



Original Research Article

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Isolation and Identification of Phenol Degrading Bacteria from Effluent Treatment Plant of Coir Industry, Kerala, India

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Abstract

Phenol and its components are extremely toxic and can easily be isolated from different industrial sewage such as oil refinery, petrochemical industry and mines, especially collier and chemical factories. The presence of these compounds in the environment could cause environmental pollution, especially in water resources. Phenol and its components are majorly toxic to the environment. It is hazardous when exposed to the organism's surface. Phenol-degrading bacteria exist widely in the environment, and they are usually isolated from phenol contaminated site. The aim of the study was to isolate and identify the phenol degrading bacteria from effluent treatment plant of coir industry. Bacterial strains capable of degrading phenol were isolated from enrichments containing 50 mg/L of phenol. From this 50 ppm sample, the strains were transferred and grown in selective sorbitol media. Five potential strains were selected and were subjected to biochemical characterization and were identified as *Brucella* sp., *Aquaspirillum* sp., *Erwinia* sp., *Aeromonas* sp. and *Moraxella* sp. The cultures were subjected to phenol tolerance level of minimum medium containing different concentrations of phenol (200 ppm to 1000 ppm). As the result of the study, it was clear that the selected 5 bacterial strains were able to survive and gave maximum degradation up to 800 mg/L phenol. From the preliminary analysis, the most potent among the selected were *Brucella* sp. and *Aeromonas* sp. This study will help to find out the phenol degraders from effluent treatment plant which can be isolated and cultured in large scale and also can be used in waste management.

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Introduction

For a past few years the rate of environmental issues have risen considerably due to various industrial activities. The sources to which these pollutants reach also include water bodies, which is the crucial life source of every living matter. The biodegradation of aromatic substances in the environment with their toxic property and their ability to persist in the environment for a long period of time is now a major concern. Factories, industries and firms that deal with production of coke, coir, leather, paper etc, release large amounts of toxic

components to the water bodies in an overwhelming capacity. Toxicity of phenolic compounds inhibits biological treatment or even eliminates sensitive micro-organisms from biological wastewater treatment process and significantly reduces the biodegradation of the other components (Yan et al., 2006).

Phenols and their derivatives commonly exist in the environment. These compounds are used as the components of dyes, polymers, drugs and other organic substances. The presence of phenols in the ecosystems is also related with production and degradation of

numerous pesticides and the generation of industrial and municipal sewages. Some amount of phenols is also formed during natural processes.

These compounds may be substituted with chlorine atoms, may be nitrated, methylated or alkylated. Both phenols and catechols are harmful ecotoxins. The consumption of water containing high phenolic compounds may lead to cancer as it is a susceptible carcinogen. The exposure of phenol and its derivative compounds to human and animals causes liver and kidney damage, central nervous system impairment, diarrhoea and excretion of dark urine (Olujimi et al., 2010; Khare, 2011).

Materials and methods

Samples from effluent treatment plant of paper industry were collected and serially diluted. Microbial enrichment was done using nutrient broth with different phenol concentrations (10, 20, 30, 40, 50 ppm). From the 50 ppm culture, organisms were collected and added to sorbitol agar medium with varying concentrations of phenol (200, 400, 600, 800 and 1000 ppm).

Estimation of total phenol

Estimation of total phenol was carried out the method of (Bray and Thorpe, 1954).

Observation of total growth

The growth rates of the microbes were observed by spectrophotometric analysis.

Table 1. Biochemical tests for bacterial isolates.

Strains	Gram staining	Oxidase test	Catalase test	Citrate utilization	Methyl red	Voges Proskauer	Indole test	Motility	Urea hydrolysis	Gelatin hydrolysis
<i>Brucella</i> sp.	-cocci	+	+	-	-	-	-	-	-	+
<i>Aquaspirillum</i> sp.	-cocci	+	+	-	-	-	-	-	-	+
<i>Erwinia</i> sp.	-rod	+	+	-	-	-	-	-	-	+
<i>Aeromonas</i> sp.	-rod	+	-	-	-	-	-	+	-	+
<i>Moraxella</i> sp.	-cocci	+	+	+	-	-	-	-	-	-

Total phenol and growth were recorded according to its incubation periods. This study was proceeded in order to find out growth rate of bacteria by using media of concentrations from 200ppm to 1000ppm. Neumann et al. (2004) adapted *Pseudomonas* strains to high concentrations of phenol (1000 mg/L) and further biodegradation was carried out at a concentration of 500 mg/L. They opinioned that the cultures could grow by utilizing phenol as a source of carbon and energy. The

Identification of isolates

Biochemical characterization was done based on Bergey's Manual of Determinative Bacteriology (1994) and Cappuccino and Sherman (1992).

Results and discussion

Phenol is highly water soluble, and its presence in the water imparts a carbolic odour to the receiving water bodies and can have baleful effects on aquatic as well as terrestrial flora and fauna including human beings (ATSDR, 2008). Hence removal of phenol from the discharged sewage and effluent is highly necessary. Conventional methods for treatment of phenol have their own set of disadvantages; hence biological method is the current choice. Reports on possibility of treatment of phenol bearing waste water using microorganisms are also available (Nandish, 2005). Till date, research communities have focused only on the isolation of microorganisms from the sites contaminated with industrial effluents containing phenol and use them for the treatment of the industrial wastewater.

In the present study, serially diluted effluent of coir industry was transferred to 10, 20, 30, 40 and 50 ppm phenol having minimal media. From this 50 ppm sample, the strains were transferred and grown in selective sorbitol media. Five potential strains were selected and were subjected to biochemical characterization and were identified as *Brucella* sp, *Aquaspirillum* sp, *Erwinia* sp., *Aeromonas* sp. and *Moraxella* sp. (Table 1).

growth rate and the total phenol degradation for the microbes were given in tables 2 to 6.

In 200 ppm medium it was observed that *Brucella* sp. showed highest phenol degradation at 48hrs (0.0057) followed by *Aquaspirillum* sp. (0.0119), *Erwinia* sp. (0.0205), *Aeromonas* sp. (0.0132) and *Moraxella* sp. (0.0162). The least degradation was showed by *Aeromonas* sp. for 24 hrs (0.0880) incubation. The

growth rate was observed highest for *Aquaspirillum* sp at 72 hrs of incubation (0.0817) followed by *Aeromonas* sp. (0.0777), *Brucella* sp. (0.0768), *Moraxella* sp. (0.0757) and *Erwinia* sp.

Table 2. Total phenol and growth of coir effluent sample (200ppm).

Strains	Total phenol (720nm)				Growth (600nm)			
	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
<i>Brucella</i> sp.	0.0144	0.0057	0.0220	0.0101	0.0631	0.0063	0.0768	0.0708
<i>Aquaspirillum</i> sp.	0.0461	0.0119	0.0190	0.0086	0.0634	0.0072	0.0817	0.0752
<i>Erwinia</i> sp.	0.0687	0.0205	0.0153	0.0094	0.0630	0.0068	0.0725	0.0618
<i>Aeromonas</i> sp.	0.0880	0.0132	0.0265	0.0153	0.0579	0.0054	0.0777	0.0739
<i>Moraxella</i> sp.	0.0737	0.0162	0.0206	0.0135	0.0540	0.0051	0.0757	0.0755

Table 3. Total phenol and growth of coir effluent sample (400ppm).

Strains	Total phenol (720nm)				Growth (600nm)			
	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
<i>Brucella</i> sp.	0.0647	0.0139	0.0168	0.0087	0.0477	0.0064	0.0658	0.0324
<i>Aquaspirillum</i> sp.	0.0482	0.0103	0.0144	0.0093	0.0576	0.0067	0.0744	0.0694
<i>Erwinia</i> sp.	0.0863	0.0100	0.0218	0.0108	0.0598	0.0056	0.0713	0.0329
<i>Aeromonas</i> sp.	0.0888	0.0065	0.0178	0.0134	0.0587	0.0054	0.0754	0.0355
<i>Moraxella</i> sp.	0.0067	0.0052	0.0277	0.0120	0.0623	0.0051	0.0709	0.0574

Table 4. Total phenol and growth of coir effluent sample (600ppm).

Strains	Total phenol (720nm)				Growth (600nm)			
	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
<i>Brucella</i> sp.	0.0772	0.0170	0.0181	0.0106	0.0534	0.0083	0.0755	0.0672
<i>Aquaspirillum</i> sp.	0.0194	0.0021	0.0169	0.0132	0.0613	0.0079	0.0774	0.0706
<i>Erwinia</i> sp.	0.0832	0.0126	0.0259	0.0172	0.0554	0.0065	0.0680	0.0673
<i>Aeromonas</i> sp.	0.0702	0.0089	0.0209	0.0078	0.0582	0.0078	0.0688	0.0488
<i>Moraxella</i> sp.	0.0507	0.0093	0.0096	0.0080	0.0566	0.0090	0.0694	0.0434

Table 5. Total phenol and growth of coir effluent sample (800ppm).

Strains	Total phenol (720nm)				Growth (600nm)			
	24	48	72	96	24	48	72	96
<i>Brucella</i> sp	0.0971	0.0108	0.0250	0.0111	0.0577	0.0051	0.0734	0.0681
<i>Aquaspirillum</i> sp	0.0314	0.0153	0.0230	0.0103	0.0574	0.0080	0.0740	0.0630
<i>Erwinia</i> sp	0.0163	0.0092	0.0187	0.0124	0.0557	0.0052	0.0833	0.0762
<i>Aeromonas</i> sp	0.0890	0.0226	0.0248	0.0192	0.0561	0.0065	0.0686	0.0647
<i>Moraxella</i> sp	0.0820	0.0230	0.0188	0.0140	0.0555	0.0058	0.0715	0.0488

Table 6. Total phenol and growth of coir effluent sample (1000ppm).

Strains	Total phenol (720nm)				Growth (600nm)			
	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
<i>Brucella</i> sp.	0.0923	0.0140	0.0149	0.0091	0.0554	0.0076	0.0718	0.0694
<i>Aquaspirillum</i> sp.	0.0233	0.0077	0.0188	0.0076	0.0559	0.0084	0.0592	0.0465
<i>Erwinia</i> sp.	0.0563	0.0063	0.0333	0.0061	0.0552	0.0070	0.0649	0.0677
<i>Aeromonas</i> sp.	0.0572	0.0069	0.0200	0.0064	0.0549	0.0072	0.0640	0.0631
<i>Moraxella</i> sp.	0.0652	0.0134	0.0190	0.0106	0.0594	0.0060	0.0738	0.0434

In 400ppm medium *Moraxella* sp showed highest phenol degradation at 48 hrs (0.0052) followed by *Aeromonas* sp (0.0065), *Erwinia* sp. (0.0100), *Aquaspirillum* sp. (0.0103) and *Brucella* sp. (0.0139).The least degradation was observed for *Aeromonas* sp. (0.0888) at 24hrs incubation. The growth rate was observed highest for *Aquaspirillum* sp. at 72 hrs of incubation (0.0817)

followed by *Aeromonas* sp. (0.0777), *Brucella* sp. (0.0768), *Moraxella* sp. (0.0757) and *Erwinia* sp. (0.0725).

In 600ppm medium, *Aquaspirillum* sp showed highest phenol degradation at 48hrs incubation (0.0021) followed by *Aeromonas* sp. (0.0089), *Moraxella*

sp(0.0093), *Erwinia* sp. (0.0126) and *Brucella* sp. (0.0170). The growth rate was observed to be highest for *Aquaspirillum* sp. at 72 hrs of observation (0.0817) followed by *Aeromonas* sp. (0.0777), *Brucella* sp. (0.0768), *Moraxella* sp. (0.0757) and *Erwinia* sp. (0.0725).

In 800ppm medium, *Erwinia* sp showed highest phenol degradation at 48hrs incubation (0.0092) followed by *Brucella* sp. (0.0108), *Aquaspirillum* (0.0153), *Aeromonas* sp (0.0226), and *Moraxella* sp. (0.0230). The least phenol degradation was shown by *Brucella* sp (0.0971) at 24 hrs of incubation. The growth rate was observed to be highest for *Aquaspirillum* sp at 72 hrs of incubation (0.0817) followed by *Aeromonas* sp. (0.0777), *Brucella* sp. (0.0768), *Moraxella* sp. (0.0757) and *Erwinia* sp. (0.0725). Shen and Wang (1993) conducted experiments on simultaneous chromium reduction and phenol degradation in a co-culture of *Escherichia coli* and *Pseudomonas putida*. They also reported similar growth pattern with a short lag period, followed by an exponential growth phase and then a declining growth phase. In 1000ppm medium the phenol degradation rate was highest for *Erwinia* sp. (0.0061) followed by *Aeromonas* sp. (0.0064), *Aquaspirillum* sp. (0.0076) *Brucella* sp. (0.0091) and *Moraxella* sp. (0.0106). The growth rate was observed highest for *Moraxella* sp. at 72 hrs of incubation (0.0738) followed by *Brucella* sp. (0.0718), *Aquaspirillum* sp. (0.0592), *Erwinia* sp. (0.0649) and *Aeromonas* sp. (0.0640).

As the result of the study, it was clear that the selected 5 bacterial strains were able to survive and gave maximum degradation up to 800 mg/L phenol. Regina et al. (2004) studied the behavioural patterns of hydrocarbon utilizing bacteria in media containing different concentrations of crude oil. They showed that lower doses of crude oil were more highly utilized than higher doses. They accounted this for the high growth response in the low dose of crude oil. From the preliminary analysis, the most potent among the selected were *Brucella* sp and *Aeromonas* sp. This study will help to find out the phenol degraders from effluent treatment plant which can be isolated and cultured in large scale and also can be used in waste management.

Conclusion

The coir-retting environment harbours a variety of potential microbes which can degrade phenols. The growth and adaptability of these bacteria in environment containing phenolic compounds have induced their

biodegradation potential (Reshma et al., 2004). Phenolics, one of the major pollutants, are discharged in the waste water from the various industries such as phenolic resin and pharmaceuticals, oil refineries, petrochemical plants, textile industry, ceramic plants, steel plants, and coal conversion processes. The degradation performance of selected strains was examined for different phenol concentrations. Serial exposure to increasing level of phenol concentration was used to determine the resistance of isolated strains. Acclimatization of the microorganisms overcomes the substrate inhibition problems that normally occurred in phenol biodegradation at high concentration (Lob and Tar, 2000). The experiment aimed to find the highest tolerance level of phenol concentrations and found that it was able to survive and degrade up to 800 mg/L phenol. The growth of bacteria and phenol concentration in the media showed the inverse proportion with each other. From this preliminary analysis, the most potent were *Brucella* sp and *Aeromonas* sp. They showed high degradation potential in most of the higher phenol concentrations.

Effluent from waste treatment plant of Coir industry contains a variety of substances including phenolic components. Serial exposure to increasing level of phenol concentration can be used to determine acclimatisability of a particular isolate. Highly acclimatisable microbes are those which are able to degrade phenol at high concentration and at greatest rate will be the best phenol degrader candidates. Future studies should be carried out to isolate more potent useful microbes.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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