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Phenotypic Characterization and Evaluation of Phenol-Degrading Indigenous Bacteria Isolated from UAE Seawater

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Abstract: Growth of bacteria on phenol as a sole carbon source has been investigated. Four different bacterial colonies have been recovered on the agar plates and their biochemical and phenotypic characterization revealed that they are mainly Gram negative motile bacteria with negative methyl red test but with positive catalase test. O.D. reading at 600 nm and UV absorbance at 273 nm determined the growth and/or phenol degradation by the tested bacteria. Incubation of the 4 tested bacteria in Mineral Salts Medium (MSM) supplemented with 0.2% (w/v) (2000 mg/L) of phenol for 21 days at 28°C indicated the removal of 1989.16 mg/L of phenol by strain KS8 as compared to 1984.0, 1981.28 and 1922.88 mg/L by strains KS6, KS5 and Aw2, respectively. Removal of phenol by indigenous microbial communities to marine environment was shown to be successful with a considerable role can be exploited by strain KS8 in degradation of toxic compounds such as phenol.

Key words: Bacteria, degradation, optical density, phenol, seawater, mineral salts medium

INTRODUCTION

The pollution with petroleum, heavy metals, xenobiotics organic compounds and other contaminants is a growing environmental concern that harms both terrestrial and aquatic ecosystems. Bioremediation as a cleanup method and through the exploitation of the activities of microorganisms would degrade or attenuate such contaminants. Because phenolic compounds are widely distributed in the environment from various industrial as well as natural sources, bacteria with a potential to grow on toxic compounds such as phenol as a sole source of carbon and energy were tested here. Several aerobic microorganisms that degrade phenol have been isolated (Ahamad and Kunhi 1996, Folsom *et al.* 1990, Futamata *et al.* 2001, Fries *et al.* 1997, Yang and Humphrey 1975) with the Pseudomonads were the most widely distributed bacteria known for the biodegradation of phenolic compounds (Saadoun, 2011). Degradation of phenol by other bacteria such as *Ochrobactrum* sp. and *Streptomyces* sp. (Antai and Crawford 1983, EL-Sayed *et al.*, 2003, Saadoun 2011) molds (Alexieva *et al.*, 2007) and filamentous fungi were reported.

The aim of this work is to isolates phenol degrading bacterial strains indigenous to marine habitats located on the coastal area of the United Arab Emirates, to investigate the phenol degradation potential of the recovered isolates and to describe the biochemical characteristics of the most potent degraders.

MATERIALS AND METHODS

Location, sampling and sample processing: Water samples were collected from two different oil spilled sites inflicted by shipping activities in Khorfakkan (Kw) (Emirate of Sharjah/UAE) and Ajman Port (Aw) (Emirate of Ajman/UAE). The water samples were collected in 500 mL autoclaved Duran bottles. Surface hydrocarbon sediment from a depth of 5-10 cm was also collected from Khorfakkan Port (Ks) and kept in a pre-sterilized plastic container. The samples were assigned numbers for referencing and the source, type and condition of each sample was recorded. All collected samples were transferred to the laboratory and stored in the refrigerator at 4°C for further analysis.

Determination of bacterial population and isolation of bacteria: For seawater samples, sub samples of 10 mL of seawater were mixed thoroughly in 40 mL of sterile Mineral Salts Medium (MSM) of Leadbetter and Foster (1958) while for the surface hydrocarbon sediment sample, sub sample of 1g was suspended in 50 mL of sterile MSM. The final pH of the medium was adjusted to 7.2. The samples were incubated at 28°C with shaking at 100 rpm for 7 days and then serially diluted up to 10^{-6}. Aliquots of 0.1 mL from each dilution were spread over the surface of nutrient agar (Himedia India) plates. The plates were incubated at 28°C for 2 days and thereafter the appearance of different bacterial colonies on the surface of agar plates was observed. The morphological features

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such as colour, size form, margin and elevation of each colony were determined. Gram stain test was performed for each isolate. The bacterial strains were characterized biochemically with the following biochemical tests were used in the bacterial isolate's identification process: catalase; indole formation; methyl red; urea; H_2S gas production and bacterial motility (Cappuccino and Sherman, 1996).

Growth on phenol: Laboratory detection and monitoring of phenol degradation is accomplished either by direct phenol concentration measurements in the culture media or by the appearance of one or more of its products after degradation. Direct detection of phenol can be done chemically by using reagents like Fehlings reagent or Folin-Ciocalteu reagent and then measured spectrophotometrically at 400 nm. These procedures are time consuming and are not preferred. Gas chromatography is another way to follow up the concentration of phenol in a culture. The widely used and easy to be applied method for monitoring phenol concentration is done by ultraviolet-spectrophotometry at 273 nm (Kar *et al*., 1997) and which will be applied here in this investigation.

Bacterial colonies were inoculated into 50 mL sterile Mineral Salts Medium (MSM) supplemented with 0.2% (w/v) of phenol and incubated at 28°C and 100 rpm for 21 days. The growth response of each of the bacterial isolate on phenol was initially determined at 0, 3, 9, 15 and 21 days by physical appearance (turbidity) and measuring the Optical Density (OD) at 600 nm using UV-Visible spectrophotometer (Jenway 6320D). To determine phenol concentration in the shake batch culture flasks, 2 mL aliquots from bacterial cultures were withdrawn at 0, 3, 9, 15 and 21 days interval then phenol absorbance was measured spectrophotometrically at 273 nm (Kar *et al*., 1997) using the same spectrophotometer. Control flasks contain only the MSM supplemented with 0.2% (w/v) of phenol. Degradation was estimated as a difference between the initial and final concentration of the phenol content in the medium.

RESULTS AND DISCUSSION

Bacterial population: The maximum recovered colonies on agar plates from the polluted water samples were 6 (4 from Ajman and 2 from Khorfakkan) different types of bacterial colonies. However, 8 different types of bacterial colonies were recovered from surface hydrocarbon sediment sample (Table 1). A total of 14 distinct bacterial isolates were obtained, each with distinct morphological features.

Table 1: Morphological and biochemicala characterization of the recovered bacterial isolates

Sampling site	Isolate[b]	Morphological properties of the isolated bacterial cultures			
		Size	Color	Margin	Elevation
Khorfakkan	KS1	Large	Green	Curled	Flat
	KS2	Small	White	Entire	Flat
	KS3	Medium	White creamy	Curled	Flat
	KS4	Small	White	Curled	Raised
	KS5[a]	Small	Transparent	Entire	Flat
	KS6[a]	Small	White	Entire	Flat
	KS7	Small	Transparent	Entire	Raised
	KS8[a]	Medium	Green	Curled	Raised
	Kw1	Small	Light green	Entire	Flat
	Kw2	Large	Green	Curled	Flat
Ajman	Aw1	Medium	Orange	Entire	Flat
	Aw2[a]	Small	Transparent	Entire	Flat
	Aw3	Small	White	Entire	Flat
	Aw4	Very small	Transparent	Curled	Flat

[a]All isolates were Gram negative, motile bacteria with negative methyl red test but with positive catalase test. Isolates KS8 was negative for indole test when compared to positive results of the other isolates. However, KS8 isolate was positive for the urea test when compared to the negative results of the other isolates [b]Ks: Oil sediment from Khorfakkan harbor; Kw: Oil polluted water from Khorfakkan; Aw: oil polluted water from Ajman Port

All isolated bacteria were generally described based on their size, color, margin and elevation. The highest number of bacterial isolates obtained directly from the samples collected from Khorfakkan's sedimented hydrocarbons with 8 isolates out of 14 and then Ajman's oil spilled seawater with 4 isolates and Khorfakkan oil spilled seawater with 2 isolates (Table 1).

Those isolates namely Aw2, KS5, KS6 and KS8 that showed an excellent growth response on phenol were further biochemically characterized. All the four isolates were Gram negative, motile bacteria with negative methyl red test but with positive catalase test. Isolates KS8 was negative for indole test when compared to positive results of the other isolates. However, KS8 isolate was positive for the urea test when compared to the negative results of the other isolates (Table 1).

The prevalence of this group of bacteria in hydrocarbon-polluted seawater reflects their potential in utilizing toxic compounds, thus, cleaning up these sites (Cork and Krueger, 1991). Therefore, the different bacterial isolates of this study were visually evaluated for their growth on phenol as a toxic organic compound using turbidity method. Pakula *et al*. (1999) identified 2 strains of *Pseudomonas* from a biological petroleum-refining wastewater purification plant with high effectiveness of phenol removal. Saadoun (2011) reported the removal of 42-102 mg/L of phenol by different *Pseudomonas* sp. which seems to play a significant role in decomposition of toxic compounds such as phenol.

Table 2: Absorbance of cell free broth of the four bacterial isolates at 273 nm

	Absorbance (273 nm) at 0-21 days					
Isolate	0	3	9	15	21	Degraded phenol (mg/L)
KS5	2.030 (100)	0.933 (45.961)	0.296 (14.582)	0.139 (6.848)	0.019 (0.936)	1981.28*
KS6	2.001 (100)	1.303 (30.20)	0.777 (38.831)	0.545 (27.237)	0.016 (0.800)	1984.00
KS8	2.032 (100)	0.555 (27.313)	0.419 (20.621)	0.100 (4.922)	0.011 (0.542)	1989.16
Aw2	2.075 (100)	1.101 (53.061)	0.667 (32.145)	0.398 (19.181)	0.080 (3.856)	1922.88

*Numbers between parentheses represent percentage of remaining phenol in each bacterial culture

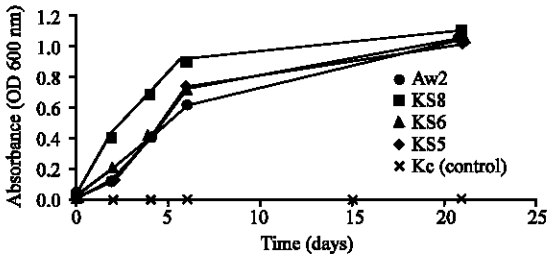

Fig. 1: Growth of bacterial isolates on phenol as reflected by Optical Density (OD) measured at 600 nm

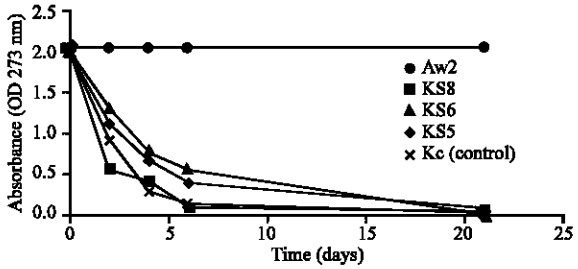

Fig. 2: Phenol degradation as reflected by Optical Density (OD) measured at 273 nm

Growth on phenol: Figure 1 shows the absorbance at 600 nm to measure the growth of some tested bacterial isolates on 0.2% (w/v) (2000 mg/L) phenol. All tested isolates showed a percentage increase of >99% in turbidity after 21 days of incubation and when compared to initial turbidity at day 0. This was also reflected in their ability to remove phenol with 1922.88, 1981.28, 1984.00 and 1989.16 mg/L for isolates Aw2, KS5, KS6 and KS8, respectively (Table 2). The unidentified recovered bacterial isolates showed >99.0% increase in turbidity (Fig. 1) and able to remove phenol with >1920 mg/L after 21 days (Table 2). Ahamad and Kunhi (1996) reported that *P. stutzeri* stain SPC2 can utilize up to 1200 ppm of phenol as a sole source of carbon and energy after a stepwise increase in phenol concentration with approximately 250 mg/L (~20.8%) remaining in the culture after 30 h of incubation. Tobajas *et al.* (2007) found that phenol at intermediate concentrations (10-45 mg/L) partially eliminated by Saadoun (2011) reported the removal of removal of 42, 84, 90 and 102 mg/L by *P. putrefaciense*, *P. fluorescense*, *P. cepacia* and *P. acidovorans*, respectively. Our study shows that isolate KS8 can utilize upto 2000 ppm phenol as a sole source of carbon and energy with 0.542% (~10.84 ppm) remaining in the culture after 21 days of incubation (Table 2). Those characterized isolates that showed a considerable growth on 2000 mg/L of phenol with the ability to remove ~1920-1990 mg/L of phenol after 21 days of incubation is shown in Fig. 2.

CONCLUSION

Removal of phenol by indigenous microbial communities in marine environment was shown to be successful in naturally remediating phenol contamination. This study clearly shows that oil contaminated seawater can be principle source for potent phenol degrading bacteria.

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