Bacterial Diversity in a polluted Manmade Lake Nearby Industrial City, Riyadh, Saudi Arabia

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Abstract: A manmade lake (pond) nearby the second industrial city at the south of Riyadh, Saudi Arabia was studied for its Bacterial community inhabiting this extreme environmental polluted area; This lake is fed by different types of wastewater ranged from treated wastewater generated from facilities of the industrial city and rainfall draining system; Physico-Chemical parameters of water has been measured including temperature of 31°C, pH of 7.6 to 7.8, TDS of 7700 ppm, conductivity of15590 µS/cm and selected heavy metals of dominance sequence: Ni > As >Cr > Fe > Zn > Cu >Mn> Co >Pb> Cd.Nitrates, carbonates and sulfates clearly existed at levels that Bactria can utilize for their energy, which were as an average of 25, 252 and 787 mg/L respectively. A total of 16 isolates were identified by using state-of-art RiboPrinter®system which is based on pattern of DNA bands. *Aeromonas* sp. was dominant with 15% followed by *Rhodococcusequi* and *Pseudomonasputida* with 12% each and then *Lactobacillusparacasei*, *Enterobactercoloacae* and *Brevibacillusbrevis* with 8% each.

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1. Introduction

Microbial communities inhabiting aquatic environments varies according to the water composition, depending mainly on the physiochemical parameters including temperature, salinity, DO, turbidity, pH and nutrient loads (Magdy, 2011; Mangla and Singh, 2011). Contamination sources also play a great role in changing the microbial diversity such as PAHs, heavy metals and wastes of commercial and industrial processes; where as studies have revealed that microbial communities shows an obvious decrease in diversity and number. Also, the subjected microorganisms can have a different physiological and genetic characteristics which enable them to survive under such stressful conditions (Marie, 2005). These extreme environments are a promising source of resistant microorganisms that can be significantly applied to biotechnological processes like bioremediation, biomineralization, bioleaching, biodegradation and metal detoxification (Satchanska *et al*., 2005; Memory *et al*., 2011). This study aims to record the Bacterial communities inhabiting a manmade polluted lake (pond) nearby the second industrial city of Riyadh, Saudi Arabia and to record the diversity of Bacteria in such harsh environment for further applications in biotechnology.

2. Material and Methods

2.1. Site description and sampling

The manmade lake (pond)is located at (N 24° 32′ 16and E 46° 57′ 10) nearby the second industrial city at the south of Riyadh, Saudi Arabia. It is of an estimated area of 25 Km2 and 20 m depth, where it is used to be a location of exploring and mining activities for raw materials. This lake might be created accidentally through dumping of wastewater generated from facilities of the industrial city in addition to the rainfall draining system. No actual documentations have recorded the history of this pond. Samples has been collected from 4 different sites as to be representative to the location of study, samples were collected from the polluted lake on 16th March 2013 in sterile borosilicate glass bottles with screw cap; pH and temperature was measured immediately after taking the samples.

2.2. Physiochemical parameters

pH and temperature was measured using portable HI 9026 pH/Temp. meter (HANNA®Instruments), conductivity and TDS was measured by (Ultrameter II™ MYRON L COMPANY); Heavy metals (Fe, Ni, Zn, Cu, As, Cr, Mn, Co, Cd and Pb) were analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), cations (Na+, K+, Ca2+, Mg2+) were determined by atomic absorption spectrophotometer (AAS) and anions ($HCO3^-$, Cl^-) were analysed by volumetric method. Sulphate and Nitrate were measured by colorimetric and ionic chromatography, respectively.

2.3. Bacterial identification

Performed by automated ribotyping using a robotized instrument (Riboprinter® Microbial Characterization System, Qualicon, Du Pont, Wilmington, DE, USA), proprietary reagents (Qualicon, Inc.) and the Riboprinter® System Data Analysis Program. Only strains identified as Bacteria were streaked on Nutrient agar and incubated for 1 day at 37°C to produce single-colony growth. The primary inoculum was touched with the end of a proprietary inoculation device (Stickpick; Qualicon, Inc.), which was used to inoculate 200 µl for Gram negatives and 40 µl for Gram positives of sample buffer. Thirty microliters of the mixture was transferred to the sample carrier and heated to 80 °C in the RiboPrinter heating station (Qualicon, Inc.). Five microliters of each lysing agent was then added, the total DNA was restricted with Eco RI, then separated and transferred to a membrane followed by hybridization and the sample carrier was transferred to the automated analyzer. The remainder of the procedure was conducted in the automated analyzer over 8 h. The output consisted of a densitometric scan depicting the distribution of the restriction fragments and their molecular weights and was saved in the Riboprinter computer.

3. Results and discussion

3.1. Physiochemical analysis

Results of physicochemical parameters presented in Table 1. showed pH ranged from 7.6 to 7.8and temperature of 31°C but it is reported that temperature of this area reaches up to 60°C in summer and - 2°C in winter; noticeably, TDS measured was very high ranging from 7630 to 7686 mg/L similar to that of many high temperature resources like hot springs which ranges from 6,000 to 10,000 mg /L (Wright, 1991). The high level of TDS probably resulted in elevated rates of electrical conductivity, which ranged from 15590 to 15710 µS/cm. Nitrates, carbonates and sulfates were found to be at an average of 25, 252 and 787 mg/L respectively, levels that Bactria can utilise for their energy, growth and multiplication. The heavy metals concentration analysis showed in Table 2indicated the industrial wastewater pollution with heavy metals and it was of a sequence: Ni > As >Cr > Fe > Zn > Cu >Mn> Co >Pb> Cd. But despite the presence of toxic heavy metals such as Cd and Pb in low levels 0.1 and 0.2 ppb respectively; their accumulation over time can cause a hazardous environmental threat on both biota and man (Igbinosa*et al*., 2012).

Table 1 shows physico-chemical parameters of wastewater from four sites of polluted lake, Riyadh, Saudi Arabia. Table 2 shoes heavy metal concentrationsof wastewater from four sites of polluted lake, Riyadh, Saudi Arabia.

3.2. Bacteriological analysis

A total of 16 species has been detected by Riboprinter and shown in Figure 1., whereas Aeromonas sp. was dominant with 15% followed by two other common species *Rhodococcusequi* and *Pseudomonas putida*with 12% each and three other less common ones including *Lactobacillus paracasei*, *Enterobactercoloacae* and *Brevibacillusbrevis* with 8% each and then comes the least common species *Streptococcus pneumonia*, *Pseudomonasstutzeri*, *Thermoanaero bacterium* sp., *Vibrio vulnificus*, *Bacillus thuringiensis*, *Hydrogenphagaflava*, *Idiomarinaabyssalis*, *Klebsiellapneumonia*, *Pelobacterseleniigenes* and *Paenibacillus*sp. with 4% each. Some of these bacterial species are already been used in biotechnological applications including two species of Pseudomonas;*P.putida*and *P. stutzeri*. *P. putida*is a bacteriumwell known for its great metabolic versatility and capability of growing in aquatic systems, soils and rhizosphere(Timmis, 2002; Dos Santos *et al*., 2004) and its ofstrain KT2440 (Al-Sum *et al*., 2012) is certified to be a biosafety strain and it iswidely used in the fields of agriculture, Bioremediation, Biocatalysts and Bioplastics production (Molina *et al*., 2000; Bloemberg and Lugtenberg, 2001; Wackett, 2003; Jiménez et al., 2004; Pieper et al., 2004; Dos Santos *et al*., 2004; Kim *et al*., 2006). *P. stutzeri*is a denitrifying soil bacterium, used in Bioremediation for its ability to degrade carbon tetrachloride (Hirad, *et al*., 2013; Sepulveda *et al*., 1999; Lalucat, *et al*., 2006).

*Lactobacillus paracasei*found naturally on milk, meat and vegetables because of its ability to ferment hexoses, pentoses and gluconate into lactic acid; strains of this bacterium are being used in milk and cheese production (Al-Arfaj, *et al.,* 2012).

Bacillus thuringiensis(Bt) a soil bacterium commonly used as a biological insecticide as its strains are capable of producing crystal proteins called δ-endotoxins which have an Insecticidal action (Madigan *et al*., 2005; Roh*et al*., 2007).

*Enterobacter cloacae*is a bacterium that has been used in an experiment to degrade Pentaerythritoltetranitrate (PETN),which is a powerful explosive used in blasting caps and detonators, as the strain used PB2 was found to utilize PETNas a sole source of nitrogen for growth(Abu-Thyab, *et al*., 2012; Peter *et al*., 1996; Milton and Rachakonda, 2005). Members of genus *Paenibacillus* has been identified from different environments including water systems, soil and rhizosphere; where some has been used in agriculture and horticulture for their ability to fix nitrogen such as *P. polymyxa*, while others like *Paenibacillus* sp. JDR-2 is used in various biotechnological applications because it is a rich source of chemical agents (McSpadden, 2004;Lal and Tabacchioni, 2009).

Members of genus *Thermoanaero bacterium* has been detected from deep surface oil wells, hot springs and geothermally heated water outlets. A wide range of thermostable enzymes have been either extracted or cloned from this group of organisms. For example, polysaccharide-hydrolysing enzymes such asthermostableendoxylanases,this is used in biomass conversion and paper industry (Isaac *et al*., 2001).

Table 1. Physico-chemical parameters of wastewater from four sites of polluted lake, Riyadh, Saudi Arabia.

	PH	Conductivity (µS/cm)	TDS (mg/L)	ANIONS (mg/L)				CATIONS (mg/L)			
				HCO_3^-	NO_3^-	Cl^-	SO_4^-	Ca^{++}	Mg^{++}	K^+	Na^+
Site 1	7.72	15710	7686	229	25.2	4083	816	485	205	94.3	2210
Site 2	7.68	15600	7649	259	26.3	4171	840	480	188	96.3	2228
Site 3	7.80	15690	7685	275	25.1	4438	578	475	215	6.4	2284
Site 4	7.78	15590	7630	244	22.8	4260	912	460	213	70.2	2310

Table 2. Heavy metal concentrationsof wastewater from four sites of polluted lake, Riyadh, Saudi Arabia.

Analyte (ppb)	Site 1	Site 2	Site3	Site 4
Cr	16.2	6.90	9.01	8.21
Mn	5.11	2.90	6.32	5.73
Fe	12.8	11.1	14.5	14.0
Co	1.90	1.70	2.10	2.10
Ni	82.5	71.9	89.0	86.2
Cu	6.42	5.11	7.80	8.10
Zn	6.51	6.62	8.40	11.8
As	41.5	38.8	49.6	61.4
Cd	0.11	0.11	0.11	0.12
Pb	0.21	0.20	0.30	0.23

Figure 1 shows the detected bacterial species using state-of-art RiboPrinter®.

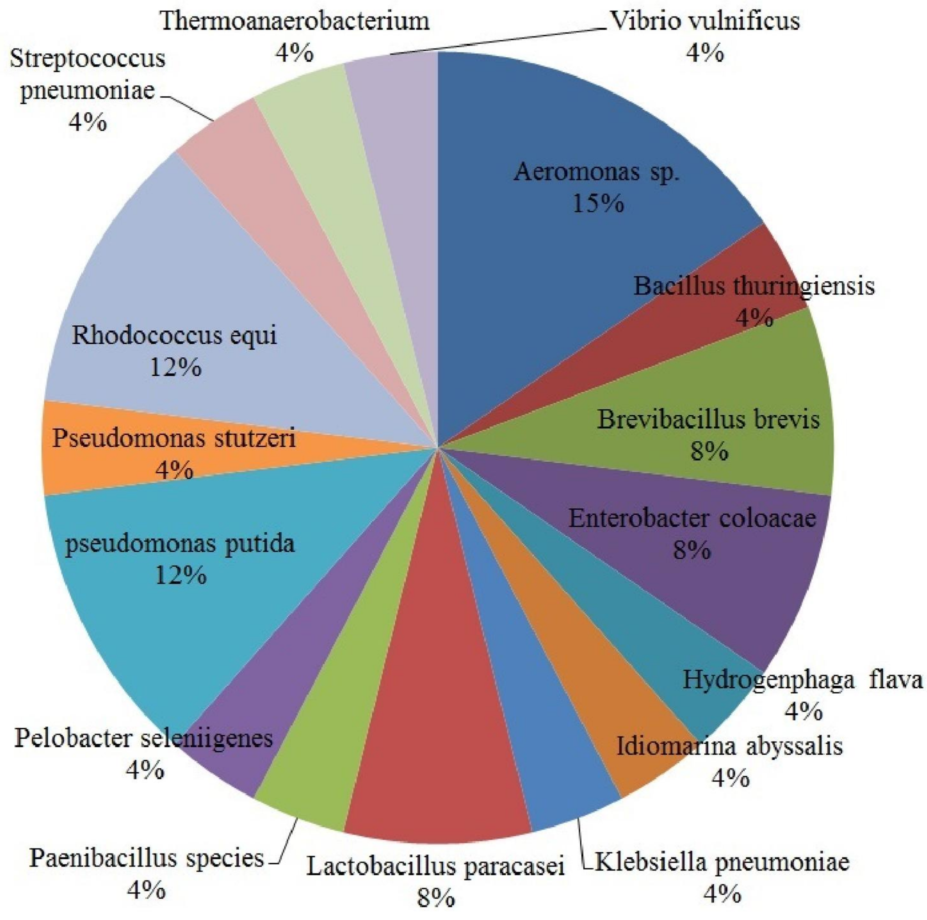

Figure 1. Detected bacterial species using state-of-art RiboPrinter®

4. Conclusion

Extreme polluted environments is considered to be a promising area of study as the results of this work confirmed that under this harsh conditions bacterial diversity can be a valuable source for resistant species that can be utilized for their enzymes and bioactive products. Also their genes can be tapped in further biotechnological approaches and applications.

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