DOI: https://doi.org/10.36094/sc.v88.2022.Phenol_Bioremediation_by_a_Bacterium.Chakraborty.199

Sci. and Cult. 88 (5–6) : 199-202 (2022)

Phenol Bioremediation by a Bacterium Isolated from Waste of Pharmaceutical Industry

***Abstract*: Phenol is toxic substance that present in the pharmaceutical industry waste. None of the eco friendly conventional methods are available to degrade it except Bioremediation. We have isolated a potent Gram negative bacterial strain that shows phenol-degradation by exhibiting emulsification-activity and hydrophobicity-activity. There is a significant correlation between emulsification activity and bacterial adherence to hydrocarbons (BATH) assay activity in case of phenol biodegradation. These results define significant phenol removal ability of the strain. Therefore, the isolated bacterial strain could serve as a potential candidate for phenol bioremediation in the phenol-contaminated environments.**

Fundamental elements of life like air, water and land are being contaminated continuously due to rising population, urbanization and industrialization[1] . Solvents, heavy metals are widely found in industries such as metallurgical, chemical, pharmaceuticals, mining, and smelting[2,3]. These also cause water pollution that released into agricultural environment[4].

Phenol is considered as a highly hazardous chemical and has the toxic properties, as per the United States' priority pollutants list. For the widespread production and industrial use of phenols particularly in oil refineries, cooking plants, pharmaceutical and plastic industries, it becomes a hazardous pollutant. These compounds are discharged from industrial aqueous effluents. Even at low concentrations, effluent containing phenolic chemicals endangers fish life if dumped in water bodies. Numerous aquatic organisms, including bacteria, plants, and fish, provide a risk of mutagenic, teratogenic, and carcinogenic outcomes in phenol-polluted environments. It is reported that the level of phenol concentration is reduced after physical and chemical purification methods[5]. These techniques were effective in the removal phenol, but were time-consuming and expensive. As a result, efficient, cost-effective, and environmentally friendly technologies for degrading phenol in the environment are required. Activated sludge has been used to remove phenolic pollution for many years. In addition to the physico-chemical removal, microbial remediation of phenolic pollutants from wastewater plays an essential role[5]. Biodegradation of phenols is now the most popular method for reducing phenol compounds since it is both environmentally beneficial and cost-effective.Several microorganisms have been discovered for phenol degradation. There are very limited microorganisms which can use phenol as the sole source of carbon for their growth. *Rhodococcus erythropolis, Serratia marcescens , Pseudomonas putida, , Bacillus subtilis, Bacillus brevis, , Arthrobactercitreus, Alcaligenesfaecalis, Sphingomonas*, and *Acinetobacter* are among the bacteria that have been reported to grow using phenol. In the remediation of contaminated environments, the microbial species that are native proven to to be more adaptive and competent of outperforming non-indigenous bacteria. As a result, novel phenol-degrading bacteria must be discovered in order to bioremediate phenol-contaminated environment in diverse region of the universe [6,7] .

The main objective of the current study is to isolate naturally occurring bacteria from industrial waste soil, screen the isolate as a phenol detoxifier, and determine the basis of their resistance so that they can be used for detoxification in a bioremediation scheme.

Materials and Methods: *Isolation of toxic metals tolerant bacteria:* Soil sample was collected from the pharmaceutical industrial waste located in Kolkata, India. Soil samples were transferred to sterilized plastic bags, labeled, sealed, immediately transported to the laboratory, and kept at 4 °C until use in subsequent experiments. 1g of soil sample was added to 100 ml of nutrient broth (NB) (Himedia, India) containing 100 mg $L^{-1}$of phenol in conical flasks and kept at 30 ± 2 °C for 72 h at 160 rpm. Then this broth was serially diluted, and 10^{-3} (CFUH $\approx 10^7$/ml) dilution was considered and plated on NA plates amended with100 mg $L^{-1}$of phenol, and kept at 30 ± 2 ^{0}C for 72 h. The colonies on the plates were purified. Isolated single colony was stored for further study[8] .

Phenol Degradation: The effect of various phenol quantities (50, 100, 200, and 300 mg L^{-1}) on the isolated bacterial growth was analyzed. Nutrient broth medium was added with varied amounts of phenol for this purpose.On a rotary shaker set to 180 rpm, the flasks were kept at 30°C for 3 days. After 3 days, growth was examined using a UV–visible spectrophotometer to measure turbidity (OD at 600 nm) (Systronics *UV*-160)[5].

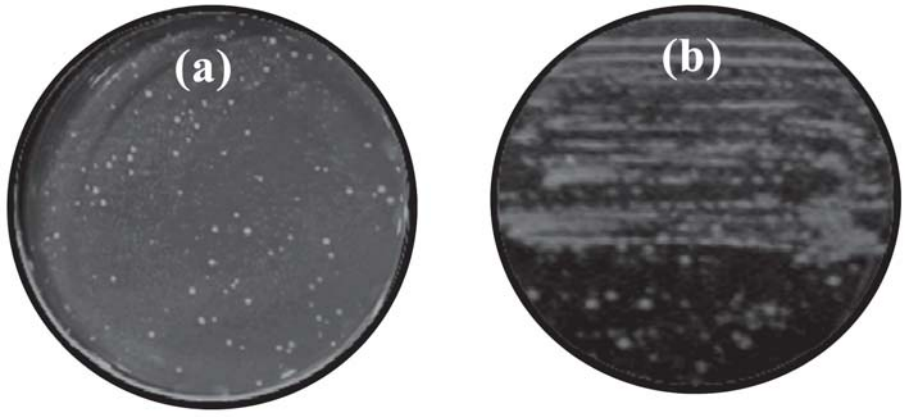

Figure1: (a) Isolation of phenol tolerant bacteria (b) Single colony of the isolated phenol tolerant bacteria

The 2,4 dichloroquinon-4-chloroimide dyes (Gibb's reagent) were used inanalysis of phenol removal. 150 mL LB medium with mg L^{-1} phenol and 150 mL LB medium without phenol (Control) were kept for 72h at 30±2°C on a rotary shaker set to 180 rpm. These media were centrifuged at 6000 g for 10 minutes, then supernatants were mixed with 30 mL Na_2HCO_2 and 20 mL Gibb's reagent, and the colours obtained at 630 nm were quantified [5].

Mechanism for Phenol Degradation: The activity of emulsification was assessed by vortexing equal amounts of hexadecane and broth of cell free culture for 2 minutes and letting the mixture sit for 24h, by dividing the height of the emulsified layer (mm) by the overall height of the liquid column, the emulsification activity was estimated (mm). As previously mentioned, the bacterial adhesion to hydrocarbon was assessed by the reported method[5].

To determine the phenol degradation, cell surface hydrophobicity of the isolated bacteria was measured [5]. Bacteria were grown overnight and centrifuged at 6,000 g at 4 °C for 10 minutes, washed two times, and resuspended in PBS. OD600 was 0.5. (OD Initial). The bacterial cell suspension was then homogenized with 1 mL xylene (Sigma-Aldrich) and pre-incubated at 30 °C for 10 minutes. To separate the two phases (water and xylene), the mixture was vortexed for 2 minutes and then kept at 37 °C for 20 min. After the aqueous phase extraction, the absorbance were taken at 600 nm (OD Time).

The hydrophobicity of the cell surface was estimated using the formula below:

- Cell surface hydrophobicity = $[1- (OD_{Time}/OD_{Initial})] \times 100$
- Cell surface hydrophobicity was categorised as high (51-100%), medium (30-50%) and low (0-29%).)

Results: *Isolation and partial characterization of the isolated bacteria:* Visual examination of the growth of the bacteria under phenol supplemented (100mg L^{-1}) nutrient agar medium after 72h of incubation showed that the colonies were phenol tolerant bacteria that was collected from soil sample. From there one potent bacterium was streaked and single colony was selected for further study (Figure1a and 1b).

Partial Characterization of the Isolated Bacteria: The phenol resistant bacterium isolated from waste was identified on the basis of the Gram staining method. The bacterial strain is a Gram-negative rod-shaped bacterium. This procedure was performed three times and selective media was used for the determination of the Gram reaction.

Tolerance of Phenol: Isolated heavy metals tolerant bacterium was screened for phenol tolerance as this soil was collected from pharmaceutical industry waste where phenol is usually available in the sewage in high concentration. In presence of increasing phenol concentration (50, 100, 300 mgL^{-1}), the bacterium grow considerably in virtue of its tolerance towards the phenol (Figure 2a). This bacterial strain can tolerate upto 300 mg L^{-1} of phenol concentration.

Phenol Detoxification: The level of phenol degradation by the strain was measured. The results were shown in Figure 2b. This strain exhibited a significant phenol degradation rate. The strain in consideration is a high phenol degrader. At a phenol content of 50mg L^{-1} at a temperature of 30 ± 2 ^{0}C temperature, the phenol degradation rate was 37.2 percent (Figure 2b). When the initial phenol content was greater than 200 mg L^{-1}, an inhibitory effect was seen, indicating that phenol degradation was slowed.

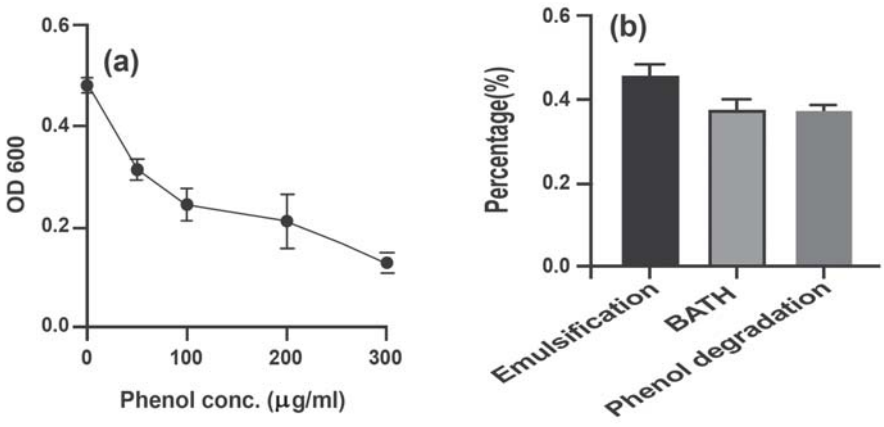

Figure 2: (a) The effect of different concentrations (0,100,200,300 mg L^{-1}) of phenol on the growth of isolated bacteria.(b) Activity (%) of Emulsification, BATH and phenol degradation by the isolated bacteria.

*Emulsification and BATH activity***:** The activity of emulsification and BATH were examined for the strain. The results for these two tests are shown in Figure 2b. Emulsification activity was 45.6% and hydrophobicity activity is 37.6 % which define significant phenol tolerant ability of the strain.There is a definite correlation between emulsification activity and BATH activity in phenol biodegradation.These two properties of the strain confirmed the phenol biodegradation activity.

Discussion: When phenol is present as the solitary source of carbon and energy, a number of *Acinetobacter* strains have been observed to thrive. It is reported that, cells of *Acinetobacter* could only degrade phenol at minimum dosage. The strain *A. calcoaceticus* PA can tolerate high concentration of phenol [1,6]. Adav and Lee reported that some isolated strains were totally inhibited at a phenol concentration [9]. From waste water, Karimi isolated phenol-degrading yeasts. Some yeast genera, such as *Rhodotorula*, *Trichosporon*, and *Candida*, have been found to be able to metabolise phenolic substances as their only supply of carbon and energy[5].

According to Karimi, yeast with high emulsification activity may perform better in the degradation of phenol[5]. Hassanshahian et al. discovered that *Y. lipolytica* PG-20 and PG-32 strains showed significant cell surface hydrophobicity and were able to significantly lower surface tension. Based on hydrocarbon substrates, these strains demonstrated sufficient emulsification activity [10] . It is reported that, isolated crude oil degrading bacteria have a direct link between cell surface hydrophobicity and alkane breakdown[5]. Those reports supported our recent result of emulsification and hydrophobicity for the phenol degradation.

Conclusions: The rising concentration of dangerous organic solvent phenols in pharmaceutical industry waste soil necessitates the development of environmentally acceptable methods for their removal from the environment.

Emulsification, BATH activity and phenol degradation percentage results also indicated that isolated bacteria has a very good organic solvent detoxifier. Because native microbial species were more adaptable in polluted habitats than non-indigenous microorganisms, their preponderance aided bioremediation of phenol-contaminated environments. Bioremediation of hazardous metals and phenol-contaminated settings may be possible using isolated bacteria. Field trials, on the other hand, are required to assess its efficacy and cost-effectiveness in the real world.

Acknowledgements: We are thankful to Chancellor, Techno India University, West Bengal for providing us the infrastructure to perform these experiments. ❐

SOHINI CHAKRABORTY*, ARNAB GANGULI*, SANDHIMITA MONDAL[1]**

*Department of Microbiology,
Techno India University, West Bengal,
EM-4 Sector-V, Salt Lake City,
Kolkata - 700091, West Bengal, India.
e-mail: sohinee2014@gmail.com

*Department of Microbiology,
Techno India University, West Bengal,
EM-4 Sector-V, Salt Lake City,
Kolkata - 700091, West Bengal, India.
e-mail: ganguli.arnab009@gmail.com

**Department of Microbiology,
Techno India University, West Bengal,
EM-4 Sector-V, Salt Lake City,
Kolkata - 700091, West Bengal, India.
e-mail: sandhimita@gmail.com

****Corresponding Author**

Received: 16 September, 2021

Revised: 20 December, 2021

Accepted: 10 February, 2022

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