Isolation of a Most Potent Bacterial Strain from Soil for Bioremediation of Phenol

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Abstract Phenol is present as the basic structural compound of many synthetic organics. This pollutant generates from different sources such as herbicides, wood preservatives, petroleum industries, pharmaceuticals, etc. US Environmental Protection Agency marked it as the priority pollutant. The objective of this study is to isolate a potent bacterial strain from soil which is capable to remove phenol from wastewater. For this, soil sample was collected from the hospital area and enriched with 500 ppm phenol for 10 days. After serial dilution of the soil sample, colonies were developed in petri plate on nutrient agar medium. The isolated colonies were transferred to the slant and screening was done to select the most potent strain in liquid nutrient medium containing 500 ppm of phenol. The most efficient strain, P25, was able to reduce almost ~99.44% of phenol concentration in 24 h, 37 °C temperature, pH 6.8. The isolated strain was acclimatized in MSM (minimum salt medium) for 2 months and habituated to remove 700 mg/L phenol concentration. Simultaneously the strain was biochemically characterized and identified by 16S rDNA sequence analysis.

Keywords Phenol · Pollutant · Minimum salt medium (MSM) · 16S rDNA

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D. Ramkrishna et al. (eds.), *Advances in Bioprocess Engineering and Technology*,
Lecture Notes in Bioengineering,
https://doi.org/10.1007/978-981-15-7409-2_29

1 Introduction

Many pollutants that are very hazardous for the nature are discharged from chemical, pharmaceuticals and oil refinery industries including many aliphatic and aromatic hydrocarbons (Taghreed and El-Naas 2014). Phenol is one of them, which has the capacity to damage the gastrointestinal tract, irritation of respiratory tracts, muscle tremors. Damage of liver, kidney, and nervous system are the adverse effects of phenol. Also phenol is very much hazardous to the aquatic ecosystems (Szczyrba et al. 2016). Therefore, it is mandatory to treat the phenol and phenolic wastes properly before disposal into the nature (Szczyrba et al. 2016; Kumar et al. 2005). The maximum permissible level of phenol in land water is 1 ppm according to the Central Pollution Control Board (CPCB) and IS:2490-1974 (Cheela et al. 2014; Lathasree et al. 2004; Saravanan et al. 2009). As per World Health Organization (WHO), the safety limit of phenol in drinking water should not exceed 1 mg/L (Bakhshi et al. 2011; Saravanan et al. 2008; Wang et al. 2010).

Several treatment methods are there to treat phenol such as adsorption, chlorination, ozonation as well as many physicochemical methods (Szczyrba et al. 2016; Tamer et al. 2010). Due to high cost effect of these methods and production of toxic intermediate compounds, involvement of biological processes is necessary in the treatment of phenol (Szczyrba et al. 2016). These biological treatments may be biosorption, biodegradation, bioaccumulation, etc., involving bacteria, algae, fungi, etc. Many such studies have been done previously as Mohanty and Jena (2017) did his experiment on biodegradation of phenol using *Pseudomonus* sp. NBM11 that was able to degrade up to 1000 ppm phenol completely in the temperature ranging between 30 and 32 °C and pH 6.8–7.2. Another study was done by Parvathy and Prabhakumari (2017) involving *Pseudomonas aeruginosa*, isolated from industrial soil to remove catechol.

The current study has been carried out aiming to isolate the most potent bacterial strain to degrade phenol and the identification of the strain.

2 Materials and Methods

2.1 Soil

Soil was collected from the local hospital area.

2.2 Reagents

Phenol-(analytical grade), 4-amino antipyrene, potassium ferricyanide, ammonium chloride and ammonium hydroxide solution. Our targeted concentration of phenol was prepared by mixing the properly weighed phenol to distilled water.

2.3 Bacterial Media

The nutrient agar medium with following composition was used for cultivation of bacteria.

Peptone—0.5%, beef extract—0.3%, agar—3.0%.

2.4 Isolation and Screening of Most Potent Bacterial Strain

The bacterial strain capable of removing phenol was isolated by soil enrichment and serial dilution plate count method. The soil was enriched with 500 ppm phenol for 10 days. After serial dilution and plate count isolated colonies were transferred in individual slant. A total of 29 isolated colonies were transferred to slant.

Screening was done to select most potent strain. Each of the isolated colonies was transferred to liquid medium and incubated 37 °C for 24 h in the presence of 500 ppm phenol. After fermentation, the fermented broth was centrifuged and the clear supernatant was used for spectrophotometric estimation of residual phenol content.

2.5 Estimation of Residual Phenol Content

Residual phenol concentration was measured in spectrophotometer at 510 nm wavelength followed by APHA method. Residual phenol content was calculated from the standard curve made with the known concentrations of phenol.

2.6 Morphological, Biochemical, and Phylogenetic Characterization of the Isolated Bacterial Strain

Morphological and biochemical characterization of the isolated bacterial strain was done as per Bergey's Manual of Determinative Bacteriology (Holt et al. 1993). Phylogenetic assay was made by 16s rDNA method.

3 Results and Discussion

3.1 Isolation and Screening of Most Potent Bacterial Strain

After serial dilution of the enriched soil sample and transfer of the diluted soil sample into nutrient agar medium, 29 isolated colonies were obtained. Colony characteristics of the isolated colonies are shown in Table 1.

After screening, it was found that the strain marked as P25 showed maximum phenol removal capability (~99.44%) (Table 1), so it was selected for further study.

3.2 Morphological, Biochemical, and Phylogenetic Characterization of the Isolated Bacterial Strain

Morphological characterization of the isolated bacterial strain P25 is shown in Tables 2 and 3. Gram characteristics and spore characteristics are shown in Figs. 1, 2, and 3. Physicochemical characteristics are shown in Table 4. The phylogenetic tree is shown in Fig. 4.

Table 1 Screening of the most potent bacterial strain

Sample strain number	Colony size	Colony pigmentation	Colony form	Colony margin	Colony elevation	% of phenol removal
P1	Moderate	White	Circular	Serrate	Flat	83.16
P2	Moderate	White	Irregular	Serrate	Flat	82.86
P3	Small	White	Irregular	Serrate	Flat	80.75
P4	Large	White	Circular	Entire	Flat	80.15
P5	Moderate	White	Circular	Entire	Flat	84.36
P6	Small	Light yellow	Circular	Entire	Raised	81.35
P7	Small	White	Circular	Serrate	Umbonate	80.15
P8	Pinpoint	White	Circular	Serrate	Flat	80.45
P9	Small	Light yellow	Circular	Serrate	Flat	79.25
P10	Moderate	White	Circular	Serrate	Raised	77.44
P11	Moderate	White	Circular	Entire	Flat	82.86

(continued)

Table 1 (continued)

Sample strain number	Colony size	Colony pigmentation	Colony form	Colony margin	Colony elevation	% of phenol removal
P12	Moderate	Light yellow	Circular	Entire	Flat	82.56
P13	Large	Yellowish	Irregular	Entire	Flat	84.06
P14	Small	Reddish yellow	Circular	Undulate	Flat	84.06
P15	Small	White	Circular	Serrate	Flat	83.76
P16	Moderate	White	Irregular	Serrate	Raised	84.36
P17	Large	White	Circular	Serrate	Raised	84.06
P18	Moderate	Yellowish	Circular	Entire	Umbonate	84.06
P19	Small	Light yellow	Circular	Entire	Raised	83.46
P20	Pinpoint	White	Irregular	Entire	Flat	64.51
P21	Small	Light yellow	Irregular	Serrate	Raised	80.15
P22	Small	White	Circular	Serrate	Flat	83.46
P23	Moderate	White	Circular	Entire	Flat	83.16
P24	Large	Light yellow	Circular	Serrate	Flat	98.84
P25	**Large**	**White**	**Circular**	**Serrate**	**Flat**	**99.44**
P26	Large	Light yellow	Irregular	Serrate	Raised	98.83
P27	Large	Light yellow	Circular	Serrate	Flat	98.68
P28	Large	White	Circular	Serrate	Flat	98.83
P29	Large	White	Circular	Entire	Flat	98.48

Bold indicates the corresponding organism removes maximum amount of phenol

Table 2 Colony characterization of the isolated bacterial strain

Colony characteristics	P25
a. Size	1 mm
b. Opacity	Opaque
c. Surface growth	Smooth
d. Edge	Sharp
e. Consistency	Good
f. Pigmentation	Nil

Table 3 Growth characterization on slant

Colony characteristics	D25
a. Opacity	Opaque
b. Surface growth	Smooth
c. Consistency	Sharp
d. Color	Good
e. Pigmentation	Nil

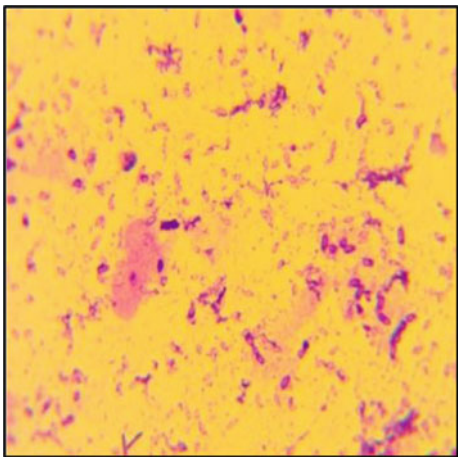

Fig. 1 Simple staining

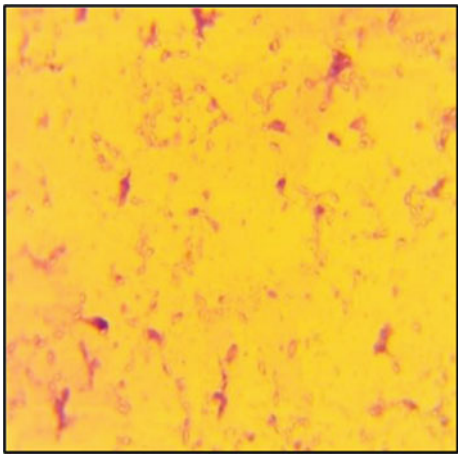

Fig. 2 Gram staining

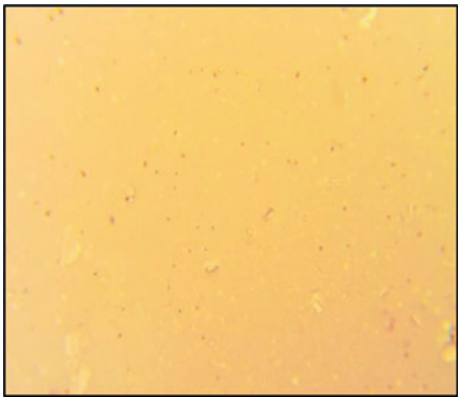

Fig. 3 Spore staining

Table 4 Physicochemical characteristics of the isolated bacterial strain P25

Parameters	Characteristics of P25	
1. Ammonia from arginine	+	
2. Arginine used as sole source of energy	+	
3. Nitrate reduction	+	
4. Catalase reduction	−	
5. Carbohydrate fermentation	**Acidity**	**Gas formation**
a. Fructose	+	+
b. Arabinose	−	+
c. Galactose	+	+
d. Xylose	Not done	Not done
e. Glucose	+	+
f. Lactose	+	+
g. Raffinose	+	+
h. Sucrose	−	+
i. Maltose	+	+
j. Dextrin	+	+
k. Salicin	+	+
l. Mannitol	+	+
m. Glycerol	+	+
n. Inositol	−	+
6. Indole formation	+	
7. Litmus milk test	+	
8. Starch hydrolysis test	−	
9. Urease test	Incubation	
10. Voges—Proskauer test	Incubation	
11. Growth under anaerobic condition	Not done	
12. Growth at different temperatures	+	
13. Growth at extreme pH and NaCl concentration	+	

4 Conclusion

The isolated most potent bacterial strain was identified as *Brevibacillus formosus* strain NRRL NRS-863, which was able to reduce almost 99.44% of phenol. However, the mechanism of removal of phenol by the isolated strain i.e. whether the strain degrades it or accumulates it is not known till now. Our further study will reveal it.

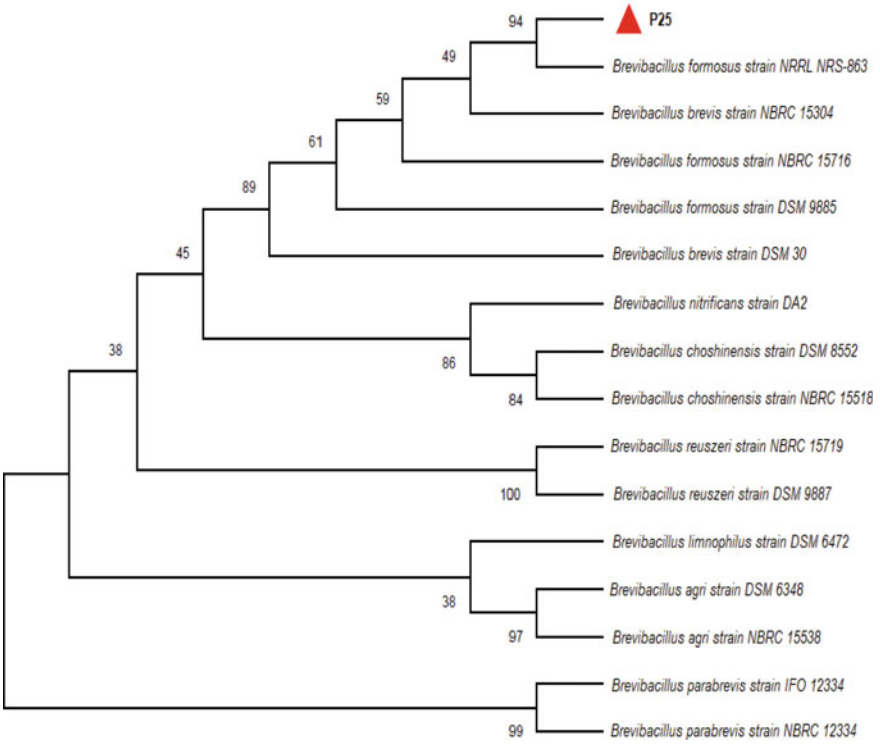

Fig. 4 Phylogenetic tree of the strain P25

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