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CULTURED DIVERSITY OF PETROLEUM HYDROCARBON DEGRADING BACTERIA OF CHILIKA LAGOON, ODISHA, INDIA

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ABSTRACT

Chilika Lake situated on the East coast of India, is the Asia's largest brackish water Lagoon. It is a wetland of ecological importance and is listed in the Ramsar convention of 1982. The lake is rich in its floral and faunal diversity besides being a repository to numerous endangered and vulnerable species. Lake Chilika is also a habitat for Irrawady dolphin and numerous migratory birds. Chilika Lake is an important tourist destination where motorized boats travel along the lake and is also the main source of livelihood for local residents. Oil discharges from the motorized boats being used for tourism and fishing are polluting the Lagoon environment. The present study aims at determining the cultured diversity of petroleum hydrocarbon degrading bacteria of Chilika Lagoon. Twenty samples were collected from four different sectors of the Lagoon and were enriched for petroleum hydrocarbon degrading bacteria. Eighteen bacterial strains were isolated and eleven of them were found to be degrading petroleum hydrocarbons.

Keywords: Chilika Lagoon, oil pollution, petroleum hydrocarbon degrading bacteria, 16S rRNA gene sequence analysis.

Introduction:

Chilika Lake, a wetland of international importance is situated in the state of Odisha, India. It is positioned between 19° 28′ to 19° 57′ N and 85° 5′ to 85° 36′ E. The Lake is divided into four sectors namely Northern, Southern, Central and outer channel based on the hydrological parameters. Lake Chilika harbors numerous islands and prominent among them are Nalabana, Kalijal, Somolo, Honeymoon, Break-fast, Birds and Rajahansa. The Lake is known for its immense biological diversity and is a home to several globally threatened floral and faunal species. It is an important tourist destination and is also majorly associated with the socio economic prospects of the region (www.chilika.com).

During the recent times, the Lake system is subjected to severe anthropogenic disturbances which are a matter of prime concern (Baliarsingh *et al*., 2014). Pollution caused by discharges from motor boats used for fishing and tourism, unregulated and indiscriminate fishing activities, discharges of chemicals and solid wastes, encroachments and climatic changes have severely affected the ecological parameters of the Lake and thereby the biodiversity.

The discharge of petroleum hydrocarbons in the Lagoon is a serious threat as it disrupts the functioning of the ecosystem and is lethal to all forms of life (Schafer *et al*., 2009; Das & Chandran, 2010). The microbial degradation of hydrocarbons and their prospective role in the removal of oil discharges from the marine and terrestrial ecosystems is well known (Rahul *et al*., 2015). The present study aims at isolating potential petroleum hydrocarbon degrading bacteria from Chilika Lagoon and assessing their hydrocarbon degrading capabilities.

Material and methods:

Collection of samples

Five water samples (with traces of oil) each from the four sectors namely Northern, Southern, Central and outer channel were collected in sterile screw cap polypropylene bottles of 15 ml capacity in July 2016, brought to the laboratory and were transferred to enrichment broth with in 4 days of sample collection.

Enrichment and isolation of petroleum degrading bacteria

Water samples with traces of oil were inoculated into 100 ml conical flasks containing 50 ml mineral salts media [consisting ($g.l^{-1}$): KH_2PO_4 (0.5), $MgSO_4.7H_2O$ (0.2), NH_4Cl (0.6), NaCl (10), $CaCl_2.2H_20$ (0.05), 5 ml of ferric-citrate solution (0.1 %, w/v)] with petrol 2 % (v/v) as sole carbon source for selective enrichment of petroleum hydrocarbon degrading bacteria and were incubated at 28 °C under shaking at 100 rpm for three days. Petrol was sterilized by filtration though a 0.20 µm PTFE membrane (Millipore). Enrichment cultures of petroleum degrading bacteria were purified by repeated streaking on nutrient agar (HiMedia) plates and incubated at 28±2 °C. Single colonies were sub-cultured onto fresh agar plates till identical colonies were observed on two successive plates. The isolated strains were inoculated in the mineral salts medium with petrol (2 %, v/v) as carbon source in order to ensure they retain their capacity to grow on petrol as sole source of carbon and energy.

16S rRNA gene amplification and sequencing

Genomic DNA was extracted and purified from the isolated strains according to the method of Marmur (1961). 16S rRNA gene amplification was performed using a DNA thermal cycler (Eppendorf) and sequencing of the same was done as described previously (Rahul *et al*., 2014). Identification of phylogenetic neighbors and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (Kim *et al*., 2012).

Petroleum hydrocarbon degradation (Gravimetric analysis)

To determine the petroleum hydrocarbon degradation capability, the isolated strains were grown in 250 ml conical flasks containing 100 ml mineral salts media [consisting ($g.l^{-1}$): KH_2PO_4 (0.5), $MgSO_4.7H_2O$ (0.2), NH_4Cl (0.6), NaCl (10), $CaCl_2.2H_20$ (0.05) with petrol 5 % (v/v) as carbon source and were incubated at 28±2 °C under shaking at 100 rpm for two weeks. Un-inoculated flask served as control. Cells were harvested by centrifugation (10000 g for 15 min at 4 ºC). The petrol in the

supernatant was extracted with 50 ml of dichloro methane (DCM) and collected by centrifugation at 10000 g for 10 min at 4 ºC (Rahul *et al*., 2015).

The % of degradation was calculated as follows

Weight of residual petrol = Weight of beaker containing 50 ml of DCM + residual petrol – Weight of beaker containing 50 ml of DCM

Amount of petrol degraded = Weight of petrol added in the media – Weight of residual petrol

% degradation = Amount of petrol degraded / Amount of petrol added X 100

Results:

Out of twenty (20) samples collected from the four sectors of the Lake, ten (10) samples gave positive enrichments for petroleum degrading bacteria in mineral salts medium with 2 % (v/v) petroleum as sole source of carbon and energy under aerobic conditions (Table 1). Repeated streaking on nutrient agar plates resulted in the isolation and purification of eighteen (18) pure cultures from ten (10) positive enrichments and strain numbers were designated to them. Eleven (11) out of eighteen (18) strains have retained their capacity to utilize petroleum as sole carbon source (Table 1) and they were further studied for their abilities to degrade petrol.

Phylogenetic relatedness of 18 strains was analyzed based on 16S rRNA gene sequence similarity using Ez Taxon-e server. The strains were identified as belonging to the genera *Serratia*, *Enterobacter*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Deinococcus*, *Acinetobacter*, *Microbacterium*, S*hewanella Arthrobacter*, *Delftia*, *Marinobacter*, *Exiguobacterium*, *Micrococcus* and *Citrobacter*. Though *Serratia* sp. CL1, *Enterobacter* sp. CL8, *Paenibacillus* sp. CL5, *Deinococcus* sp. CL15, *Microbacterium* sp. CL14, *Shewanella* sp. CL10 and *Delftia* sp. CL11 were isolated from the positive enrichment samples, they were not utilizing petroleum hydrocarbons as sole source of carbon and energy.

Gravimetric analysis was used to determine the petroleum degradation ability of the isolates. Though gravimetric analysis is not as sensitive as GC analysis, it is comparatively helpful method for the initial determination of degradation. *Bacillus* sp. CL4, *Exiguobacterium* sp. CL7, *Arthrobacter* sp. CL2 exhibited highest degradation rates in the range of 60-78% in a period of two weeks. *Citrobacter* sp. CL18, *Micrococcus* sp. CL16, *Acinetobacter* sp. CL6, *Bacillus* sp. CL9 degraded petroleum hydrocarbons in the range of 48-56%. The other strains degraded petroleum hydrocarbons in the range of <40 %. GCMS analysis and microcosm studies have to be performed to further assess the ability of the isolated strains in bioremediation studies.

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Sample No	Sample collection site	Enrichment for petroleum degrading bacteria (+/-)	Strain number of pure culture (s) obtained	Percentage (%) 16S rRNA gene sequence similarity with the nearest type strain (Based on EzTaxon search results)	Petroleum hydrocarbon degradation ability (+/-)
NS1	Northern Sector	-	-	-	-
NS2		-	-	-	-
NS3		+	CL1, CL8	CL1: 99.3% - *Serratia ureilytica* NiVa 51^T	-
				CL8: 99.1%- *Enterobacter massiliensis* JC163^T	-
NS4		-	-	-	-
NS5		-	-	-	-
CS6	Central Sector	+	CL3, CL5	CL3: 99.8% *Bacillus butanolivorans* K9^T	+
				CL5: 100% *Paenibacillus chungangensis* CAU 9038^T	-
CS7		-	-	-	-
CS8		+	CL9, CL13, CL15	CL9: 99.1% *Bacillus oleivorans* JC228^T	+
				CL13: 98.7% *Pseudomonas toyotomiensis* HT-3^T	+
				CL15: 100% *Deinococcus mumbaiensis* CON-1^T	-
CS9		+	CL6	CL6: 99.9% *Acinetobacter kookii* 11-0202^T	+
CS10		-	-	-	-
SS11	Southern Sector	-	-	-	-
SS12		-	-	-	-
SS13		+	CL14	CL14: 98.9% *Microbacterium lindanitolerans* MNA2^T	-
SS14		+	CL10, CL17	CL10: 99.2% *Shewanella donghaensis* LT17^T	-
				CL17: 100% *Acinetobacter gerneri* 9A01^T	+
SS15		-	-	-	-
OC16	Outer Channel	+	CL2, CL11, CL12	CL2: 100% *Arthrobacter humicola* KV-653^T	+
				CL11: 99.6% *Delftia lacustris* 332^T	-
				CL12: 99.2% *Marinobacter xestospongiae* UST090418-1611^T	+
OC17		+	CL7	CL7: 99.8% *Exiguobacterium profundum* 10C^T	+
OC18		+	CL16,CL18	CL16: 99.3% *Micrococcus lactis* DW152^T	+
				CL18: 98.7% *Citrobacter pasteurii* CIP 55.13^T	+
OC19		+	CL4	CL4: 98.4% *Bacillus okhensis* Kh10-101^T	+
OC20		-	-	-	-