25	4	22	4	28	4	21	4
	- -	29	26	30	26	33	26	33	26	31	26
pH	6.6-8.5	7.8^{****}	6.9^{****}	7.4^{***}	6.7^{***}	8.7^{***}	7.5^{***}	10.4^{***}	8.2^{***}	8.4^{**}	7.4^{**}
EC (µs/cm)	-	413^{****}	214^{****}	444^{****}	267^{****}	861^{****}	574^{****}	350^{****}	193^{****}	775^{****}	435^{****}
TSS (mg/L)	<500 mg/L	1920^{****}	363^{****}	2300^{****}	483^{****}	2150^{****}	425^{****}	2120^{****}	398^{****}	2670^{****}	491^{****}
TDS (mg/L)	1000	296^{****}	213^{****}	296^{****}	220^{****}	608^{****}	398^{****}	105^{***}	87^{***}	541^{****}	323^{****}
COD (mg/L)	150-400	212^{****}	76^{****}	396^{****}	260^{****}	153^{****}	17^{****}	269^{****}	133^{****}	235^{***}	99^{***}
BOD_5 (mg/L)	80-250	176^{****}	39^{****}	246^{****}	78^{****}	174^{****}	14^{****}	223^{****}	68^{****}	169^{****}	40^{****}
BOD_5 : COD	-	0.83	0.51	0.62	0.30	1.14	0.82	0.83	0.51	0.72	0.40
Salinity (ppt)	-	0.2^{**}	0.1^{**}	0.2^{**}	0.1^{**}	0.5^{***}	0.3^{***}	0.3^{**}	0.2^{**}	0.4^{**}	0.3^{**}
Turbidity (NTU)	5	38^{****}	4^{****}	51^{***}	5^{***}	76^{****}	5^{****}	61^{****}	3^{****}	123^{****}	4^{****}
Arsenic (As)	0.05 mg/L	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Cadmium (Cd)	0.01 mg/L	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Chromium (Cr)	0.05 mg/L	Nd	Nd	1.8	Nd	Nd	Nd	1.7^{****}	0.05^{****}	0.9^{****}	0.02^{****}
Lead (Pb)	0.05 mg/L	Nd	Nd	0.17	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Nickel (Ni)	0.02 mg/L	0.08	0.07	1.8	0.25	0.18	0.08	1.0	0.5	0.5	0.22

The wastewaters became colourless and odourless after the biotreatment (Figure 1 a,b).

Figure 1. Photographic image of **a)** the untreated wastewaters(Domestic wastewater – DWW; Hospital wastewater – HWW; Textile wastewater – TWW; Pharmaceutical wastewater – PWW and Mixed wastewater – MWW) and **b)** the same wastewaters after biotreatment.

3.1.1 BOD, COD and heavy metals estimation

The biological oxygen demand (BOD_5) for domestic, hospital, textile, pharmaceutical and mixed wastewaters were reduced to 39, 78, 14, 68 and 40 mg/L, respectively after the biotreatment (Table 1). The values of COD for these wastewaters were also reduced to 76, 260, 17, 133 and 99 mg/L, respectively after the biotreatment. The BOD_5 /COD ratio for these wastewaters after biotreatment were reduced to 0.51, 0.30, 0.82, 0.51 and 0.40, respectively. A BOD_5 /COD ratio value within the range of 0.3-0.6 normally indicates that these wastewaters are biodegradable [52]. The lowest value of this ratio (0.3) was recorded for the hospital wastewater demonstrating that it was the most recalcitrant (least amenable to biological treatment)

wastewater tested. The heavy metal chromium was detected in the untreated wastewaters, i.e. hospital (1.8 mg/L), pharmaceutical (1.7 mg/L) and mixed (0.9 mg/L) wastewaters which was exceeding the NEQs limit (< 0.05 mg/L). Lead too was present solely in the untreated hospital wastewater (0.17 mg/L). Nickel was present in the untreated domestic (0.08 mg/L), hospital (1.76 mg/L), textile (0.19 mg/L), pharmaceutical (1 mg/L) and mixed (0.5 mg/L) wastewaters (Table 1). The raw untreated hospital wastewater was found to have more heavy metals (Cr, Pb, Ni) than the other types of untreated wastewaters tested. After treatment, the chromium could no longer be detected in the hospital wastewater that confirmed efficacy of the biotreatment. Chromium was reduced to the NEQ limit (< 0.05 mg/L) in pharmaceutical (0.05 mg/L) and mixed (0.019 mg/L) wastewaters after biotreatment (Table 1). Lead which was only present in the hospital wastewater was not detected after biotreatment. The values of Nickel were reduced to 0.07, 0.25, 0.08, 0.5 and 0.22 mg/L after the biotreatment of domestic, hospital, textile, pharmaceutical and mixed wastewaters, respectively. In previous works, slight reduction in colour, temperature, pH, EC, BOD_5, COD, TSS, TDS and heavy metals ions present in textile, sewage and electroplating wastewaters after the bioremediation have been reported [41, 53, 54]. Our results agree well with this previous work and demonstrate the strong potential of native isolates for the removal of a wide range of pollutants in complex wastewaters.

3.2 Isolation and screening of bacteria

In total, 37 bacterial strains were isolated from domestic, hospital, textile, pharmaceutical and mixed wastewaters. Eight bacteria from the domestic wastewater (D1-D8), nine from the hospital wastewater (H1-H9), six from the textile wastewater (T1-T6), six from the pharmaceutical wastewater (P1-P6) and eight from the mixed wastewater (M1-M8) were isolated. Eleven bacteria, isolated from domestic, hospital, textile, pharmaceutical and mixed wastewaters, had the potential to decolourise the preliminary tested domestic wastewater in comparison with all other bacterial isolates under study. The percentage decolourisations of these bacterial strains isolated from domestic (D5, D6, D7 and D8), hospital (H6), textile (T4, T5, and T6), pharmaceutical (P1) and mixed wastewaters (M5 and M8) were > 50% (Figure 2).

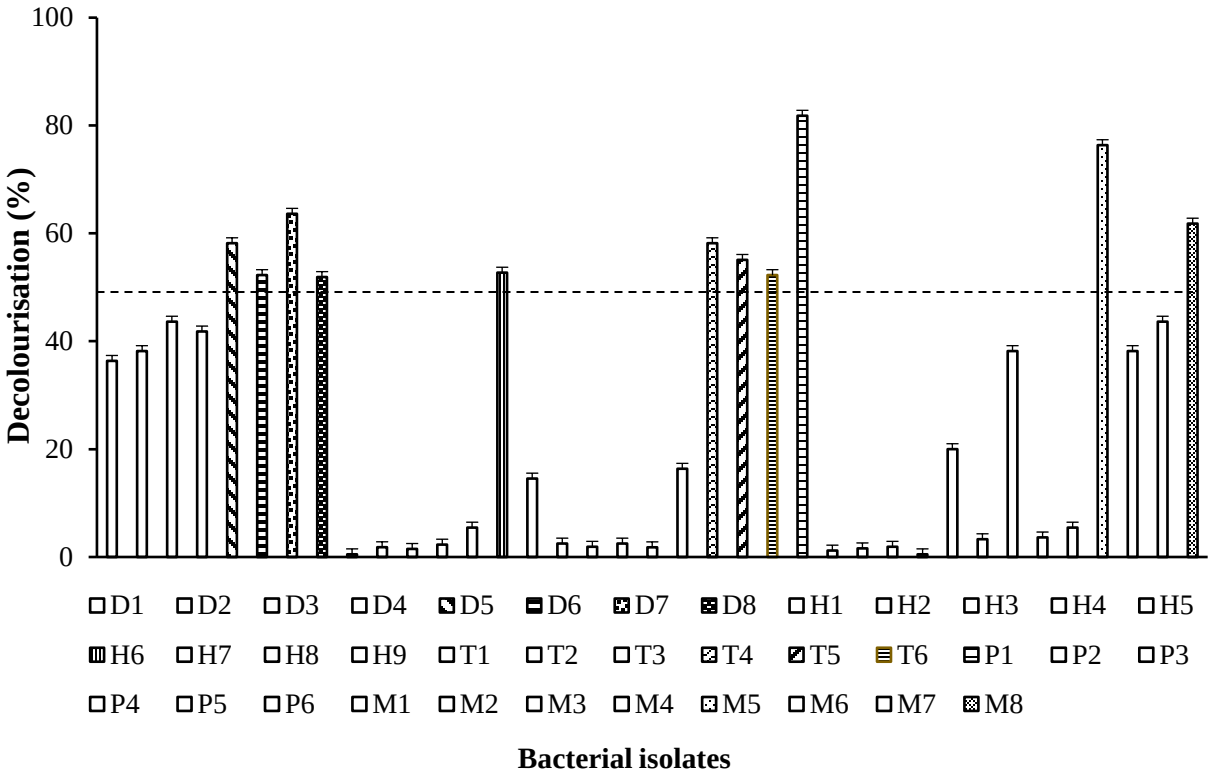

Figure 2. Bar-plot indicating the decolourisation potential (%) of the 37 bacterial isolates in an initial screening against domestic wastewater. The dashed line indicates 50%.

After final screening, three bacterial strains showed more than 70% decolourisation potential against all wastewaters, *i.e.* D6, D7 and P1 (Figure 3). The isolate D6 exhibited 71, 93, 70, 83 and 73 % decolourisation of domestic, hospital, textile, pharmaceutical and mixed wastewaters, respectively. The isolate D7 showed 74, 91, 70, 83 and 73 % decolourisation of domestic, hospital, textile, pharmaceutical and mixed wastewaters, respectively. The isolate P1 showed 82, 92, 71, 77 and 75 % decolourisation of domestic, hospital, textile, pharmaceutical and mixed wastewaters, respectively. Authors have previously reported a varying range of decolourisation potential (14 – 90 %) from six bacteria isolated from textile wastewater used to treat colour contamination owing to specific dye compounds such as, azo, anthraquinone and indigoid dye groups [55]. Traditionally, authors including those above have tested isolates only against synthetic components of textile wastewaters. Thus, the true action and potential of isolates to work in a complex treatment scenario remains to be ascertained. In our work, we

tested the decolourisation potential in raw complex wastewaters to determine the potential remediation of these isolates in an environment that is more representative of the real environmental conditions from wastewater in Pakistan.

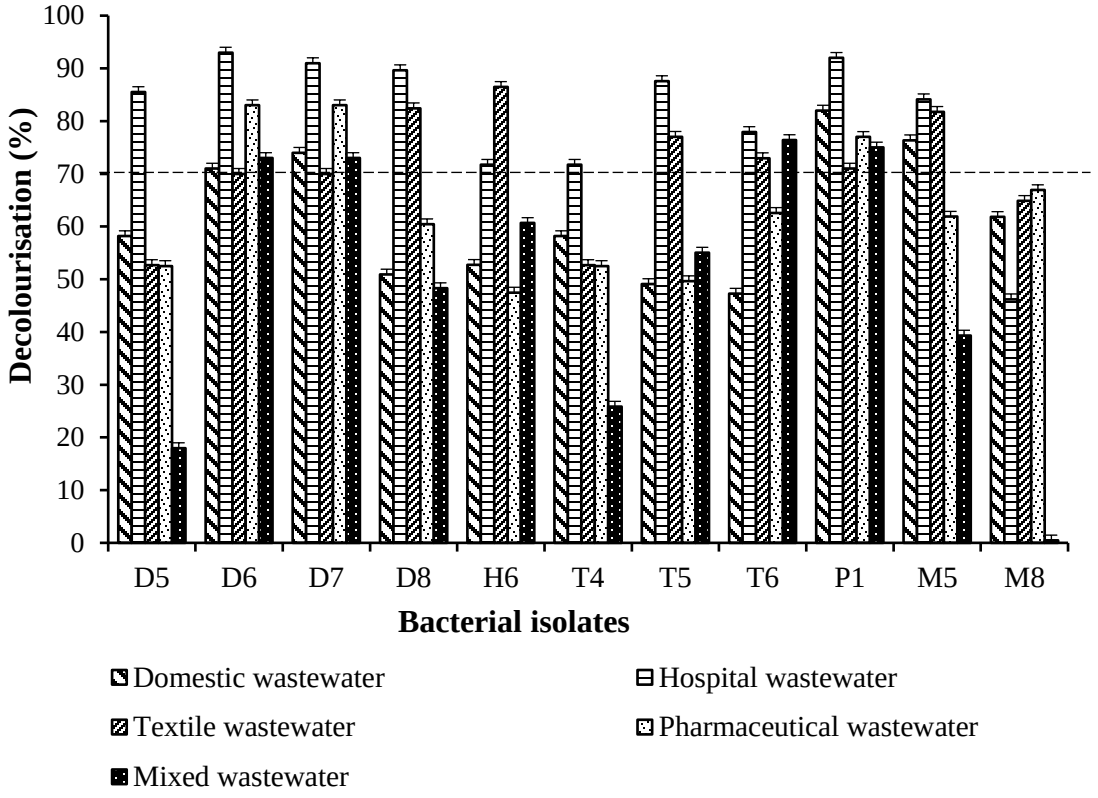

Figure 3. Bar-plot indicating the decolourisation potential (%) of the 11 bacterial isolates in a final screening against five wastewater sources. The dashed line indicates 70%.

3.3 Identification of the screened bacterial isolates

BLAST analysis indicated that strain D6 was a *B.* species with 100% homology to *B. paramycoides* (see Supplementary Material, Table S1). *B. pseudomycoides* is one of nine novel species which are said to possess distinct and distinguishable sequences from the other known species of *B. cereus* group [56]. BLAST analysis indicated that strain D7 was also a *B.* species with 99.86% homology to *B. paramycoides* (see Supplementary Material, Table S2). Thus, we can summarise that D6 and D7 isolates share a high degree of similarity. Authors have previously found other *Bacillus* spp. to be associated with bioremediation of toxic effluents containing

cyanide [57] and alkylphenols [58]. However, this strain has never reported before for any bioremediation work. BLAST analysis indicated that strain P1 was an *Alcaligenes* species with 97.47% homology to *A. faecalis* (see Supplementary Material, Table S3). *A. faecalis* was also reported as a biocontrol agent [59]. However, their role in bioremediation of wide ranged wastewaters has never reported before.

3.4 Testing decolourisation potential of isolated bacteria

All three strains showed a wide range of decolourisation potential of the domestic (> 84 %), hospital (> 93 %), , textile (> 80 %), pharmaceutical (> 87 %) and mixed (> 83 %) wastewaters (Figure 4).

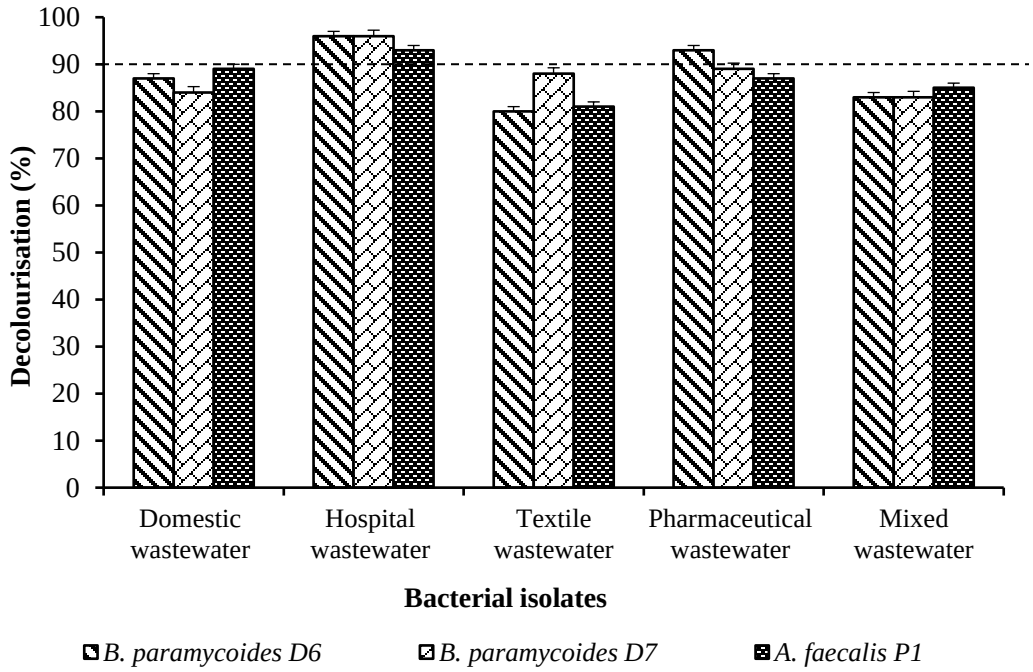

Figure 4. Decolourisation potential (%) of the bacterial isolates (D6, D7 and P1) against domestic, textile and mixed wastewaters.

Previously researchers have worked on the decolourisation of colour contaminants present only in textile wastewaters [60-64]. However, to our knowledge, this present study has demonstrated the highly efficient decolourisation potential of these three bacterial strains for

complex hospital, pharmaceutical industrial, domestic and mixed wastewaters that are frequently discharged in Pakistan.

3.4.1 Organic compounds degradation

Given widespread heavy metal concentrations in hospital effluents (Cr, Pb, Ni), higher decolourisation potential (> 93 %), BOD_5 removal (91 - 68 %) and COD removal (89 - 52 %) and biodegradability index (0.3), we chose to further analyse the consituents of this wastewater. Six pharmaceutic organic pollutants were identified in the untreated hospital wastewater effluent (considered as control) (see Supplementary Material, S1.3, Table S4). The compounds were hydroxybenzene (phenol) RT = 26.72, 2-Hydroxybenzoic acid (salicylic acid) RT = 6.51, 1,3,7-Trimethylpurine-2,6-dione (caffeine) RT = 7.96, 2-arylpropionic acid family member (naproxen) RT = 9.16, 1-Octadecene (octadecene) RT = 28.65 and 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin 2-one (diazepam) RT = 38.06. These pollutants belong to aromatic, metabolite, stimulant, NSAID (non-steroidal anti-inflammatory drug), organic and sedative groups, respectively.

The GCMS analysis of hospital wastewater treated with each of the three isolates revealed significant reduction in the concentrations and peak intensities for the organic compounds described above that were identified in uninoculated hospital wastewater (Figure 5 a-d). This suggests that the screened bacterial strains may have the potential to uptake the constituents of wastewater effluent as energy and food (C, N) source [65]. The capability of *A. faecalis* to use phenol as a carbon source has been previously reported [66]. This supports our results showing the degradation of phenol. Phenol (876 µg/L in the uninoculated hospital wastewater) is known to be toxic even in trace amounts [67, 68] was 100% degraded (not detectable) in wastewater samples treated by *B. paramycoides* (D6) and *B. paramycoides* (D7). Recently, phenol degradation in synthetic wastewaters has been reported in a bacterial consortium reactor within a period of 55 days [69]. However, our two monocultures were successful in achieving complete degradation in 48 hrs. In addition, phenol was degraded by approximately 57 % by *A. faecalis* (P1) treated wastewater.

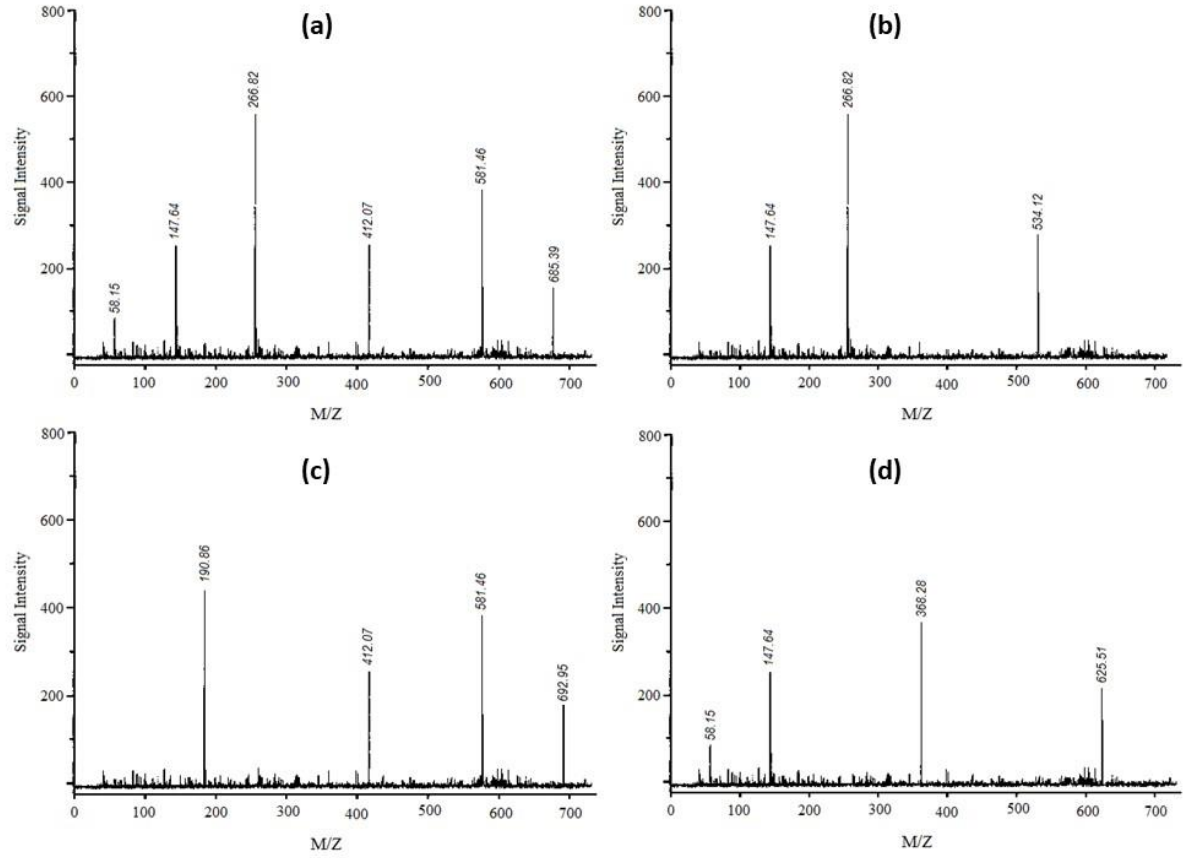

Figure 5. Mass spectra **(a).** untreated hospital wastewater (control) **(b).** *B. paramycoides* (D6) treated hospital wastewater **(c).** *B. paramycoides* (D7) treated hospital wastewater **(d).** *A. faecalis* (P1) treated hospital wastewater

Salicylic acid is one of the emerging most concentrated pollutant (exceeding the 1 µg/L) which is recalcitrant in wastewater [70]. The compound was not detected in the wastewater treated with the *B. paramycoides* (D7), suggesting that it was degraded 100 % from the hospital wastewater by this isolated bacterium. Additionally, the salicylic acid concentration found in uninoculated hospital wastewater sample (48 µg/L) was reduced up to 85 % and 69 % in the treated samples with *B. paramycoides* (D6) (7 µg/L) and isolate *A. faecalis* (P1) (15 µg/L), respectively. The possible reason for the observed reduced percentage degradation may be because of its role as a derivatising agent to degrade the organic compounds into simpler molecules so it may be used in the mechanism of derivatisation within the complex mixtures of wastewater [71]. Caffeine molecule as pharmaceutic is present in all raw hospitals wastewaters which is a high-priority environmentally hazardous pollutant [72]. Caffeine can be removed

through conventional wastewater treatment processes (as much as 97%) [73]. Here we observed 100 % degradation of caffeine (from 7 µg/L in the uninoculated sample) using wastewater treated with both *B. paramycoides* (D7) and *A. faecalis* (P1) and up to 43 % in *B. paramycoides* (D6) treated wastewater. Thus, demonstrating the efficacy of this low-cost and low-tech solution for priority contaminant removal.

Naproxen (23 µg/L) was degraded 100 % in *B. paramycoides* (D6) and *A. faecalis* (P1) bacterial treated wastewaters. The removal of Naproxen was previously reported in monoculture studies [74, 75], slow sand filtration [76, 77] and in soil aquifer treatment [78] which are biological systems. The role of isolates *B. paramycoides* (D6) and *A. faecalis* (P1) as monoculture was found successful in its removal from treated wastewaters. *B. paramycoides* (D7) treated wastewater also demonstrated successful removal (up to 74 %). We also observed 96 - 100 % removal of the following priority contaminants from all three treated wastewaters: octadecene and diazepam.

Some of the compounds [Triclopidine (11 µg/L), Tetradecene (35 µg/L), Griseofulvin (28 µg/L), Lidocaine (122 µg/L) and Butalbital (54 µg/L)], that were not found in the untreated water but were identified in the treated water (see Supplementary Material, S1.3, Table S4). There are two suppositions for the existence of these compounds in the treated hospital wastewaters. First, it could be due to the transformation of metabolites by parent compounds [79]. Secondly, it could be a matrix effect in the complex control hospital wastewater that has led to suppression of the signals for these compounds in the mass spectrometer [80, 81]. Therefore, it could be an analytical reason that may have caused a shift on the LODs [82, 83] due to which the LOD of these compounds, which were not being detected in the untreated hospital wastewater, were shifted for a LOD that could be detected in treated wastewater.

3.5 Metal tolerance limits

At 100 mg/L concentration of metal salts, the three isolates showing > 70 % in particular metal salts were selected to be tested at higher concentrations (see Supplementary Material, Figure S26). For isolate *B. paramycoides* (D6), $CaCl_2$ and $MgSO_4$ metal salts were selected that showed overall maximum growth of 78 and 70 % at 300 mg/L concentrations, respectively. For isolate *B. paramycoides* (D7), $PbNO_3$ metal salt was selected that showed maximum growth of 82

% at 300mg/L concentration. For isolate *A. faecalis* (P1), $PbNO_3$, Na_2MoO_4, $CaCl_2$, $MgSO_4$ and $K_2Cr_2O_7$ metal salts were selected that showed maximum growth of 65, 90, 73, 73 and 75 % at 300mg/L concentration of these metal salts, respectively (Figure 6 a-c).

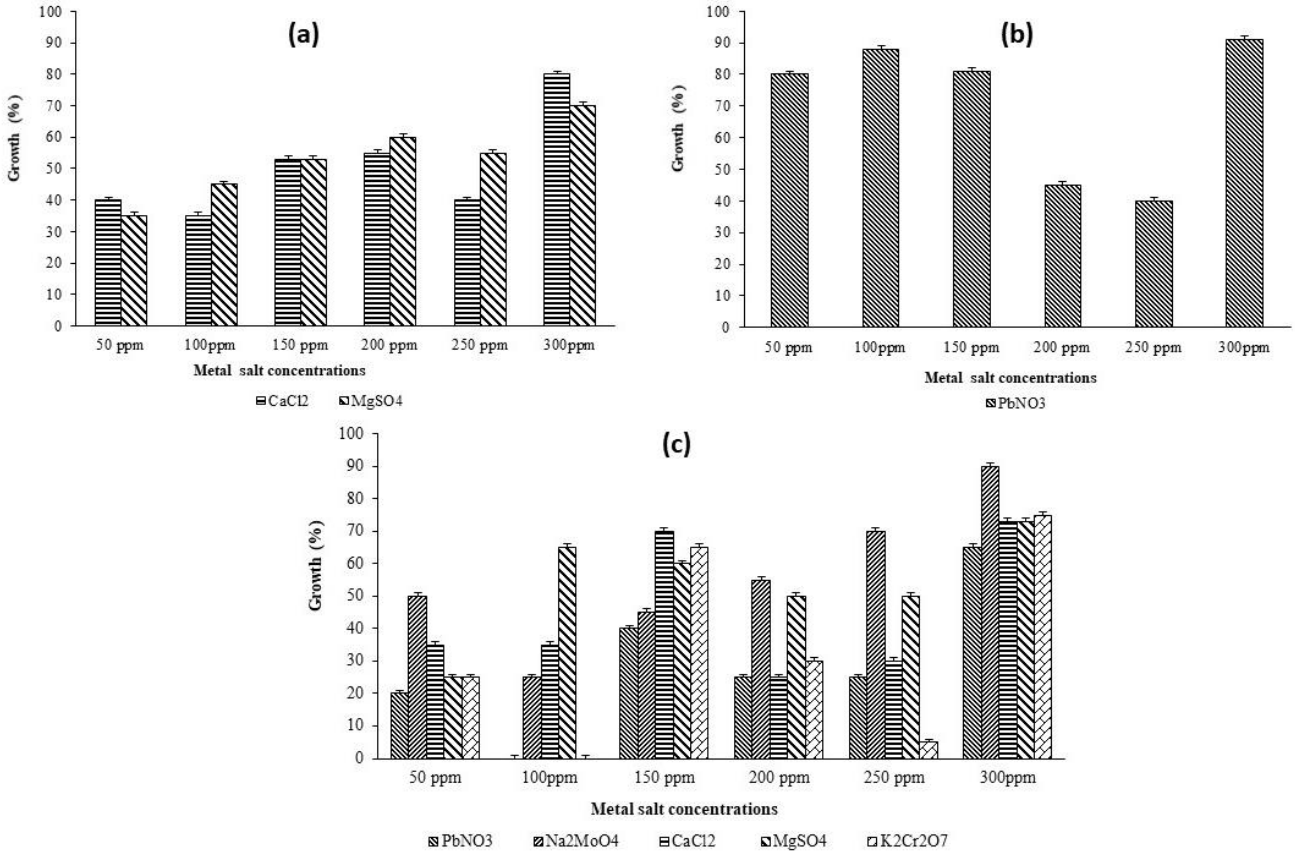

Figure 6. Maximum tolerance limits of bacterial isolates to metals salts solutions at different concentrations **(a)** *B. paramycoides* (D6)*,* **(b)** *B. paramycoides* (D7), **(c)** *A. faecalis* (P1).

This has confirmed that all three strains have the potential to tolerate these metals efficiently along with remediating the organic compounds from wastewaters even in co-existence with heavy metals. It also supported our results (Section 3.1.1) that these isolates have potential to adsorb the heavy metals to remove them from wastewaters. The high metals concentration is really a big challenge for wastewater treatments as it leads to the inhibition of the microbial populations etc. These strains were resistant to high metal concentrations and thus tolerated the harsh environments of these complex wastewaters.

4. Conclusions

In this study, we isolated three bacterial strains (Isolates D6, D7 and P1) from different wastewater (domestic, hospital, textile and pharmaceutical) sources which we identified as *Bacillus paramycoides* (D6), *B. paramycoides* (D7) and *Alcaligenes faecalis* (P1). These isolates were capable of decolourising (> 93%) and degrading (BOD_5 removal: 91-68 % and COD removal: 89 - 53 %) hospital wastewater. In addition, these mono-cultures achieved 100% removal of multiple pharmaceutical compounds (Phenol, Salicylic acid, Caffeine, Naproxen, Octadecene and Diazepam) and high metal tolerance ($PbNO_3$, Na_2MoO_4, $CaCl_2$, $MgSO_4$ and $K_2Cr_2O_7$ up to 300 mg/L). The present work provides us a low-cost and low-tech mono-culture system alternative to bioremediate complex matrices of hospital wastewater to support the crop irrigation and achievement of clean water and sanitation in developing countries like Pakistan.

Conflicts of interest

There are no conflicts to declare.

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Supplementary Material

S1.1 Optimal growth conditions

S1.1.1 Methodology

The estimation of optimal growth conditions with maximum decolourisation potential for the three bacterial isolates was first tested with the following parameters; incubation time, temperature and inoculum concentration. For incubation time, 100 mL of autoclaved domestic wastewater was inoculated with each of these three screened isolates separately (10 % inoculum) in conical flasks placed in a shaking incubator at 120 rpm (PMI Labortechnik GMBH, WIS-20R) [58]. The flasks were incubated at 37 °C for 24, 48, 72 and 96 hours. For testing the optimal temperature, the inoculum (10 %) of screened isolates was added to autoclaved domestic wastewater (100 mL) in conical flasks (250 mL) for 24 h. Flasks were incubated at 30, 37, 44, 51 and 58 °C in a shaking incubator (PMI Labortechnik GMBH, WIS-20R). For the inoculum concentration, a loop full of bacterial colony from plate of each bacterial strain was added separately in autoclaved distilled waters (100 mL). The optical density (OD) was adjusted to 1 at 545 nm wavelength using UV/VIS spectrophotometer (A&E Labmed, AE-S80) in order to maintain equal number of bacterial cells of each inoculum prior to incubation. The inoculum concentrations tested were 5, 10, 15, 20, 25 and 30 % [59]. The bacterial cell count per mL of each screened isolate was also determined using a haemocytometer slide bridge (Neubaur improved HBG, Marinefield, Germany). The determined optimum inoculum concentration (10 % for each isolate), optimum incubation time (48 h for each isolate) and optimum temperature (*B. paramycoides* spp. at 37 ℃ and *A. faecalis* at 51 ℃) were used as the experimental conditions to perform the subsequent decolourisation tests.

S1.1.2 Result

The strain D6 showed the maximum decolourisation at 37 ℃ while lowest at 51℃. The same pattern was observed for D7 (Figure S1). The strains D6 and D7 were proven to be perfect mesophile in nature as they showed maximum decolourisation of 50 and 73 % at 37 ℃. The strain P1 showed maximum decolourisation of 78 % at 51 ℃. The present result indicated the presence of species at 51 °C and showed its existence as a thermophile. A big difference in decolourisation was observed between 51 and 58 degrees for all isolates even the thermophile. Perhaps some hydrolysis or production of toxic compounds may responsible for that.

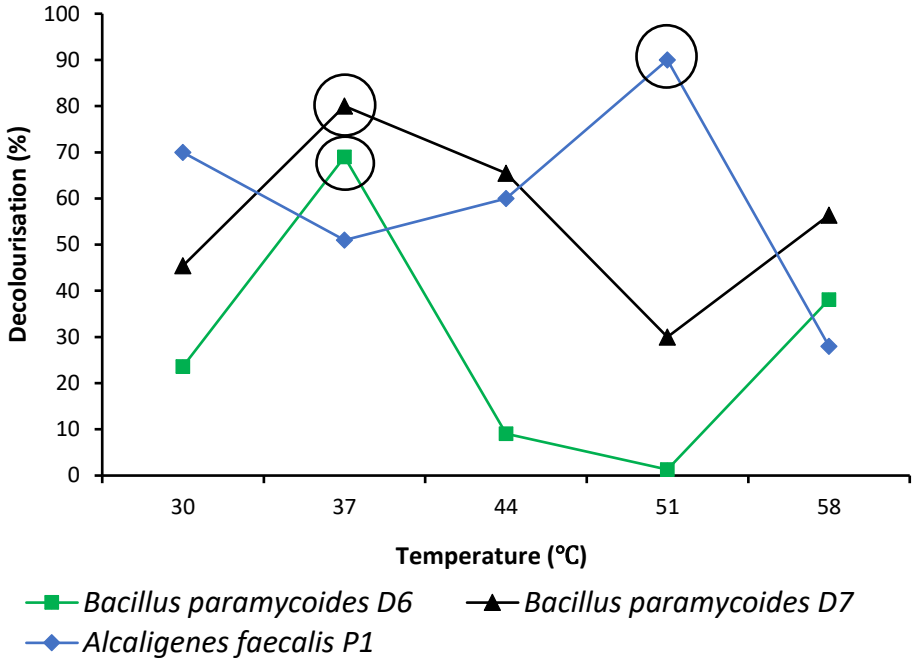

Figure S1. Optimisation I: Temperature (℃).

After 48 hours of incubation, the strains D6, D7 and P1 showed maximum decolourisation of 88, 88, 89 % against domestic wastewater, respectively (Figure S2).

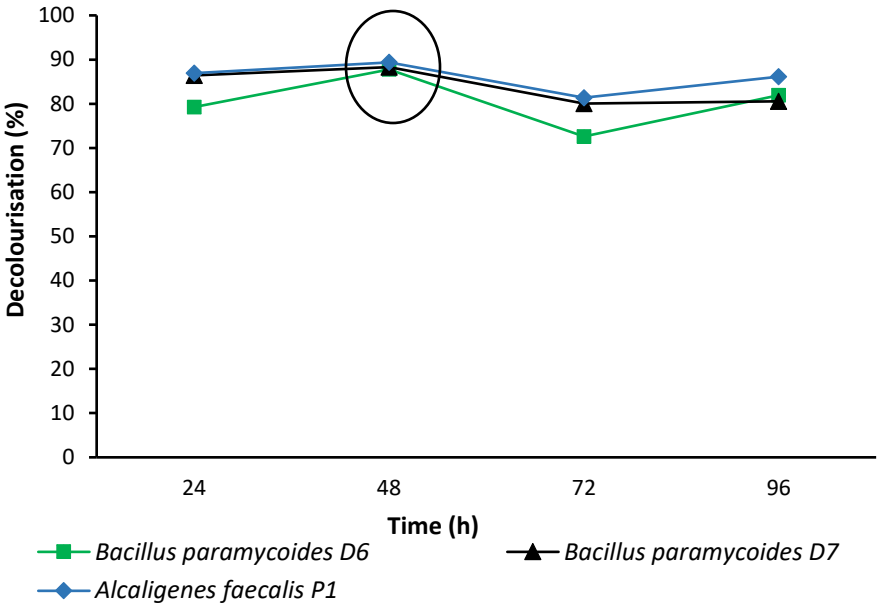

Figure S2. Optimisation II: Incubation time (h).

The bacterial cell count calculated for strains D6, D7 and P1 (at OD = 1) were 4.675×10^7 cells/mL, 5.15×10^7 cells/mL and 7.6×10^7 cells/mL respectively. The strains D6, D7 and P1 showed maximum percentage decolourisation of 60, 68 and 70 for 10% of inoculum concentration (Figure S3).

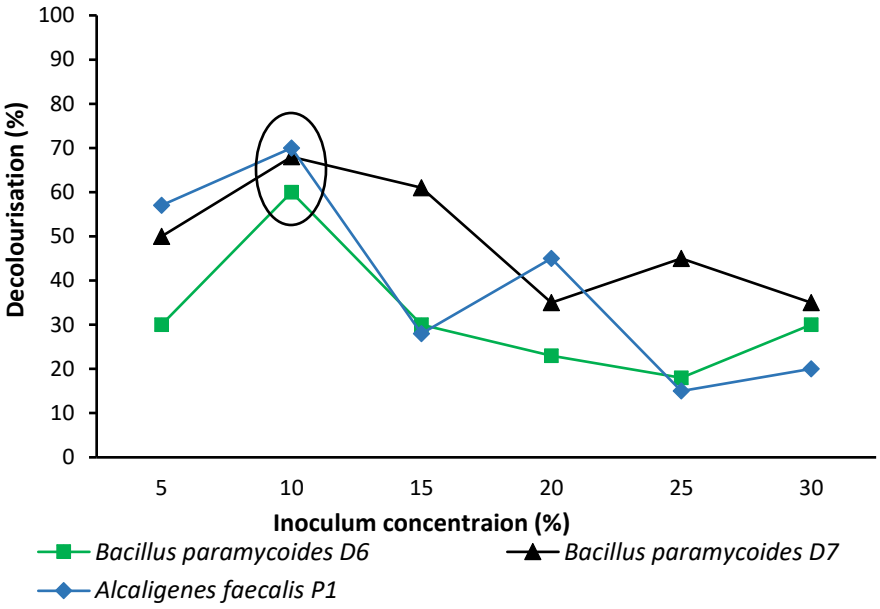

Figure S3. Optimisation III: Inoculum concentration (%).

S1.2 Identification of isolates

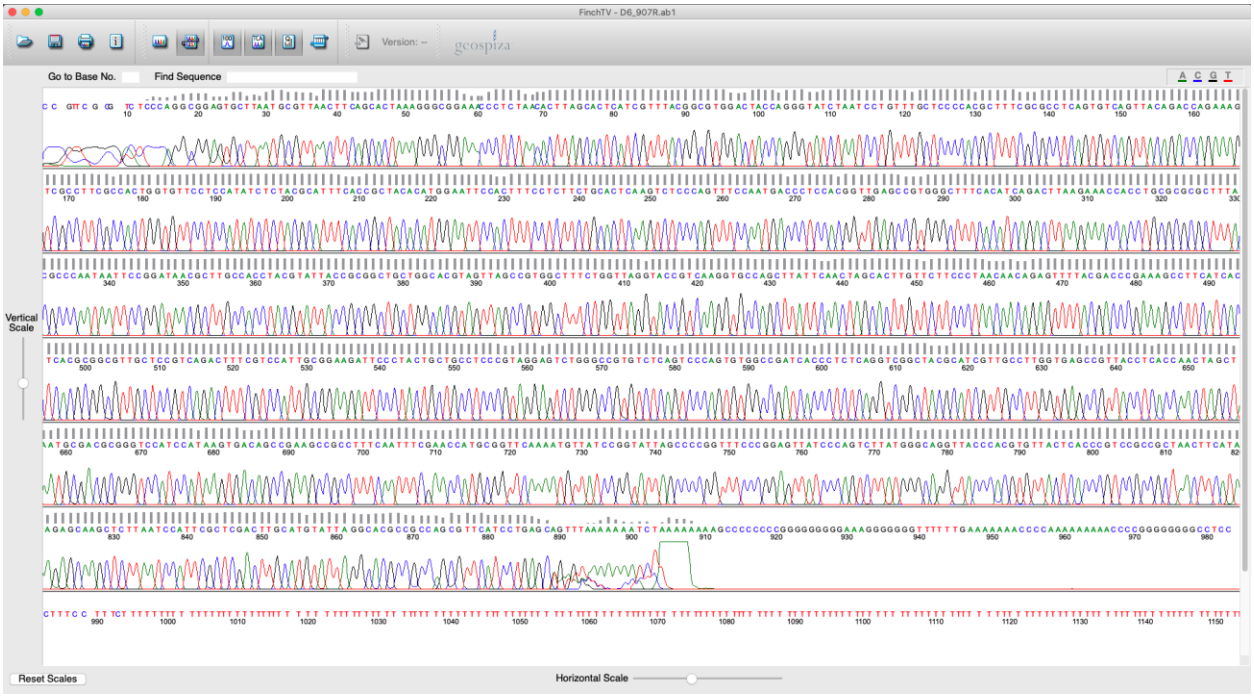

Figure S4. D6 785F raw chromatogram.

D6 785F – 1359 nucleotides

TAGTCGATGAGTGCTAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGC
CTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA
TGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAG
GGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTG
GGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGAC
TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACA
CACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTC
TCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGC
CGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAG
TCGGTGGGGTAACCTTTTTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTCTAAAAGG
GGGAGACCCAAAAAAGGCGCCCTTCCGGGGTTGGAACCCTACCCTGAGGCGCCCAAACCCTGGGGGG
CAAACCCGGGTTAAGGGGCGTGGGGGTCCCCCGCCCGGGGGCCAAGGAGTGCCGGGGGGGAAAGGC
GGTCGGCCGTTAAGGGAGGGAAGTAACAGAGCTAAAGAAAGACACCCCGGCGGGGGGAGAGCGGGGG
GGGAGGAATACCAAAAGAATAGGACACGCGGGGCGCAGCATAACCGACAACAAAAAGCTCGAAGATATA
ACTCCCCGCCCAATAAACATAACCCAGGCGGGGGGCAGGCACATACCCGCCCACAATACAGAGAGAGG
CTCCCCTCCCGCCGCACGGAAGACAAAAGGAACAAAGTGGGTGGACCGCCTCCTCCCCCCACCCGGA
GAACAAGGGGAGGAAGGGCGCTCCACCCCCCCCCCACGCCCCGGGGGGCCCCAGTTACTATACCTATT
TTTTTTGGGGATAATTAAAAAGACTCCTCCCCCCGCCCGCCACCCCAAAAGAAAGGGGGGGGGGTATGC
TTGTCGGGTGGATCTTTCAACCCCCCCTTCACCCCCTTTTTAGGGCGGGGGGCTTATTGTTGAGGGGGG
GGTGTGTTGGGGGAGGACCCCCAAAACACCCTACAAAAGAAAGGTGGTTTTTTTTAAA

Figure S5. D6 785F raw sequence.

D6 785F – 670 nucleotides

TAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGG
CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCG
AAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGG
GAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAAC
CGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATG
GACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTA
GGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACC
TTTTTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGAAGTC

Figure S6. D6 785F cleaned sequence.

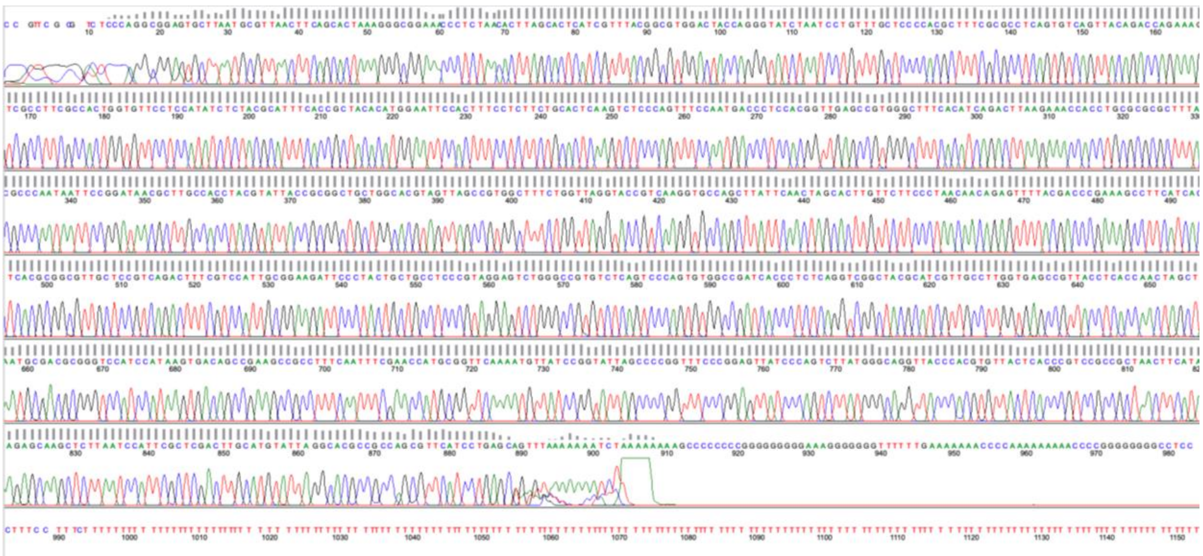

Figure S7. D6 907R raw chromatogram.

D6 907R – 1208 nucleotides

CCGTTCGCGTCTCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAAC
ACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCG
CCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCA
CCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTT
GAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAA
CGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGT
CAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCA
TCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTA
GGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCC
TTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCG
CCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAG
TCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCC
ATTCGCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGCAGTTTAAAAAAATCTAAAAAA
AAGCCCCCCCCGGGGGGGGGAAAGGGGGGGTTTTTTGAAAAAAACCCCAAAAAAAAAACCCCGGGGGG
GGCCTCCCTTTCCTTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
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TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

Figure S8. D6 907R raw sequence.

D6 907R – 875 nucleotides

CCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATC
GTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTT
ACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGA
ATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTT
TCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCT
TATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGC
GTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCG
TGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTA
CCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAA
CCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTT
ACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTG
CATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGC

Figure S9. D6 907R cleaned sequence.

D6 907R – 875 nucleotides

CCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATC
GTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTT
ACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGA
ATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTT
TCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTA
CGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCT
TATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGC
GTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCG
TGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTA
CCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAA
CCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTT
ACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTG
CATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGC

Figure S10. D6 907R cleaned sequence.

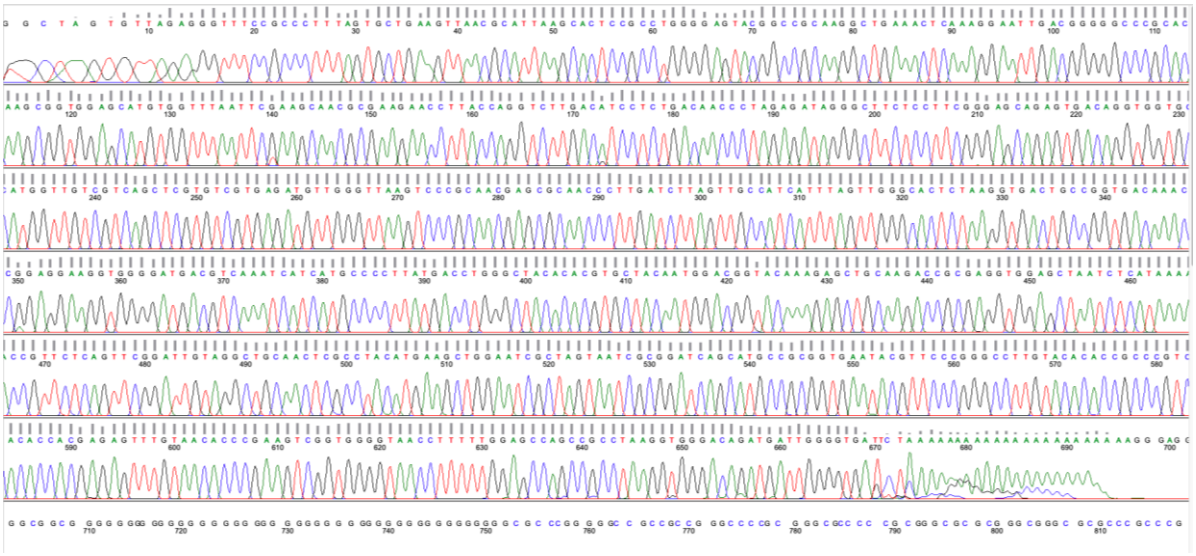

Figure S11. D7 785F raw chromatogram.

D7 785F – 1681 nucleotides

GGCTAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTA
CGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
TCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTT
CGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC
GCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACA
AACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACA
ATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATT
GTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATAC
GTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTA
ACCTTTTTGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTGATTCTAAAAAAAAAAAAAAAAAAA
AAAAGGGAGGGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGCG
CCCGGGGGCCGCCGCCGGGCCCCGCGGGCGCCCCCGCGGGCGCGCGGGCGGGCGCGCCCGCCCG
CCGCGCCCGCGCGCGCCCCGGCCGGGCGCCCGCCCCCGCGGGGGCGCCGCGGGCGCCCGCCGC
GGCGGGCCGGCGGCCCGCCCGCCGGCCGCCCCGGCGGGGCCCGGGGCGCGGGGGCGCCGCCCGC
CGCCGCGCCCCGGCCGCGGGGGGGGGGGGGCCCCGCCCGCCGCCCCCCGGGGCGGGCGGGCGCC
GGCCCGCGCGCGGGGCGCCCCCCGCCCGCGGCGGGGCGGCGCCCCCGCGCCGCGGCGGGGGGCC
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GGGGGGGGGGGGCGCCGCGCGCGCGCGCCCCCGCCGGGGCGGGGGCGGGGCGCCCGCCGGG
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CCCCCCGCCCCGGCGCGGGGGGGGGGGGGGGGGCGCCCGCGGGCCGCGCCGGCGCGGGCGCGCGC
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GCCGGCGCGGCGGCGCCGCCCCCCCGCGGCGGCGGGGGCCGCGCGCGGGCGCGGCGGCGCCCGC
CCCGCCGCGCCCCGGCCGCCGCGCCGCGGCGCGGGGGCGGCCGCGCCCGGCCCGGGGGGGCGCG
GCGCCCGCGGCGCCGCGCGCGGCGCGGCGCGGGCGCGACGGAAAAGAGAGGGGGGGGGGGGA
GGGGGCGCGGCGCGGCGGGCGCGGGCCGCGGCCGCGCGGCGGGCGCGCGGCGGCGCGGGGGGG
GGGGGGGGGGGGGGGGGGGGGCGCGCGCGGGG

Figure S12. D7 785F raw sequence.

D7 785F – 661 nucleotides

GTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGC
AAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGC
AACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGGGAG
CAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGA
GGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACG
GTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCT
GCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACCTTTT
TGGAGCCAGCCGCCTAAGGTGGGACAGATGATTGGGGTG

Figure S13. D7 785F cleaned sequence.

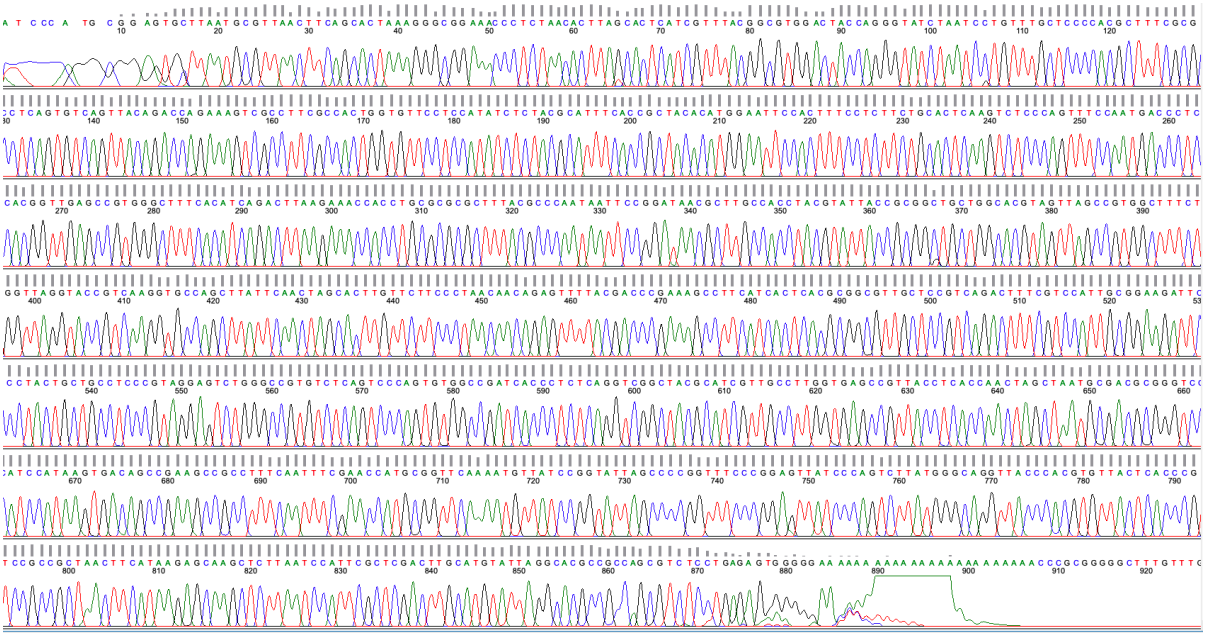

Figure S14. D7 907R raw chromatogram.

D7 907R – 1640 nucleotides

ATCCCATGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTC
ATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCA
GTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATG
GAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGC
TTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCT
ACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGC
TTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGG
CGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCC
GTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTT
ACCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGA
ACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGG
TTACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTT
GCATGTATTAGGCACGCCGCCAGCGTCTCCTGAGAGTGGGGGAAAAAAAAAAAAAAAAAAAAAAAACCC
GCGGGGGCTTTGTTTGGGGGTTTTTTGCCCCCCCCGAAAAAAAAAAACGGGGGGGGGGAAAAACCCCCC
AAACCCCCCAAGATAAGGGGCTTGCCCCCGGGGAGAGGAAAAAAAAAGGAAAGAGGGGGTGTTTTTTC
CCCCCAAAAAGAAAAAAAGAATAAGAGTAAATCCACCCCCCCCACCCCCCCCCCTGGCCCCTTTTTTTTT
TTCCTTTTTTTTTTTTGAGAAAGAAAAAAAGAGCCGGGGGCGGGGCGAAACGCGAAGAAAAGGGGGGG
GGGGGGGGGGAACCACATAATCTCACCCCCCCCCCCCCCATCCCCCTGTTGGATACAAACATGTCCCCAA
ATTGAGGGGGGGGGAGGGGAGGAGAAAAAAAAAAAAAAAAAGAAAGGGGGGGGGGGGGGGGGGGGG
GCAGAGCCATTCTCAAATGAATGGACGTTTGAAAGTTGTGGCCCCTCCCAAACTCACAAAAAAAAAAAAG
ATAAGGTATAAAATATGTAAGTCCCCCCGGCACACTGCCCGGCCACCCTACAATGATGGCATTGGTTCCCT
GGGTGCCGCCCGCCCCCCCCCCCCCCCCCCCCCCCACCCAAAAACACACAAAGAAAAAATAAAATAAAAAT
TTTTTTGTGTTTGTTTTTTTGTTTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGAAAAAAAAAGAA
GGAAGGAGGGAAGAGAGAATAAAATAAAAAAAAATGGGGTTGTA

Figure S15. D7 907R raw sequence.

D7 907R – 860 nucleotides

CAGGAGACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAA
GTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACC
GGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTAT
GGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGA
CCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAG
GGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCG
TAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGA
AAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTG
GGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTC
ATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAG
AGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCG
TGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGG
TTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCA

Figure S16. D7 907R cleaned sequence.

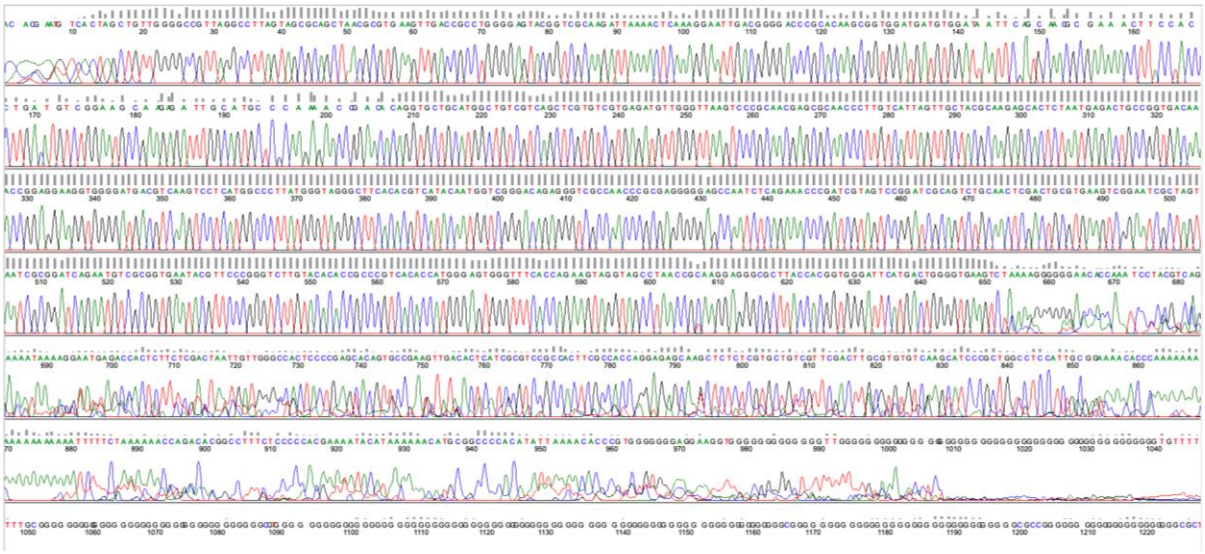

Figure S17. P1 785F raw chromatogram.

P1 785F – 1712 nucleotides

ACACGAATGTCACTAGCTGTTGGGGCCGTTAGGCCTTAGTAGCGCAGCTAACGCGTGAAGTTGACCGCC
TGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGT
GGATAATTCAGCAACGCGAAACTTCCACCTGATGTCGGAAGCAAGAGATTGCATGCCCAAAACCGACACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC
TTGTCATTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGGACAGAGGGTCG
CCAACCCGCGAGGGGGAGCCAATCTCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACT
GCGTGAAGTCGGAATCGCTAGTAATCGCGGATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTAC
ACACCGCCCGTCACACCATGGGAGTGGGTTTCACCAGAAGTAGGTAGCCTAACCGCAAGGAGGGCGCT
TACCACGGTGGGATTCATGACTGGGGTGAAGTCTAAAAGGGGGGAACACCAAATCCTACGTCAGAAAATA
AAAGGAATGAGACCACTCTTCTCGACTAATTGTTGGGCCACTCCCCGAGCACAGTGCCGAAGTTGACAC
TCATCGCGTCCGCCACTTCGCCACCAGGAGAGCAAGCTCTCTCGTGCTGTCGTTCGACTTGCGTGTGTC
AAGCATCCCGCTGGCCTCCATTGCGGAAAACACCCAAAAAAAAAAAAAAAAAAATTTTTCTAAAAAACCAGA
CACGGCCTTTCTCCCCCACGAAAATACATAAAAAACATGCGGCCCCACATATTAAAACACCCGTGGGGGG
GAGGAAGGTGGGGGGGGGGGGGTTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
GGGGGGGGGTGTTTTTTTGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGCGTGGGGGG
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GGGGCCCGGGCCCCCCCCCCCCCCCCCCCCCGGCCGGGCGGGGGCGCCGCCCCCCCCGCCCCGGC
CCGGCGGGGGGGGGGGGGGGGGGGGCGGCGCCCGCCGCGCCCCCCCCCCCCCCCCCCCCCGCC
GCGCGGGGGGCGGGGGGGGGGCCGCGGCCCGCCCCCCCCGCGCCGCGCGGCCCGCTCCCGCCC
CCCCCCGCGCGCGGGGCCCGCGCGGCCGCGGCGCGGGGTCGGAGGCCGCCGCGCCCCCGCCCCCC
CCCCGCCCCCCCCCCGCCGCCGGCGCGGCGACGCGCCGCCCCCGCGCGGGCCGCCGCGCGCCCGC
CCGCGGGCGGGGGGGGGCGGCCCCGCGCCGCGCGCGCCGGCCGGCGCGCCGAACGCCCGGGCGC
CGGGGCCGCGGCGGCGCGGGGGGGGCGCGCGCCGGGGCGCGCCCG

Figure S18. P1 785F raw sequence.

P1 785F – 640 nucleotides

CTAGCTGTTGGGGCCGTTAGGCCTTAGTAGCGCAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACG
GTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATAATTCAG
CAACGCGAAACTTCCACCTGATGTCGGAAGCAAGAGATTGCATGCCCAAAACCGACACAGGTGCTGCAT
GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCATTAGT
TGCTACGCAAGAGCACTCTAATGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGT
CCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGGACAGAGGGTCGCCAACCCGCG
AGGGGGAGCCAATCTCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTC
GGAATCGCTAGTAATCGCGGATCAGAATGTCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCG
TCACACCATGGGAGTGGGTTTCACCAGAAGTAGGTAGCCTAACCGCAAGGAGGGCGCTTACCACGGTG
GGATTCATGACTGGGGTGAAGTC

Figure S19. P1 785F cleaned sequence.

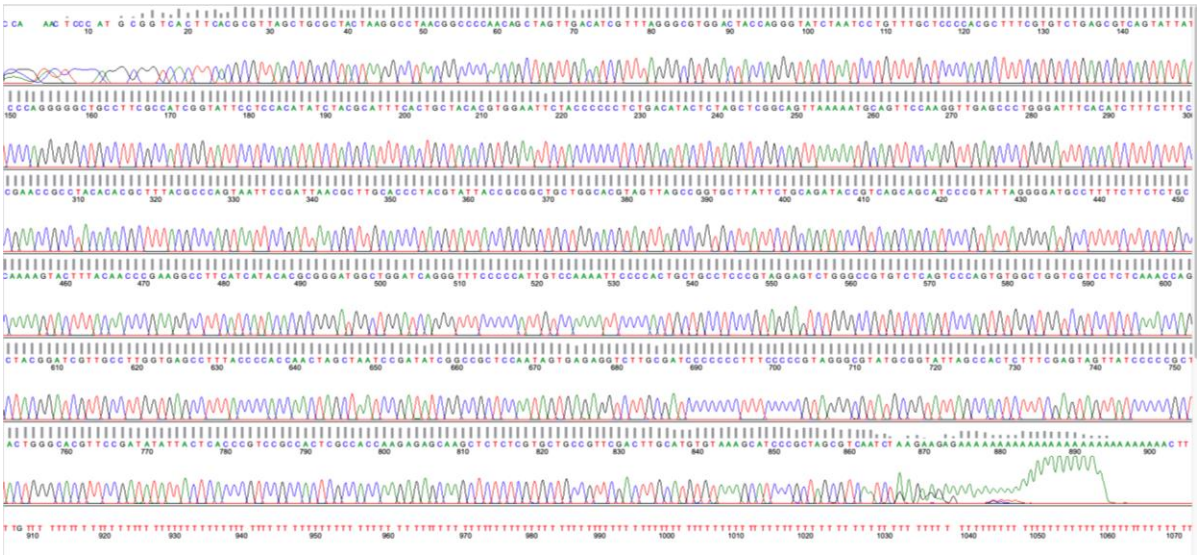


Figure S20. P1 907R raw chromatogram.

P1 907R – 1627 nucleotides

CCAAACTCCCATGCGGTCACTTCACGCGTTAGCTGCGCTACTAAGGCCTAACGGCCCCAACAGCTAGTT
GACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGTCTGAGCG
TCAGTATTATCCCAGGGGGCTGCCTTCGCCATCGGTATTCCTCCACATATCTACGCATTTCACTGCTACAC
GTGGAATTCTACCCCCCTCTGACATACTCTAGCTCGGCAGTTAAAAATGCAGTTCCAAGGTTGAGCCCTG
GGATTTCACATCTTTCTTTCCGAACCGCCTACACACGCTTTACGCCCAGTAATTCCGATTAACGCTTGCAC
CCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTATTCTGCAGATACCGTCAGCAGCATC
CCGTATTAGGGGATGCCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCG
GGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGC
CGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTT
TACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCC
CCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATA
TATTACTCACCCGTCCGCCACTCGCCACCAAGAGAGCAAGCTCTCTCGTGCTGCCGTTCGACTTGCATG
TGTAAAGCATCCCGCTAGCGTCAATCTAAGAAGAGAAAAAAAAAAAAAAAAAAAAAAAAAAAACTTTTGTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTA
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAATTTT
TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTATTTTTTTT
TTTTTTTTCTTTTTT

Figure S21. P1 907R raw sequence.

P1 907R – 851 nucleotides

GGTCACTTCACGCGTTAGCTGCGCTACTAAGGCCTAACGGCCCCAACAGCTAGTTGACATCGTTTAGGG
CGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGTCTGAGCGTCAGTATTATCCCA
GGGGGCTGCCTTCGCCATCGGTATTCCTCCACATATCTACGCATTTCACTGCTACACGTGGAATTCTACCC
CCCTCTGACATACTCTAGCTCGGCAGTTAAAAATGCAGTTCCAAGGTTGAGCCCTGGGATTTCACATCTTT
CTTTCCGAACCGCCTACACACGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTACGTATTACCGC
GGCTGCTGGCACGTAGTTAGCCGGTGCTTATTCTGCAGATACCGTCAGCAGCATCCCGTATTAGGGGATG
CCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGGCCTTCATCATACACGCGGGATGGCTGGATCA
GGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCC
AGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTTGCCTTGGTGAGCCTTTACCCCACCAACTA
GCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTTGCGATCCCCCCCTTTCCCCCGTAGGGCGTAT
GCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTACTGGGCACGTTCCGATATATTACTCACCCGTC
CGCCACTCGCCACCAAGAGAGCAAGCTCTCTCGTGCTGCCGTTCGACTTGCATGTGTAAAGCATCCCGC
TAGCGTCAATC

Figure S22. P1 907 cleaned sequence.

D6 Assembled Contig – 1484 nucleotides

GACTTCACCCCAATCATCTGTCCCACCTTAGGCGGCTGGCTCCAAAAAGGTTACCCCACCGACTTCGGG
TGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCT
GATCCGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAACGGT
TTTATGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCC
AGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTA
GAGTGCCCAACTAAATGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTC
ACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAG
GGTTGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCG
CTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTA
ATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACT
ACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCG
CCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTC
TGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGA
AACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGC
TGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGT
TCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTT
TCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGT
GGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAAT
GCGACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATG
TTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCAC
CCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCC
GCCAGCGTTCATCCTGAGC

Figure S23. D6 Assembled Contig – GenBank accession number MT477810.

D7 Assembled Contig – 1475 nucleotides

CACCCCAATCATCTGTCCCACCTTAGGCGGCTGGCTCCAAAAAGGTTACCCCACCGACTTCGGGTGTTA
CAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATC
CGCGATTACTAGCGATTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAACGGTTTTA
TGAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGCACGTGTGTAGCCCAGGT
CATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGT
GCCCAACTAAATGATGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAGGGTT
GTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTG
TGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGC
GTTAACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCA
GGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTT
CGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCA
CTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACC
ACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGC
ACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTT
CCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGT
CCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCC
GATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGA
CGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATC
CGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGT
CCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTCGACTTGCATGTATTAGGCACGCCGCCA
GCGTCTCCTG

Figure S24. D7 Isolate assembled contig GenBank accession number MT477812.

P1 Assembled Contig – 1438 nucleotides

GACTTCACCCCAGTCATGAATCCCACCGTGGTAAGCGCCCTCCTTGCGGTTAGGCTACCTACTTCTGGTG
AAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCGACATTCTGA
TCCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGATCGGGT
TTCTGAGATTGGCTCCCCCTCGCGGGTTGGCGACCCTCTGTCCCGACCATTGTATGACGTGTGAAGCCC
TACCCATAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCATTA
GAGTGCTCTTGCGTAGCAACTAATGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGA
CACGAGCTGACGACAGCCATGCAGCACCTGTGTCGGTTTTGGGCATGCAATCTCTTGCTTCCGACATCA
GGTGGAAGTTTCGCGTTGCTGAATTATCCACATCATCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTG
AGTTTTAATCTTGCGACCGTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTGCGCTACTAAGGCCTAAC
GGCCCCAACAGCTAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCAC
GCTTTCGTGTCTGAGCGTCAGTATTATCCCAGGGGGCTGCCTTCGCCATCGGTATTCCTCCACATATCTAC
GCATTTCACTGCTACACGTGGAATTCTACCCCCCTCTGACATACTCTAGCTCGGCAGTTAAAAATGCAGTT
CCAAGGTTGAGCCCTGGGATTTCACATCTTTCTTTCCGAACCGCCTACACACGCTTTACGCCCAGTAATT
CCGATTAACGCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTATTCTGCAG
ATACCGTCAGCAGCATCCCGTATTAGGGGATGCCTTTTCTTCTCTGCCAAAAGTACTTTACAACCCGAAGG
CCTTCATCATACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTC
CCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCG
TTGCCTTGGTGAGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAGGTCTT
GCGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACTCTTTCGAGTAGTTATCCCCCGCTA
CTGGGCACGTTCCGATATATTACTCACCCGTCCGCCACTCGCCACCAAGAGAGCAAGCTCTCTCGTGCT
GCCGTTCGACTTGCATGTGTAAAGCATCCCGCTAGCGTCAATC

Figure S25. P1 Isolate assembled contig GenBank accession number MT477813.

Table S1. Top 10 BLAST hits for isolate D6.

Hit Number	Description	Max Score	Total Score	Query Cover	Percentage Identity	Accession number
1.	*B. paramycoides* strain MCCC 1A04098 16S ribosomal RNA, partial sequence	**2743**	**2743**	**100%**	**100.00%**	**NR_157734.1**
2.	*B. tropicus* strain MCCC 1A01406 16S ribosomal RNA, partial sequence	**2737**	**2737**	**100%**	**99.93%**	**NR_157736.1**
3.	*B. nitratireducens* strain MCCC 1A00732 16S ribosomal RNA, partial sequence	**2737**	**2737**	**100%**	**99.93%**	**NR_157732.1**
4.	*B. luti* strain MCCC 1A00359 16S ribosomal RNA, partial sequence	**2737**	**2737**	**100%**	**99.93%**	**NR_157730.1**
5.	*B. albus* strain MCCC 1A02146 16S ribosomal RNA, partial sequence	**2737**	**2737**	**100%**	**99.93%**	**NR_157729.1**
6.	*B. cereus* strain CCM 2010 16S ribosomal RNA, partial sequence	**2732**	**2732**	**100%**	**99.87%**	**NR_115714.1**
7.	*B. cereus* ATCC 14579 16S ribosomal RNA (rrnA), partial sequence	**2732**	**2732**	**100%**	**99.87%**	**NR_074540.1**
8.	*B. proteolyticus* strain MCCC 1A00365 16S ribosomal RNA, partial sequence	**2726**	**2726**	**100%**	**99.80%**	**NR_157735.1**
9.	*B. cereus* strain IAM 12605 16S ribosomal RNA, partial sequence	**2721**	**2721**	**99%**	**99.86%**	**NR_115526.1**
10.	*B. wiedmannii* strain FSL W8-0169 16S ribosomal RNA, partial sequence	**2721**	**2721**	**100%**	**99.73%**	**NR_152692.1**

Table S2. Top 10 BLAST hits for isolate D7.

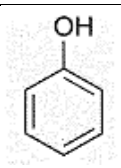
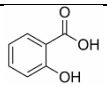
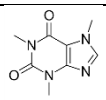
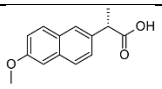
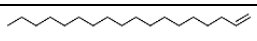
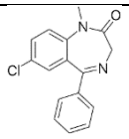
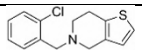
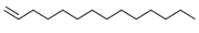
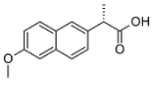
Hit Number	Description	Max Score	Total Score	Query Cover	Percentage Identity	Accession number
1.	*B. paramycoides* strain MCCC 1A04098 16S ribosomal RNA, partial sequence	**2715**	**2715**	**100%**	**99.86%**	**NR_157734.1**
2.	*B. tropicus* strain MCCC 1A01406 16S ribosomal RNA, partial sequence	**2710**	**2710**	**100%**	**99.80%**	**NR_157736.1**
3.	*B. nitratireducens* strain MCCC 1A00732 16S ribosomal RNA, partial sequence	**2710**	**2710**	**100%**	**99.80%**	**NR_157732.1**
4.	*B. luti* strain MCCC 1A00359 16S ribosomal RNA, partial sequence	**2710**	**2710**	**100%**	**99.80%**	**NR_157730.1**
5.	*B. albus* strain MCCC 1A02146 16S ribosomal RNA, partial sequence	**2710**	**2710**	**100%**	**99.80%**	**NR_157729.1**
6.	*B. cereus* strain CCM 2010 16S ribosomal RNA, partial sequence	**2704**	**2704**	**100%**	**99.73%**	**NR_115714.1**
7.	*B. cereus* ATCC 14579 16S ribosomal RNA (rrnA), partial sequence	**2704**	**2704**	**100%**	**99.73%**	**NR_074540.1**
8.	*B. cereus* strain IAM 12605 16S ribosomal RNA, partial sequence	**2702**	**2702**	**99%**	**99.86%**	**NR_115526.1**
9.	*B. cereus* strain NBRC 15305 16S ribosomal RNA, partial sequence	**2702**	**2702**	**99%**	**99.86%**	**NR_112630.1**
10.	*B. cereus* strain JCM 2152 16S ribosomal RNA, partial sequence	**2702**	**2702**	**99%**	**99.86%**	**NR_113266.1**

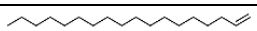
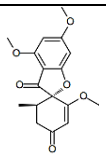
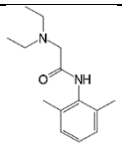
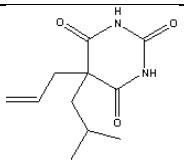
Table S3. P1 Isolate Top 10 Blast hits.

Hit Number	Description	Max Score	Total Score	Query Cover	Percentage Identity	Accession number
1.	*A. faecalis* strain NBRC 13111 16S ribosomal RNA, partial sequence	**2473**	**2473**	**99%**	**97.47 %**	**NR_113606.1**
2.	*Alcaligenes aquatilis* strain LMG 22996 16S ribosomal RNA, partial sequence	**2455**	**2455**	**99%**	**97.20%**	**NR_104977.1**
3.	*A. faecalis* strain IAM 12369 16S ribosomal RNA, partial sequence	**2429**	**2429**	**99%**	**96.99%**	**NR_043445.1**
4.	*Alcaligenes endophyticus* strain AER10 16S ribosomal RNA, partial sequence	**2388**	**2388**	**100%**	**96.39%**	**NR_156855.1**
5.	*A. faecalis subsp. parafaecalis* strain G 16S ribosomal RNA, partial sequence	**2364**	**2364**	**96%**	**97.17%**	**NR_025357.1**
6.	*Alcaligenes pakistanensis* strain NCCP-650 16S ribosomal RNA, partial sequence	**2320**	**2320**	**96%**	**96.67%**	**NR_145932.1**
7.	*A. faecalis subsp. phenolicus* strain J 16S ribosomal RNA, partial sequence	**2303**	**2303**	**99%**	**95.25%**	**NR_042830.1**
8.	*Paenalcaligenes suwonensis* strain ABC02-12 16S ribosomal RNA, partial sequence	**2204**	**2204**	**97%**	**94.76%**	**NR_133804.1**
9.	*Parapusillimonas granuli* strain Ch07 16S ribosomal RNA, partial sequence	**2200**	**2200**	**99%**	**94.36%**	**NR_115804.1**
10.	*Pusillimonas ginsengisoli* strain DCY25 16S ribosomal RNA, partial sequence	**2200**	**2200**	**100%**	**94.07%**	**NR_116103.1**

S1.3 Organic Compounds Degradation

Table S4. *Analysis of untreated and treated hospital wastewater through GCMS.*

Wastewater samples	Pollutants	Major Group/Class	Chemical structure	Retention time (min)	Confirmation ion (m/z)	Conc. (µg/L)
Untreated HWW (Control)						
	Phenol	Aromatic		26.72	58.15	876
	Salicylic acid	Metabolite		6.51	147.64	48
	Caffeine	Stimulant		7.96	266.82	7
	Naproxen	NSAID		9.16	412.07	23
	Octadecene	Organic		28.65	581.46	185
	Diazepam	Sedative		38.06	685.39	14
Treated HWW (*B. paramycoides* D6)						
	Salicylic acid	Metabolite		6.51	147.64	7
	Caffeine	Stimulant		7.96	266.82	4
	Ticlopidine	Fibrinolitic		31.95	534.12	11
Treated HWW (*B. paramycoides* D7)						
	Tetradecene	Organic		7.08	190.86	35
	Naproxen	NSAID		9.16	412.07	6

	Octadecene	Organic		28.65	581.46	19
	Griseofulvin	Antibacterial		46.18	692.95	28
Treated HWW (*A. faecalis* P1)						
	Phenol	Aromatic		26.72	58.15	381
	Salicylic acid	Metabolite		6.51	147.64	15
	Lidocaine	Anesthetic		20.26	368.28	122
	Butalbital	Barbiturate		30.88	625.51	54
Internal standards						
	Acenaphthene	*Na*		24.27	156.18	*Na*
	Phenanthrene	*Na*		29.74	184.25	*Na*

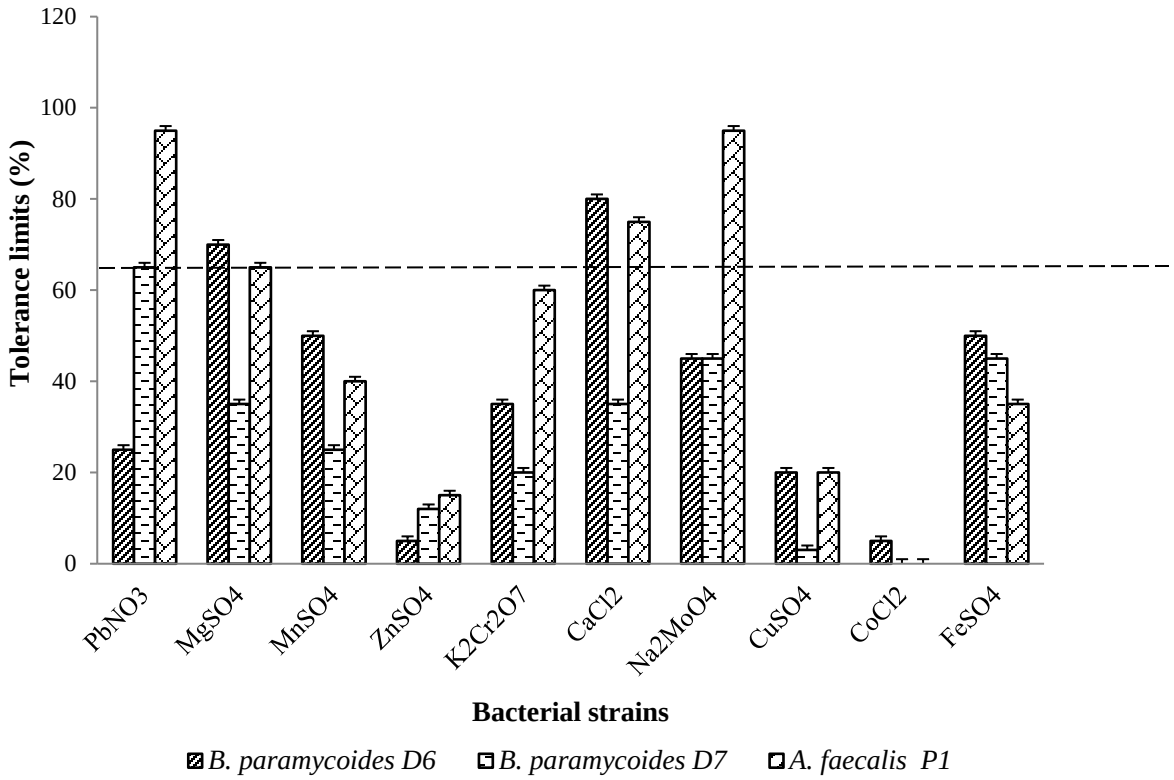


Figure S26. Tolerance limits of bacterial isolates against metal salts (>65%).