

## HYDROCARBON-UTILISING MICRO-ORGANISMS FROM DONA PAULA BAY, GOA

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### ABSTRACT

*Twenty-three hydrocarbon-utilising bacteria and one yeast were isolated, using enrichment techniques, from water and sediment samples. Vibrio and Pseudomonas were the predominant genera. Of the different organisms screened, Bacillus, Candida and Arthrobacter sp. exhibited the widest range of hydrocarbon-utilising profiles. Arabian Sea crude and kerosene supported the growth of most of the isolates.*

### INTRODUCTION

Accidental oil spills, bilge dumping from tankers and offshore blow outs are the main causes of oil pollution at sea. An estimated one million metric tons of oil are spilled into the waters of the world annually. The problem of oceanic oil pollution and the necessity of removing the oil from the refinery waste water prior to discharge have focused attention on the biodegradability of various petroleum components.

Virtually all kinds of hydrocarbons, crude oils and refined petroleum products are vulnerable to microbial degradation under favourable conditions (Davis, 1967; Treccani, 1962; ZoBell, 1964; 1969). Therefore, it seems necessary to obtain information on the abundance of micro-organisms capable of growing on crude oils in the marine environment.

The present work was undertaken to isolate, identify and study the hydrocarbon-utilising micro-organisms and their potential to utilise various crude oils and pure hydrocarbons.

## MATERIALS AND METHODS

The hydrocarbon substrates—for example, kerosene, motor oil, furnace oil, aviation turbine fuel, raw naphtha, gasoline and diesel—were obtained from the Indian Oil Corporation, Bombay. Arabian Sea crude was obtained from Bharat Petroleum refinery, Bombay. Petroleum tar was collected from local beaches (Dwivedi & Parulekar, 1974). The *n*-pentane, *n*-hexane, cyclohexane, *n*-heptane, benzene, xylene, toluene, naphthalene and anthracene were of analytical grade (obtained from BDH, Sarabhai Chemical Co. and E. Merck).

Water and sediment samples were collected in glass containers from Dona Paula Bay, Goa, approximately 3 km offshore. Glass containers used for collecting samples were chromic acid washed and steam sterilised at 121 °C for 30 min.

The enrichment medium consisted of basal salt solution with petroleum tar (0.1 %) or one of the refined oils such as motor oil (1 % v/v) or kerosene (1 % v/v). The basal salt solution per litre of distilled water was made of 33.0 g NaCl, 7.0 g of MgSO<sub>4</sub>, 0.75 g of KCl, 1.0 g of NH<sub>4</sub>NO<sub>3</sub>, 3 ml of KH<sub>2</sub>PO<sub>4</sub> (10%), 7 ml of K<sub>2</sub>HPO<sub>4</sub> (10%) and 1 ml of trace metal solution (Stanley, 1973).

The enrichment medium was inoculated with sea water (1 %) or sediment (0.1 %) samples. Flasks were incubated at room temperature (28–30 °C) on a rotary shaker for between 8 and 15 days and were checked daily for any increase in turbidity and/or emulsification of the oil used. In order to obtain consistent growth, the cultures were transferred to the same medium at least three times at 8-day intervals.

The enriched microbial populations were streaked on the nutrient agar medium (Colwell & Walker, 1973). Plates were incubated for 48 h at room temperature. Isolated cultures were maintained on nutrient agar slants.

The isolates were studied for morphological and biochemical characteristics (Cruickshank *et al.*, 1975) and then identified following the methods of Shewan *et al.* (1960) and Buchanan & Gibbons (1975).

In order to study their hydrocarbon-utilising profiles, the isolates were individually subcultured three times in 5 ml of basal medium with 1 ml of motor oil:kerosene (1:1 v/v) and incubation was carried out at room temperature for between 8 and 15 days. The growth of each culture was followed in three subcultures in different hydrocarbon substrates.

For volatile pure hydrocarbons, the agar method was used. Each isolate was streaked on basal agar. Filter sterilised hydrocarbons were added to filter paper placed in the lid of a petri dish. Plates were incubated at room temperature for between 8 and 15 days and were checked for growth daily.

## RESULTS

Using the enrichment technique, twenty-three strains of bacteria and one yeast were

isolated from water and sediment samples (Table 1). Morphological and biochemical characteristics of the isolates are shown in Table 2.

Of the different oils tested, the Arabian Sea crude and kerosene were preferentially utilised. In the case of the pure hydrocarbons studied, pentane, benzene, naphthalene and anthracene accelerated the growth of most of the isolates.

TABLE 1  
GROUPS OF THE ISOLATES OBTAINED ON DIFFERENT CARBON SOURCES FROM SEA WATER AND SEDIMENT SAMPLES

Carbon source	Sample	Type of isolate					
		<i>Pseudomonas</i> <i>sp.</i>	<i>Vibrio</i> <i>sp.</i>	<i>Bacillus</i> <i>sp.</i>	<i>Candida</i> <i>sp.</i>	<i>Arthrobacter</i> <i>sp.</i>	Unidentified
Motor oil	Sea water	1	10	—	—	—	1
	Sediment	5	3	—	—	1	—
Tar	Sediment	—	—	1	—	—	1
	Sediment	—	—	—	1	—	—

The present study revealed that none of the hydrocarbons tested was completely oxidised by micro-organisms during the period of incubation (8–15 days). However, emulsification of the oil and increased turbidity of the medium indicated that the oil was being used by micro-organisms as a source of carbon and energy (Tables 3 and 4).

#### DISCUSSION

Each isolate grew on one of the oils or pure hydrocarbons tested. Considerable diversity in the hydrocarbon-utilising pattern of the isolates was observed. This may be due to differences in the composition of each oil (Nagy & Colombo, 1967; Liu, 1973) or to the lack of a particular enzyme system necessary for the metabolism of hydrocarbon.

The limited growth of some isolates on hexane, toluene and xylene may be due to their toxic effect on the cell membrane (Foster, 1962; Cooney & Walker, 1973).

The results suggest the prevalence of bacteria, in the Dona Paula Bay, capable of utilising different oils and a variety of aliphatic, cyclic and aromatic hydrocarbons.

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TABLE 2  
MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE ISOLATES

Group of organisms	Number of isolates	Gram stain and morphology	Glucose	Sucrose	Maltose	Lactose	Methyl red	Voges proskauer	Motility	H <sub>2</sub> S	NO <sub>3</sub> reduction	Starch	Indol	Oxidase	Catalase	Urease	Gelatin	Hugh & Leifson Oxidative Fermentative
<i>Pseudomonas</i> sp.	6	- ve cocco bacilli	-	-	-	-	-	-	+	(4) <sup>a</sup>	(5)	-	(1)	+	+	(1)	-	-
<i>Vibrio</i> sp.	13	- ve cocco bacilli	+	(12)(12)(12)(10)	-	-	-	-	+	(3)	+	(3)	(8)	+	+	(1)	-	+
<i>Bacillus</i> sp.	1	+ ve sporulating rod	+	+	+	+	+	-	+	+	-	-	-	-	-	-	+	-
<i>Candida</i> sp.	1	+ ve oval, presence of pseudomycelia	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	+
<i>Arthrobacter</i> sp.	1	- ve long slender rods to cocci	-	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-
Unidentified	2	- ve cocco bacilli	-	-	-	-	-	-	+	-	(1)	+	-	-	+	(1)	(1)	+

+ = All isolates giving positive results.

- = All isolates giving negative results.

<sup>a</sup> = Number of isolates giving positive results.

TABLE 3  
OIL UTILISATION BY THE ISOLATES

Group	Number of isolates	Kerosene	Motor oil	Arabian Sea crude	Furnace oil	Aviation turbine fuel	Raw naphtha	Gasoline	Diesel	Tar
<i>Pseudomonas</i> sp.	6	(5)*	(3)	(6)	(4)	(4)	(5)	(2)	(3)	(2)
<i>Vibrio</i> sp.	13	(13)	(4)	(12)	(5)	(4)	(5)	(1)	(5)	(3)
<i>Bacillus</i> sp.	1	+	+	+	+	+	+	+	+	+
<i>Candida</i> sp.	1	+	+	+	+	+	+	+	+	+
<i>Aerobacter</i> sp.	1	+	+	+	+	+	+	-	+	+
Unidentified	2	+	+	+	+	+	+	+	+	+

+ = All isolates giving positive results.  
 - = All isolates giving negative results.  
 \* = Number of isolates giving positive results.

TABLE 4  
UTILISATION OF INDIVIDUAL HYDROCARBONS BY THE ISOLATES

Group	Number of isolates	n-Pentane	n-Hexane	Cyclohexane	n-Heptane	Benzene	Xylene	Toluene	Naphthalene	Anthracene
<i>Pseudomonas</i> sp.	6	(5)*	(2)	-	(5)	(3)	(1)	(1)	+	+
<i>Vibrio</i> sp.	13	+	(3)	(6)	(12)	(12)	(7)	(5)	+	+
<i>Bacillus</i> sp.	1	+	+	+	+	+	+	+	+	+
<i>Candida</i> sp.	1	+	+	+	+	+	+	+	+	+
<i>Aerobacter</i> sp.	1	+	+	+	+	+	+	-	+	+
Unidentified	2	+	+	(1)	+	+	+	(1)	+	+

+ = All isolates giving positive results.  
 - = All isolates giving negative results.  
 \* = Number of isolates giving positive results.

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