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DELINEATING PHENOL DEGRADING BACTERIA FROM COIR RETTING CONTAMINATED SOILS OF SOUTHERN REGIONS OF CHERTHALA

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ABSTRACT
The coir retting industries in Southern regions of Cherthala provides a combative environment for the multiplication of specialized microbes with high phenol biodegradation potential. The present study aimed at isolation and characterization of phenol degrading bacteria from coir retting contaminated soils. After a series of screening enrichment techniques in MSPM at 37ºC, two bacteria were found to have the highest efficiency of pronounced growth in phenol stress conditions. Also, these bacteria have shown to be able to remove 22.5µM of phenol concentration that was measured by modified Mordaque et al, 1999 protocol at 24, 48 and 72 hours of incubation with a regular interval of 24 hours . The strong phenol degrading bacterial isolates were identified and the sequences were deposited in genbank as *Lysini bacillus sp strain GAKVM (*MT476862.1) *and Paenibacillus sp strain. GA2KVM (*MT509865.1). In the concentrations of 22.5µM of phenol, MT476862.1was able to degrade 74.44% and MT509865.1were able to degrade 74.77% within 72 hours of incubation at 37ºC.

KEYWORDS: Coir retting, Biodegradation, Enrichment techniques, MSPM.

INTRODUCTION
Urbanization and explosion of population with pollution from anthropogenic activities affected the ecosystem accompanied by an economic loss (Pradeep et al; 2014). Those rapid industrialization activities strike the environment of the coastal regions of India and make several serious problems. Among the small-scale industries coir retting industries rank first (Nirmala. E et al; 2002).

The coastal regions of Kerala witnessed the coir retting for ages. Coir retting created an impact that caused a drastic reduction in the wealth and health of fish (Anoop. M, 2022) The U.S. Environmental Protection Agency (EPA) established a phenol concentration of 0.5 mg/l as the limit for wastewater discharge into natural water bodies and land or municipal sewerage systems. For drinking water, EPA has prescribed a guideline concentration of 1 µg/l (Sal and Boaventura, 2001). In future technologies for bioremediation, microbial systems might be the potential tools to deal with the environmental pollutants (Nair et al, 2008). By the biological degradation microorganisms and enzymes are capable of converting phenol into nontoxic intermediates of tricarboxylic acids via Ortho or Meta pathway (Powlowski and Shinglar, 1994).

The present study is an attempt to find out the most efficient bacterial isolates which can utilize phenol as a carbon source and thereby making an effective degradation to phenol concentrations especially in water bodies where the range of phenol concentration is well above the permissible limits as exhibited by the water bodies in coir retting ground. The studies on different kinetic parameters may brought out better understanding of complex interactions between substrate concentrations and the performance of bacteria through its growth. Some bacteria presents diauxic growth in case of effective degradation of phenol and associated toxic organic compounds (Anoop M, 2018)

MATERIALS AND METHODS
Sampling and growth media
For isolation of phenol-degrading bacteria, the soil samples were collected from the coir retting zone of southern regions of Cherthala, in sterilized screw capped tubes, brought to the laboratory within 24 hour and refrigerated. Two types of growth media were used in the present study: The minimal salt medium (MSM) and nutrient agar medium. The nutritionally- rich medium, which is primarily used for the growth of bacteria contained 5g Nacl, 3g beef extract, 5g peptone. 15g Agar-agar in 1000 ml double distilled water. The MSM contains KH_2PO_4 (1g), $MgSO_4$ $7.H_2O$ (0.5g),

$CaCl_2.2H_2O$ (0.00005mg), $(NH4)_2SO_4$ (1g) in 1000 ml distilled water.

Isolation of bacterial strain by enrichment method

After a series of subculture, the inoculums from the flask were streaked onto nutrient agar plates, incubated at 30^0C overnight. The steps were repeated and only the isolates exhibiting pronounced growth on phenol were stored for further characterization.

Identification of phenol degrading bacteria

The isolates were identified based on morphological observation and biochemical characterization (Garrity G.M et al, 2005).

Confirmation of the identity of the bacterial strains through 16s r DNA analysis (Suheir I. et al, 2017)

DNA was isolated using standard isolation kit (HiPer® Bacterial Genomic DNA Extraction kit). Amplification of 16SrRNA gene was accomplished using the universal primers. The obtained sequences undergone BLAST and a phylogenetic tree was constructed through test/UPGMA method of mega 7 version software. Bootstrap method was also performed to check the reliability of the phylogram.

Assay for Percentage of Biodegradation of Phenol by the isolated bacteria

The removal of 22.5µm phenol concentration were determined with modified 4- amino antipyrine method. (Mordaque et al, 1999).

RESULTS

Isolation and characterization of phenol degrading bacteria

Two phenol degrading bacteria obtained from coir retting zones that collected from Cherthala. These different bacteria were subjected to the various biochemical tests (Table 1). It was observed that all were Gram- positive, non-motile, *Bacilli* (Fig 1).

Confirmation of the identity of the Phenol degrading strains

BLAST analysis reported that isolated bacteria were Lysini *bacillus sp strain* and *Paenibacillus sp strain*. The sequences from the isolated bacteria, aligned with standard strain sequence with Clustal W tool mega 7 version software. The phylogenetic tree was constructed using sequences of comparable regions of the 16S rRNA gene sequences through test/UPGMA method of mega 7 version software. The phylogenetic trees of these two isolated strains were illustrated in (Fig 3 & 4). The GenBank deposit accession numbers were recorded as MT509865 and MT476862.

Assay for Percentage of Biodegradation of Phenol

The two phenol isolated strains show more than 65% of degradation by modified 4-amino anti pyrine method (Mordaque et al, 1999). The colour variation (Fig 2) revealed the efficiency of the phenol degradation by the isolated strains.

Table 1: Result on the analysis of different biochemical parameters for the identification of bacteria.

Biochemical test	***Microorganism***	
	Strain 1	***Strain 2***
Gram staining	*+ve*	*+ve*
Endospore staining	*-ve*	*-ve*
Motility assay	*-ve*	*-ve*
Methyl red test	*+ve*	*-ve*
Urease test	*+ve*	*+ve*
Catalase test	*+ve*	*+ve*
Indole test	*-ve*	*-ve*
Oxidase test	*+ve*	*-ve*
Carbohydrate fermentative test Glucose Mannose Sucrose Lactose	 *+ve* *-ve* *+ve* *+ve*	 *-ve* *-ve* *-ve* *-ve*
Identified strain	*Paenibacillus sp.*	*Lysinibacillus sp*

(a) *Paenibacillus sp* **(b) *Lysinibacillus sp***

Fig. 1: Phenol degrading on the agar plates.

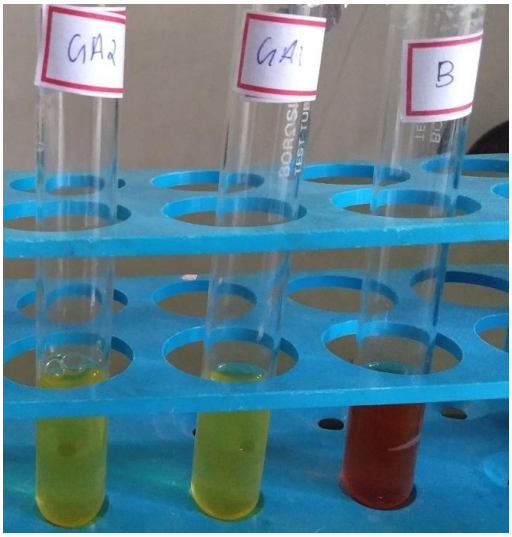

Fig. 2: Efficiency of biodegradation of the isolated strains notified by 4-amino anti pyrine method.

16s identification of Selected Bacterial isolates

Confirmation of the identity of the selected bacterial isolates with 16s rDNA analysis have revealed that the bacteria used in the study were presented to be *Paenibacillus sp* and *Lysinibacillus sp* strains .The sequence obtained after BLAST analysis were deposited and the accession numbers were represented for *Lysini bacillus sp strain GAKVM (*MT476862.1) *and Paenibacillus sp strain. GA2KVM (*MT509865.1).

On performing BLAST analysis for the phylogenetic analysis of the selected isolates, the taxonomic linage was obtained and the bacteria strains were found.

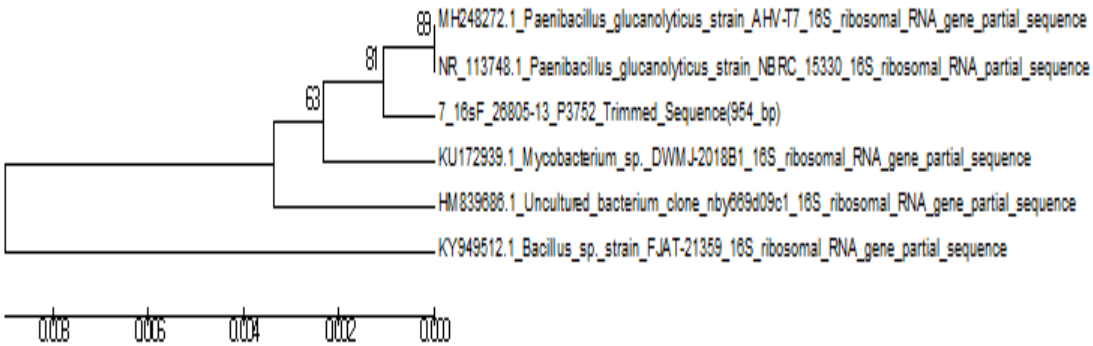

Fig. 3: 16s Phylogenetic Analysis of Phenol degrading bacteria Paenibacillus sp. isolated through Soil Enrichment Culture.

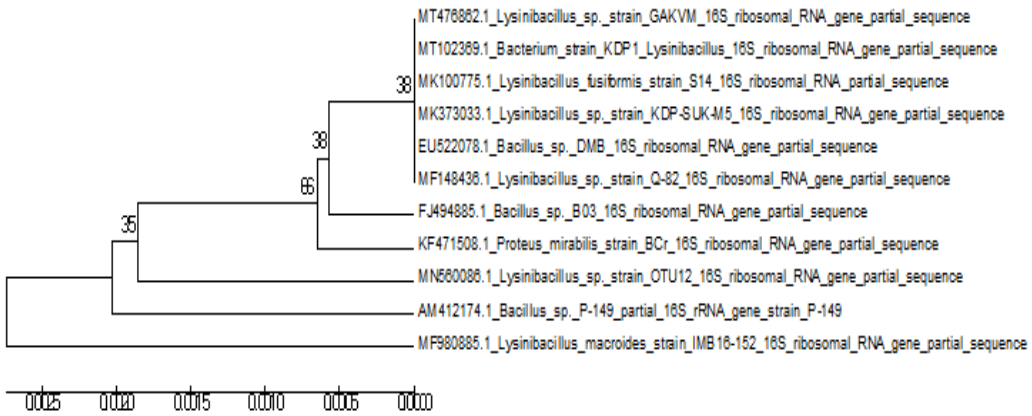

Fig. 4: 16s Phylogenetic Analysis of Phenol degrading bacteria *Lysini bacillus sp*. isolated through Soil Enrichment Culture.

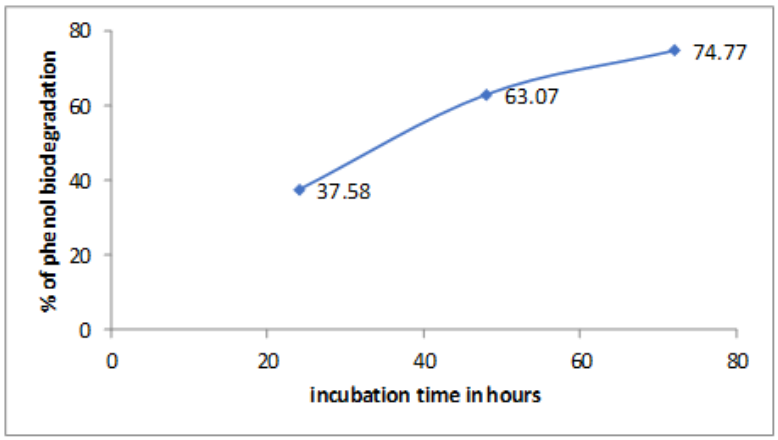

Fig 5:- Activity of *Lysini bacillus sp strain GAKVM* on 22.5 µM Phenol concentration.

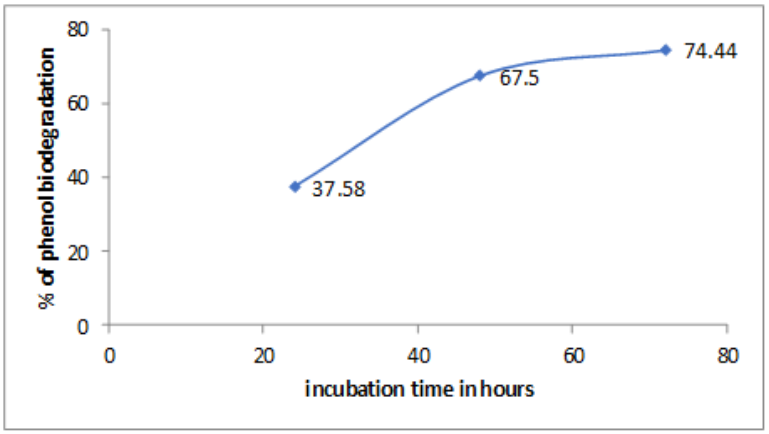

Fig. 6: Activity of *Paenibacillus sp strain. GA2KVM* on 22.5 µM Phenol concentration.

The results demonstrated the activity of *Paenibacillus sp strain. GA2KVM* and *Lysini bacillus sp strain GAKVM* on 22.5 µM Phenol concentration was effective as *Lysini bacillus sp strain GAKVM* degraded 37.58% of phenol in 24 hours and continued to degrade upto 74.77% in 72 hours of incubation and *Paenibacillus sp strain. GA2KVM* degraded 37.58 % in 24 hours and 74.44% in 72 hours of incubation.

DISCUSSION

Phenol and Poly phenols can be considered as aromatic compounds and are also presented as intermediates in the biodegradation of certain natural and industrial compounds. Theconsistent contamination to our environment can be considered as a biological disaster and biodegradation can be prescribed as an effective strategy for remediation (B Leena Grace& S Viveka. 2009). When we consider the degradation of toxic aromatic compounds, biodegradation is prescribed the

best as it ensures complete mineralisation of toxic aromatic pollutants.

In this study, two phenol degrading bacteria were isolated from coir retting contaminated regions and the isolated strains presented distinguished degrees of potential towards the provided carbon source phenol. The direct isolation method provides the platform to isolate the dominant members from the microbial communities of the source. However the two isolated strains have better degradation ability and reveals healthier growth in phenol stress conditions.

The 22.5µM concentrations of phenol showed different interactions with the bacteria *Paenibacillus Sp strain GAKVM*. The degradation ability of the mentioned bacteria from 24-72 hours of incubations suggested that the bacteria is a good candidate in degrading 74.77% of phenol within 72 hours which clearly indicated the competitive potentiality of the isolate in phenol contaminated environments.

Lysinibacillus Sp strain GA2KVM too have spotted more than 70% of degradation to 22.5µM phenol within 72 hours. The data presented crystal clearly that both the isolates are capable of degrading toxic aromatic compound phenol effectively and were an efficient candidate in further future researches in the field of biodegradation of toxic organic compounds.

Many reports through isolation and characterization of phenol degrading bacteria from coir retting contaminated zones (Reshma et al, 2011). Extensive biodegradation studies have described the effectiveness of Bacillus sp in the removal of many environmental pollutants from contaminated sites including petroleum industries (Das et al, 2010).

CONCLUSION

Biodegradation is the best effective strategy that can be considered as it deals with complete mineralization of the toxic compounds. The Bacterial strains isolated from coir-retting zones showed tolerance to high phenol concentration. This clearly reveals the adaptability of the cultures to survive in such unique ecosystems. Our study provided useful guidelines in evaluating potential phenol biodegrades isolated from environment. This work has provided a useful guideline in evaluating potential phenol bio degraders isolated from environment.

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