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# Characterization of *Bacillus nealsonii* strain KBH10 capable of reducing aqueous mercury in laboratory-scale reactor

Asifa Farooqi, Ghufranud Din, Rameesha Hayat, Malik Badshah, Samiullah Khan and Aamer Ali Shah

## ABSTRACT

The environmental release of mercury is continuously increasing with high degree of mobility, transformation and amplified toxicity. Improving remediation strategies is becoming increasingly important to achieve more stringent environmental safety standards. This study develops a laboratory-scale reactor for bioremediation of aqueous mercury using a biofilm-producing bacterial strain, KBH10, isolated from mercury-polluted soil. The strain was found resistant to 80 mg/L of $HgCl_2$ and identified as *Bacillus nealsonii* via 16S rRNA gene sequence analysis. The strain KBH10 was characterized for optimum growth parameters and its mercury biotransformation potential was validated through mercuric reductase assay. A packed-bed column bioreactor was designed for biofilm-mediated mercury removal from artificially contaminated water and residual mercury was estimated. Strain KBH10 could grow at a range of temperature (20–50 °C) and pH (6.0–9.0) with optimum temperature established at 30 °C and pH 7.0. The optimum mercuric reductase activity (77.8 ± 1.7 U/mg) was reported at 30 °C and was stable at a temperature range of 20–50 °C. The residual mercury analysis of artificially contaminated water indicated 60.6 ± 1.5% reduction in mercury content within 5 h of exposure. This regenerative process of biofilm-mediated mercury removal in a packed-bed column bioreactor can provide new insight into its potential use in mercury bioremediation.

**Key words** | *Bacillus nealsonii*, bioreactor, bioremediation, mercuric reductase enzyme, mercury, transformation

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## HIGHLIGHTS

- Bioaccumulation of aqueous mercury in the food web is a matter of grave concern.
- Enzymatic reduction of mercury by resistant bacteria reduces its potential toxicity.
- *Bacillus nealsonii* possess the inbuilt mechanisms of necessary adaptation.
- Mercury content was reduced up to 60.6 ± 1.5% within 5 h of exposure in packed-bed bioreactor.
- Biofilm-mediated mercury remediation is efficient, regenerative and cost-effective.

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## INTRODUCTION

Mercury (Hg) is a potentially toxic metal that exists naturally in the Earth's crust. Despite its toxic nature, mercury is widely used in several industrial sectors and released to the environment in finishing waste. The anthropogenic release of mercury accounts for its release in air, water and soil. Mercury released in aquatic bodies is alarming due to its transformation into methyl mercury (MeHg) resulting in biomagnification through the ecosystem. The overall high mobility of mercury facilitates its environmental cycling and bioaccumulation at various levels of food chain that eventually poisons human beings (Tchounwou *et al.* 2012). The increasing release of recalcitrant mercury continues to be a matter of grave concern to the public health and environment. Before discharge, the industrial waste is treated for undesirable concentrations of mercury to minimize its release to the environment. However, the physicochemical means of mercury remediation are costly, and produce a large volume of biomass and mercury-rich residues, which in turn creates disposal problems. The contemporary use of biological tools for remediation has simultaneous advantages of eco-friendliness and inexpensive approach along with greater flexibility to environmental fluctuations (Fulke *et al.* 2020; Chang *et al.* 2020).

Biotransformation of mercury to its least toxic form is an effective and alternative method to traditional remediation techniques. The ability of bacteria to reduce mercury is among the most widely found microbe-mediated enzymatic transformation of toxicants to non/less-toxic forms. Mercury resistant bacteria harbor *mer* operon, a gene cluster that detoxifies ionic mercury ($Hg^{2+}$) to elemental mercury ($Hg^0$) through enzymatic reduction (Naguib *et al.* 2018) and attains resistance to potentially toxic mercury. Such mercury-resistant bacteria can be applied beneficially in the detoxification and bioremediation of mercury. In addition to molecular mechanism of mercury resistance, biofilm formation also provides considerable resistance against unfavorable conditions. Bacterial biofilm established on inert support matrix in a bioreactor brings enzymatic transformation of mercury to minimize its toxicity (Gluszcz *et al.* 2008). The biofilm formation can substantially enhance the remediation potential of mercury-resistant strains.

In the current study, we isolated mercury-resistant bacteria followed by determination of their tolerance limit to mercury, screening for biofilm production, mercury volatilization and characterization of the most putative strain KBH10 for optimum growth and mercury biotransformation potential. Furthermore, a packed-bed reactor was set up employing *Bacillus nealsonii* strain KBH10 for bioremediation of mercury through biofilm-mediated enzymatic reduction.

## MATERIALS AND METHODS

### Sampling site

Soil samples were collected from Korangi Industrial Area (KIA), Karachi, Pakistan (24°48′37.5″N, 67°06′52.6″E). The KIA is comprised of 8,500 acres and approximately 4,500 industries, trading and commercial units such as engineering, steel, textile, automobile, pharmaceutical, and chemicals are located in this area. The temperature of this area fluctuates throughout the year from 25 °C to 47 °C with a pH range of 7.5 to 8.5. The soil samples were collected from approximately 10 inches depth after removing the upper layer with a sterile spatula in Nalgene sample bottles. Samples were transported at 4 °C to Applied Environmental and Geomicrobiology (AEG) Research Laboratory, Department of Microbiology, Quaid-i-Azam University, Islamabad, following standard microbiological protocols and analyzed within 24 h after collection.

### Sample analysis and isolation of mercury-resistant bacteria

Physicochemical properties of the soil samples including pH, temperature and moisture content were noted. Samples were prepared for elemental analysis of copper (Cu), lead (Pb), nickel (Ni), cadmium (Cd), cobalt (Co) and mercury (Hg) by *Aqua regia* leach method as previously described (Din *et al.* 2020) and analyzed through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent – 7800, USA). Mercury-resistant bacteria were isolated through selective enrichment in mercury-amended nutrient broth followed by serial dilution according to the method as previously described (Din *et al.* 2019). The colonies, which differed in shape, color and margins, were sub-cultured on nutrient agar plates supplemented with 20 mg/L concentration of mercury chloride ($HgCl_2$) as an inducer for the expression of mercury-resistant genes.

### Mercury tolerance assay

Bacterial isolates were streaked on nutrient agar plates amended with increasing concentration of $HgCl_2$ in

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multiples of ten up to 100 mg/L to assess their maximum tolerance to $HgCl_2$. A nutrient agar plate with no mercury was streaked for each strain as control. Plates were kept at 30 °C for 72 h and growth was observed after every 24 h. The highest concentration of mercury allowing an apparent growth was considered as the mercury tolerance limit. The tolerance limit was further confirmed by challenging the bacterial strain with the same concentration of $HgCl_2$ in nutrient broth incubated for 24 h in a shaking incubator at 120 rpm and 30 °C. Growth was measured in terms of optical density at 600 nm ($OD_{600}$) via spectrophotometer (Agilent – 8453, USA).

### Quantitative assay for biofilm formation

The biofilm formation was quantified to identify the most putative biofilm-producing strain. The mercury tolerant bacterial isolates were grown overnight in Tryptone Soya Broth (TSB) at 30 °C and 120 rpm in a shaking incubator. Twenty microliter broth culture was added to a flat-bottom 96-well microtiter plate containing 180 μL of freshly prepared TSB and incubated at 30 °C for 24 h. The wells with TSB only and no bacterial culture were considered as control. After incubation, the plates were gently washed three times with phosphate buffer saline (pH 6.7) to eliminate planktonic cells. Then 300 μL methanol was added to each well for 15 minutes, followed by staining with safranin (0.1% w/v) for 20 minutes. The extra stain was discarded, and wells were gently washed twice with PBS. The stained cells adhering to wells were resolubilized by adding glacial acetic acid (33% v/v) and optical density was measured at 490 nm.

### Mercury volatilization assay

Mercury resistant isolates producing biofilm were tested for $HgCl_2$ volatilization according to the method as previously described (Nakamura *et al.* 1999). The bacterial isolates were grown for 24 h in nutrient broth supplemented with 50 mg/L of $HgCl_2$. After incubation, 5 mL of culture broth was centrifuged for 20 minutes at 12,000 × *g*. The pellet was washed twice with PBS and re-suspended in 50 μL of freshly prepared 0.07 M phosphate buffer (pH 7.0) containing EDTA (0.5 mM), sodium thioglycolate (5 mM), magnesium acetate (0.2 mM) and $HgCl_2$ (50 μg/mL) in a microtiter plate. The plate was covered with X-ray film and incubated at 30 °C for 60 min in dark. Subsequently, the X-ray film was developed and observed for white patches produced due to the reaction of volatile mercury with the silver coating on X-ray film.

Based on mercury tolerance, biofilm formation and mercury volatilization, the most putative strain (KBH10) among all isolates was selected for further study.

## Characterization of strain KBH10

### Identification and phylogenetic analysis

Strain KBH10 was grown on nutrient agar plates at 30 °C for 24 h and observed for its colony morphology. Gram's reaction was determined using a Gram staining kit (Carlo Erba, France) and cellular morphology was examined under a light microscope (Nikon YS100, Japan). The genomic DNA was extracted using a genomic DNA extraction kit (Norgen Biotek Corp, Canada) and analyzed through a NanoDrop 2000 (Fisher Scientific, USA). The 16S rRNA sequence analysis was carried out at Alpha Genomics, Islamabad, Pakistan, by amplifying the V4 region with universal primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporas *et al.* 2011) using standard polymerase chain reaction conditions (Din *et al.* 2019). The obtained sequence, together with closely related sequences from the GenBank BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/), was aligned together in MEGA-X software and a phylogenetic tree was constructed by adopting the Neighbor-Joining method. The partial 16S rRNA sequence of KBH10 was submitted to NCBI and can be obtained under sequence number MT180167.

### Optimization of temperature and pH

The effect of temperature and pH on growth was determined by incubating 1% inoculum ($OD_{600}$ 0.08–0.1) of strain KBH10 in 50 mL nutrient broth at a range of temperatures (20, 25, 30, 35, 40, 45 and 50 °C) and pH (6, 7, 8 and 9) for 48 h in a shaking incubator at 120 rpm. Growth was checked every 24 h by measuring its OD at 600 nm through a spectrophotometer.

### Mercuric reductase assay

Mercuric reductase (MR) assay was performed according to the method as previously described with slight modification (Freedman *et al.* 2012). The cells of strain KBH10 in the mid-log phase were harvested through centrifugation at 12,000 × *g* for 20 min at 4 °C and stored at −20 °C. Frozen cells (200 mg/mL) were re-suspended in a buffer of 20 mM

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sodium phosphate (pH 7.5), 0.5 mM EDTA, and 1 mM β-mercaptoethanol; then lysed by sonication for 6 × 30 s cycles using an ultrasonic bath (Spectralab, Italy). The cell lysate was centrifuged at 12,000 × *g* for 10 min and supernatant was collected in clean Eppendorf tubes. Mercuric reductase assay of the obtained crude cell extract was performed in 80 mM sodium phosphate buffer (pH 7.4) containing 1 mM β-mercaptoethanol, 200 μM NAD(P)H and 50 μM $HgCl_2$ in a final volume of 800 μL. Assay was performed on range of temperatures (20, 30, 40 and 50 °C) at which the strain was able to grow. Assay temperature was controlled using a water-jacketed cuvette holder connected to a water bath. A 4 μL of crude extract was added to the 800 μL assay buffer, incubated for 1 min, and mercury-dependent NAD(P)H oxidation was monitored as a decrease in absorbance at 340 nm using a UV-visible spectrophotometer (UV-3600 Shimadzu, Japan). Total protein concentration in crude cell extract was determined by Lowry's method (Lowry *et al.* 1951). Mercury-dependent NADH oxidation rate was calculated from the absorbance curve and expressed as mU (1U = μmol of NADH oxidized/min/mg of total protein).

### Packed-bed column bioreactor for mercury remediation

Strain KBH10 was evaluated for its mercury remediation potential by adopting the methods as previously described with slight modification (Gluszcz *et al.* 2008). Biofilm of strain KBH10 was developed on sterilized white pebbles (6–10 mm) by growing for 72 h in 500 mL nutrient broth in a static incubator at 30 °C. A control was run in parallel with sterilized white pebbles in culture-free nutrient broth. The biofilm formation was analyzed by sonicating 4–5 pebbles in PBS and CFU/mL was calculated by serial dilution method on a mercury-amended nutrient agar plate. A packed-bed glass column reactor was designed for mercury bioremediation with 53.5 cm length and an internal diameter of 4.5 cm (Figure 1). The total capacity of the column was calculated. The column was loaded with biofilm-coated white pebbles and 500 mL of 10 mg/L $HgCl_2$ solution prepared in deionized water. A similar column setup was used as a control containing sterilized pebbles with no biofilm. At 0 h, a 10 mL sample was collected from the control and test columns followed by successive samples collected at 1 h intervals up to 24 h and analyzed for mercury concentration through ICP-MS.

### Statistical analysis

Three individual replicates were performed for each experiment and values were presented as mean ± S.D. One-way ANOVA followed by post-Tukey's multiple comparisons were used for the significant results ($p < 0.05$) during mercury removal experiments. GraphPad Prism version 7 was used for statistical analyses.

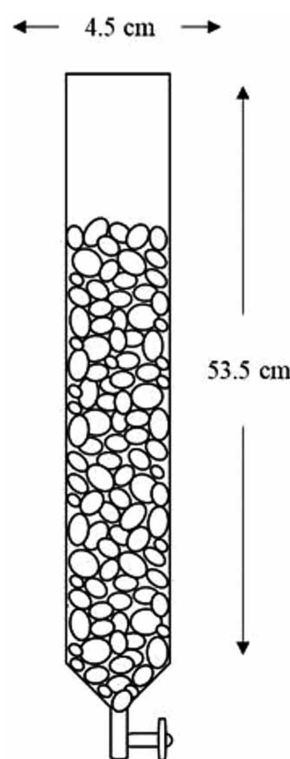


**Figure 1** | Packed-bed reactor using a glass column of length 53.5 and internal diameter 4.5 cm. The total capacity (V) of the column was 850 mL. The average particle size was 6–10 mm.

## RESULTS AND DISCUSSION

### Sampling site and isolation

Mercury being recognized as a potent and widely distributed toxicant in the global environment is of major concern in the present-day world. It is actively applied in various industries and released in finishing waste, which in turn pollutes the industrial sites and atmosphere. The metal analysis of our study sample indicated high concentrations of Cu, Pb, Ni, Cd, Co and Hg in comparison to the control sample. Table 1 shows the physicochemical properties and elemental analysis of soil samples collected from Korangi industrial area (Karachi), Pakistan. The high mercury concentration in groundwater and vegetables grown on-site signify the mercury contamination of the sampling site (Haq *et al.* 2005). A total of 18 bacterial strains, designated

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**Table 1** | Elemental analysis of Korangi mud sample

| | Metal concentration | | | | | |
|---|---|---|---|---|---|---|
| | μg/Kg | mg/Kg | | | | |
| Sample | Mercury | Copper | Lead | Nickel | Cobalt | Cadmium |
| Korangi | 712 ± 15 | 798 ± 63 | 240 ± 8 | 983.0 ± 44 | 29 ± 2 | 13 ± 2 |
| Control[a] | 103 ± 7 | 3 ± 0.09 | 0.4 ± 0.06 | 2 ± 0.1 | 1 ± 0.1 | 0.2 ± 0.1 |

[a]Garden soil.

as KBH1 to KBH18, were isolated by selective enrichment with 20 mg/L of $HgCl_2$ and recognized as separate strains with distinct cultural characteristics on nutrient agar medium. The high degree of mercury resistant bacteria indicates that a rich microbial community is inhabiting the sampling site of the industrial zone. Strain KBH10 was selected as the most putative strain on the basis of mercury tolerance, biofilm production, and mercury volatilization (Table 2).

## Mercury tolerance assay

All the 18 bacterial isolates were screened for their tolerance against increasing concentration of mercury. A gradual decrease in growth was observed with increase in $HgCl_2$ concentration on nutrient agar. Strain KBH10 was the only strain that could grow efficiently in the presence of 80 mg/L of $HgCl_2$ as shown in Table 3, whereas no growth was observed above this concentration. A number of researchers have reported mercury-resistant bacteria from polluted sites with *Bacillus* and *Pseudomonas* as the

**Table 2** | Results of screening experiments for comparative analysis in order to choose the most putative strain

| No. | Strain | Mercury tolerance (mg/L) | Biofilm OD | Hg volatilization |
|---|---|---|---|---|
| 1 | KBH1 | 40 | 0.4 ± 0.06 | ND |
| 2 | KBH2 | 60 | 0.5 ± 0.06 | Weak positive |
| 3 | KBH3 | 20 | 0.5 ± 0.11 | Negative |
| 4 | KBH4 | 20 | 0.4 ± 0.08 | ND |
| 5 | KBH5 | 40 | 0.5 ± 0.1 | Negative |
| 6 | KBH6 | 40 | 0.3 ± 0.1 | ND |
| 7 | KBH7 | 20 | 0.5 ± 0.08 | Negative |
| 8 | KBH8 | 40 | 0.5 ± 0.11 | Weak positive |
| 9 | KBH9 | 40 | 0.7 ± 0.13 | Weak positive |
| 10 | KBH10 | 80 | 1.0 ± 0.05 | Strong positive |
| 11 | KBH11 | 40 | 0.3 ± 0.07 | ND |
| 12 | KBH12 | 20 | 0.3 ± 0.08 | ND |
| 13 | KBH13 | 60 | 0.4 ± 0.03 | ND |
| 14 | KBH14 | 40 | 0.5 ± 0.06 | Weak positive |
| 15 | KBH15 | 40 | 0.4 ± 0.04 | ND |
| 16 | KBH16 | 40 | 0.3 ± 0.05 | ND |
| 17 | KBH17 | 20 | 0.4 ± 0.07 | ND |
| 18 | KBH18 | 40 | 0.6 ± 0.04 | Weak positive |

ND, Not Determined.

**Table 3** | Bacterial isolates with their maximum tolerance to mercury on nutrient agar plates

| Bacterial Isolates | $HgCl_2$ concentration (mg/L) 0 | 20 | 40 | 60 | 80 |
|---|---|---|---|---|---|
| KBH1 | ++++ | ++++ | +++ | ++ | – |
| KBH2 | ++++ | ++++ | ++++ | +++ | – |
| KBH3 | ++++ | ++++ | ++ | + | – |
| KBH4 | ++++ | ++++ | ++ | + | – |
| KBH5 | ++++ | ++++ | +++ | ++ | + |
| KBH6 | ++++ | ++++ | ++++ | ++ | + |
| KBH7 | ++++ | ++++ | ++ | + | – |
| KBH8 | ++++ | ++++ | +++ | + | – |
| KBH9 | ++++ | ++++ | ++++ | ++ | – |
| KBH10 | ++++ | ++++ | ++++ | ++++ | +++ |
| KBH11 | ++++ | ++++ | +++ | ++ | – |
| KBH12 | ++++ | ++++ | + | + | – |
| KBH13 | ++++ | ++++ | ++++ | +++ | – |
| KBH14 | ++++ | ++++ | +++ | ++ | + |
| KBH15 | ++++ | ++++ | +++ | ++ | – |
| KBH16 | ++++ | ++++ | ++++ | + | – |
| KBH17 | ++++ | ++++ | + | – | – |
| KBH18 | ++++ | ++++ | +++ | ++ | + |

Growth: Excellent (++++), Good (+++), Fair (++), Poor (+), No Growth (–).

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most representative genera (Mindlin *et al.* 2005; Dash *et al.* 2014).

## Quantitative assay for biofilm production

All the isolates were screened for biofilm formation in 96-well microtiter plate. Among all the isolates, strain KBH10 was observed as an efficient biofilm producer in terms of optical density (Figure 2). Bacteria adapt to harsh conditions of metal stress by presenting various resistance mechanisms. The production of exopolysaccharides (EPS) and biofilm is one of the resistance mechanisms that contributes to increase the survival chance of bacteria in the metal-polluted site by adoring metal elements (Yin *et al.* 2019). In quantitative biofilm assay, *Bacillus nealsonii* strain KBH10 was found as a strong biofilm producer among the rest of the isolates. The role of biofilm in resistance against heavy metal has been addressed by several researchers in their reports (Nocelli *et al.* 2016).

## Mercury volatilization assay

Mercury volatilization potential of selected biofilm-producing strains was determined using the simplified X-ray film method. Strain KBH10, with maximum mercury tolerance, was observed to produce determined foggy formation on X-ray film among all tested isolates (Figure 3). The distinguished foggy spots on X-ray film indicates mercury transformation from $Hg^{2+}$ into $Hg^0$ with subsequent volatilization (Nakamura *et al.* 1999). The reduction of mercury from its oxidized to volatile form is the premium resistance mechanism that mercury-resistant bacteria showcase and facilitated by the *merA* gene (Naguib *et al.* 2019). Our results coincide with the study (Chatziefthimiou *et al.* 2007) reporting three strains indicative of mercury volatilization that were among the most mercury-resistant isolates in their culture collection. Several bacterial genera resistant to mercury have also been reported for their mercury volatilization potential (Moller *et al.* 2011).

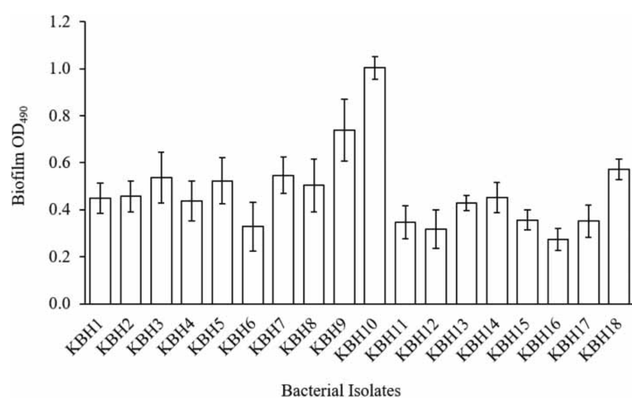


**Figure 2** | Quantitative analysis of biofilm production by bacterial strains isolated from mercury polluted soil samples.

**Figure 3** | Mercury volatilization by biofilm-producing mercury-resistant bacteria KBH2 to KBH18 from left to right on X-ray film in the presence of 50 μg/mL of $HgCl_2$. The intensity of foggy spots indicates the production of Hg0.

## Characterization of strain KBH10

Strain KBH10 was observed as Gram-positive rods under the microscope whereas the colony appeared dull gray, opaque with a rough matted surface, larger in size and with irregular perimeters. Figure 4 shows the phylogenetic tree presenting the evolutionary distance of strain KBH10 with closely related strains obtained from NCBI. The 16S rRNA gene sequencing indicated a close genetic relationship of strain KBH10 to *Bacillus nealsonii* with a boot strap value of 90. Strain KBH10 exhibited growth at a range of temperature (20–50 °C) and pH (6.0–9.0) with optimum at 30 °C and pH 7.0 (Supplementary Figures 1 and 2). The optimized growth condition of strain KBH10 could be helpful in determining optimum conditions during the application of the strain. The optimum growth conditions usually reflect the physicochemical properties of the sampling site (Kotwal *et al.* 2018). The temperature of our site was 45 °C at the time of sample collection, which may be a reason for the adaptation of microbiota up to 50 °C. Mercury-resistant bacteria adapted to high temperatures are also suggestive of the enzymatic transformation of mercury and its mobilization in geothermal environments (Chatziefthimiou *et al.* 2007).

## Mercuric reductase assay

The activity of mercuric reductase (MR) from *B. nealsonii* strain KBH10 was calculated in order to determine its role in conversion of $Hg^{2+}$ to $Hg^0$ under standard assay conditions. MR activity of the crude extract of strain KBH10 was observed at a temperature range of 20–50 °C with no statistically significant difference in MR activity ($p$-value = 0.1424). Figure 5 shows the Hg-dependent NAD(P)H oxidation at temperatures suitable for growth of strain

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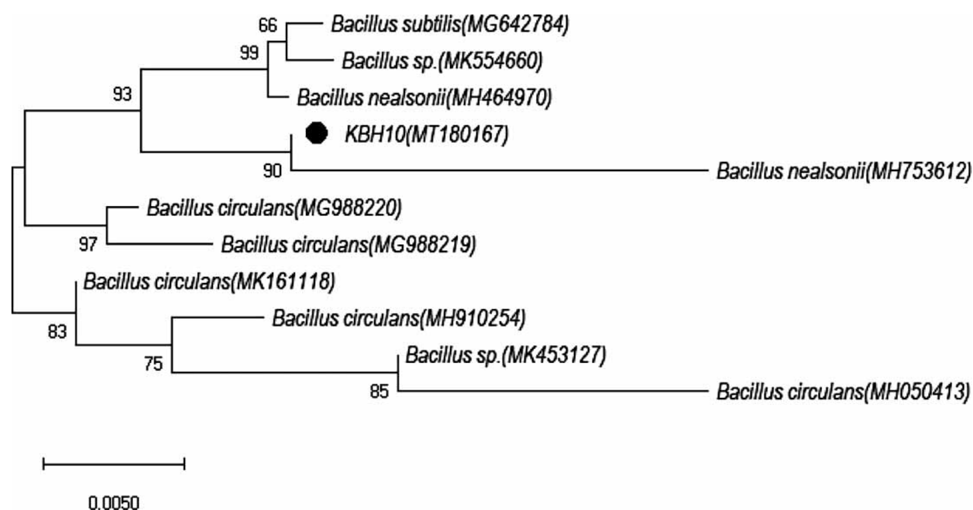


**Figure 4** | Phylogenetic tree of bacterial strain KBH10 constructed by the Neighbor-Joining method. Number at nodes shows the level of bootstrap support based on data for 1,000 replications. Bar = 0.005 substitutions per nucleotide position and numbers in bracket represent GenBank accession numbers.

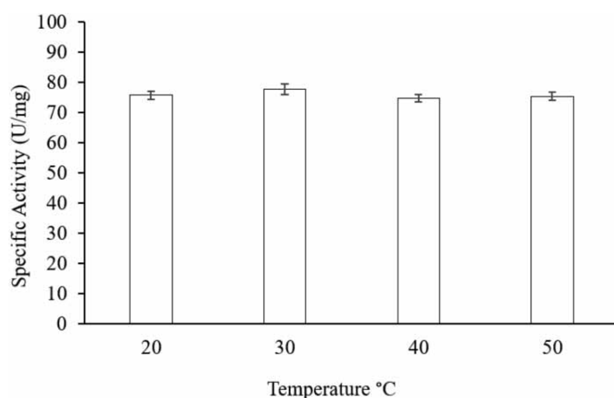


**Figure 5** | Effect of temperature on MR activities. Specific activities of crude cell extracts were determined for strain KBH10 at different temperatures (20–50 °C).

KBH10 with maximum MR activity $77.8 \pm 1.7$ U/mg at 30 °C. No substantial MR activity was observed in control. The total protein concentration in crude extract from strain KBH10 was estimated as 0.334 mg/mL using a BSA standard curve ($R^2 = 0.996$). Mercury resistant bacteria carry *merA* gene a component of *mer* operon, coded for mercuric reductase enzyme which reduces mercury to its least toxic form i.e. $H^0$ and thus confer resistance to bacteria (Sotero-Martins *et al.* 2008). In some of the previous reports, the scientists have reported maximum MR activity under both mesophilic as well as thermophilic conditions. The thermal stability of their mercuric reductase enzyme was established at a temperature range of 30–50 °C with gradual inhibition of enzyme activity up to 80 °C (Nakamura *et al.* 1999; Sayed *et al.* 2014). The mercury resistant *B. nealsonii* KHB10 expressed resistance to considerable concentration that could be the possible reason for its high MR activity. The reducing ability of mercury resistant *Bacillus* sp. is accredited for their excellent bioremediation potential (Zulaika & Sembiring 2013). The mercury volatilization and high MR enzyme activity suggests that *B. nealsonii* strain KBH10 can be recruited as an efficient tool for bioremediation of aqueous mercury.

## Packed-bed column bioreactor for mercury removal

Biofilm-mediated mercury removal was carried out by treating artificially contaminated water samples containing 10 mg/L $HgCl_2$ in a column bioreactor packed with pebbles coated by biofilm of *B. nealsonii* strain KBH10 ($4.4 \times 10^2$ CFU/mL). ICP-MS analysis of samples indicated that $60.6 \pm 1.5\%$ mercury was removed within 5 h with no further decrease in subsequent samples.

Although the biotic enzymatic reduction of $Hg^{2+}$ to elemental $Hg^0$ is an important pathway for detoxification of mercury in natural ecosystems (Chang *et al.* 2021), it is restricted to the bioavailable mercury only and no further reduction is possible, due to the unavailability of mercury to the bacteria. Only $2 \pm 0.06\%$ of mercury content was decreased in samples from the control setup (Figure 6). Decrease in the mercury content of the treated water indicates mercury removal by immobilized cells of *B. nealsonii* KBH10. Our results coincide with the findings of Pepi *et al.* (2011) and Mahbub *et al.* (2016) identifying mercury-resistant bacterial strains with substantial mercury volatilization within a few hours to several days. Biofilm forming bacteria possess a dual role in mercury

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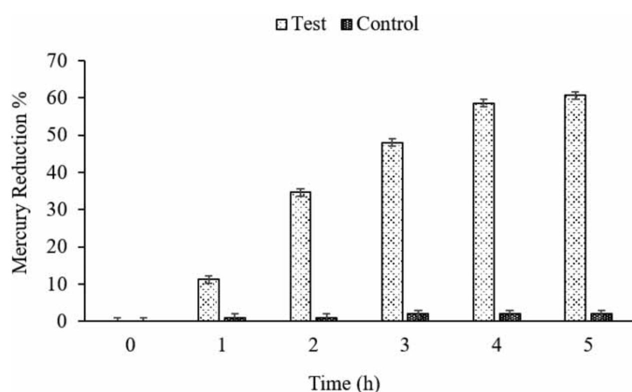


**Figure 6** | Mercury removal efficiency of *Bacillus nealsonii* KBH10 treating water supplemented with 10 mg/L of $HgCl_2$. Values were calculated as mean and bar represents standard deviation ($n = 3$).

bioremediation by utilizing its EPS for mercury entrapment with subsequent volatilization through expression of *mer* operon (Dash *et al.* 2017). Immobilization of biofilm-producing bacteria in a packed-bed bioreactor can efficiently reduce mercury content in wastewater (Dzairi *et al.* 2004; Leonhauser *et al.* 2006). The immobilized cells are advantageous over a free cells for bioremediation of various types of wastes (Mitra & Mukhopadhyay 2016). The biofilm-mediated regenerative method adapted in this study could used for efficient removal of mercury in wastewater over a long period of time that will not only lower the operational cost but also boost the remediation strategy.

## CONCLUSION

Identifying a suitable mercury-resistant strain is a prerequisite to the technological development of mercury bioremediation strategies. We demonstrate biofilm-mediated mercury treatment in a packed-bed column using mercury-resistant strain *Bacillus nealsonii* strain KBH10. It is a simple, rapid and regenerative approach that could prove a valuable addition to the previously available bioremediation approaches and could help in minimizing mercury in industrial wastewater.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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