A Study on Antimicrobial Property of Phenol Tolerant Bacteria Isolated From Indian Mangrove Forest

S. Silambarasan, V. Hari Prasad, R. Balaji and Jayanthi Abraham*
School of BioSciences and Technology, VIT University,
Vellore-632014, Tamil nadu.
* jayanthi.abraham@gmail.com

First author: Mr. S.Silambarasan, M.Sc.,
School of Bio Sciences and Technology, VIT University,
Vellore-632 014. Tamil Nadu, India

Co author: Mr. V. Hari Prasad, M.Sc.,
School of Bio Sciences and Technology, VIT University, Vellore-632 014. Tamil Nadu, India.

Co author: Mr. R. Balaji, M.Sc.,
School of Bio Sciences and Technology, VIT University, Vellore-632 014. Tamil Nadu, India.

Corresponding author:
Dr. Jayanthi Abraham, M.Phil., Ph.D., Assistant Professor (Senior),
School of Bio Sciences and Technology, VIT University,Vellore- 632 014. Tamil Nadu, India. Email: jayanthi.abraham@gmail.com, Mobile: +91- 9843580709

Abstract: The objective of the present study was to screen for phenol tolerant bacteria and analyse its antimicrobial activity. The soil samples were collected from Pichavaram mangrove ecosystem situated along the southeast coast of South India. Phenols are one of the most widely distributed classes of chlorinated chemicals in the environment. For cleanup of large areas of phenol contaminated environments, bioremediation seems to be a promising approach. Four phenol tolerant bacterial species were isolated. In the presence of high concentration of phenol, both *Pseudomonas aeruginosa* and *Bacillus cereus* were found to tolerate higher concentration of phenol during this study on phenol degradation. From the four Bacterial isolates *Pseudomonas aeruginosa* was selected and tested for its antimicrobial activity against various pathogens. The antibiotic extraction of *Pseudomonas aeruginosa* has been shown significant antimicrobial activity against *Streptococcus pyogenes, Salmonella typhi,* and *Klebsiella pneumoniae* but against *Vibrio cholerae* no zone of inhibition was noticed. [Report and Opinion 2010;2(9):27-32]. (ISSN: 1553-9873).

Key words: antimicrobial activity; bacterial isolates; mangrove soil; phenol tolerant

1. Introduction

Mangroves are unique inter-tidal ecosystems of the tropics, which support genetically diverse groups of aquatic and terrestrial organisms. India is one among the 12 mega-biodiversity countries and 25 hotspots of the richest and highly endangered eco-regions of the world (Venkataraman, 2003). The mangroves in India are productive ecosystems that are very sensitive to environmental changes (Krishnamurthy, 1993). Pichavaram mangrove is one of the rare mangrove forests in southeast coast of India. It represents 14 exclusive mangrove species (Kannupandi and Kannan, 1998). Microbes perform various activities in the mangrove ecosystem like photosynthesis (Vethanayagam and Krishnamurthy, 1995) nitrogen fixation (Toledo et al. 1995), methanogenesis (Mohanraju and Natarajan, 1992), agarolysis (Shome et al. 2000), production of antibiotics and enzymes (arylsuphatase, L-glutaminase, chitinase, Lasparaginase, cellulose, proteasae, phosphatase), etc. which result in the high productivity (Kathiresan and Bingham, 2001). Phenol are commonly employed chemicals that are used by many industries such as coke, refineries, and manufacturers of resin, pharmaceuticals, pesticides, dyes, plastics, explosives, and herbicides, as well as occurring in their wastewaters (Lenke et al. 1992). Therefore, presently an attempt was made to isolate the bacterial species from soil samples collected from pichavaram mangrove forest and test them for their phenol tolerance and antimicrobial activity.

2. Materials and Methods

2.1 Sample collection and isolation of bacteria

The soil samples were collected from Pichavaram mangrove ecosystem situated along the

southeast coast of India. They were serially diluted in sterile distilled water and plated on nutrient agar plates. The plates were then incubated at 37ºC for 48 hrs.

2.2 Identification and characterization of the bacterial isolates

Morphological characters such as shape and color of the colonies were examined. Grams staining and motility were also done. Isolates were biochemically analyzed for the activities of oxidase, catalase, MR-VP test, starch hydrolysis and gelatin hydrolysis, indole production, hydrogen sulphide test, nitrate reduction, TSI, urease, sugar fermentation and citrate utilization. The results were compared with Bergey's Manual of Systematic Bacteriology.

2.3 Determination of phenol tolerant

All isolates were separately tested for their tolerance to phenol. About 75 ml of the nutrient broth was prepared and it is sterilized by autoclaving and to this phenol was added at the concentrations of 2.66 mM, 2.76 mM, 2.86 mM, 2.96 mM and the pH were adjusted to 7. Then the culture was inoculated and incubated at room temperature for 48 hrs. After microbial growth was observed by turbidity and the cell densities were estimated at 600 nm using spectrophotometry (Bastos et al. 2000).

2.4 Antimicrobial activity assay

The antibiotic fraction was prepared by using solvent extraction method. In this study we used benzene as a solvent. The *Pseudomonas aeruginosa* isolated from mangrove soil sample was separately tested for their antimicrobial activity against various pathogens. The culture was grown in selective broth for 3 to 5 days at 28± 2°C and it was centrifuged at 5000 rpm and supernatant was collected. The pH of the supernatant was adjusted to 2.0 with concentrated HCL and then it was extracted with an equal volume of benzene using separating funnel and collected the benzene layer. Then the benzene layer was subjected to evaporation in the water bath till near wetness stage. After the evaporation, resuspended the residues in the methanol and stored in the refrigerator. Antimicrobial activity was assayed by well diffusion method against pathogenic organisms like *Streptococcus pyogenes*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Vibrio cholerae*. The pure cultures of pathogenic organisms were sub cultured on Muller-Hinton broth at 35°C on rotary shaker at 200 rpm. Wells of size 6 mm have been made on Muller-Hinton agar plates using gel puncture. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Using micropipette, 20µl of the sample of antibiotic fraction was poured on each four wells on all plates. After incubation at 35°C for 18 hrs, the different levels of zone of inhibition were measured.

3. Results and Discussion

The four different phenol tolerant bacterial species were isolated from Pichavaram mangrove forest and screened for antimicrobial activity against various pathogens. The isolates were identified as *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Shigella dysenteriae* and *Bacillus cereus*. The morphological and biochemical characteristics of bacterial isolates are represented in Table 1. The results were similar to the previous works, there are different groups of bacteria which get nourished by detritus and in turn help the mangrove ecosystem in different ways. These bacteria perform various activities in the mangrove ecosystem like photosynthesis, nitrogen fixation, methanogenesis, agarolysis, production of antibiotics and enzymes which result in the high productivity (Kathiresan and Bingham, 2001).

Phenol is one of the pollutants in waste water which is highly toxic. Phenol is a toxic organic component often found in wastes from oil refinery, plastic, coke, petroleum refining, dyestuff, pharmaceuticals and petrochemical industries. Recent literature on the methods of removal of phenol and their compounds from waste water focuses on adsorption and microbial biodegradation process (Zhong-Cheng et al. 1994). Certain species of *Pseudomonas* under controlled conditions of pH, temperature and in the presence of some specific nutrients can degrade phenol. *Pseudomonas* strain, capable of degrading pentachlorophenol has been isolated around tannery soil and characterized as *Pseudomonas aeruginosa* (Suseela et al. 1991). The bacterial Isolates showed rapid phenol degradation, as measured by spectrophotometer. Increase of growth rate in nutrient broth with various concentration of phenol was observed due to efficient tolerant of phenol with a maximum time at 48 hours. The bacterial growth in the nutrient broth with various concentration of phenol, optical density (OD) were measured at 600 nm values is varied from 2.66 mM to 2.96 mM represented in Table 2, Figure 1. *Pseudomonas aeruginosa* and *Bacillus cereus* showed highest OD value at 2.86 mM and 2.96 mM concentration of phenol respectively. *Shigella dysenteriae* showed maximum tolerance to phenol at 2.76 mM concentration where as *Micrococcus luteus* at 2.96 mM concentration.

Polychlorinated biphenyls (PCBs) are one of the most widely distributed classes of chlorinated chemicals in the environment. The toxicities and carcinogenicities of some PCB congeners make them

a serious environmental and health problem. For cleanup of large areas of PCB-contaminated soils and aquatic environments bioremediation seems to be a promising approach (Pieper and Reineke, 2000). Phenol and its derivatives are aerobically biodegraded by two main metabolic pathways, initiated either by ortho or meta cleavage (Leonard and Lindley, 1998; Muller and Babel, 1994). The enzymes phenol hydroxylase and catechol 1, 2 (ortho) or 2, 3 (meta) dioxygenase catalyze the first and second steps of phenol degradation respectively (Gaal and Neujahr, 1979).

Very few studies have been done on the antimicrobial activity of mangroves (Christopherson et al. 1998). The mangrove environment is a potent source for isolation of antibiotic producing actinomycetes (Ratnakala and Chandrika, 1995).

The antimicrobial activity of *Pseudomonas aeruginosa* was investigated using well diffusion method against various pathogenic organisms, the highest antimicrobial activity was observed against *Streptococcus pyogenes* followed by *Salmonella typhi* (Figure 2). The lesser activity was found against *Klebsiella pneumonia* and no zone of inhibition against *Vibrio cholerae*. The mean of four replicates of the diameter of inhibition zones (mm) around each well with antibiotic fraction solution is represented in Table 3. It could be interpreted from the results that within 48 hrs through phenol tolerant process efficient phenol degradation capabilities over a wide range of phenol concentrations under aerobic condition that may offer to employ these organisms for phenol microbial biodegradation process along with antimicrobial property are clear indication of the distribution of antimicrobial substances.

Table 1. Morphological and biochemical characteristics of bacterial strains isolated from mangrove soil sample

Characteristics	Bacterial strains			
	I	II	III	IV
Gram staining	-ve	+ve	-ve	+ve
Shape	Rod	Cocci	Rod	Rod
Motility	M	NM	NM	M
Indole test	-ve	-ve	-ve	-ve
Methyl red test	-ve	-ve	+ve	-ve
Voges proskauer test	-ve	-ve	-ve	+ve
Citrate utilization test	+ve	-ve	-ve	-ve
Catalase test	+ve	+ve	+ve	+ve
Oxidase test	+ve	-ve	-ve	-ve
TSI test	KS/NCB	NP	KS/AB	NP
Urease test	-ve	+ve	-ve	+ve
Nitrate reduction test	+ve	-ve	+ve	+ve
H_2S test	-ve	-ve	-ve	-ve
Starch test	-ve	-ve	-ve	+ve
Gelatin Liquefaction	+ve rapid	+ve slow	-ve	+ve rapid
Sugar fermentation				
(a) Glucose	-ve	-ve	+ve	+ve
(b) Sucrose	-ve	-ve	+ve	+ve
(c) Lactose	-ve	-ve	-ve	-ve

Key: +ve = Positive, -ve = Negative, KS = Alkaline Slant, NCB = No Change Butt, AB = Acid Butt, NP = Not Performed, M = Motile, NM = Non motile, I = *Pseudomonas aeruginosa*, II = *Micrococcus luteus*, III = *Shigella dysenteriae*, IV = *Bacillus cereus.*

Table 2. OD value of phenol tolerant bacteria isolated from mangrove soil sample

Concentration of phenol (mM)	Bacterial isolates OD value at 600 nm			
	Pseudomonas aeruginosa	*Micrococcus luteus*	*Shigella dysenteriae*	*Bacillus cereus*
2.66 mM	0.295	0.210	0.166	0.331
2.76 mM	0.339	0.288	0.384	0.345
2.86 mM	0.567	0.332	0.277	0.412
2.96 mM	0.490	0.339	0.339	0.463

Table 3. Antimicrobial activity of *Pseudomonas aeruginosa* against various pathogenic bacteria

S.NO	Pathogenic Bacteria	Zone of diameter (mm) mean of four replicates
1	*Streptococcus pyogenes*	15.5 mm
2	*Salmonella typhi*	10.6 mm
3	*Klebsiella pneumoniae*	0.8 mm
4	*Vibrio cholera*	Absence

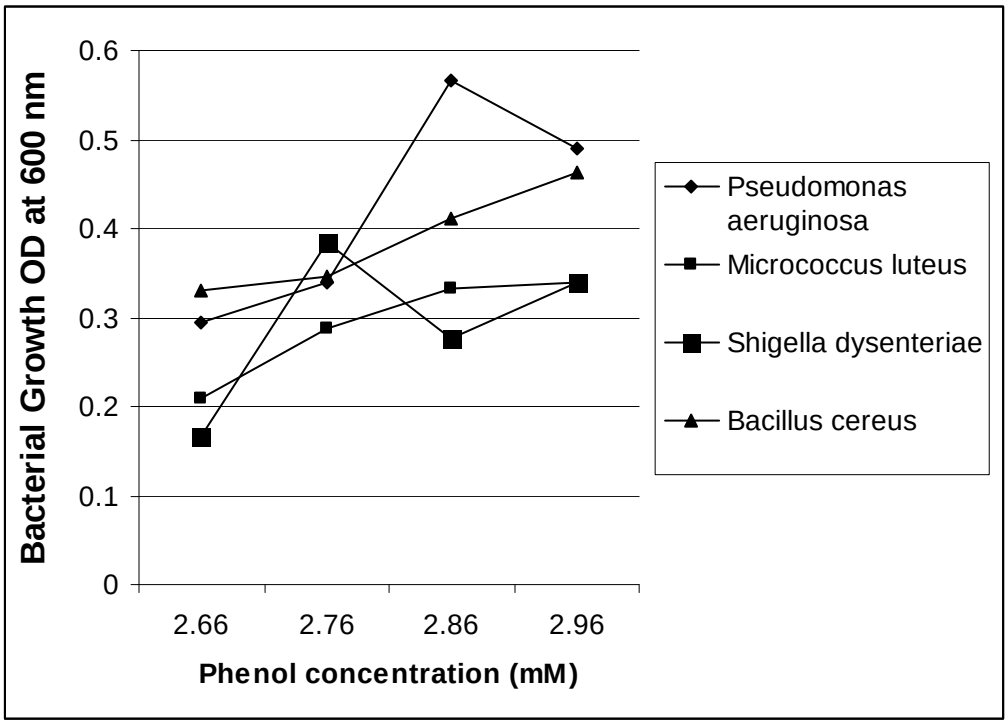
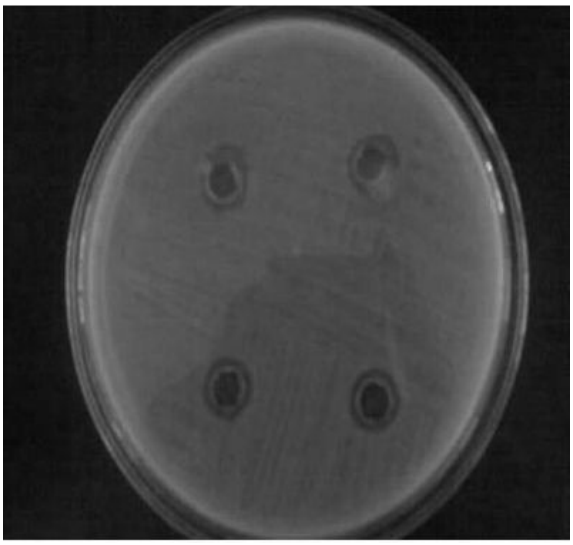
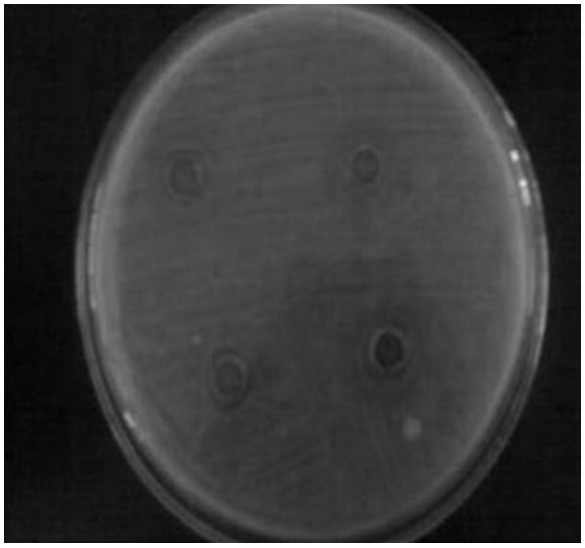


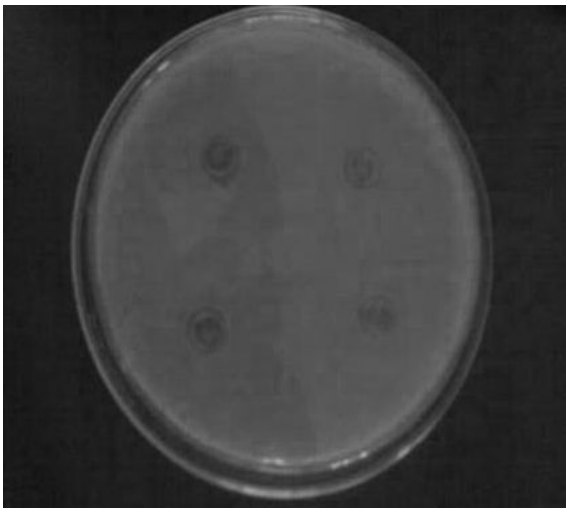
Figure 1. Phenol tolerant bacterial species isolated from mangrove soil sample.



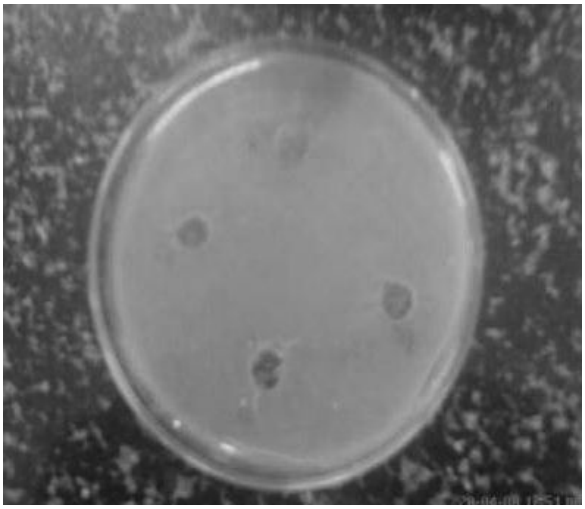
Pseudomonas aeruginosa against *Streptococcus pyogenes*



Pseudomonas aeruginosa against *Salmonella typhi*



Pseudomonas aeruginosa against *Klebsiella pneumoniae*



Pseudomonas aeruginosa against *Vibrio cholera*

Figure 2. Antimicrobial activity of *Pseudomonas aeruginosa* against various pathogenic bacterial strains shown by well diffusion method.

Correspondence to:
Dr. Jayanthi Abraham, M.Phil., Ph.D.,
Assistant Professor (Senior),
School of Bio Sciences and Technology,
VIT University,Vellore- 632 014. Tamil Nadu, India.
Mobile: +91- 9843580709
Email: jayanthi.abraham@gmail.com

References

[1] Bastos AER, Tornisielo VL, Nozawa SR, Trevors JT, Rossi A. Phenol metabolism by two microorganisms isolated from Amazonian forest soil samples. J Ind Microbiol Biotechnol 2000:24:403-9.

[2] Christopherson C, Crescente O, Frivad JC, Gram L, Nielsen J, Nielsen PH, Rahabaek L. Antibacterial activity of marine derived fungi. Mycopathol 1998:143:135-8.

[3] Gaal A, Neujahr HY. Induction of phenol-metabolizing enzymes in *Trichosporon cutaneum.* J Bacteriol 1979:37(1):13-21.
[4] Krishnamurthy K. The mangroves. ENVIS Newsletter 1993:1:1-3.
[5] Kannupandi T, Kannan R. Hundred years of Pichavaram mangrove forest. In: An Anthology of Indian mangroves. In: ENVIS Special Publication, Annamalai University, India 1998:66.
[6] Kathiresan K, Bingham BL. Biology of mangroves and mangrove ecosystems. Advances in Marine Biology. Academic Press UK: 2001:81-251.
[7] Lenke H, Pieper DH, Bruhn C, Knackmuss HJ. Degradation of 2,4-dinitrophenol by two *Rhodococcus erythropolis* strains, HL 24-1 and HL 24-2. Appl Env Microbiol 1992:58 (9):2928-32.
[8] Leonard D, Lindley N. Carbon and energy flux constrains in continuous cultures of *Alcaligenes eutrophus* grown on phenol. Microbiology 1998:144:241-48.
[9] Mohanraju R, Natarajan R. Methanogenic bacteria in mangrove sediments. Hydrobiologia 1992:247:187-93.
[10] Muller R, Babel W. Phenol and its derivatives as heterotrophic substrates for microbial growth and energetic comparison. App Microbiol Biotech 1994:42:446-51.
[11] Pieper DH, Reineke W. Engineering bacteria for bioremediation. Curr Opi Biotechnol 2000:11:262-70.
[12] Ratnakala R, Chandrika V. Microbial production of antibiotics from mangrove ecosystem. CMFRI Special Publcation 1995:61:117-22.
[13] Shome BR, Shome R, Ahlawat SPS, Verma ND. Agar depolymerizing (agarolytic) bacteria isolated from mangrove soil samples of Andaman. Curr Sci 2000:79:696-97.
[14] Suseela GR, Basu SK, Nandy SC. Degradation of pentachlorophenol by *pseudomonas aeruginosa.* Ind J Environ Health 1991:33(4):425-32.
[15] Toledo G, Bashan Y, Soeldner A. Cyanobacteria and black mangroves in Northwestern Mexico: colonization, diurnal and seasonal nitrogen fixation on aerial roots. Can J Microbiol 1995:41:999-1011.
[16] Venkataraman K. Natural aquatic Ecosystems of India, Thematic Working Group. The National Biodiversity Strategy Action Plan, India 2003:1-275.
[17] Vethanayagam RR, Krishnamurthy K. Studies on anoxygenic photosynthetic bacterium *Rhodopseudomonas* sp. from the tropical mangrove environment. Ind J Mar Sci 1995:24:19-23.
[18] Zhong-Cheng HU, Rozer AI, William AK, Ron LC. Adsorption and biodegradation of pentachlorophenol by polyurethane immobilized flavobacterium. Environ Sci Technol 1994:28:497-596.

Date: 27/08/'10.