Indigenous *Kurthia* sp. degrading phenol isolated from a petrochemical dumpsite and its derivatives identified by gas chromatography-mass spectrometry

R Bindu, R Soundarya, Chakra S Prashantkumar, SM Pruthvi, Devaraja Gayathri*

Department of Studies in Microbiology, Davangere University, Shivagangothri, SH-76, Davangere-577007, Karnataka State, India

Received 27 November 2025; revised 6 February 2026; accepted 26 March 2026

Abstract:

Phenol is a toxic aromatic compound widely released into the environment through oil refineries, petrochemical industries, and industrial effluents, posing serious risks to ecosystems and human health. Given its persistence and toxicity even at low concentrations, environmentally sustainable methods for its removal are highly sought. Biological remediation using indigenous microorganisms offers a cost-effective and eco-friendly substitute to conventional physicochemical methods. In the present study, aerobic phenol-tolerant bacterial isolates were obtained from petrochemical dumpsite soil and characterised for their capacity to grow in MSM supplemented with phenol. The isolate was identified as *Kurthia populi* GB1 based on morphological, biochemical, and 16S rRNA gene sequence analysis. The phenol tolerance of the isolate was assessed at various concentrations, while the influence of pH and temperature on growth was investigated under aerobic conditions, and phenol transformation products were tentatively identified using gas chromatography-mass spectrometry. The isolate demonstrated a tolerance to phenol concentrations reaching 500 mg/l, with optimal growth conditions identified at a pH of 6 and temperatures of 25 - 37°C. GC-MS analysis revealed the existence of

*Corresponding author: Email: gayathridevaraja@gmail.com

various low-molecular-weight compounds, indicating potential phenol transformation activity; however, conclusive degradation pathways need to be determined. The results emphasise the promise of indigenous bacteria in phenol bioremediation efforts.

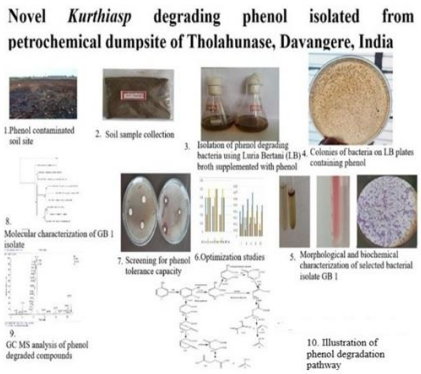
***Keywords*: *Bacillus tropicus*, bioremediation phenol tolerance, chromatography-mass spectrometry, *Kurthia* sp.**

***Classification number*: 3.5**

Highlights

- Phenol-degrading bacteria was identified by gene sequencing (16S rRNA) as *Kurthia populi* GB-1.
- *Kurthia populi* GB1, an indigenous isolate that was isolated from petrochemical dumpsite and known to degrade phenol and its derivatives.
- GC-MS analysis of cell free extract of *Kurthia populi* GB-1 detected Phenol, 3-methyl, acetic acid, 2-pyrolidinone, 3-aminopiprndin-2-one, 1H-pyrazole-1-carboxaldehyde,v4-ethyl-4,5-dihydro-N,N-dihydro-5-propyl, 2-naphthaleamine,3,4-dihydro-N,N-dimethyl, 2H-octahydropyridol [1,2-a] pyrazine-1-one, 4-Nitrobenzyl alcohol, [(5-Amino-3-cyclopropyl-1-methylpyrazol,4yl)-2-one sulfanyl] formonitrile as the major constituents.
- This indigenous species can be used as a microbial source for the remediation of phenol-polluted environments.

Graphical abstract



1. Introduction

Phenol is one of the most commonly used aromatic compounds in petrochemical, paper and pulp, textile, dye, pharmaceutical, resin, plastic, pesticide, coal processing, and oil refinery industries [1]. Phenol is commonly found contaminating soil and aquatic ecosystems due to its significant industrial use and improper disposal practices, especially around petrochemical dumping grounds and zones of industrial wastewater discharge. The Agency for Toxic Substances and Disease Registry (ATSDR) has listed phenol among the top hazardous substances, ranking it within the priority chemicals of environmental concern [2]. The United States Environmental Protection Agency (EPA) has therefore recommended that phenol concentration in wastewater should not exceed 1 mg/l because of its high toxicity even at low concentrations [3, 4].

Phenol along with its derivatives is known for its environmental persistence and capacity for bioaccumulation, thereby posing a considerable threat to terrestrial and aquatic ecosystems [5]. Phenolic compounds, including cresols, alkyl phenols, pentachlorophenol, and bisphenol-A, are acknowledged for disrupting the biological oxygen balance in aquatic systems and causing toxicological harm to fish, birds, and other living organisms [6, 7]. Exposure to phenol can cause skin lesions, eye burns, respiratory distress, and gastrointestinal disorders, whereas chronic exposure may result in immune system dysfunction, neurological disorders, organ failure and carcinogenic effects [8-10]. Furthermore, the presence of phenol in the food chain poses substantial risks; addressing the issue of phenol contamination is crucial for safeguarding public health and preserving the environment [11].

A number of standard approaches have been extensively used for the removal of phenol from polluted environments, which mainly involve physical, chemical, and advanced oxidation methods [12]. Physical techniques including

adsorption, solvent extraction, ion exchange, hot gas stripping, and photocatalysis have been documented as effective; however, they tend to be energy-intensive and costly [13]. Chemical treatment methods using oxidizing agents such as hydrogen peroxide, potassium permanganate, and sulphur dioxide commonly create secondary toxic by-products, which contribute to additional environmental pollution [14]. Although advanced oxidation processes (AOPs) like UV/H_2O_2 treatment, Fenton reactions, ozonation, and wet air oxidation are effective, they require substantial operational costs, elevated temperatures or pressures, and may still yield incomplete mineralisation or create carcinogenic intermediates [15, 16]. Thus, the practical application of these methods is limited, particularly for large-scale or in-situ remediation.

In contrast, biological remediation has emerged as an eco-friendly, cost-effective, and sustainable alternative for the treatment of phenol-contaminated environments [17]. Indigenous microorganisms found in contaminated environments are frequently naturally adapted to toxic conditions and exhibit improved metabolic abilities to effectively tolerate and degrade phenol. Both aerobic and anaerobic microorganisms are capable of degrading phenol through established metabolic pathways that involve the formation of catechol, followed by ortho- or meta-cleavage processes facilitated by catechol dioxygenases [18, 19]. Although anaerobic degradation of phenol has been reported, its effectiveness is relatively low and restricted to specific microbial groups [20].

The extraction of indigenous phenol-tolerant bacteria from soil at petrochemical dumpsites is thus significantly beneficial, as these organisms are already adapted to elevated phenol levels and extreme environmental conditions, rendering them ideal candidates for *in-situ* bioremediation [21, 22]. Several studies have proved that bacteria isolated from industrially contaminated soils show superior phenol degradation efficiency compared to laboratory strains

[23]. However, reports on phenol-degrading bacteria belonging to the genus *Kurthia* are very limited.

In this context, the current study was planned to isolate, characterise and evaluate indigenous phenol-tolerant aerobic bacteria from petrochemical dumpsite soil near Davangere University, Karnataka, India. The results of this study aim to contribute towards the development of an efficient and sustainable microbial strategy for the bioremediation of phenol-polluted environments.

2. Materials and methods

2.1. Chemicals

All bacteriological culture media and chemicals were obtained from HiMedia Laboratories (India). All reagents utilised in this research were of analytical grade. Luria-Bertani (LB) medium was composed of (per liter): 10 g tryptone, 5 g yeast extract, and 5 g NaCl. The minimal salt medium (MSM) included (per liter): 0.5 g K_2HPO_4, 0.1 g $CaCl_2$, 0.2 g NaCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $MnSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, and 1.0 g NH_4NO_3, prepared with deionised/distilled water.

2.2. Collection of soil sample

For the isolation of phenol-degrading bacteria, the test soil sample was procured from the petrol pump dumping site at Tholahunase village located in the vicinity of Davangere University, India. Immediately after the sample arrived at the laboratory in a zip-lock bag, it was processed, and a small portion was preserved under aseptic precautions [24, 25].

2.3. Isolation and enrichment of phenol tolerant/degrading bacteria

The test soil sample (10 g) was inoculated into two different conical flasks containing 100 ml each of Luria-Bertani (LB) broth; one was

supplemented with phenol at 500 mg/l and the other was without phenol addition. The culture flasks were incubated at room temperature on rotary shaker (120 prm) for 3-5 days. The inoculum concentration was adjusted to 0.1 OD (540 nm) [26]. 0.1 ml of this culture was further inoculated to freshly prepared LB culture plates containing phenol discs. After incubation plates were observed for the growth. Phenol-tolerant isolates were selected for further study. Those bacterial isolates showing tolerance to phenol were selected for the study and duplicates were maintained on nutrient agar slants (1% glycerol/500 µg/ml) phenol under refrigeration [27].

2.4. Molecular characterisation of phenol-degrading bacteria using 16S rRNA typing.

Using the potential bacterial isolate GB1, DNA was extracted [28]. The DNA was precipitated, using 50 ml of sterile deionised water. The extracted DNA sample was subjected to PCR amplification using universal 16S rRNA primers 27F(5'AGAGTTTGATCMTGGCTCAG3') and 1492 R (5'GGTTACCTTGTTACGACTT-3') [29].

PCR was conducted for the amplification of 16S rRNA using the following conditions as per J. Sambrook, et al. (2001) [30] with slight modifications. 50 ml of PCR mixture in 10 mM Tris-HCl (pH 8), KCl 50 mM, $MgCl_2$ 1.5 mM, 0.2 mM dNTPs, each primer (0.5 pmol), 1.25 U of Taq polymerase (Sigma-Aldrich, Germany), and DNA template (10 ng). PCR amplification was conducted using an ABI thermal cycler (Applied Biosystems, USA). The entire procedure was programmed as below:

Denaturation of DNA at 95°C for 5 min, 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 10 min. The amplification was ensured using gel electrophoresis. The resulting PCR amplified products were purified with the Mol Bio 250 Prep Clean up kit. Later,

it was sequenced using an ABI 3130XL genetic analyser (Applied Biosystems) [31].

2.5. Phylogenetic analysis

16S rRNA sequences (bacteria and archaea type strains) reference material was analysed for homology using the BLAST-N search programme and closely related species/strains showing maximum relatedness (97-100%) were taken as the nearest matches. The present sequences, along with the related genus, were retrieved from NCBI GenBank (www.ncbi.nlm.nih.gov) and a phylogenetic tree was constructed after pairwise alignment using ClustalW. Using the Kimura two-parameter model, a maximum-likelihood tree was generated [32], and the reliability of the phylogenetic tree was further evaluated using MEGA 11.0 software [33, 34].

2.6. Optimisation parameters for phenol degradation:

Phenol degrading bacteria can convert phenol into simpler compounds and this biochemical transformation is influenced by various physical, chemical, and nutritional parameters [27].

2.7. Effect of pH and temperature

An optimum pH is necessary to show maximum phenol degradation. The influence of pH on phenol degradation by potential indigenous bacteria was assessed in LB medium fortified with phenol at various pH values (2, 4, 6, 8, and 10). The experiments were carried out for two days at 37ºC and the results were recorded [35]. Temperature plays a crucial role in the utilisation of nutrients including phenol and metabolism for the growing bacteria. The effect of incubation temperature on the growth of potential phenol-degrading bacterium GB1 was studied at various temperatures (4, 24 and 37ºC) and the growth was measured [36].

2.8. Identification of phenol derivatives from GC-MS analysis

For the purpose of determining the intermediate metabolites of phenol degradation by the potential *Kurthia populi* GB1 isolate, GC-MS analysis was performed. For this, the *Kurthia populi* GB1 bacterial isolate was inoculated into MSM containing phenol at a concentration of 2.0 mM and incubated at room temperature/200 rpm. The broth was centrifuged (10000×g) at 4°C/10 min, the cell-free supernatant was acidified to pH 2.0 (1N HCl). Later, extraction was done with an equal volume of ice-cold ethyl acetate [37]. This mixture was vortexed to form two distinct layers; this process was repeated three times to get the extract for phenol degradation products analysis by GC-MS analysis. The organic layer was concentrated by air drying; subsequently, 4.0 µl of this concentrated filtered sample (0.22 µm, Millipore, USA) was introduced into the GC-MS. The analyses were performed using the acquisition general method (0 eV) with a TSQ 9000 gas chromatograph containing a capillary column of 30-m length, an ID of 0.25 mm, maximum temperature 350°C, equipped with a flame ionisation detector. The sample extract was processed in splitless mode. The column temperature was initially held at 100°C for 5 min, which was gradually raised to 250°C at a rate of 10°C per minute. The carrier gas (helium) flow rate in the column was 1.0 ml/min, and MS transfer line temperature was maintained up to 280°C [38]. Compound identification was carried out by comparing mass spectra with those in the NIST library. Identifications are considered tentative and based on spectral similarity and retention time. No internal standards were used.

3. Results

3.1. Screening, morphological and biochemical characterisation of indigenous phenol-degrading bacteria

Potential indigenous phenol-degrading bacteria were isolated from petrochemical dumpsite soil by pour plate method, in minimal salt medium (MSM) supplemented with phenol as the sole carbon and energy source. A total of 16 phenol-tolerant bacterial isolates were initially screened. The isolated bacteria were identified using morphological characteristic features and biochemical tests. Later, they were enriched at high concentrations of phenol (500 mg/l) for a maximum of 15 days. Out of 16 phenol-tolerant bacterial isolates, only three isolates were found to be potential. For further studies, we selected the phenol-degrading bacterial isolate GB1 with the highest activity. Bacteria isolated from petrochemical dumpsite showed distinct phenotypical and biochemical features. The isolate formed circular, smooth, opaque colonies on MSM agar plates and was maintained for subsequent experiments. Microscopic examination revealed that isolate GB1 was Gram-positive and rod-shaped. The isolate tested positive for catalase activity, methyl red production, and the Voges-Proskauer test, but showed negative results for KOH, indole, and citrate utilisation tests. The results were consistent with previously described members of the genus *Kurthia*.

3.2. Molecular identification and phylogenetic analysis

The 16S rRNA gene sequence of the GB1 isolate showed similarity to members of the genus *Kurthia*, with the closest match being *Kurthia populi* with GenBank accession no. KM613131.2 based on BLAST analysis against the NCBI database. The 16S rRNA gene sequence of isolate GB1 has been deposited in GenBank under the accession number PX951428. In MEGA version 12.0, phylogenetic analysis was executed using the maximum likelihood method alongside the Kimura two-parameter model. The resulting phylogenetic tree (Fig. 1) demonstrated that isolate GB1 clustered within the *Kurthia* genus, grouping closely with *Kurthia populi*. Sequence similarity analysis revealed that

isolate GB1 shared 92.73% similarity with *Kurthia populi* (KM613131.2). Therefore, the isolate is conservatively designated as *Kurthia populi* GB1.

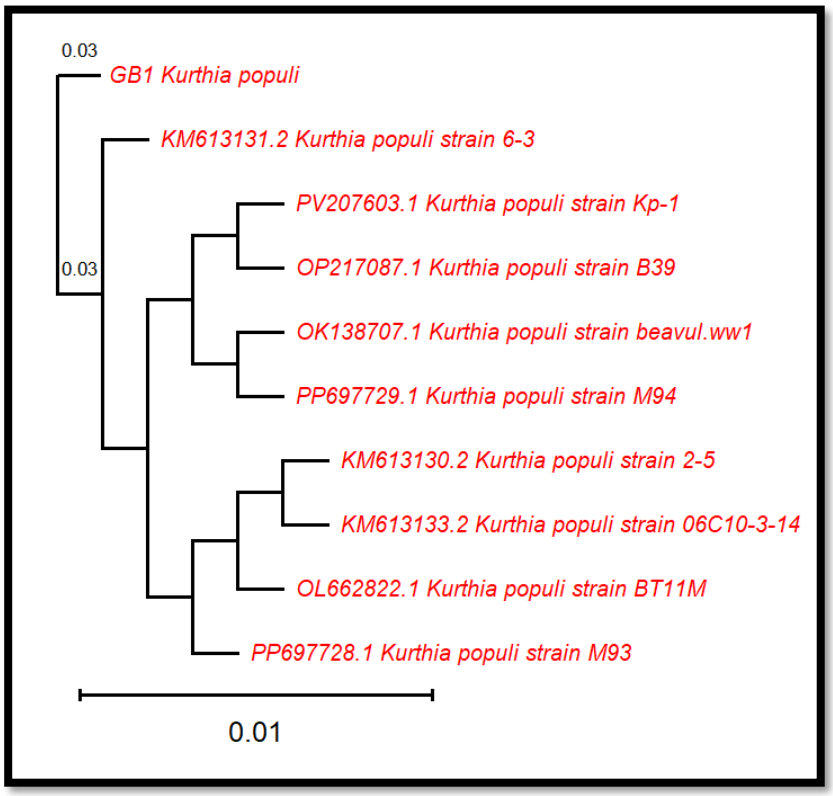


Fig. 1. Phylogenetic relationship of isolate GB-1 based on 16S rRNA gene sequences. Neighbour-joining phylogenetic tree showing the relationship between isolate GB-1 and closely related *Kurthia populi* strains retrieved from the NCBI GenBank database. The tree was constructed using the Kimura two-parameter model. Bootstrap values (expressed as percentages of 1,000 replicates) are shown at branch points. The scale bar represents 0.01 substitutions per nucleotide position.

3.3. Effect of pH and temperature on growth under phenol-amended conditions

To ascertain the optimal physiological conditions, the effects of pH and temperature on the growth of isolate *Kurthia populi* GB1 in phenol-amended environments were analysed. Since phenol is weakly acidic in aqueous solution, differences in pH can influence both bacterial growth and phenol tolerance. Growth of isolate *Kurthia populi* GB1 was observed over a pH range of 5-8; however, the maximum growth was recorded at a pH of 6. At pH values lower

and higher than 6, a gradual reduction in growth was observed, indicating reduced tolerance under altered pH conditions. Temperature optimisation studies demonstrated that isolate *Kurthia populi* GB1 showed growth over a temperature range of 24-37 °C, with observable growth throughout this interval. These results indicate that *Kurthia populi* GB1 is capable of tolerating phenol-infused conditions across a moderate temperature range.

3.4. Phenol tolerance of isolate Kurthia populi GB1

The selected *Kurthia populi* GB1 isolate and the reference strain Bacillus tropicus (MTCC 10650), used as a control phenol-degrading bacterium and available in the Department of Microbiology, Davangere University, India, were cultured on MSM agar with phenol discs (100 to 500 mg/l) under aerobic conditions. Phenol tolerance at concentrations up to 500 mg/l was exhibited by *Kurthia populi* GB1, when compared to *Bacillus tropicus*. This indicates that the native species *Kurthia populi* GB1 isolated from petroleum dumpsite soil was able to tolerate high concentrations of phenol and also possessed the ability to degrade phenol. However, these observations do not directly quantify phenol degradation or removal. Fig. 2 illustrates the phenol tolerance.

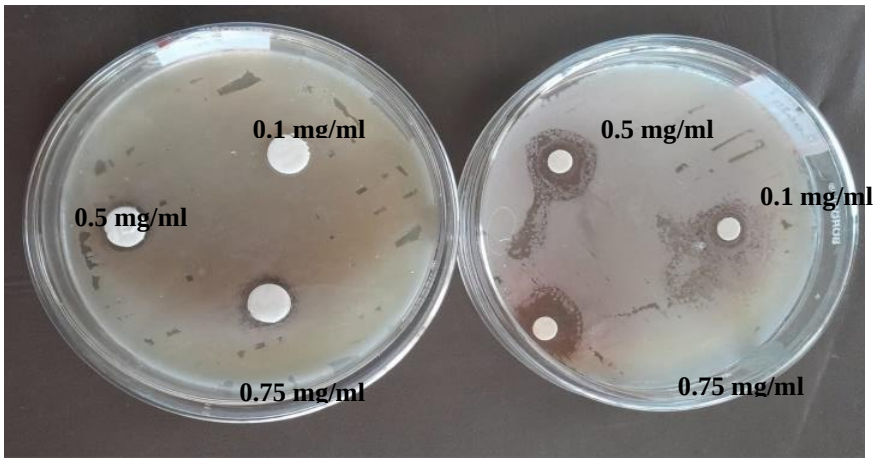


Fig. 2. Phenol tolerance assay using disc diffusion method on MSM agar supplemented with different concentration of phenol (A) *Kurthia populi* GB1; (B) *Bacillus tropicus* (control). The assay qualitatively demonstrates bacterial tolerance/sensitivity to phenol.

3.5. GC-MS analysis of Kurthia populi GB1 for phenol degradation products

The GC-MS chromatogram analysis of phenol biodegradation by *Kurthia populi* GB1 showed that GC-MS is one of the best-known techniques for identifying the constituents of volatile compounds, aromatic compounds, esters, alcohols, branched long-chain hydrocarbons, and acids. The GC-MS analysis of cell-free supernatant ethanolic extract of phenol-degrading bacteria revealed the presence of bioactive compounds that were identified using the NIST library and determined based on the retention time, molecular mass, molecular formula, and peak height. Thus, these identifications should be considered tentative, and their direct role as phenol degradation intermediates cannot be conclusively established. The values of the above-mentioned parameters are represented in Table 1 and Fig. 3. Overall, GC-MS results support the occurrence of phenol transformation activity by isolate *Kurthia populi* GB1 under aerobic conditions. The results are therefore presented as screening-level evidence to complement phenol tolerance and growth observations.

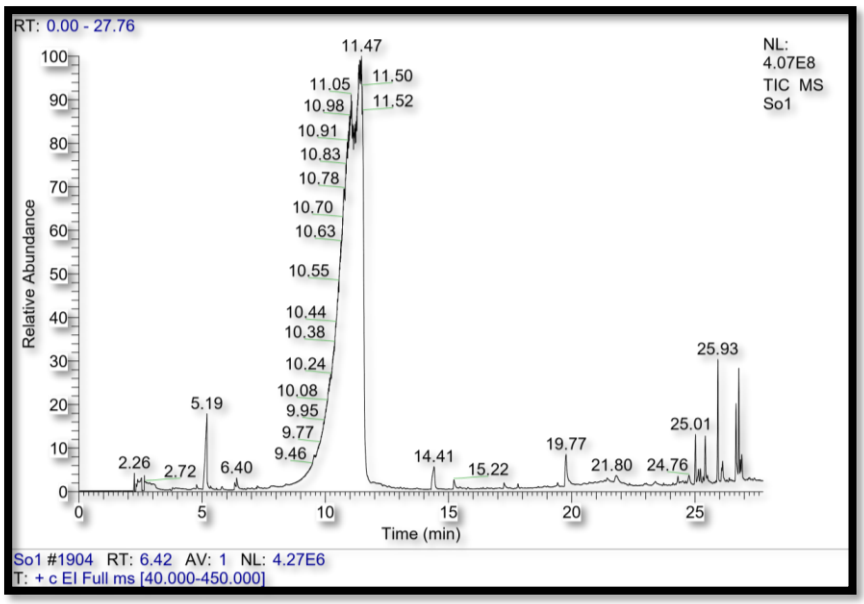
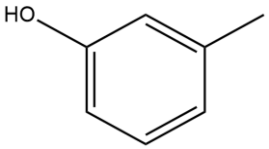
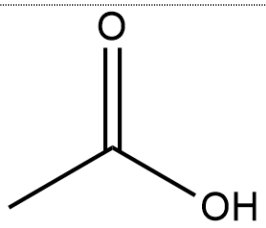
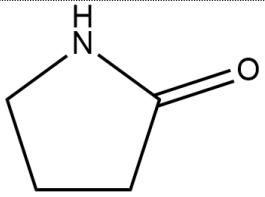
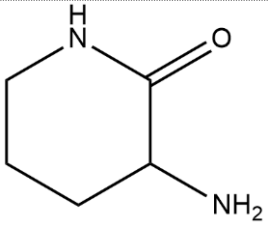
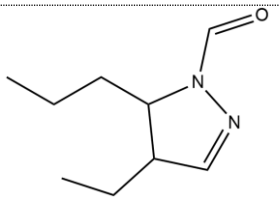
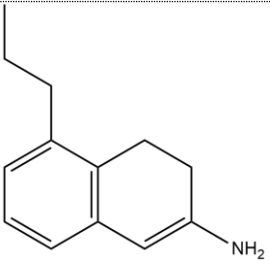
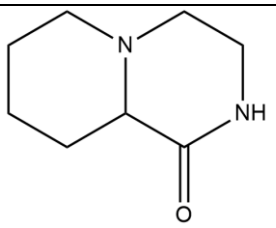
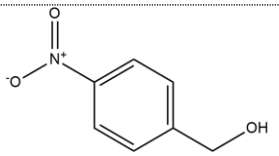
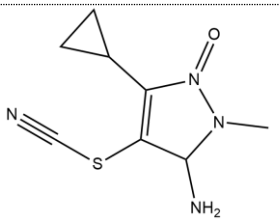


Fig. 3. GC-MS chromatogram of phenol-treated culture extract of isolate GB-1. Representative GC-MS chromatogram obtained from cell-free extracts of

isolate *Kurthia populi* GB1 grown in MSM supplemented with phenol. Detected peaks correspond to low-molecular-weight organic compounds tentatively identified based on mass spectral library matching (NIST).

Table 1. Tentative compounds detected by GC-MS analysis of phenol-treated culture extracts of isolate *Kurthia populi* GB1.

No.	Retention time (min)	Tentatively identified compound	Molecular formula	Molecular weight (g/mol)	Peak Area (%)	Structure
1	2.80	3-Methylphenol	C_7H_8O	108.05	0.77	
2	5.18	Acetic acid	$C_2H_4O_2$	60.02	4.79	
3	14.40	2-Pyrrolidinone	C_4H_7NO	85.05	1.33	
4	19.77	3-Aminopiperidin-2-one	$C_5H_{10}N_2O$	114.07	2.04	
5	25.00	1H-Pyrazole-1-carboxaldehyde, 4-ethyl-4,5-dihydro-5-propyl	$C_9H_{16}N_2O$	168.12	3.17	
6	24.75	2-Naphthalenamine, 3,4-dihydro-5-propyl	$C_9H_{16}N_2$	173.12	0.72	

7	25.90	2H-Octahydropyrido[1,2-a] pyrazine-1-one	$C_{12}H_{15}N$	154.11	7.86	
8	26.78	4-Nitrobenzyl alcohol	$C_7H_7NO_3$	153.04	7.24	
9	26.90	[(5-Amino-3-cyclopropyl-1-methylpyrazol-4-yl)-2-one-sulfanyl] formonitrile	$C_8H_{10}N_4S$	194.06	1.97	

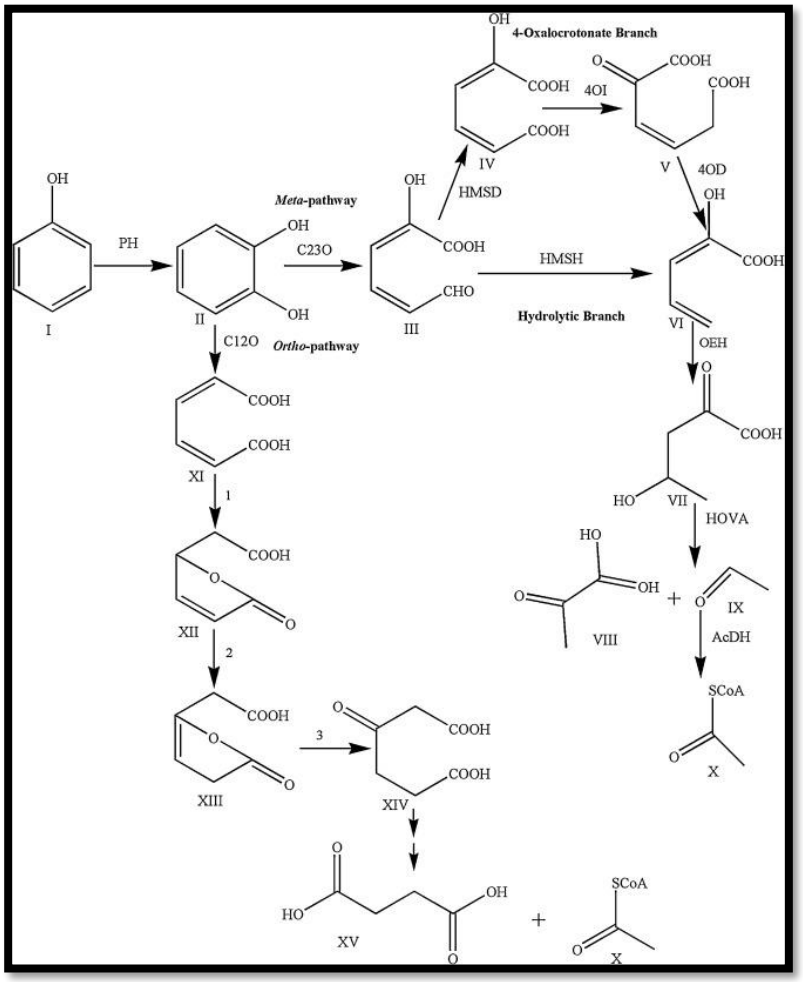


Fig. 4. General schematic representation of aerobic phenol degradation pathways (ortho- and meta-cleavage) as reported in previous studies [40]**. The figure represents established pathways.**

Under aerobic conditions, it is commonly reported that the degradation of phenol involves an initial hydroxylation step converting phenol into catechol, followed by enzymatic ring cleavage and the assimilation of the resulting intermediates into the tricarboxylic acid (TCA) cycle [39]. The schematic pathway (Fig. 4) indicates that catechol may undergo ortho-cleavage via catechol 1,2-dioxygenase or meta-cleavage through catechol 2,3-dioxygenase, resulting in intermediates such as succinate, acetyl-CoA, pyruvate, and acetaldehyde that enter central metabolism [39]. These pathways are well-established mechanisms in the literature, yet they were not experimentally confirmed for isolate *Kurthia populi* GB1 in this study.

4. Discussion

Phenol bioremediation plays a pivotal role in the elimination of phenol in the polluted environment. There is an urgent need for sustainable, green, and eco-friendly approaches to safeguard living beings from chemical hazards. The environment is affected by different types of pollution; among them, one major source is oil spillage and petrochemical contamination with phenol and phenolic compounds. Biodegradation or bioremediation is the ideal methodology to remediate phenol by the intervention of microorganisms, as it is cost-effective and simple enough to adopt. Certain bacterial isolates oxidise phenol to CO_2 and H_2O during their metabolic reactions and use them as a single source of carbon and energy. Although a good number of micro-organisms are capable of degrading phenol under aerobic conditions, only a few phenol-degrading anaerobic bacteria have been reported [36, 40, 41].

In the present study, an aerobic, phenol-tolerant bacterial isolate designated GB1 was recovered from a petrochemical dumpsite and identified as *Kurthia populi* GB1 based on morphological and biochemical characteristics. The isolate has the ability to grow in minimal salt medium supplemented with

phenol as the sole carbon and energy source, suggesting that isolate *Kurthia populi* GB1 has metabolically adapted to phenol-contaminated environments. Long-term exposure drives the selection of tolerant or metabolically adapted strains.

Kurthia populi GB1 tolerated phenol up to 500 mg/l under aerobic conditions at pH 6 and 25-37°C. When compared to other studies, comparable tolerance ranges have been reported for *Acinetobacter calcoaceticus* (400-800 mg/l), *Bacillus* spp. (300-600 mg/l), *Rhodococcus pyridinivorans* (500 mg/l), and *Stenotrophomonas maltophilia* (up to 2000 mg/l) under optimised conditions. Unlike these reported strains, which are usually isolated from either activated sludge or industrial bioreactors and require nutrient supplementation [40, 42]. In contrast, *Kurthia populi* GB1 was isolated from a petrochemical dumpsite soil and maintained phenol tolerance under minimal medium conditions, which suggests good environmental adaptability for in-situ applications, although direct kinetic comparisons are required and will be performed in future studies. GC-MS analysis also confirmed phenol transformation into lower-molecular-weight metabolites, supporting its metabolic capability. These features indicate that *Kurthia populi* GB1 may be more suitable for low-cost, site-specific bioremediation applications rather than maximal laboratory efficiency [43, 44].

Phenol tolerance was assessed at varying pH values and temperatures and it was observed that the results are consistent with the earlier reported findings. Most of the aerobic phenol-degrading bacteria isolated from soil exhibit optimal activity at temperatures around 30 °C and near-neutral pH values ranging from 6 to 8. Isolate *Kurthia populi* GB1 showed optimal growth and phenol tolerance at pH 6 and temperatures between 25 and 37°C, which is consistent with the reported species such as *Pseudomonas resinovorans* strain P-1, *Pseudomonas fluorescens*, and *Rhodococcus pyridinivorans*, which support our claim of

ecological relevance of *Kurthia populi* GB1 for phenol-contaminated soil environments [27, 45].

To detect and understand the by-products or secondary metabolites produced after the degradation of phenol, we performed GC-MS analysis of cell-free extracts from phenol-treated cultures, which detected several low-molecular-weight compounds. Aerobic phenol degradation in bacteria commonly begins by hydroxylation of phenol to catechol via phenol hydroxylase, followed by ring cleavage through either the ortho- or meta-pathway mediated by catechol 1,2-dioxygenase or catechol 2,3-dioxygenase, respectively [45-47]. This results in the formation of intermediates that can subsequently be directed into the tricarboxylic acid cycle through the formation of compounds such as succinate, pyruvate, or acetyl-CoA [48]. While these pathways are well established in several phenol-degrading bacteria, including *Pseudomonas*, *Acinetobacter*, and *Stenotrophomonas* species [45, 49-51], the compound identification in this study was based on mass spectral library matching using retention time and molecular mass and should therefore be considered tentative. Accordingly, a schematic representation of a proposed aerobic phenol transformation pathway has been included to contextualise the GC-MS findings as evidence of phenol transformation activity rather than as definitive proof of a specific degradation route.

Future studies should focus on quantitative phenol degradation kinetics. The observed tolerance to phenol and metabolic activity of *Kurthia populi* GB1 under aerobic conditions suggest further investigation to uncover its full potential. Future studies should focus on kinetic modelling, along with genomics and transcriptomics analyses for the confirmation of degradation pathways and the application of the strain under contaminated field conditions, which will help to evaluate the feasibility of using *Kurthia* spp. in practical bioremediation applications.

5. Conclusions

This investigation showcases the pragmatism of isolating indigenous phenol-tolerant bacteria from soil affected by petrochemical pollution and highlights *Kurthia populi* GB1 as a notable candidate for further exploration. The work provides preliminary, laboratory-scale evidence supporting the adaptive potential of native soil bacteria in phenol-impacted environments. The findings are nevertheless limited by the absence of quantitative degradation kinetics, enzyme-level validation, and field-scale assessments. Consequently, our future research is aimed at validating metabolic pathways at genetic and enzymatic levels and assessing performance under conditions that are environmentally relevant before bioremediation applications can be pursued.

CRediT author statement

R Bindu: Conducting the Investigation and Formal analyses; R Soundarya and SM Pruthvi: Execution of Investigation and Manuscript preparation; Prashantkumar S Chakra: Writing, Reviewing and Data analysis; Devaraja Gayathri: Conceiving the idea, Designing the experiment and Supervision.

This article does not contain studies with humans or animals.

COMPETING INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] N.K. Pazarlioğlu, A. Telefoncu (2005), “Biodegradation of phenol by *Pseudomonas putida* immobilized on activated pumice particles”, *Process Biochemistry*, **40(5)**, pp.1807-1814, DOI: 10.1016/j.procbio.2004.06.043.

[2] S.M. Dankaka, J.B. Muhammad, S. Usman, et al. (2023), “Phenol biodegradation by *Acinetobacter baumannii* and *Citrobacter sedlakii* isolated

from petroleum-contaminated environments", *Case Studies in Chemical and Environmental Engineering*, **8**, DOI: 10.1016/j.cscee.2023.100468.

[3] B. Alizadeh, M. Delnavaz, A. Shakeri (2018), "Removal of Cd(II) and phenol using cross-linked magnetic EDTA/chitosan/TiO_2 nanocomposite", *Carbohydrate Polymers*, **181**, pp.675-683, DOI: 10.1016/j.carbpol.2017.11.095.

[4] S. Mohammadi, A. Kargari, H. Sanaeepur, et al. (2015), "Phenol removal from industrial wastewaters: A short review", *Desalination and Water Treatment*, **53(8)**, pp.2215-2234, DOI: 10.1080/19443994.2014.883327.

[5] A.A. Gami, M.Y. Shukor, K.A. Khalil, et al. (2014), "Phenol and its toxicity", *Journal of Environmental Microbiology and Toxicology*, **2(1)**, pp.11-23, DOI: 10.54987/jemat.v2i1.89.

[6] W. Duan, F. Meng, H. Cui, et al. (2018), "Ecotoxicity of phenol and cresols to aquatic organisms: A review", *Ecotoxicology and Environmental Safety*, **157**, pp.441-456, DOI: 10.1016/j.ecoenv.2018.03.089.

[7] M.D. Baquerizo, A. Gallardo, F. Covelo, et al. (2015), "Species-specific effects of biocrust-forming lichens on soil nutrients and microbial communities", *Functional Ecology*, **29(8)**, pp.1087-1098, DOI: 10.1111/1365-2435.12403.

[8] W. Raza, J. Lee, N. Raza, et al. (2019), "Removal of phenolic compounds from industrial wastewater using membrane-based technologies", *Journal of Industrial and Engineering Chemistry*, **71**, pp.1-18, DOI: 10.1016/j.jiec.2018.11.024.

[9] S.J. Kulkarni, J.P. Kaware (2013), "Review on research for removal of phenol from wastewater", *International Journal of Scientific and Research Publications*, **3(4)**, pp.1-5.

[10]. L.G.C. Villegas, N. Mashhadi, M. Chen, et al. (2016), "Techniques for phenol removal from wastewater: A review", *Current Pollution Reports*, **2**, pp.157-167, DOI: 10.1007/s40726-016-0035-3.

[11] W.W. Anku, M.A. Mamo, P.P. Govender (2018), "Phenolic compounds in water: Sources, toxicity and treatment methods", *Phenolic Compounds - Natural Sources, Importance and Applications*, pp.419-443, DOI: 10.5772/66927.

[12] M. Jin, M. Long, H. Su, et al. (2017), "Magnetically separable maghemite/montmorillonite composite as a Fenton-like catalyst for phenol degradation", *Environmental Science and Pollution Research*, **24**, pp.1926-1937, DOI: 10.1007/s11356-016-7866-8.

[13] T. Singh, A.K. Bhatiya, P.K. Mishra, et al. (2020), "Degradation of phenolic wastes: Phenols and cresols", *Abatement of Environmental Pollutants*, Elsevier, pp.203-243, DOI: 10.1016/B978-0-12-818095-2.00011-4.

[14] D.J. Naghan, A. Azari, N. Mirzaei, et al. (2015), "Photocatalytic degradation of phenol using ZnO nanocatalyst under UV-C irradiation", *Bulgarian Chemical Communications*, **47(D)**, pp.14-18.

[15] A. Yazdanbakhsh, H. Daraei, M. Davoodabadi (2015), "Performance of advanced oxidation processes for phenol degradation from water", *Environmental Science: An Indian Journal*, **11(3)**, pp.83-88.

[16] A. Tungler, E. Szabados, A.M. Hosseini (2015), "Wet air oxidation of aqueous wastes", *InTechOpen*, DOI: 10.5772/60935.

[17] S. Sivasubramanian, S.K.R. Namasivayam (2015), "Optimization of medium components for phenol degradation by EM-1", *Biosciences Biotechnology Research Asia*, **12(2)**, pp.1471-1480.

[18] M. Mahiuddin, A.N.M. Fakhruddin (2012), "Degradation of phenol via meta-cleavage pathway by *Pseudomonas fluorescens* PU1", *International Scholarly Research Notices*, Article ID 960708.

[19] D. Gayathri, K.J. Shobha (2015), "Biodegradation of 2,4'-dichlorobiphenyl by *Pseudomonas* isolates GSa and GSb", *Indian Journal of Experimental Biology*, **53**, pp.665-671.

[20] N.V. Pradeep, S. Anupama, K. Navya, et al. (2015), "Biological removal of phenol from wastewater: A mini review", *Applied Water Science*, **5**, pp.105-112, DOI: 10.1007/s13201-014-0176-8.

[21] D. Park, D.S. Lee, Y.M. Kim, et al. (2008), "Bioaugmentation of cyanide-degrading microorganisms in full-scale coke wastewater treatment", *Bioresource Technology*, **99(6)**, pp.2092-2096, DOI: 10.1016/j.biortech.2007.03.027.

[22] A. Hussain, S.K. Dubey, K.K. Shandilya, et al. (2013), "Anaerobic degradation of phenolic wastewater: Batch study", *Environmental Research Journal*, **7(1)**, pp.1-8.

[23] P. Sachan, S. Madan, A. Hussain (2019), "Isolation and screening of phenol-degrading bacteria from pulp and paper mill effluent", *Applied Water Science*, **9**, DOI: 10.1007/s13201-019-0994-9.

[24] A. Bhattacharya, A. Gupta, A. Kaur, et al. (2018), "Microbial remediation of phenol: A sustainable approach", *Current Organic Chemistry*, **22(4)**, pp.370-385, DOI: 10.2174/1385272821666171121160602.

[25] Z. Liu, W. Xie, D. Li, et al. (2016), "Biodegradation of phenol by *Acinetobacter calcoaceticus* PA", *International Journal of Environmental Research and Public Health*, **13(3)**, DOI: 10.3390/ijerph13030300.

[26] P.K. Somaraja, D. Gayathri, N. Ramaiah (2013), "Molecular characterization of 2-chlorobiphenyl-degrading *Stenotrophomonas maltophilia* GS-103", *Bulletin of Environmental Contamination and Toxicology*, **91**, pp.148-153, DOI: 10.1007/s00128-013-1044-1.

[27] B.V. Mohite, S.P. Pawar, A. Morankar (2011), "Biodegradation profile of phenol-degrading bacteria from oil-contaminated soil", *Bulletin of Environmental Contamination and Toxicology*, **87**, pp.143-146, DOI: 10.1007/s00128-011-0322-z.

[28] S.S. Patil, H.M. Jena (2023), "Kinetic study of dimethyl phthalate biodegradation by *Micrococcus* sp. KS2", *Scientific Reports*, **13**, 3900, DOI: 10.1038/s41598-023-29256-x.

[29] J.T. Hollibaugh, N. Bano, H.W. Ducklow (2002), "Distribution of Nitrosospira-like ammonia-oxidizing bacteria in polar oceans", *Applied and Environmental Microbiology*, **68(3)**, pp.1478-1484, DOI: 10.1128/AEM.68.3.1478-1484.2002.

[30] J. Sambrook, E.F. Fritsch, T. Maniatis (2001), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, 46p.

[31] C.T. Swamy, D. Gayathri, T.N. Devaraja, et al. (2016), "Plant growth-promoting potential of *Enterobacter cloacae*", *Journal of Basic Microbiology*, **56**, pp.1369-1379.

[32] M. Barik, C.P. Das, A.K. Verma, et al. (2021), "Metabolic profiling of phenol biodegradation by *Rhodococcus pyridinivorans*", *International Biodeterioration & Biodegradation*, **158**, DOI: 10.1016/j.ibiod.2020.105168.

[33] M. Kimura (1980), "A simple method for estimating evolutionary rates of base substitutions", *Journal of Molecular Evolution*, **16**, pp.111-120, DOI: 10.1007/BF01731581.

[34] J. Felsenstein (1985), "Phylogenies and the comparative method", *The American Naturalist*, **125(1)**, pp.1-15, DOI: 10.1086/284325.

[35] S.D. Priyadharshini, A.K. Bakthavatsalam (2016), "Optimization of phenol degradation by *Chlorella pyrenoidosa*", *Bioresource Technology*, **207**, pp.150-156, DOI: 10.1016/j.biortech.2016.01.138.

[36] S. Bera, A.S. Roy, K. Mohanty (2017), "Biodegradation of phenol by a native mixed bacterial culture", *International Biodeterioration & Biodegradation*, **121**, pp.107-113, DOI: 10.1016/j.ibiod.2017.04.002.

[37] Y. Liu, W. Wang, S.B. Shah, et al. (2020), "Phenol biodegradation by *Acinetobacter radioresistens* APH1", *Applied Microbiology and Biotechnology*, **104**, pp.427-437, DOI: 10.1007/s00253-019-10271-w.

[38] A. Bhattacharya, A. Gupta, A. Kaur, et al. (2015), "Phenol degradation by *Acinetobacter* sp. B9", *Chemistry and Ecology*, **31(7)**, pp.607-621, DOI: 10.1080/02757540.2015.1050464.

[39] W. Zhou, W. Guo, H. Zhou, et al. (2016), "Phenol degradation by *Sulfobacillus acidophilus* via meta-pathway", *Microbiological Research*, **190**, pp.37-45, DOI: 10.1016/j.micres.2016.05.005.

[40] T. Deng, H. Wang, K. Yang (2018), "Phenol biodegradation by *Citrobacter* under hypersaline conditions", *Water Science and Technology*, **77(2)**, pp.504-510, DOI: 10.2166/wst.2017.543.

[41] N. Ahmad, I. Ahmed, M. Iqbal, et al. (2015), "Characterization and identification of phenol-degrading bacteria isolated from industrial waste", *Pakistan Journal of Agricultural Sciences*, **52(1)**, pp.193-201, ISSN (Print): 0552-9034; ISSN (Online): 2076-0906.

[42] P.K. Somaraja, D. Gayathri, N. Ramaiah (2013), *Molecular characterization of 2-chlorobiphenyl-degrading Stenotrophomonas maltophilia*

GS-103, Bulletin of Environmental Contamination and Toxicology, **91**, pp.148-153, DOI: 10.1007/s00128-013-1044-1.

[43] I. Jerin, M.S. Rahi, T. Sultan, et al. (2021), "Diesel degradation efficiency of *Enterobacter*, *Acinetobacter* and *Cedecea* species isolated from petroleum waste dumping sites", *Archives of Microbiology*, **203**, pp.5075-5084, DOI: 10.1007/s00203-021-02469-2.

[44] Z. Moghiseh, A. Rezaee, S. Dehghani, et al. (2019), "Phenol degradation in a microbial electrochemical system under alternating current: Metabolic pathway study", *Bioelectrochemistry*, **130**, DOI: 10.1016/j.bioelechem.2018.12.002.

[45] E.V.P. Pandeeti, D. Siddavattam (2011), "Purification and characterization of catechol 1,2-dioxygenase from *Acinetobacter* sp. DS002 and cloning of partial *catA* gene", *Indian Journal of Microbiology*, **51**, pp.312-318, DOI: 10.1007/s12088-011-0123-4.

[46] S. Shi, Y. Qu, H. Zhou, et al. (2015), "Cometabolic degradation of carbazole by a phenol-cultivated *Arthrobacter* sp. W1", *Bioresource Technology*, **193**, pp. 281-287, DOI: 10.1016/j.biortech.2015.06.106.

[47] S.A. Hasan, S. Jabeen (2015), "Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species", *Biotechnology & Biotechnological Equipment*, **29(1)**, pp.45-53, DOI: 10.1080/13102818.2014.991638.

[48] J.R. Mason, R. Cammack (1992), "The electron-transport proteins of hydroxylating bacterial dioxygenases", *Annual Review of Microbiology*, **46**, pp.277-305, DOI: 10.1146/annurev.mi.46.100192.001425.

[49] N.N. Tuan, H.C. Hsieh, Y.W. Lin, et al. (2011), "Bacterial degradation pathways of long-chain alkylphenols involving phenol hydroxylase and catechol dioxygenase genes", *Bioresource Technology*, **102(5)**, pp.4232-4240, DOI: 10.1016/j.biortech.2010.12.067.

[50] P.K. Somaraja, D. Gayathri (2016), "Biodegradation study of a novel biphenyl-degrading bacterial isolate GS-008", *Indian Journal of Science and Technology*, **9(11)**, DOI: 10.17485/ijst/2016/v9i11/109813.

[51] D. Gayathri, J.K. Shobha (2016), "Bioremediation of 2,4-dichlorobiphenyl by a consortium of *Pseudomonas* isolates: A laboratory-scale study", *Journal of Environmental Biology*, **37**, pp.145-152.