8. Isolation and Characterization of aliphatic hydrocarbon degrading bacteria from Godavari River

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Abstract

In the present investigation, the water sample was collected from Godavari River and isolated 10 bacterial cultures. All the cultures are reacted against the various salt concentration and aliphatic hydrocarbon compounds and found that Aliphatic compound Degrader (ACD) 4 & 8 showed salt tolerance phenomenon against 16% NaCl Concentration. While most of the bacterial cultures are resistant against Ethyl acetate, Hexane & Adipic acid.

Introduction

Environmental pollution with petroleum and petrochemical products (complex mixtures of hydrocarbons (Including aliphatic and aromatic)) has been recognized as one of the most serious current problems (1, 2, 4). If this occurs, hydrocarbons may reach the water table before becoming immobilized in the soil. They spread horizontally on the ground-water surface and continue to partition into ground water, soil pore space air, and to the surfaces of soil particles. Bioremediation has become an alternative way of remediation of oil polluted sites, where the addition of specific microorganisms can improve biodegradation efficiency (3, 11, 14). Biodegradation efficiency depends on microorganisms, capable of producing enzymes that will degrade the target compound. Factor such as temperature, pH, and nutrient status are of importance as moderators (09). In the present investigation, Godavari River water sample was used to isolate efficient aliphatic degrading bacteria in situ.

Materials and Methods

Collection of water sample

Water sample was collected from Godavari river of Aurangabad region. The sterile container was used to collect the water sample for analysis of physical, chemical and bacteriological examination in situ.

Physico-chemical Analysis of water sample

Samples has been subjected to various analyses including water temperature at the time of sampling using a mercury thermometer; pH value, total dissolved solids (TDS), electric conductivity (EC) (APHA).

Isolation of microorganisms

The collected water sample was used to isolate the different microorganisms from the Godavari River water sample. The water samples were collected from three different of Godavari River. All isolates were isolated according to their ability to degrade aliphatic compounds. Each of the selected thirteen bacterial isolates was sub-cultured in nutrient agar incubated at 30°C for 1-2 days. Pure colonies of sub-cultured bacterial isolates were stored in the refrigerator.

Enrichment of isolated culture

The isolated were enriched by using nutrient broth incubated at 35±2°C for 24-48 hrs at rotary shaker incubator having 120 rpm.

Chemicals: In the present investigation, three aliphatic compounds were studied against the isolated strain from Godavari River such as Ethyl acetate, Hexane and Adipic acid.

Salt Tolerance Test: The aliphatic degrading bacterial isolates were assayed to grow on different sodium chloride (NaCl) concentrations (1, 4, 8, 12, 16 and 18%).

Result and Discussion

Microbial analysis

The microbiological study of the water samples is shown in Table 1.

Table 1 Biochemical characteristics of selected bacterial species.

Test / Characteristics	ACD-1	ACD-2	ACD-3	ACD-4	ACD-5
Catalase	+	+	+	+	-
Indole	-	+	+	+	+
Nitrate reductase	-	-	+	+	-
Urease	-	-	-	+	+
Methyl red	+	+	-	-	-
Casein hydrolase	+	+	+	+	+
Vogus- proskauer test	+	-	+	-	+
Starch hydrolysis	+	+	+	+	+
Citrate Utilization	-	-	+	-	-

D-Glucose fermentation	+	+	+	+	+
Sucrose fermentation	+	+	+	+	+
Lactose fermentation	-	+	+	+	+
Maltose fermentation	-	+	+	+	+
Mannitol fermentation	+	+	+	+	+
Milibiose	+	-	+	-	+
Rhamnose	+	+	+	+	+
Cellobiose TDP TDT	+ 70°C 60 min	- 60°C 60 min	- 60°C 55 min	+ 65°C 60 min	+ 55°C 40 Min

Hence it confirms that the Godavari River water is not useful for the drinking purpose. The collected Godavari River water sample (5%) was inoculated into nutrient broth and incubated at 33°C for 24 h on rotary shaker incubator having 150 rpm.

Loopful of enriched broth samples was streaked on nutrient agar plate and incubated for 24 h at 30°C. After incubation, morphological characters of different colonies were observed. The identification carried out by different biochemical tests such as citrate utilization, glucose utilization, millibiose utilization, arabinose utilization, rhamnose utilization, lactose utilization and so on using Bergey's manual of determinative bacteriology. There are five morphologically distinct isolates (ACD-1, ACD -2, ACD -3, ACD-4 and ACD -5) were randomly selected from the nutrient agar plate. They differ in their ability to hydrolyze large molecules like carbohydrates and proteins. These variations are due to the differences in the types of enzymes that bacteria possess

In citrate utilization test, citrate is the only carbon source available for the bacteria. If the bacteria able to use citrate for their growth then the pH of the media will increase and the media will turn a bright blue in color indicates the test is positive. The isolate ACD-3 showed positive test while remaining three isolate are citrate negative. The isolates showed ability to ferment different carbon sources such as D-glucose, sucrose, mannitol and rhamnose (Table 1).

According to Atlas, the higher level of temperature affects the qualitative as well as quantitative structure of microbial communities and this was found in several studies that environmental factors adversely affect on growth, morphology and biochemical activities of microorganisms, resulting in decreased biomass and diversity (07).

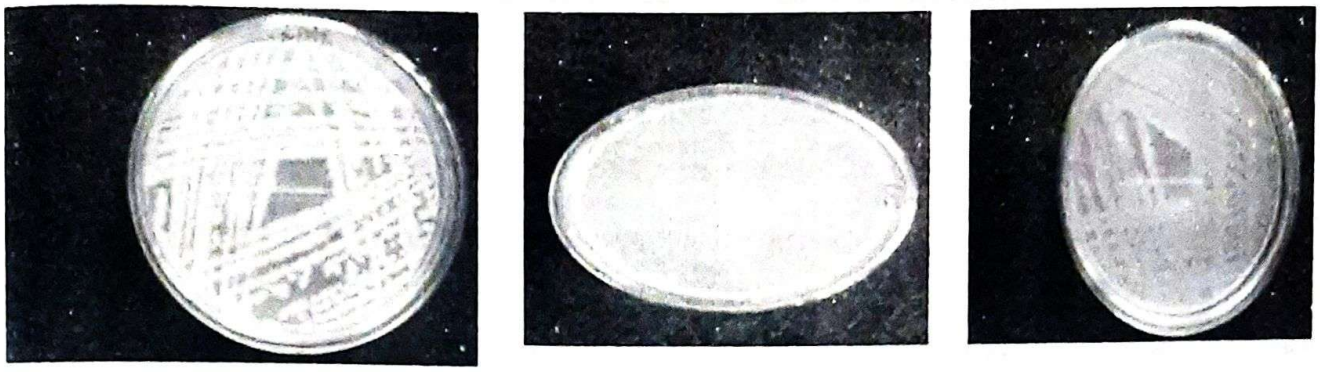

Figure: 1 The bacterial isolates of Godavari River water sample on nutrient agar medium.

Physico-chemical examination of collected Godavari River water sample

In almost all the collected water samples from Godavari River having the pH ranges from 8.1 to 8.3 while temperature 33±3°C was recorded. According to Atlas, the variable pH and fluctuating temperature of River responsible to develop a variable biodiversity (01). According to APHA the fluctuations in pH ranges may leads to an increase or decrease in the toxicity of poisonous compound in water bodies.

Dissolved oxygen present in drinking water adds taste and it is highly fluctuating factor in water (3). In present study, the observed dissolve oxygen, biological oxygen demand and chemical oxygen demand was 0.53 mg/l, 0.30 mg/l and 3.95 mg/l respectively. The maximum allowed value of chemical oxygen demand is 10 mg/l in drinking water. The insignificant DO, BOD and COD was obtained, hence it confirms that the presence of microorganisms and organic compounds are in lower amount (40).

According to WHO, a limiting value of Total dissolved solids (TDS) for potable water is 500 mg/l. In the present investigation this limit was not crossed on either side by any of the samples under study. The TDS of the river water was 3.32 ppm. These values are acceptable for domestic use and agricultural purposes. Increase content of TDS elevates the density of water and such a medium increase in osmoregulation. An overwhelming value of TDS has also increased the conductivity values of the water sample (9).

Table 2. Physiochemical analysis of the Godavari River water sample.

Tests	Concentrations
Color	Colourless
pH	8.1±0.2
Temperature	33±3°C
BOD	0.30±0.03 mg/l
COD	3.95±0.10 mg/l

DO	0.53±0.03 mg/l
Chlorine	0.6674±0.05 g/l
Sulphate	0.93± 0.02 mg/l
TDS	3.32±0.07 ppt
Conductivity	6.79±0.10 ms

Conductivity is a numeric measure of the capacity of an aqueous solution to pass electric current. The pure water is not expected to conduct the electricity. The ability depends on the presence of ions, the total concentration, mobility, valency and temperature (09, 12). The conductivity of the collected water sample was recorded about 6.79±0.10 ms. According to Atlas, the high level of conductivity should be expected since ions such as sodium ions (Na^+), chloride ions (Cl^-) and nitrates (NO_3^{2-}) are present in high concentration level. Chloride is one of the important anions which determine the total salinity of water (13). In present investigation, the chlorine concentration in Godavari water was 0.6674±0.05 g/l. High chloride concentration in the water indicates the presence of organic waste, primarily of animal origin (06, 10).

Aliphatic compounds were used against the organisms for to check the efficiency of growth in the nutrient broth. The 0.1% of the aliphatic compounds were used against the isolated organisms. The obtained data are given below.

Table 3: Aliphatic compound degradation by plate assay method

Bacterial isolates	Aliphatic compounds		
	Ethyl acetate	Hexane	Adipic acid
ACD-1	+	+	+
ACD-2	+	+	+
ACD-3	+	+	-
ACD-4	+	+	+
ACD-5	+	-	+
ACD-6	+	+	+
ACD-7	-	+	+
ACD-8	+	-	+
ACD-9	-	+	+
ACD-10	+	+	+

All the isolates showed, actively degrade the used aliphatic compounds and grow in the nutrient broth were analyzed by turbid-metrically. The positive sign indicates the growth of the isolates and negative sign indicates the retardation of the bacterial isolate.

Table 4: Salt tolerance of the bacterial isolates.

Bacterial isolates	Concentration of NaCl (%)					
	1	4	8	12	16	18
ACD-1	+	+	+	-	-	-
ACD-2	+	+	+	+	-	-
ACD-3	+	+	-	-	-	-
ACD-4	+	+	+	+	+	-
ACD-5	+	+	+	-	-	-
ACD-6	+	+	+	+	-	-
ACD-7	+	+	+	-	-	-
ACD-8	+	+	+	+	+	-
ACD-9	+	+	+	-	-	-
ACD-10	+	+	+	-	-	-

Salt tolerance phenomenon was studied against the ten different microorganisms. Showed the various growth pattern of the isolate on the different percentage of the salt concentration.

Conclusion

The collected bacterial isolates were showed degradation property against the aliphatic compound such as Ethyl acetate, Hexane and Adipic acid. Hence for control the toxicity of the water microbial cultures should be used for maintaining the environmental imbalance.

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