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SCREENING AND ISOLATION OF SOIL BACTERIA CAPABLE OF PHENOL DEGRADATION

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ABSTRACT

Phenol is a toxic pollutant that is produced naturally as well as synthetically in the environment. Several studies were conducted worldwide to isolate microorganisms, including bacteria and fungi that are capable of degrading phenols. In this study, bacterial species from soil samples were screened and isolated for their phenol degradation ability. Selected colonies were screened for phenol tolerance at various phenol concentrations ranging from 10 to 140 mM/ml phenol concentrations. Eight isolates were found to be potent phenol-tolerators and were identified as *Bacillus* species. Of these, three *Bacillus cereus* strains named RM4, RM23 and RC29 showed maximum degradation to high concentrations of phenol. The strain RM23 could degrade 65.11% of phenol content in the MSPM medium containing 10 mM/ml phenol. RM4 showed a potential degradation of 33.33% in 40mM/ml concentration. It also exhibited degradation at 120 mM/ml concentration. Maximum degradation showed by RC29 was 38 % at 60 mM/ml. The optimum p^H, temperature and incubation period for maximum phenol-degradation was found to be p^H 7, temperature 37^0C, and three days of incubation.

Keywords: phenols; biodegradation; bacteria; *Bacillus*

1. INTRODUCTION

Phenolic compounds are widespread in the environment. They are formed naturally during the decomposition of various substances of plant and animal origin, mainly polymers containing aromatic rings. Phenolic acids, such as O-coumaric acid, caffeic acid, ferulic acid, P-coumaric acid, P-hydroxy benzoic, syringic and vanillic acids are commonly found in plant tissues and the soil in which the plants are cultivated. Phenolic compounds are the typical by-products of any industrial process, such as the manufacture of dye and dye intermediate industries, plastics, drugs, explosives, antioxidants, paper and petroleum industries, and rubber production and processing industries. Phenols itself is an established disinfectant and germicide used by household cleaners. These are discharged along with the effluents from several categories of industries such as textiles, woollen mills, coke ovens, pulp and paper industries, iron and steel plants, petrochemicals, paint industries, resins, cosmetics, oil-drilling and gas extraction units,

pharmaceuticals, coal washeries, refractory industries etc. and enters into various environmental matrices.

Phenols may be released into the soil or sediments during the manufacturing process when spills occur during loading, transport and leaching from hazardous waste sites and landfills. Phenols that leach through the soil to groundwater spend at least some time in that soil as they travel to groundwater. Organic contamination prompted by high organic content in aquatic ecosystems and eutrophication are the two sorts of substantial and long-lasting pollution dangers of water bodies. Organic pollutants are derived from farm water, industrial effluents, urban run-off and domestic sewage (Muthanna & Afaf, 2018). Phenols are toxic, carcinogenic, mutagenic and teratogenic. Unfortunately, phenols and phenolic compounds have now become a common environmental pollutant found in potable water, soil or sediments and ambient air. Eleven phenolic compounds are included in the list by the United States Environmental Protection Agency (USEPA, 2014) and European Commission (EC) as priority pollutants. Other national-level regulatory bodies, such as the Ministry of Environment and Forests (MoEF) and the Central Pollution Control Board (CPCB) under the Government of India, also listed phenols and phenolic compounds as the priority pollutants. In India, the regulatory actions for phenols and phenolic compounds were contemplated under the Environmental (Protection) Rules, 1986, under which several environmental standards for the discharge of phenols and phenolic compounds in industrial effluent have already been notified. A large number of phenolic compounds are subjected to regulations for air and water pollutants around the world. Many are persistent in the environment. Therefore, it is essential to control and monitor the use of phenols and their proper treatments.

Many microbes are involved in the toleration and degradation of phenols and phenolic compounds. It was identified that the enzyme system of several species of microbes, including algae, fungi, and bacteria, is actively involved in the degradation of phenols. More studies are to be conducted in this area to discover new species or strains causing degradation. Those species can be developed to effectively treat the phenols released into soil and water for a better life. Removal of phenols from the environment improves the quality of natural vegetation. Industrial waste carrying different types of phenolic compounds may transform the normal vegetation, which finally leads to the destruction of the native flora and fauna and thereby imbalances the ecosystem. The accumulation of these toxic chemicals may create mutations in the genes of native species. It may also change the physiochemical and biological properties of the biotic and abiotic systems (soil, water).

Modern environmental biotechnology, together with microbial enzymatic systems for the effective degradation of phenols, can make a dramatic change in the treatment of environmental pollutants like phenols. A lot of microorganisms were found to be tolerant to phenol. Microbial processes can be used for environmental protection and improvement. Bio-separations and bioreactor technologies help in the process of environmental cleaning. Environmental and economic benefits in manufacturing, monitoring and waste management are offered by the field of environmental biotechnology. Treatment methods such as activated carbon adsorption, ion exchange, chemical oxidation, liquid-liquid extraction, etc., are effective, but they often suffer from drawbacks such as high cost. Hence, bio-degradation is considered an environment-friendly and cost-effective alternative method.

Phenol bio-degradation in soil may take place under both aerobic and anaerobic conditions. Microorganisms (bacteria, fungi, yeasts, algae) use phenols as the sole carbon source for the growth of the organisms. Most of the cases are reported from bacterial species. The degradation process was catalysed by a variety of enzymes such as oxygenases, hydroxylases, peroxidases, oxidases, laccases, and tyrosinases. Degradation under aerobic conditions is initiated by oxygenation. The aromatic ring is initially monohydroxylated by a mono-oxygenase phenoldehydroxylase at an ortho position to the pre-existing hydroxyl group to form catechol. This includes *Pseudomonas, Bacillus, Acinetobacter, Streptomyces, Desulfobacterium,* and *Geobacter*, among others. Anaerobic degradation of phenol is less advanced than the aerobic process. The first step in this pathway is the carboxylation of phenol at the para position to 4-hydroxybenzoate by the enzyme 4-hydroxybenzoate carboxylase. *Thauera aromatica* K172 and *Clostridium species* are examples of anaerobic degradation of phenols (Schie & Young, 2007). Klekner & Kosaric, 1992 investigated the fact that 2,4-dimethylphenol was converted to an isomer of dimethylbenzenediol by a species of *Chlorella*. It was also capable of degradation and dechlorination of 2-chlorophenol. *Scenedesmus* species, after an adaptation of a period of 5 days, showed quick degradation of 2,4-dinitrophenol at a concentration of about 190 $mg1^{-1}$. All algae tested by Klekner & Kosaric, 1992 for the study has a mechanism for the degradation of phenolic compounds.

The diverse group of microbial enzymes responsible for the treatment of paper mill effluent are lignin peroxidase, manganese peroxidase and laccase (Malaviya & Rathore, 2007). Microbes generally treat such effluents through processes that include enzymes and biosorption. Degradation through benzene ring cleavage using the enzymes present in the microalgae was studied by Muthanna & Afaf in 2018.

Jim & Reyes, 2008 studied microbial degradation of chlorinated phenols under aerobic and anaerobic conditions. Many strains of halo-respiring bacteria can eliminate ortho-chlorines. The *Desulfobacterium hafniense* can eliminate para and meta-chlorines. Once dechlorinated, the phenolic carbon skeletons are wholly converted into methane and carbon dioxide by anaerobic microbes in the environment.

A soil bacterium named *Xanthomonas flavus* MTCC 9130, isolated from a contaminated site, degraded phenol when provided as the sole carbon and energy source in the medium (Lowry, 2009). A study by Krastanov, 2013 presented the best achievements in degrading phenolic compounds by bacteria and yeasts. *Pseudomonas species*, *Candida tropicalis*, and *Trichosporon cutaneum* are found to be highly efficient. *Acinetobacter sp* RTE1.4 was isolated by Paisio *et a*l., 2013 from effluents of a chemical industry that degrade phenols at a wide range of pH and temperature through a degradative pathway. A bacterium, *Pseudomonas sp* SA01, was identified and examined to have the most significant potential for degrading phenol and was isolated from pharmaceutic disposal wastewater (Shourian, 2009). *Pseudomonas putida* is the most popular organism for the degradation of phenols. Phenolics are also degraded by many Basidiomycetes and Ascomycetes in the soil (Min, 2015).

Isolation and identification studies conducted by Kafilzadeh & Mokhtari in 2013 from the mangrove sediments in the Persian Gulf (Asaluyeh) discovered the presence of bacteria with a high ability in phenol degradation. They isolated *Pseudomonas putida, Acinetobacter sp,*

Bacillus thuringiensis, *Brevibacterium iodinum* and *Staphylococcus aureus* species out of the nine samples collected from the Persian Gulf. The bacteria were cultured in different phenol concentrations (0.3,0.5,0.7,0.9,1,2 and 3gl^{-1} phenol concentrations) to evaluate their growth ability and investigated that *Pseudomonas putida* and *Acinetobacter sp* can grow in 0.9 to 2.0 gl^{-1} phenol concentrations.

Paper factory effluent treated with free and immobilised cells of *Alcaligenes sp* d2 exhibited a maximum of 99% reduction in phenol and 40% reduction in chemical oxygen demand after 32 and 20 hours of treatment (Nair, 2008). *Bacillus subtilis* sp3, a PHB-producing bacteria, was found to degrade phenols, which can also be used for the laboratory-scale treatment of paper mill effluent containing phenolic compounds (Nair & Prakash, 2017).
The presented study is an attempt to isolate and identify indigenous soil bacteria capable of degrading phenols.

2. MATERIALS AND METHODS

All the chemicals used for the experiment are from HIMEDIA and Nice chemicals. The glassware used was manufactured by Borosil and Nice. Materials used include polythene covers, sterile loops, test tubes and racks, measuring cylinders, conical flasks, standard flasks, micropipettes, sterile tips, petri-plates, non-absorbant cotton, and various growth media. The equipment and instruments used include a shaking incubator, Laminar Air Flow chamber, incubator, p^H meter, weighing balance, hot air oven, autoclave, microscopes and spectrophotometer.

2.1 Soil sample collection

Soil samples were collected in sterile polythene plastic covers from an industrial area of Kottayam district in Kerala. Soil samples were randomly collected from six sites of three different industrial units in the study area. Collected samples from each unit were taken into the laboratory within 24 hours after pooling for further studies.

2.2 Estimation of phenols and preparation of standard graphs

As a part of the preliminary analysis, the phenolic content in the soil samples was tested using the 4-Aminoantipyrine method (4-AAP method, APHA,1978). Aminoantipyrine reacts with phenol in the presence of Potassium ferricyanide to give a red-coloured compound called antipyrine, whose absorbance was recorded at 500nm. The concentration of this dye is directly proportional to the concentration of phenol in the sample. Different aliquots of the stock phenol solution were prepared, and the assay was carried out. The readings were recorded, and a standard graph was prepared.

2.3 Screening of bacterial colonies for higher phenol-tolerance

The soil enrichment method was used for the study. Each soil samples (10 gm each) were added to 90 ml of sterile distilled water containing 5mM/ml concentration of phenol and was kept in a shaking incubator for 24 hours at 150 rpm speed and 37^0C temperature (Nair, 2007). All soil samples were then serially diluted and pour-plated into a nutrient agar medium containing 10mM/ ml phenol concentration and incubated for two days at 37^0C. The bacterial colonies that showed growth were then transferred into subsequent higher concentrations of phenol-containing agar medium (20mM, 30mM, 40mM, etc.) using a sterile loop and incubated. This process was

then continued up to phenolic concentrations in which no bacterial colonies showed growth. Those bacteria showing maximum tolerance to high concentrations of phenols were selected as phenol-tolerating strains and were isolated for the purpose of the study.

2.4. Screening of bacterial colonies for phenol-degradation

The phenol-tolerating strains were then screened for maximum degradation of phenols. The Minimal salt medium that contains phenol (MSPM) as the only source of carbon was used for degradation studies. 2 ml inoculum of the selected phenol-tolerating strains was then inoculated into 250 ml conical flask containing 50 ml of sterile MSPM (p^H 7) having various concentration of phenol (10, 40, 60, 80, 100, 120 and 140 mM/ml phenol) and were incubated in a shaking incubator for five days at 150 rpm speed and 37^0C temperature. The medium without inoculum was set as the control. The degradation of phenol was measured at regular intervals of time (48, 72 and 120 hrs). The initial and final concentration of phenol in the samples and control was measured using 4-AAP assay. The percentage of degradation was calculated using the following equation;

$$\text{Percentage phenol-degradation} = \frac{C_i - C_f}{C_i} \times 100$$

Where C_i = initial concentration of phenol in the medium.

C_f = final concentration of phenol in the medium.

The strain that shows maximum degradation to high concentrations of phenol was selected as the phenol-degrading strain.

2.5 Isolation and identification of phenol-degrading strains

Bacterial colonies that showed maximum degradation to high concentrations of phenols were selected as phenol-degrading strain. These bacterial colonies were isolated. Pure cultures of the colonies were obtained and stored in the lab for further studies. Cultures were stored in both agar slants and nutrient broth. Periodical sub-culturing of the isolates was done every two weeks. The isolated bacterial colonies were identified through microscopic and biochemical characteristics. The colony morphology was studied. Growth of colonies in MYPA medium (Mannitol Egg Yolk Polymixin Agar medium) inoculated with bacterial strain was also studied (FDA BAM,1998).

2.5.1. *Gram staining* (Gephardt,1981)

A loop of bacterial suspension was placed on a clean glass slide and spread to an even-thin film. Air-dried or heat-fixed the organism. Crystal violet stain is added over the fixed culture and kept for 1 minute. The excess stain is rinsed with tap water. Iodine solution is added and kept for 1 minute to fix the dye. The slide is then rinsed in tap water, and a few drops of decoloriser are added. Wash the slides in tap water and then counterstained with a few drops of safranin stain and keep for 30 seconds. Wash the slides in tap water and allow them to air dry or blot dry. The slides are then examined under the microscope.

2.5.2 *Endospore staining* (Schaeffer and Fulton.,1933)

A drop of bacterial suspension is allowed to air dry or heat fix the organism on a clean glass slide and covered with blotting paper. Saturated the blotting paper with Malachite green stain solution and steamed it for 5 minutes, keeping the paper moist, and added more dye as required. Alternatively, the slides were then steamed over boiling water. The slides were then washed in tap water and counterstained with safranin for 30 seconds. Then, the slides were washed in tap water and blot dry. Examine the slides under a microscope for the presence of endospores that appear bright green in colour.

2.5.3 *Motility test*

The motility of the bacterial cells isolated was checked using the hanging drop method. A drop of the culture is placed in the centre of a cover slip. A cavity slide is placed over the coverslip in such a way that the cover slip sticks to the ring on the slide. The glass slide is lifted and turned around. The suspension now hangs on the lower surface of the cover slip and is then observed under the microscope.

2.5.4 *Morphological and Microscopic Characters*

The colony features of the bacterium were taken for the study. It includes cell shape, size, arrangement, texture, colour, and spores of the isolated bacterium.

2.5.5 *Biochemical characterization of the bacterial strains*

The biochemical properties of the strains were tested based on their carbohydrate utilization abilities. Many bacterial species in incubation exhibit metabolic changes, which are indicated by a colour change in the media, which can be either interpreted visually or after the addition of a particular reagent. A rapid test identification kit of HIMEDIA laboratories called the HiBacillus identification kit (product code KB013) was used for this study. The bacterial cultures were inoculated into the test kit wells and were incubated for 24-48 hours at 37^0C. The results were then recorded. Another selective and differential media called MYPA medium (FDA, 1998) was used for the identification processes. The bacterial cultures were inoculated into MYPA medium and were incubated for 24-40 hours at 37^0C and observed for pink-coloured colonies.

3. RESULTS AND DISCUSSION

The three industrial sites from which soil samples were collected for the study were named Site I as PLY, Site II as RM, and Site III as RC, respectively.

3.1 Estimation of phenol

Estimation was done by 4-AAP assay. A standard graph was plotted with concentration of phenol in the X-axis and absorbance values measured at 500 nm in the Y-axis. More or less, a straight line was obtained. From the standard graph, the phenol content was estimated to be 13 µg/ml, 10 µg/ml, and 15 µg/ml, respectively, for samples PLY, RC and RM.

3.2 Screening and isolation of phenol-tolerating strains

Each culture plate was inoculated with dilutions 10^{-3}, 10^{-4} and 10^{-5} of the soil samples. Hundreds of bacterial colonies were produced in each culture plate. Colonies with similar characteristics and appearance in the plates were then transferred into the next higher concentrations of phenol

in the medium (Nutrient Agar medium). The number of colonies in the plates was found to be decreasing at higher phenol concentrations (Nair, 2007). A phenol concentration of 5 mM, 10 mM, 20 mM, 30 mM,….., 100 mM was applied. It was observed that, above 80 mM/ml concentration of phenol, no bacteria showed growth. It may be due to the substrate inhibition of bacterial growth by phenol (Banerjee & Ghoshal, 2010). From the screening studies, 70 mM/ml phenol concentration was found to be the optimum substrate concentration at which bacterial colonies showed growth. Among all the bacterial colonies, eight bacterial colonies showed visible growth at 70mM/ml concentrations. The colonies were then pure cultured and stored. These strains were named PLY7, PLY13, RM4, RM5, RM23, RC8, RC23 and RC29 respectively.

3.3 Screening of phenol-degrading bacterial strains

The phenol-degrading capacity of each isolated phenol-tolerating strain were then studied in MSPM. It was observed that three out of eight strains are good phenol-degraders. The growth and degradation rate exhibited by strains PLY7, PLY13 and RM5 were not consistent and were excluded from the study. Those strains that exhibited a degradation rate of above 10% were selected in this study. The strains RC8 and RC23 showed a degradation rate above 10% up to a phenol concentration of 40 mM/ml. Above 40mM/ml phenol, RC8 and RC23 showed poor degradation rate. The strain RM23 gave a degradation rate of 65.11% at 10 mM/ml phenol concentration. At 40 and 100 mM/ml concentrations, it exhibited a degradation rate of 11.44 and 15.19%. RM4 showed a degradation rate of 33.33% and 11.40% at a concentration of 80 mM/ml and 120 mM/ml phenol. At 60 and 120 mM/ml phenol, RC29 exhibit 38% and 10.08% degradation. Even though all the phenol-tolerating strains showed growth in MSPM at higher concentrations (above 120 mM/ml phenol), the rate of degradation was found to be decreasing rapidly. High substrate concentration is an inhibition to degradation. This may possibly be due to the substrate-level inhibition mechanism. At low concentrations of phenol, all the five strains showed a higher rate of degradation. Optimum degradation at optimum concentration of phenol was exhibited by RC29. The strain RM23 has a range of degradation. At low phenol concentration (10 mM/ml), it showed maximum growth and maximum degradation in minimum time.

Since bacteria require a carbon source for their growth and metabolism, the phenol present in the MSM (minimal salt medium) serves as the only source of carbon. Conditions such as p^H, temperature, number of incubation days, and substrate concentration were optimized to obtain maximum phenol degradation. p^H value of 7 was observed to be the optimum p^H for maximum degradation. The optimum temperature was identified to be 37^0C. Above three days of incubation, the rate of degradation falls off. The optimum concentration of substrate was found to be different for each strain. For RM4, the optimum phenol concentration was 80 mM/ml, which gave a degradation rate of 33.33%. It is also the most efficient strain in degrading phenol. It also showed degradation at 120 mM/ml. A concentration of 60 mM/ml was optimum for RC29 and 10 mM/ml for RM23. The results of RM4, RM23 and RC29 were consistent with the triplicate study. Therefore, these three strains can be promoted as efficient phenol-degrading bacterial strains (Fig.1)

(a) (b) (c)

Fig.1. Plates showing pure cultures of (a) RM4, (b) RM23 and (c) RC29

3.4 Identification of phenol-degrading bacterial strains

All the bacterial strains were subjected to Gram staining and observed under the microscope. They were found to be positive for Gram staining. All are rods. The bacterial strains were found to be producing endospores when stained and observed under the microscope. Bacterial cells with green-coloured and partially red and green-coloured cells were found. All of the isolated strains are motile when tested for motility except RM23. The colony characters of RM4 and RC29 are almost similar. Colonies are rough, flat, opaque with jagged edges and slightly yellowish- fuzzy in appearance. The strain RM23 produced dark pink-coloured round colonies.

Various biochemical fermentation tests for sugars were done. It includes glucose, mannittol, catalase, MR-VP, indole and citrate test. Even though the bacteria exhibit similar colony features, their biochemical characteristics were found to be different. Based on the assumption that the strains belonged to the genus Bacillus, a rapid identification test kit for the Bacillus species was conducted using HiBacillus test kit and the results were given in Table 1.

Table 1. **Biochemical analysis of the bacterial strains.**

Sl.no	Tests	RM4	RM23	RC29
1	Malonate	+	+	+
2	Voges Proskauer's	+	+	+
3	Citrate	+	+	-
4	ONPG	-	-	-
5	Nitrate reduction	+	+	+
6	Catalase	+	+	+
7	Arginine	-	+	+
8	Sucrose	+	-	+
9	Mannitol	-	-	-
10	Glucose	+	+	-
11	Arabinose	-	-	-
12	Trehalose	+	+	-

In addition to this, the colony growth in MYPA medium, which is a selective media used for the identification of *B. cereus* was also studied. The eight phenol-tolerating strains were inoculated into the MYPA plate. Of this, five strains named RM4, RM5, RM23, RC8, and RC29 produced pink-coloured colonies (Fig.2). The colony that produces a pink colour in the medium was identified as *B. cereus*.

Fig.2. MYPA test

From the results given above, it was clear that the three strains belong to the genus *Bacillus.* From the biochemical characterization and MYPA analysis, it was confirmed that, the three strains belong to *Bcillus cereus*. The literature analysis conducted during this study also supports the results.

Many species belonging to the genus *Bacillus* have been reported for their phenol-tolerating and degrading capabilities. Species of *Bacillus* were -isolated and identified from contaminated soil across the world. The microbes showing high tolerance to phenols are usually potent phenol degraders. *Bacillus subtilis* sp3, a PHB-producing bacteria, was isolated and used in the treatment of paper mill effluent containing phenolic compounds (Nair & Prakash, 2017). Two strains of *Bacillus cereus* (*B. cereus* MTCC 9817 and 9818) isolated from a petroleum refinery and oil exploration site showed high tolerance to phenol at a concentration of 1000 mg/L (Banerjee & Ghoshal, 2010). At an optimum p^H of 8.0, temperature 34^0C, 5% (v/v) of inoculum size, and without any co-substrate, a strain of *B.brevis* degraded 1750 mg/L of phenol in 144 hours (Arutchelvan *et al*.,2006). *Sulfobacillus acidophilus* isolated from hydrothermal vents in the Pacific Ocean has a maximum degradation rate of 2.32 mg/L/h of phenol at 38 hours. The degradation is aerobic via meta-pathway under p^H 1.8 and 45^0C temperature. *S. acidophilus* can tolerate 1300 mg/L of phenol and completely degrade 100 mg/L of phenol in 40 hours (Zhou *et al*.,2016). The peroxidase enzyme isolated and purified from *B. aryabhattai* B8W22 exhibits the highest degradation (98.47%) at p^H 5.8 with maximum activity at 30^0C and complete thermal stability at 40^0C. The enzyme showed 0.012 U/mg specific activity (Elmetwalli *et al*.,2023). *Bacillus badius* follows ortho and meta-catechol pathways. At high concentrations of substrate (phenol), the rate of degradation was more than 70%, whereas 98% was degraded at lower concentrations with an alkaline p^H of 9.0 at 37^0C (Sarwade & Gawai, 2014). From the literature studied, it was also crystal clear that the indigenous consortium of phenol-tolerating bacteria was able to survive in high concentrations of phenol (Arutchelvan et al. (2006), Banerjee & Ghoshal (2010), Castillo *et al*. (2012), Raj & Reddy (2007), Zhou *et al*. (2016)) and the species of genus *Bacillus* can tolerate a wide range of phenolic concentrations.

4. CONCLUSION

Phenol is a significant pollutant being discharged from the effluents of various industrial sources. They are very soluble in water; as a result, they can pollute a massive volume of fresh water, wastewater, groundwater, oceans and soil, negatively affecting plant germination and animal and human health (Mahgoub *et al*.,2023). Native microbial species in polluted settings were more adaptable than non-indigenous microorganisms, and their dominance aided the bio-remediation of phenol-contaminated ecosystems. A critical analysis of the literature reveals that biological treatment is economical, practical and the most promising and versatile approach as it leads to

the complete mineralisation of phenols, producing non-toxic end products (Dankaka & Abdullahi, 2021). It was also revealed from the literature survey that most of the phenol-degrading microbes belong to different groups of bacteria, of which genus *Bacillus* is of prime importance. This work has helped to isolate and identify three indigenous *Bacillus cereus* strains capable of phenol degradation from the soil. They can be promoted as efficient phenol degrading microbes. This finding is consistent with Banerjee & Ghoshal (2010), where *B. cereus* species showing high tolerance and degradation to phenol have been isolated and reported.

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Conflict of Interest Statements

The authors declare that the research was conducted in the absence of any commercial, financial or personal relationships that could have appeared to influence the work reported in this paper. The original contributions presented in the study are included in the article, and the work declared has not been published previously. Further enquiries can be directed to the corresponding author.

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