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A potent phenol degrading bacteria isolated from Cashew Industrial soil

Parvathy.G*, Prabhakumari .C & Nair Sreecha Chandran

Department of Biotechnology, CEPCI Laboratory and Research Institute Mundakkal, Kollam (India)

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Abstract: Phenol is a product of combustion of coal wood, municipal solid waste and byproducts of different pharmaceutical and chemical industries. Phenol and its derivatives are a major source of environmental pollutants. Phenol contamination poses a great threat to human health, it have been recognized as mutagenic and carcinogenic. High toxicity, carcinogenicity and wide distribution of phenol leading to great damage to human being and marine organisms. Due to their potential toxicity and perseverance in the environment, rapid removal and detoxification is urgently needed. EPA has classified phenol as a Group D contaminant. Phenol is currently removed by expensive and inefficient chemical or physical methods. Biological methods have advantage over physical and chemical methods. Bioremediation is a promising technology for the removal of phenol and also a cost effective method. Certain microbes which has an ability to utilize phenol as the sole source of carbon and produce nontoxic compounds. The present study was to isolate and characterize phenol degrading microorganisms from Cashew Industrial Soil. Ten different isolates are isolated of which three are fungus and seven are bacteria. Amoung these microbes one bacteria shows promising degradation of phenol up to 100 mg/l. Morphological and Molecular studies was done and identified as *Bacillius cereus* Batch studies are done by using the pure culture of *Bacillus cereus*, phenol degradation was setup at various pH (5, 6, 7, 8, 9) and temperature (15, 25, 35, 45, 55)^{0}C. Maximum phenol degradation was at pH 7 and temperature 35^{0}C. From this study

we can concluded that the *Bacillus cereus* is one of the efficient phenol degraders and has wide application in the field of bioremediation.

Keywords: Environmental Pollution, Bioremediation, Phenol, *Bacillus cereus*, Cashew Industry

INTRODUCTION

Environmental pollution is one of the biggest problems the world faces today and it is likely to affect the health of human populations[1,2].. Improper management of solid waste is one of the main causes of environmental pollution Wastewater from petroleum refineries, coal conversion processes, manufacture of pharmaceuticals, fertilizer and dyes contain phenolics compounds **(Table-1)**, which characterize a severe ecological problem due to their extensive use, toxicity and occurrence throughout the environment [3-5].Phenol and its derivatives are a major source of environmental pollutants [6-7]. Phenolic constitute 11 of the 126 chemicals that have been designated as priority by the United States Environmental Protection Agency [8]. Phenol in water and wastewater has been the major organic chemicals [9]. Phenol is poisonous even at a very low concentrations and the maximum limit for its tolerance for the environment is 1mg/litre. Taking up with the health issues[10] , deaths amongst adults have been reported with intake of phenol with the series[11] starting 1.5 to 33g .Phenol causes irritation of eye, swelling and finally blindness and high concentration of phenol can cause chronic exposure such as, hepatic damage, vomiting and nervous disorder. [12]. Phenol concentrations in industrial effluents[13]

Table: 1 Phenol concentration in industrial effluent

Industry	Phenol Concentration (mg L^{-1})
Cooking Operations	28 – 3900
Coal Processing	9 - 2800
Petrochemicals	2.8 – 1220
Pulp and Paper	0.1 – 1600
Gas Production	4000
Refineries	6 – 500
Pharmaceuticals	1000
Benzene Manufacturing	50

These phenolic compounds possess various degrees of toxicity and their fate in the environment is therefore important[14]. Therefore bioremediation of phenol from industrial effluents is of great importance[15, 16]. Phenol can be removed by different methods, such as physical, chemical and biological (bioremediation). Most of the physicochemical and thermal methods are costly, as they require expensive equipment and machineries and expend good amount of energy. Bioremediation is a promising method, where wastewater adapted consortium of microbial species is used for the degradation of pollutants from

water. The bioremediation method appears to be the most efficient method for the removal of phenol [17,18]. The present study was carried out for the isolation and identification of phenol degrading microbes based on molecular, morphological and cultural characters isolated from Cashew Industrial Soil and evaluation of phenol degradation potential in different temperature and pH.

MATERIALS AND METHODS

Chemicals and Reagents: Phenol used in the study was of analytical grade and purchased from Merk, India. All other chemicals were also of analytical grade which were purchased from Merk and Hi-Media laboratories, India.

Collection of Sample: Total of two different soil samples collected from two different site **(Fig 1&2)** of Cashew Industry near Kollam. All samples were properly labeled and kept in sterile sealed plastic cover at 4^0C until analysis.

Isolation of Phenol degrading microbes: To 100 ml of mineral salt medium (in 250 ml conical flask) add 100ppm phenol as carbon source then 1 gm soil sample was added. These flasks were shaken and incubated on rotary shaker for seven days at 120 rpm at 35^0C. After seven days of incubation, 10 ml of cultured broth was sub-cultured in fresh 250 ml media containing 100 ppm phenol as the carbon source and allowed to incubate in shaker for next seven days. After the incubation the isolate was plated on mineral salt medium agar containing 100ppm phenol and incubate for 24 hours. After the incubation the isolate was stored for further studies in refrigerator.

Identification of the isolate: Identification was done on one isolated phenol degrading bacteria, were characterized and identified by their morphological characteristic based on size, shape and colony morphology on nutrient agar plate. The isolates were examined by gram staining and Biochemical tests such as indole test, catalase test, oxidase test, Methyl Red Test (MR), Voges Proskauer Test (VP), Citrate Test,oxidative-fermentative (OF) test.

Determination of growth of isolate in presence of phenol: Growth of isolate in presence of phenol was determined by adding a loopful of isolate in mineral salt medium containing 100ppm phenol. The flask was incubated under shake culture condition on a rotary shaker. Samples were taken at 12 hours interval up to 96 hours. OD was measured spectrophotometrically at 600 nm. The experiment was repeated with duplicates. The optical density was used to monitor the biomass growth. [19].

Degradation of phenol by the isolate: To 100 ml of mineral salt media containing 100mg/l, a loopful 24-h-old culture of the isolate was inoculated and the Culture samples were incubated at 37°C for 8 days, at 150 rpm in rotary shaker. On the 0 to 8 th day of incubation 1 ml of culture was centrifuged at 14000 rpm for 15 minutes. Then supernatant was used for phenol estimation. For determination of phenol content, the Folin-Ciocalteau phenol reagent was used, involving the successive addition of 1 ml sodium carbonate (200 g/l) and 0.5 ml Folin-Ciocalteau phenol reagent to 10 ml sample. After 60 min at 20°C, the absorbance was measured at 725 nm against a distilled water and reagent blank [20,21].

Effect of temperature and pH on the biodegradation of phenol: A loopful 24-h-old culture of the isolate was added to 100 ml of mineral salt media containing 100mg/l of catechol and the pH and the temperature adjusted to pH ranges from (5-9) and temperature ranges from $(10,20,30,40,50)^0$C are carried out in standard flask culture experiments. These 1ml of sample were removed at different time

intervals and samples were clarified by centrifugation at 5000rpm for 10 minutes and supernatants were subjected to Folins Ciocalteus spectrophotometric method for monitoring the catechol concentration.

16S rRNA sequencing: Genomic DNA was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel). **Sequencing of 16S rRNA region using universal primers 5'**CAGGCCTAACACATGCAAGTC**3'**, **5'**GGGCGGWGTGTACAAGGC**3'**

RESULTS AND DISCUSSION

Isolation of Phenol degrading microbes: Two soil samples were collected from two different sites of Cashew Industry near Kollam. Ten different isolates are isolated, out of which one bacteria with high potential to degrade phenol was selected for further studies.

Identification of the Isolate: The potential isolate was characterized based on their gram reaction characteristics, morphological features and biochemical properties. The results showed that the isolate is a gram positive rod and the biochemical characterization are explained in the table.(**Table:2)**

Table2: Biochemical characterization of the isolate

S.No	Test	Response of the organism
1	Gramstaining	+
2	Indole	-
3	Methyl Red	+
4	Voges proskauer	+
5	Simmon citrate agar	+
6	Oxidase	-
7	Catalase	+
8	Urease	-
9	Triple Sugar iron Agar	+
10	Nitrate reduction	-
11	Glucose	+
12	Lactose	+
13	Fructose	+

Determination of growth of Isolate in presence of phenol: In the presence of phenol the isolate show growth in 12 hour and an increase in the growth up to 48 to 60 hr. After that the isolates acquires a stationary phase. The growth profile shows that the isolate which has an ability to grow in the presence of phenol (F**ig: 1**)

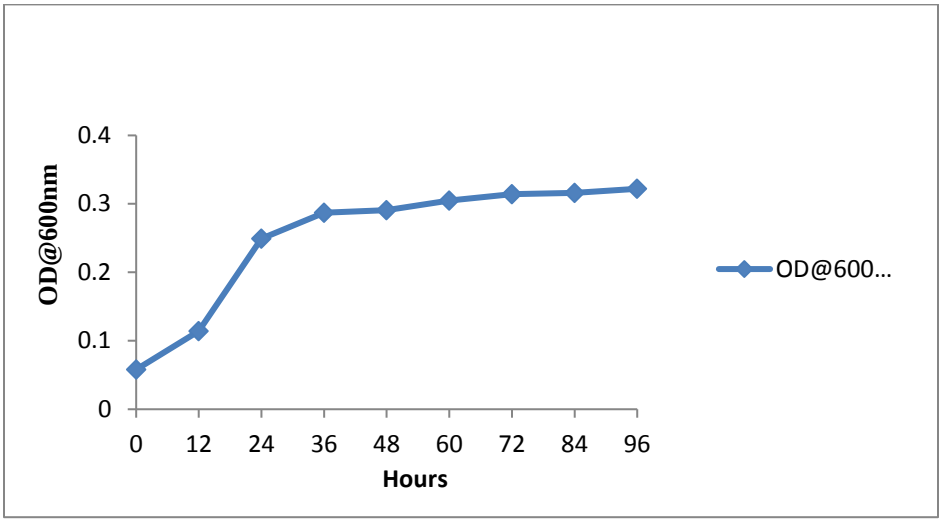

Fig 1: Growth profile of isolate in presence of phenol

Degradation of phenol by the isolate: The Isolate has an ability to degrade phenol up to 100 mg/l **(Fig:2**). 100 ppm was the initial concentration after that a rapid decrease in phenol concentration in 96 hour. This shows that the Isolate has a potential ability to degrade the phenol and has a wide application in the field of bioremediation[22].

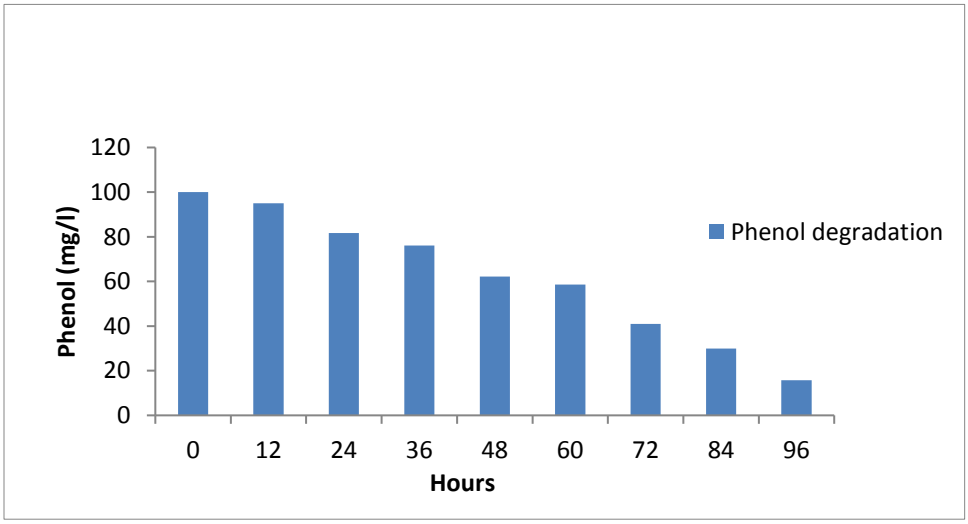

Fig.2.phenol degradation of isolate

Effect of temperature and pH on the biodegradation of phenol: To determine the effect of temperature and pH on phenol degradation the experiments were carried out at different temperatures such as 15^0C 25^0C, 35^0C, 45^0C and 55^0C at pH ranges from (5-9). The data shows that there was maximum phenol degradation takes place at room temperature of 35^0C and on further increase in temperature the rate of biodegradation decreases because the catalytic activity of the enzymes is starts to decrease beyond that temperature. So the optimum temperature for the maximum enzymatic activity is

35^0C and for pH, the results show that there was maximum phenolic degradation occurs maximum at neutral pH due to maximum utilization of carbon source **(Fig.3 and 4).**

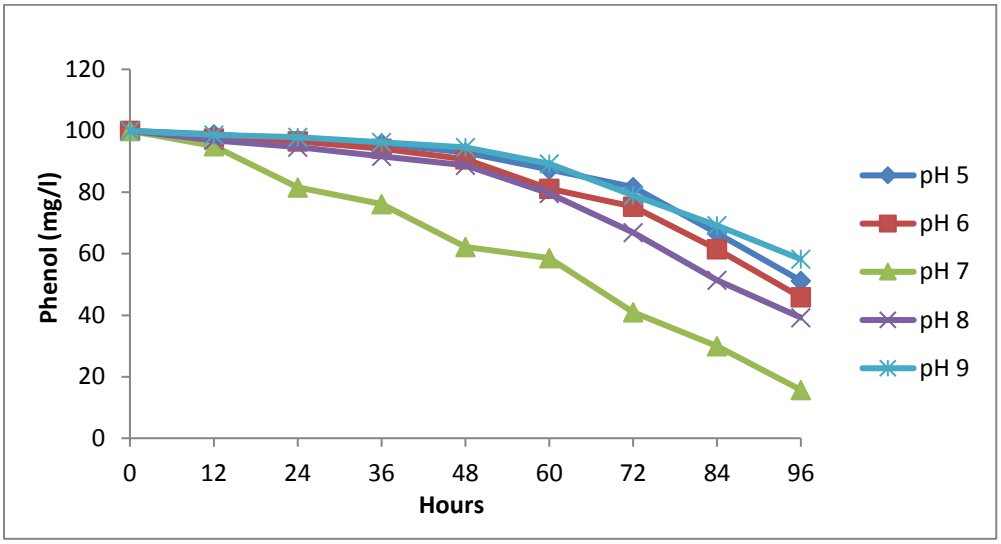

Fig.3: phenol reduction at different pH

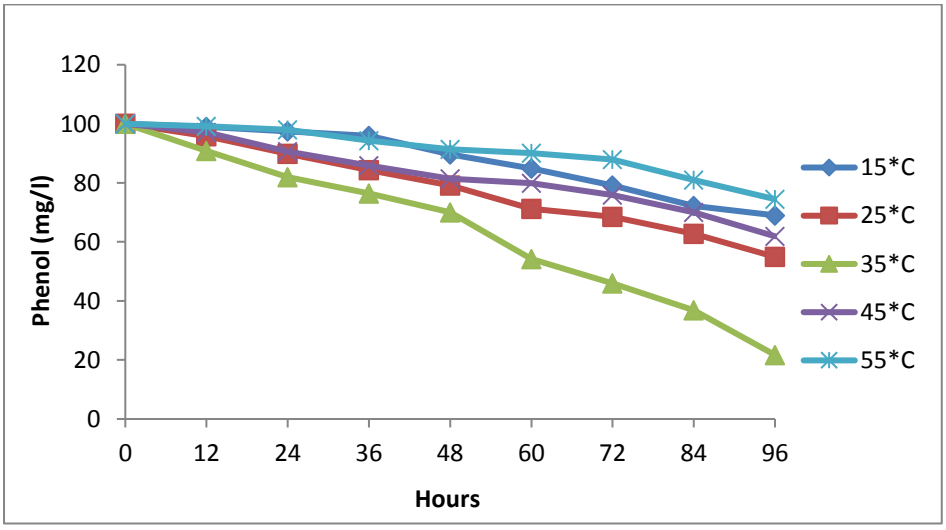

Fig.4: phenol degradation at different temperature

At acidic or basic pH there is reduction in phenolic degradation due to the fact at that culture utilize less carbon source. Viraraghavan and Rao, used the cells of Isolate .to treat the effluent of many waste water treatment plants to remove the phenol from aqueous solution. Most of the organisms, cannot tolerate the

pH values below 4.0 and above 9.0 as because the acids and bases which can easily entered in to the cell which affect the metabolic pathway and denature the proteins finally leads to lethality [24,25].

16S rRNA sequencing: The highly degrading microorganism isolated from Cashew Industrial soil were 16S rRNA sequenced. The organism was biochemically characterized, 16S rRNA sequenced and identified as *Bacillus cereus* gram positive bacteria with 424bp.

***Bacillus cereus* 424bp**

ATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGG
CTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCG
CGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGAC
GGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAA
TAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTC
AACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAAT
GCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGG
GGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTT
TAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGA
CAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACT
GCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTA
CAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGG
CTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCG

CONCLUSION

From the above study it was concluded that the phenol is one of the most important effluent of so many industries and it is harmful to the human system, so it has to be removed. Biodegradation is a simple, cost effective method for the removal of phenol and other effluents to protect the environment. In the present study we isolate the *Bacillus cereus* from Cashew Industry near Kollam for the biodegradation of phenol. The Isolate degrade phenol up to 100mg/l. The phenol degradation by *Bacillis cereus* .was maximum at room temperature of 35^0C and the degradation of phenol is maximum at neutral pH. Bioremediation is one of the most effective method for the removal of phenol and it has wide application for removing environmental pollutants.

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Corresponding author: Parvathy.G*

Department of Biotechnology, CEPCI Laboratory and Research Institute Mundakkal, Kollam (India)

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