THE ISOLATION AND CHARACTERIZATION OF HYDROCARBON UTILIZING MICROORGANISMS

THESIS

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INTRODUCTION

The microbial oxidation of hydrocarbons is a relatively new field in the science of microbiology. The fact that there is no accumulation of hydrocarbons in the form of crude oil on the surface of the earth indicates that microorganisms have the ability to remove these organic products by dissimilation, degradation, or by oxidation and utilization.

Hydrocarbon oxidation, as a subject of scientific study, provides a diverse field for microbiological endeavors, such as, taxonomical relationships of hydrocarbon-utilizing microorganisms, dissimilation mechanisms, and research in new metabolic pathways. The field of hydrocarbon oxidation is not strictly of interest to basic science; but it has, however, many potential practical applications as well. It is obvious that aspects of hydrocarbon microbiology should be closely allied with the petroleum industry. Application of knowledge gained from microbiological investigations can be used for (a) bacterial release of oil from underground oil-bearing formations, (b) bacterial ecology in prospecting for petroleum deposits, (c) the study of the decomposition of gasoline additives, and (d) the study of the formation of microbial masses at water-oil interfaces. An extremely

important application at the present time is the use of hydrocarbon-oxidizing microorganisms in the rapid and complete decomposition of large oil spills where these have occurred during marine accidents. Another even more tantalizing prospect is the use of hydrocarbons as fermentable substrates. There is nothing that could equal the possibility of a process that could convert natural gas or even fuel grade oil into animal or human feed in the form of microbial cells. In this respect hydrocarbons have several intrinsic advantages: (1) with the exception of coal and carbon dioxide, hydrocarbons are the cheapest fermentable substrates available in bulk supply, (2) a change in the molecule constitutes a weight yield bonus to the hydrocarbon in the extent to which oxygen appears in the molecule, and (3) the fact that hydrocarbons are relatively insoluble in water makes them ideal candidates for continuous fermentations employing recycling of the substrates (Foster, 1962; Span XI).

Many different types of microorganisms are capable of oxidizing hydrocarbons. In virtually every biochemical activity of microorganisms, the degree of hydrocarbon utilization is a function of two main determinative factors: (1) genus specificity (type of organisms), and (2) optimal cultural conditions for growth. The response of a particular microorganism to a specific substrate is peculiar

and unpredictable. Any hydrocarbon or organic compound can serve as a sole source of carbon and energy for a specific organism, and any of these compounds can be subjected to various chemical transformations by these microorganisms.

The utilization of hydrocarbons, especially paraffinic hydrocarbons, by a common moniliaceous mold, Botrytis cinerea, was first recognized by Myoshi (1895). The study of the consumption of methane by a pure bacterial culture (Söhngen, 1906), and by mixed cultures of soil bacteria in crude enrichment media (Kaserer, 1906) were preludes to implication of bacterial utilization of certain aliphatic alkanes. Comprehensive reviews on the isolation and characterization of hydrocarbon-utilizing microorganisms have been compiled by Zobel (1946, 1950); Beerstecher (1954); and Fuhs (1961). These early studies emphasized the identification of hydrocarbon-utilizing organisms together with their ecological distribution. More recent reviews list the organisms known to grow at the expense of certain hydrocarbons, and the extent to which each of these is able to utilize one or more of the n-alkanes or aromatic hydrocarbons (Fuhs, 1961; Lowery, Foster, and Jurtshuk, 1968; Jones and Edington, 1968; Jones, 1969; McKenna and Kallio, 1965). The hydrocarbons that microbes are capable of oxidizing range in complexity from methane

to anthracene, and include a great variety of other petroleum products such as waxes, tars, asphalts, crude oils, gasolines, kerosene, and lubricating oils (Zobell, 1959).

The organisms capable of utilizing one or more kinds of hydrocarbons now exceed two hundred species belonging to about forty genera of bacteria, actinomycetes, yeasts, and molds. The greatest number and variety of these species occur in the bacteria. Not all the organisms listed as capable of hydrocarbon-oxidation were isolated by hydrocarbon enrichment or selective culture methods (Fuhs, 1961); many of these were laboratory stock cultures originally isolated for other purposes than hydrocarbon-oxidation studies.

Different microorganisms are capable of oxidizing many different hydrocarbons; however, cells of these organisms do not necessarily grow at the expense of a particular oxidized substrate. The criterion used, therefore, to substantiate that microorganisms can grow during the oxidation of hydrocarbons becomes important. Respirometric studies, which are frequently used to measure metabolic activity and to indicate substrate degradation, cannot be used as valid criteria to show that oxidation and growth are concomitant processes. It is not unusual to encounter certain microorganisms which will grow to some extent when they are initially isolated on hydrocarbons,

but then fail later to grow after repeated exposure to the same hydrocarbon substrate or to other n-alkane hydrocarbons. This emphasizes the fact organisms require repeated exposure to the same or different hydrocarbons in order to demonstrate that they retain a continued ability to utilize these substrates. It is important to ascertain whether organisms, when first placed in a hydrocarbon environment, oxidize the substrate with concomitant growth, or that resultant growth is due to endogenous metabolism.

The dominant organisms listed as hydrocarbon-utilizers belong to the following genera: Streptomyces, Nocardia, Mycobacterium, Corynebacterium, and Brevibacterium (Foster, 1962; Kester, 1961). Organisms isolated less frequently are Pseudomonas, Flavobacterium, Achromobacter, and Azotobacter (Foster, 1962; Kester, 1961; Leadbetter and Foster, 1958; Coty, 1967). Organisms that are capable of utilizing some form of hydrocarbon, but which were originally isolated from non-hydrocarbon substrates include Alcaligenes, Bacillus, Brevibacterium, Corynebacterium, Flavobacterium, Micrococcus, Pseudomonas, Mycobacterium, Nocardia, Mycococcus, Bacterium, and Vibrio (Fuhs, 1961; Kester, 1961; Kester and Foster, 1963). The number of fungi capable of utilizing hydrocarbons as their sole carbon source is relatively small (Coty, 1967); in

most instances these fungi could not utilize for growth any alkanes with a chain length smaller than nine carbons (Lowery, Foster, and Jurtshuk, 1968; Markovets and Kallio, 1964; Scheda and Bos, 1966). The most active molds listed as hydrocarbon utilizers are Penicillium and Aspergillus (Zobell, 1959); however, other molds and yeasts have been studied for their ability to grow on these substrates (Jones and Edington, 1968; Foster, 1962; Kester, 1961).

Hydrocarbon-utilizing microorganisms have been isolated routinely from various sources in nature. The primary source is usually from soils collected in oil fields where repeated oil spillage has occurred, or from soils around oil wells or sumps that have been in contact with petroleum for long periods of time. These conditions provide for a modification in the usual soil microbial population in which a natural selection occurs for those organisms capable of oxidizing hydrocarbons. These hydrocarbon-utilizing organisms become an indigenous flora which exist in the soil, and are physically active in hydrocarbon oxidation. It is possible to isolate soil microorganisms that have never had prior exposure to hydrocarbons but which are capable of oxidizing hydrocarbon substrates. Microorganisms that have the ability to utilize hydrocarbons, however, are readily obtained from almost any soil. The

different types of organisms and the numbers of each of these isolated from a single soil sample depend on the particular hydrocarbon used for isolation, i.e. the hydrocarbon which a particular organism may use for growth is fairly selective. In the biology of microorganisms, an increase in the concentration of any single substrate will result in an increased population of organisms able to consume that substrate. This phenomenon is recognized as a "natural enrichment"; therefore, where hydrocarbons might exist in abnormal amounts, increased numbers of hydrocarbon-utilizing microorganisms can be expected to occur in the population (Foster, 1962).

Hydrocarbon utilizers are important in both the agriculture and petroleum industries. Evidence from field studies has shown that agricultural crops can be stimulated by petroleum residues. The nitrogen and organic carbon content of soils increase in areas where gas seepage occurs or hydrocarbons are in contact with the soil. Two mechanisms seem to be involved in the accumulation of nitrogen and organic carbon in soils exposed to hydrocarbons: (1) the hydrocarbon is utilized by bacteria capable of fixing atmospheric nitrogen which later results in an increase in organic carbon and nitrogen in the soil, and (2) the hydrocarbon is utilized by microorganisms incapable of fixing nitrogen; but the hydrocarbon, converted

into cells, eventually becomes available to other soil organisms which are capable of fixing atmospheric nitrogen (Davis, Coty, Stanley, 1964). Organisms which are capable of utilizing hydrocarbons and, at the same time, can fix atmospheric nitrogen include Pseudomonas, Mycobacterium, and Azotobacter (Davis, Coty, Stanley, 1964; Coty, 1967). These organisms are capable of storing lipid material as poly-β- hydroxybutyric acid. The incidence of this polymer in the hydrocarbon nitrogen-fixing bacteria follows the pattern similar to that found in non-nitrogen-fixing hydrocarbon utilizing bacteria, i.e., as the chain length of the alkane increases, the amount of polymer decreases (Coty, 1967).

The experimental approach to hydrocarbon studies and the technical difficulties in methodology are not common in studies with most other substances. Gaseous hydrocarbons in relatively large amounts in closed systems must be supplied to the organisms to insure the availability of sufficient substrate to carry out metabolic activities. Impurities often found in these gaseous substrates can be magnified quantitatively, since even a very small concentration of impurity can, with respect to the total amount, represent a considerable amount of oxidizable substrate. If the organism can utilize the impurity and the main substrate component, then misleading results might be obtained

unless critical evaluations are made of the criterion of bacterial growth (Kester, 1961).

Solubility of all of the hydrocarbons in the liquid culture medium is also of considerable technical importance since the availability of the hydrocarbon may become a limiting factor. In this respect, particularly with long-chain hydrocarbons, the organisms can oxidize some of the hydrocarbons to the homologous fatty acids that results in a soap which creates a hydrocarbon-water emulsion. A large surface area interface is thereby achieved between the hydrocarbon and liquid medium (Kester, 1961).

Information concerning the growth of hydrocarbon-oxidizing bacteria on various pure alkanes is limited, especially for Mycobacterium and Corynebacterium, and other closely related microorganisms such as Nocardia and Streptomyces. The variation in the morphology of these organisms as a result of cultivation on different alkanes, and the extent to which each organism is able to grow is significant for establishing a possible taxonomic relationship. A few investigations have used a relatively limited number of hydrocarbons to study their ability to support growth of single species.

The present study is concerned with the isolation, cultivation, and identification of microorganisms associated

with n-alkane oxidation. Selected hydrocarbons such as methane, butane, and octane are used as the sole carbon source for the initial isolation of all microbiota. Each isolate is studied on pure hydrocarbons ranging in complexity from methane to octane, decane, dodecane, and octadecane. The extent to which each of these microorganisms is capable of utilizing a particular alkane as a sole source of carbon and energy for growth is investigated. The isolated organisms are compared both culturally and morphologically on various hydrocarbons, and classified into their respective generic groups.

MATERIALS AND METHODS

In this investigation the microorganisms isolated for study were cultured from soil samples collected from different oil fields in Texas and Oklahoma; from an oil spillage near a San Marcos, Texas, service station; and from an ungrazed pasture near San Marcos, Another source of material for study was a water-oil mixture obtained from the Houston, Texas, ship channel. The number of samples studied from each location is shown:

Oil field location	Number of samples studied
Luling, Texas	13
Victoria, Texas	7
West Texas (Ozona-Bakersfield)	13
Cox-Penn, near Davis Mts, Okla.	10
Other Sources	
Service Station, San Marcos	1
Ungrazed pasture, San Marcos	1
Water-oil mixture, Houston	1

The soil samples were uniformly collected 5 cm below the surface of the ground. Each sample was placed in a sterile collecting tin and refrigerated at 5 C until laboratory investigation was begun. The water-oil mixture was collected 5 cm below the water surface in a sterile dilution bottle and refrigerated until experiments were initiated.

The basal salts medium used for all isolations and characterization procedures was "L" Medium of Leadbetter and Foster (1958). The medium had the following composition:

"L" Medium

$NaNO_3$	2.0	g.
$MgSO_4 \cdot 7H_2O$	0.2	g.
KCl	0.04	g.
$CaCl_2 \cdot 2H_2O$	0.02	g.
Na_2HPO_4	0.210	g.
KH_2PO_4	0.09	g.
$FeSO_4 \cdot 7H_2O$	0.001	g.
$CuSO_4 \cdot 5H_2O$	5.0	g.
$Na_2B_4O_7 \cdot 10H_2O$	13.0	µg.
$Mn\ SO_4 \cdot H_2O$	7.0	µg.
$ZnSO_4 \cdot 7H_2O$	70.0	µg.
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	12.0	µg.
Distilled water	1000.0	ml.

pH 7.0

For the preparation of semi-solid medium, 2.0% of water-washed agar was added to the "L" Medium solution. For each soil sample studied, methane, butane, and octane were used individually in enrichment cultures as the sole

source of carbon and energy for the primary isolations of hydrocarbon-utilizing microorganisms. The aliphatic n-alkanes (C_1 to C_4) were purchased from Matheson Gas Products; (C_5 to C_8) from Eastman Chemical Co.; (C_{10}, C_{12}, and C_{18}) from J. T. Baker Chemical Co., and were 99 mol per cent minimum purity.

Cultural Procedures

For the isolation of hydrocarbon-utilizers, 100 ml of "L" Medium was placed in 500 ml Erlenmeyer flasks. When volatile liquid hydrocarbons were used as a substrate, each flask was plugged with a single-hole rubber stopper fitted with a hollow, long-stem glass bulb. A small orifice, placed in the glass stem 2 cm above the bulb, permitted liquid hydrocarbons to volatilize into the environment and mix with the liquid medium. The hollow stem which extended to the exterior of the flask was loosely plugged with cotton which allowed aeration during incubation. The stoppered flasks and broth medium were sterilized together. Following sterilization, the hydrocarbon of choice was placed aseptically in the bulb through the orifice and, at this time, each flask was inoculated with 10 g of soil sample. The flasks were placed on a reciprocal shaker (length of stroke 14 cm; 72 strokes/min.)

and incubated at 25 C.

When gaseous alkanes were used as the hydrocarbon source in culturing organisms, 500 ml side-arm filter flasks were employed as culture vessels. The flasks were closed with rubber stoppers, and the side-arms fitted with rubber tubing and pinch clamps (the rubber tubing was plugged with sterile cotton and used as filters), which permitted the flasks to be air evacuated and refilled with the isolating gaseous hydrocarbon. The flasks containing 100 ml of "L" Medium were sterilized, inoculated with 10 g soil sample, and the air evacuated to a negative atmospheric pressure of 50 cm Hg. A ratio of 70% hydrocarbon-30% air mixture replaced the evacuated air as the carbon substrate. The inoculated flasks were placed on a reciprocal shaker and incubated at 25C. The water-oil mixture was studied in the same manner as the soil samples with the exception that 10 ml of oil-water mixture was placed in each of the culture flasks.

Turbid growth was usually observed after 3 - 4 weeks incubation. At this time 10 ml of the turbid growth material was transferred to fresh "L" Medium and isolating hydrocarbon. After turbid growth reappeared in the culture flask, 10 ml inoculum from this flask was transferred again to fresh medium and hydrocarbon. This procedure was

repeated a total of 3 times for each soil sample, and for each of the 3 isolating hydrocarbons-methane, butane, and octane.

Following final broth enrichment culture, the turbid growth material was streaked to "L" Medium agar plates, and the streak plates then incubated at 25 C in closed culture chambers containing the same hydrocarbon used in the previous enrichment culture. The developing colonies were streaked several times to fresh agar surfaces until pure cultures of each type of organism were isolated. When gaseous hydrocarbons were used as the carbon source, the culture chambers (desiccators) used in these experiments were evacuated to a negative pressure of 50 cm of Hg. A ratio of 70% hydrocarbon - 30 % air mixture replaced the evacuated air as the carbon substrate. In those procedures where liquid hydrocarbons were used as the carbon source, an open receptacle containing 5 ml of the hydrocarbon was placed in the culture chamber, and a negative atmospheric pressure of 30 cm of Hg drawn on the system. This negative pressure permitted the rapid volatilization of the hydrocarbon. After 24 hours the culture chamber was refilled with filtered air to normal atmospheric pressure.

Another technique was introduced in this study to isolate hydrocarbon-utilizers. This method involved sprinkling small quantities of soil samples over the entire

surfaces of "L" Medium agar plates. The plates were placed in atmospheres of selected hydrocarbons and incubated at 25 C. The "sprinkle plate method" is considered to have an advantage over the enrichment culture method (Foster, 1962; Kester, 1961), since there is almost no growth competition between organisms on these plates.

Some microorganisms have been found in soils which have the ability to convert concomitantly hydrocarbon and atmospheric nitrogen into cellular substance. This investigation was carried out on combined samples taken from the Luling soils to determine the prevalence of hydrocarbon-oxidizing microorganisms capable also of fixing atmospheric nitrogen. Luling soils were placed in nitrogen-free broth medium in the previously described manner in which octane was used as the sole carbon source. The nitrogen-free medium has the following composition:

Nitrogen-free Medium

$Na_2MoO_4 \cdot 2H_2O$	50.0	µg.
$CaSO_4 \cdot 2H_2O$	0.2	g.
$FeSO_4 \cdot 7H_2O$	0.006	g.
NaCl	0.01	g.
$SrCl_2$	0.01	g.
$MgSO_4 \cdot 7H_2O$	0.2	g.
KH_2PO_4	0.011	g.

Na_2HPO_4	0.189	g.
$NaHCO_3$	0.05	g.
Sucrose	5.0	g.
Distilled water	1000.0	ml.

pH 7.6

Comparative Growth on all Test Hydrocarbons

The different microorganisms obtained from all of the soil samples which had been grown on each of the 3 different isolating hydrocarbons (methane, butane, and octane) were compared for their ability to utilize others in the n-alkane series: methane, ethane, propane, butane, pentane, hexane, heptane, octane, decane, dodecane, and octadecane. This was accomplished by streaking duplicate "L" Medium agar plates with the individual organisms, and placing them with the appropriate gaseous or volatile hydrocarbon. When the long chain, less volatile, alkanes (decane, dodecane, and octadecane) were used, 0.5 ml of hydrocarbon was placed at the base of agar slant culture tubes previously inoculated with the test organism.

The amount of growth was compared for each organism on each hydrocarbon tested and the amounts recorded in the following manner:

4 = profuse growth
3 = moderate growth
2 = poor growth
1 = indefinite growth
0 = no growth

Morphology and Biochemical Studies

The morphology, cell size, Gram stain, colony characteristics, and pigmentation were studied for each isolate. These determinations were made not only on each hydrocarbon on which the organism was grown, but also on conventional Plain Nutrient Agar (DIFCO) medium. Cell morphology and size were determined microscopically using the wet mount technique and observation of the prepared Gram stain.

Standard bacteriological methods were used to assist in classifying each organism in regard to its taxonomical grouping. The biochemical tests included reactions on the following substrates: sucrose, glucose, fructose, xylose, galactose, indole, nitrate, sulfide production, gelatin, and litmus milk (Bergey's Manual, 1957; Skerman, 1967; Manual of Microb. Methods, 1957).

RESULTS

In this study, hydrocarbon-utilizing organisms were isolated from 43 separate soil samples collected from four different oil fields, and from three other locations not directly associated with oil production. A single sample of seawater-oil mixture was also included in this investigation in order to illucidate the organisms associated with hydrocarbon degradation from a marine environment.

The bacterial cultures were identified using standard bacteriological methods, and classified according to diagnostic features presented in the 7th edition of Bergey's Manual (1957). Six different sugars (glucose, sucrose, lactose, galactose, fructose, and ribose) were used routinely as diagnostic tests in the preliminary identification of these organisms. Not any of the 177 hydrocarbon-utilizing bacterial isolates were able to ferment these carbohydrates. In almost all of the tests employed only alkaline reactions were observed. Hydrogen sulfide and indole were not produced by any of the isolates. The production of nitrite from nitrate could not be used as a test to separate the different genera, since species reaction proved to be quite variable. Litmus milk remained unchanged for all of the organisms except for the different species of Pseudomonas. This genus invariably produced an alkaline-reduction litmus

reaction which was used as an identifying biochemical test for this organism. Gelatin was liquified by Pseudomonas only. This particular test was also used subsequently to separate Pseudomonas from other hydrocarbon-utilizers. In addition to these characteristic features, the pseudomonads usually produced water-soluble pigments, and were motile in young broth cultures.

The different species of Streptomyces were identified by their typical aerial mycelia and conidiospore production. This genus produced surface pellicles when grown in broth cultures, and characteristically formed large spreading colonies on agar surfaces.

Mycobacterium and Corynebacterium are culturally similar and difficult to separate into their respective genus. Differentiation and identification of Corynebacterium was based principally on characteristic pleomorphic cells which had clubbed ends, irregular staining, and palisade or Chinese-letter arrangement of cells. Species of Mycobacterium did not show these morphological features or cellular arrangement.

A. Genera of Hydrocarbon-Utilizers Isolated from Methane Enrichment Cultures.

The different genera or microorganisms isolated from

methane enrichment cultures are listed in Tables I, II, III, and IV. The soil samples used in these experiments were obtained from Victoria, Luling, West Texas, and Oklahoma oil fields, respectively. A total number of 24 organisms, representing four different genera, was isolated from all of the soil samples tested. These hydrocarbon-utilizers included 7 Pseudomonas spp., 15 Corynebacterium spp., 1 Mycobacterium sp., and 1 Streptomyces sp.

Comparison of the Growth of Methane Isolates on Various n-Alkanes.

The 24 methane isolates from different soils were cultured on different individual hydrocarbons (C_1-C_8, C_{10}, C_{12}, and C_{18}) in order to compare their ability to utilize other alkanes as carbon substrates for growth. Of the seven Pseudomonas spp. isolated, five of these could use methane only as their sole source of carbon, while the other two Pseudomonas isolates were able to grow on other hydrocarbons in varying amounts. The Pseudomonas spp. capable of growth only on the isolating hydrocarbon appear to be obligate methane utilizers.

In general, the 15 Corynebacterium spp. exhibited moderate to profuse growth on other hydrocarbons than

methane; the different isolates in this genus exhibited different substrate utilization patterns. The single isolates of Mycobacterium and Streptomyces exhibited distinct preferences for hydrocarbons C_1-C_{12}, but were unable to grow on the long chain hydrocarbon, C_{18}.

B. Genera of Hydrocarbon-Utilizers Isolated from Butane Enrichment Cultures.

In this study, butane enrichment cultures were used to isolate hydrocarbon-utilizers from oil field soils listed previously. The different genera isolated are shown in Tables V, VI, VII, and VIII. A total number of 45 organisms representing four different genera was isolated from the soil samples tested. The four genera are represented by 4 Pseudomonas spp., 25 Corynebacterium spp., 12 Mycobacterium spp., and 4 Streptomyces spp. When butane was used as the isolating alkane, a larger number of hydrocarbon-utilizers was obtained than when methane was used. It is readily apparent that butane is much more susceptible to utilization by these four different genera.

Comparison of the Growth of Butane Isolates on Various n-Alkanes.

The 45 butane isolates, representing 4 genera, were

cultured on the 11 different hydrocarbons listed. This study was carried out to show the ability of these organisms to utilize other alkanes than the hydrocarbon from which they were originally isolated. In this investigation not any of the different Pseudomonas spp. were able to utilize methane for growth when originally isolated from a butane enrichment. The other alkanes in the series were utilized in varying amounts for growth, but the three species of Pseudomonas exhibited different substrate utilization patterns. It is particularly interesting that Pseudomonas sp. ($L_{12}AC_4$) was able to grow only on even numbered alkanes.

The 25 Corynebacterium isolates were not able to use methane for growth; but with an increase in the carbon chain, C_2 through C_{12}, relatively good growth occurred. In general, most of the Corynebacterium grow best on hydrocarbons having a chain length C_4 through C_8. The fact that these Corynebacterium spp. were isolated from different oil field soils did not reflect differences in their hydrocarbon utilization patterns.

Of the 12 Mycobacterium and 4 Streptomyces isolated, not any of these were able to grow on methane. These two genera appear to be similar in their ability to grow on a specific range of hydrocarbons, i.e. the alkanes above butane and pentane.

C. Genera of Hydrocarbon-Utilizers Isolated from Octane Enrichment Cultures.

Tables IX, X, XI, and XII show the organisms isolated from octane enrichment cultures. The oil field soils used here are identical to the soils used for methane and butane enrichment cultures. From these octane enrichment cultures, a total number of 82 organisms was isolated and classified into six different genera. These genera are represented by 36 Pseudomonas spp., 25 Corynebacterium spp., 16 Mycobacterium spp., 3 Streptomyces spp., and 2 molds - Phoma and Cephalosporium. Almost four times as many organisms were isolated from octane enrichment cultures as were isolated on methane; while only twice as many were isolated on octane as were isolated on butane.

Comparison of the Growth of Octane Isolates on Various n-Alkanes.

With the exception of two Corynebacterium spp. isolated from Oklahoma soil ($P2AC_8$ and $P3A2C_8$), and a single Streptomyces ($VS3CC_8$) from Victoria soil, not any of the other 79 isolates were able to grow on methane. In general, the 36 Pseudomonas spp. had limited growth, or none at all, on the gaseous hydrocarbons C_1 through C_4, and grew only moderately well on C_5. It is apparent that all

of the Pseudomonas spp. were able to grow moderate to profuse on alkanes ranging from C_6 - C_{12}; however, the longer-chain C_{18} alkane did not support growth.

The 25 Corynebacterium spp. utilized the same hydrocarbons for growth as Pseudomonas. Most of these isolates exhibited either poor growth or none at all on C_1 - C_4 gaseous alkanes; however, all of these were capable of profuse growth on the longer chain-length hydrocarbons C_5 - C_{12}. The results show that most of the Corynebacterium spp. were capable of moderate growth on C_{18}; whereas, other genera isolated in these studies were unable to use C_{18} as a substrate. Of particular interest is that three of these organisms (L8BC_8, P2AC_8, P3A2C_8) were able to grow consistently on C_1 and other gaseous hydrocarbons (C_2 - C_4), as well as on the less volatile hydrocarbons tested.

The 16 Mycobacterium spp. and 3 Streptomyces spp., and their ability to use individual hydrocarbons, compared favorably with earlier observations. These genera could not use the alkanes below C_4 or C_5, but grew profusely on hydrocarbons C_5 - C_{12}. A single species of Streptomyces (VS3CC_8) isolated from Victoria soils was capable of growth on not only C_1 and other gaseous hydrocarbons, but also on the larger alkanes.

Two different molds, Phoma (VS1AC_8), and Cephalosporium

($V6EC_8$), were isolated from Victoria soils. Although neither of the two genera would grow to any extent on butane both could utilize C_6 - C_{12}. The fact that both organisms could be cultured repeatedly on these hydrocarbons indicates that this ability to oxidize hydrocarbons for growth is not transient.

D. Hydrocarbon-Utilizers Isolated on Various n-Alkanes from Anomalous Sources.

The different genera shown in Table XIII were isolated from the following sources:

Source	Code Prefix	Code Suffix (isolating hydrocarbon)
Houston ship channel (oil-water mixture)	HSC	C_8
Virgin pasture soil	VIRG	C_8
Soil from garage oil spillage area	Phil & LT	C_3
Laboratory isolates	L	C_8
	RAM	C_3

The Pseudomonas spp. from the marine oil-water mixture, isolated from a C_8 enrichment medium, were able to utilize C_4 through C_{12}. The pre-exposure to petroleum of the organisms in this sample material did not enhance their

ability to grow on a wider range of hydrocarbons.

The Mycobacterium spp., which had their origins from different ecological sources (see code) generally exhibited similar amounts of growth on C_4 - C_{12} hydrocarbons, with almost no growth occurring on C_3 and the shorter-chain alkanes. Two Mycobacterium (Phil and LT), isolated originally from propane enrichment, continued to use this alkane in later growth tests. Other Mycobacterium isolated from C_8 enrichment did not exhibit growth on propane substrate.

A single isolate of Streptomyces sp. (RAM101C_3), isolated from propane enrichment, grew well on all hydrocarbons tested in the series.

E. Growth on Various Hydrocarbons by Stock Cultures of Bacteria.

The laboratory stock cultures listed in Table XIV were originally isolated in 1960 from different hydrocarbon enrichments, and have been maintained in the laboratory on various nutrient media. A total number of 16 Mycobacterium spp. and 3 Corynebacterium spp. was tested for growth on 11 n-alkanes used in these experiments.

Of the 16 Mycobacterium spp., at least half were capable of growth not only on the gaseous hydrocarbons

C_1 - C_4, but on the higher alkanes as well. Almost all of these organisms could utilize for growth at least C_4 or C_5 through C_{12}. These results confirm data obtained earlier in this study. In almost all cases in which methane is utilized for growth by an organism, all alkanes from C_1 - C_{12} could also be used for growth.

The three Corynebacterium spp. did not reflect any differences in their hydrocarbon utilization pattern from other Corynebacterium tested in these studies. All of the isolates, as anticipated, grew best on hydrocarbons ranging from C_4 - C_{12}.

F. Comparison of Morphological Features and Culture Characteristics of Various Hydrocarbon-Utilizers on Different Hydrocarbons and Plain Nutrient Media Isolated on Methane.

In Part A, Tables XV, XVI, XVII, and XVIII, are listed organisms isolated from methane enrichment cultures from Victoria, Luling, West Texas, and Oklahoma soils, respectively. The Gram reaction and cell size (μm) is indicated for each organism obtained from the stained mount.

In addition, wet-mount measurements are listed for organisms grown on methane, butane, and octane hydrocarbons. A comparison is made of the cell sizes for each organism

on these different substrates. The characteristic colony size and color were obtained from growth on agar surfaces using the selected hydrocarbons as the carbon source.

In Part B, Tables XV, XVI, XVII, and XVIII are listed the methane isolates from all soil samples used in these experiments (corresponding to organisms listed in Part A above). The Gram reactions, cell sizes, and colony characteristics are shown for these same organisms cultured on Plain Nutrient Agar medium.

The following features and characteristics were observed for all of the isolates tested:

(1) the Gram reaction remained the same for a single species whether it was cultured on hydrocarbon substrates or Plain Nutrient medium.

(2) differences existed in the cellular size and morphology for a single species when grown on Plain Nutrient or hydrocarbon substrates. There was, however, a variation in the size of each organism when grown on different hydrocarbons.

G. Comparison of Morphological Features and Culture Characteristics of Various Hydrocarbon-Utilizers on Different Hydrocarbons and Plain Nutrient Media Isolated on Butane.

In Part A, Tables XIX, XX, XXI, and XXII are listed the different organisms isolated on butane enrichment cultures from the four soil samples used in other enrichment cultures in this study.

The Gram reaction, cell sizes, and colony characteristics are shown for each isolate when cultured on methane, butane, and octane carbon sources.

In Part B, the morphological features, Gram reaction, and colony characteristics are shown for the butane isolates when grown on Plain Nutrient Agar medium.

H. Comparison of Morphological Features and Culture Characteristics of Various Hydrocarbon-Utilizers on Different Hydrocarbons and Plain Nutrient Media Isolated on Octane.

In Part A, Tables XXIII, XXIV, XXV, and XXVI the morphological, cultural, and Gram reactions were determined for each of the organisms isolated on octane enrichment, and when grown on methane, butane, and octane culture media.

In Part B, the Gram reaction, cell sizes, and colony characteristics are listed for each organism isolated on octane enrichment and cultured on Plain Nutrient Medium.

I. Comparison of Morphological Features and Cultural Characteristics of Hydrocarbon-Utilizers Isolated on Various Alkanes and Cultured on Methane, Butane, and Octane Hydrocarbons and Plain Nutrient Agar Media.

In Part A, Table XXVII, these cultures were isolated originally on different alkanes and other isolation media (See Table XIII) from anomalous sources. These cultures were studied for their Gram reaction, cell size, and colony characteristics on methane, butane, and octane.

In Part B, the isolates listed were grown on Plain Nutrient Agar medium, and the different Gram reaction, morphological features, and colony characteristics indicated.

J. Comparison of Morphological Features and Culture Characteristics of Laboratory Stock Cultures Originally Isolated on Hydrocarbon Media.

In Part A, Table XXVIII, the organisms listed were originally isolated from different hydrocarbon enrichments (See Section E, Results). These stock cultures were grown

on methane, butane, and octane as the carbon source, and their Gram reaction, cell size, and colony characteristics compared.

In Part B, the organisms are described after they have been cultured on Plain Nutrient Agar medium.

TABLE I. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON METHANE FROM VICTORIA SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	V1&3BC$_1$	4	0	0	0	0	0	0	0	0	0	0
Pseudomonas	V1DC$_1$	4	0	0	0	0	0	0	0	0	0	0
Pseudomonas	V2AC$_1$	4	0	0	0	0	0	0	0	0	0	0
Pseudomonas	V5BC$_1$	3	2	2	4	4	4	4	4	4	3	2
Corynebacterium	V4BC$_1$	1	2	2	4	4	4	4	4	4	4	2
Corynebacterium	V5AC$_1$	2	4	4	4	4	4	4	4	4	4	2

TABLE II. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON METHANE FROM LULING SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	$L8EC_1$	4	0	0	0	0	0	0	0	0	0	0
Corynebacterium	L1C1	2	2	3	3	3	4	4	4	4	1	1
Corynebacterium	$L7C_1$	4	2	3	2	3	4	4	3	3	1	0
Streptomyces	$L2AC_1$	3	3	3	3	3	3	4	4	4	3	1

TABLE III. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON METHANE FROM WEST TEXAS SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Corynebacterium	W1,2,3,6, 11, 12C_1	1	4	4	4	4	4	4	4	4	4	4
Corynebacterium	W7&L2C_1	3	4	4	4	4	4	4	4	4	4	4

TABLE IV. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON METHANE FROM OKLAHOMA SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	$P9C_1$	4	4	4	4	4	4	4	4	4	4	1
Mycobacterium	$P10C_1$	2	4	3	4	4	4	4	3	4	3	1
Corynebacterium	$P1AC_1$	3	4	4	4	4	4	3	3	3	3	3
Corynebacterium	$P1BC_1$	3	4	1	1	4	4	4	4	4	4	2
Corynebacterium	$P4AC_1$	3	4	2	2	3	4	4	4	4	4	1

TABLE V. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON BUTANE FROM VICTORIA SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Mycobacterium	V3B&4AC_4	0	1	1	2	2	4	4	4	2	3	2
Mycobacterium	V5BC_4	1	2	3	3	4	4	4	4	4	4	3
Corynebacterium	V1&3AC_4	1	3	4	4	4	4	4	4	4	4	4
Corynebacterium	V2&6AC_4	1	3	3	4	4	4	2	1	1	2	1

TABLE VI. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON BUTANE FROM LULING SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	L1BC_4	2	3	3	4	4	3	3	2	2	2	2
Pseudomonas	L12AC_4	0	4	0	4	0	4	0	4	4	4	4
Corynebacterium	L2AC_4	1	2	2	3	4	4	4	4	4	4	3
Corynebacterium	L2C&7C_4	1	4	4	4	4	4	4	4	4	4	4
Corynebacterium	L3BC_4	0	3	3	3	4	3	3	3	3	2	0
Corynebacterium	L5C_4	1	2	3	4	3	3	3	3	3	3	3
Streptomyces	L3AC_4	1	2	3	4	4	4	4	4	4	4	4

TABLE VII. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON BUTANE FROM WEST TEXAS SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	W10&12SC$_4$	0	3	4	4	4	3	3	3	2	2	2
Mycobacterium	W7A&10CC$_4$	1	1	1	3	4	4	4	4	4	4	4
Mycobacterium	W7BC$_4$	0	1	1	2	3	4	4	4	4	4	3
Mycobacterium	W7,10,12, 13C$_4$	1	2	2	3	4	4	4	4	4	4	2
Mycobacterium	W11AC$_4$	1	3	3	4	4	4	4	4	4	4	4
Corynebacterium	W2,4,5C$_4$	1	1	1	2	3	3	4	4	4	4	2
Corynebacterium	W6AC$_4$	1	4	4	4	4	4	4	4	4	4	3
Corynebacterium	W6BC$_4$	1	2	3	4	4	4	4	4	4	4	3
Corynebacterium	W6CC$_4$	2	4	4	4	4	4	4	4	4	3	2
Corynebacterium	W9BC$_4$	2	2	2	4	4	4	4	4	3	2	2
Corynebacterium	W11BC$_4$	1	4	4	4	4	4	4	4	4	4	4

Table VII. (Continued)

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Streptomyces	$W9AC_4$	0	3	3	4	4	4	4	4	4	4	3

TABLE VIII. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON BUTANE FROM OKLAHOMA SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Mycobacterium	$P4C_4$	2	4	4	4	4	4	4	4	4	4	1
Corynebacterium	$P1AC_4$	3	3	3	4	2	2	2	2	2	2	2
Corynebacterium	$P1BC_4$	2	2	2	4	4	4	2	2	2	2	2
Corynebacterium	$P1CC_4$	3	4	4	4	4	4	4	4	4	4	3
Corynebacterium	$P2AC_4$	1	2	2	4	4	2	2	2	2	2	1
Corynebacterium	$P2BC_4$	1	3	4	4	3	3	2	2	2	2	2
Corynebacterium	$P3AC_4$	2	3	3	3	4	4	4	4	4	4	2
Corynebacterium	$P5AC_4$	2	4	4	4	4	4	4	4	4	4	1
Corynebacterium	$P9C_4$	3	3	3	4	3	1	1	3	2	2	2
Streptomyces	$P3BC_4$	3	3	3	2	2	2	2	1	2	4	1
Streptomyces	$P5BC_4$	1	4	4	4	4	4	4	4	4	4	1

TABLE IX. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON OCTANE FROM VICTORIA SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	$V4AC_8$	1	1	1	1	3	4	4	4	4	4	3
Pseudomonas	$V4CC_8$	0	1	1	2	2	4	4	4	4	4	2
Pseudomonas	$V5AC_8$	1	1	2	2	4	4	4	4	4	4	2
Pseudomonas	$V5BC_8$	1	1	1	1	1	1	4	4	4	4	1
Pseudomonas	$V6BC_8$	1	1	1	1	2	4	4	4	4	4	2
Mycobacterium	$VS1\&3BC_8$	0	0	0	2	4	4	4	4	4	4	2
Mycobacterium	$VS1CC_8$	1	1	1	1	4	4	4	4	4	4	3
Corynebacterium	$V1AC_8$	0	1	1	2	4	4	4	4	4	4	3
Corynebacterium	$V5CC_8$	0	1	2	2	2	4	4	4	4	4	3
Corynebacterium	$V7AC_8$	1	1	1	2	3	4	4	4	4	4	3

Table IX. (Continued)

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Streptomyces	$VS3CC_8$	3	3	3	3	4	4	4	4	2	2	2
Phoma	$VS1AC_8$	0	0	1	3	4	4	4	4	4	4	2
Cephalosporium	$V6EC_8$	0	1	1	2	1	3	3	4	4	4	1

TABLE X. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON OCTANE FROM LULING SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	LNF4C_8	1	2	2	2	2	4	4	4	3	2	2
Pseudomonas	LNF5C_8	1	1	1	1	1	2	2	4	4	2	1
Pseudomonas	L2DC_8	1	2	2	3	4	4	4	4	4	3	0
Pseudomonas	L3B&5AC_8	0	0	1	2	4	4	4	4	4	2	1
Pseudomonas	L7BC_8	0	0	0	2	3	4	4	4	4	4	0
Pseudomonas	L7CC_8	0	0	0	0	2	3	4	4	3	3	0
Pseudomonas	L8AC_8	0	0	0	0	1	3	4	4	4	4	3
Pseudomonas	L12C_8	1	2	3	4	4	4	4	4	4	3	2
Pseudomonas	L13C_8	1	2	2	2	3	4	4	4	4	3	3
Mycobacterium	L9DC_8	0	1	1	3	4	4	4	4	4	4	3
Mycobacterium	L11BC_8	0	2	2	2	3	3	4	4	4	3	0
Mycobacterium	L11CC_8	0	1	1	2	3	4	4	4	4	4	0

Table X. (Continued)

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Corynebacterium	$L2CC_8$	2	2	3	4	4	4	4	4	4	3	1
Corynebacterium	$L7AC_8$	1	2	3	3	4	4	4	4	4	4	3
Corynebacterium	$L7DC_8$	1	1	2	2	4	4	4	4	4	4	2
Corynebacterium	$L7EC_8$	0	1	2	3	4	4	4	4	4	4	4
Corynebacterium	$L8BC_8$	3	3	4	4	3	3	3	3	3	3	3
Corynebacterium	$L8CC_8$	1	1	2	2	3	4	4	4	4	4	1
Corynebacterium	$L9CC_8$	0	1	1	1	4	4	4	4	4	4	4
Corynebacterium	$L10AC_8$	0	0	0	3	4	4	4	4	4	4	3
Corynebacterium	$L10EC_8$	0	1	1	1	1	3	4	4	4	3	0
Corynebacterium	$L11DC_8$	0	0	0	0	0	4	4	4	4	4	1

TABLE XI. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON OCTANE FROM WEST TEXAS SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	W1AC_8	0	1	1	1	1	2	3	4	4	4	1
Pseudomonas	W1BC_8	0	1	2	2	3	3	4	4	4	2	1
Pseudomonas	W2C1C_8	1	2	2	3	4	4	4	4	4	4	2
Pseudomonas	W5AC_8	0	0	0	0	3	4	4	4	3	2	0
Pseudomonas	W5CC_8	1	2	2	2	3	4	4	4	4	4	2
Pseudomonas	W7&11C_8	1	1	1	2	2	3	3	3	3	3	1
Pseudomonas	W7CC_8	0	0	0	2	3	4	4	4	4	4	0
Pseudomonas	W8AC_8	0	0	0	3	4	4	4	4	4	2	0
Pseudomonas	W9A&10DC_8	0	0	1	2	4	4	4	4	4	3	1
Pseudomonas	W9&10C_8	1	2	2	2	4	4	4	4	4	3	1
Pseudomonas	W9BC_8	2	2	2	2	3	4	4	4	4	4	1
Pseudomonas	W10EC_8	0	0	0	2	4	4	4	4	3	1	1
Pseudomonas	W12EC_8	0	0	2	2	4	4	4	4	4	4	0

Table XI. (Continued)

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	WS13CC_8	0	0	0	0	3	3	4	4	2	2	1
Mycobacterium	W4BC_8	1	1	1	3	3	4	4	4	4	4	3
Mycobacterium	W10A&S13C_8	0	0	1	3	3	4	4	4	4	4	3
Mycobacterium	W11EC_8	0	0	0	3	3	4	4	4	4	4	1
Corynebacterium	W2C2C_8	1	1	2	2	2	4	4	4	4	4	3
Corynebacterium	W4AC_8	1	1	1	2	4	4	4	4	4	4	3
Corynebacterium	W4CC_8	1	2	4	4	4	4	4	4	4	4	3
Corynebacterium	W5BC_8	1	1	1	1	2	3	4	4	4	4	3
Corynebacterium	W6BC_8	1	2	2	2	2	4	4	4	4	4	3
Corynebacterium	W12AC_8	1	1	1	2	4	4	4	4	4	4	3
Corynebacterium	WS13B3C_8	1	1	2	3	4	4	4	4	4	4	2
Corynebacterium	WS13JC_8	0	0	1	1	1	4	4	4	4	4	3
Streptomyces	W10B1C_8	1	1	1	2	2	4	4	4	4	4	2

TABLE XII. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT MICROORGANISMS ISOLATED ON OCTANE FROM OKLAHOMA SOIL SAMPLES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	$P1DC_8$	0	0	0	0	3	4	4	4	4	3	0
Pseudomonas	$P1EC_8$	1	1	1	1	3	4	4	4	4	3	0
Pseudomonas	$P3A1C_8$	0	0	0	0	0	0	2	4	4	2	0
Pseudomonas	$P4C_8$	0	0	0	0	3	4	4	4	4	2	0
Mycobacterium	$P2BC_8$	1	1	1	4	4	4	4	4	4	4	2
Mycobacterium	$P5BC_8$	1	1	1	1	1	1	4	4	4	4	1
Mycobacterium	$P5DC_8$	1	3	2	1	3	3	4	4	4	4	2
Mycobacterium	$P9AC_8$	0	0	0	2	4	4	4	4	4	4	2
Mycobacterium	$P9BC_8$	0	1	1	2	2	2	2	4	3	3	1
Mycobacterium	$P10AC_8$	1	3	2	1	1	2	2	4	4	2	2
Corynebacterium	$P2AC_8$	3	4	4	4	4	4	4	4	4	4	3

Table XII. (Continued)

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Corynebacterium	$P3A2C_8$	4	4	4	4	4	4	4	4	4	4	4
Corynebacterium	$P5EC_8$	1	1	1	4	4	4	4	4	4	4	2
Streptomyces	$P1AC_8$	1	1	1	2	3	4	4	4	4	4	1

TABLE XIII. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT ORGANISMS FROM ANOMALOUS SOURCES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Pseudomonas	$HSC1C_8$	0	0	0	0	0	0	4	4	4	2	1
Pseudomonas	$HSC3C_8$	0	0	0	3	4	4	4	4	4	3	1
Pseudomonas	$HSC3BC_8$	0	0	0	0	3	4	4	4	4	3	1
Pseudomonas	$HSC5C_8$	0	0	2	4	4	4	4	4	4	4	0
Mycobacterium	$VIRG1C_8$	0	0	0	4	4	4	4	4	4	4	3
Mycobacterium	$PHIL2C_3$	2	3	3	2	2	3	4	4	4	1	1
Mycobacterium	$LT1BC_3$	1	2	3	1	4	4	4	4	4	4	1
Mycobacterium	L-22	1	1	1	3	4	4	4	4	4	4	1
Streptomyces	$RAM101C_3$	3	3	3	3	4	4	4	4	3	4	3

TABLE XIV. GROWTH ON VARIOUS HYDROCARBONS BY DIFFERENT BACTERIAL LABORATORY STOCK CULTURES

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
Mycobacterium	A107C_{16}	1	2	2	2	2	2	4	4	4	4	1
Mycobacterium	7S1C_3	3	4	4	4	4	4	3	3	4	4	2
Mycobacterium	758AC_3	1	4	4	3	1	1	1	1	1	4	1
Mycobacterium	0S13C_3	1	2	2	3	4	4	4	3	4	4	2
Mycobacterium	0C2ARC_3	1	4	4	3	1	1	1	1	1	4	0
Mycobacterium	0C2BC_3	4	4	4	4	4	4	3	2	3	4	1
Mycobacterium	0C2AC_3	4	4	4	4	4	4	3	1	2	4	1
Mycobacterium	A-33C_{16}	1	1	2	2	4	4	4	4	4	4	3
M. album	7E4AC_3	4	4	4	4	4	4	4	4	4	4	3
M. album	7E4BC_3	4	4	4	4	4	4	4	4	4	3	2
M. album	7E1B1WC_3	4	4	4	4	4	4	4	4	4	4	4

Table XIV. (Continued)

Organism	Code	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_{10}	C_{12}	C_{18}
M. rhodochrous	$7E1CC_3$	1	2	2	3	3	4	4	4	4	4	1
M. rhodochrous	$7E1CRRRC_3$	1	2	2	3	4	4	4	3	3	4	1
M. rhodochrous	$OFSC_3$	4	4	4	4	4	4	3	1	2	4	1
M. rhodochrous	$A\text{-}78C_{16}$	2	3	3	3	3	4	4	2	4	4	1
M. vaccae	$JOB5C_8$	1	1	1	3	4	2	1	2	4	4	2
Corynebacterium	$OSAE1C_3$	2	4	4	4	4	2	1	1	3	4	2
Corynebacterium	$OS1AC_3$	1	1	1	3	3	4	4	4	4	4	2
Corynebacterium	$OS1BC_3$	1	1	2	3	4	4	4	4	4	4	2

TABLE XV.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF MICROORGANISMS ISOLATED FROM VICTORIA SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Methane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony size (mm) & color
Pseudomonas	V1&3C_1	G- 0.8x1.0	0.8x1.0			3 beige
Pseudomonas	V1DC_1	G- 0.8x1.0	1.0x1.0-3.0			3 white
Pseudomonas	V2AC_1	G- 0.3x0.8	0.5x1.0			2 white
Pseudomonas	V5BC_1	G- 0.3x2.0-4.0	0.5x1.5		0.5x2.5	1.5 white
Corynebacterium	V4BC_1	G+ 0.8x1.5		0.8x4.0	0.5x3.5	3 pink
Corynebacterium	V5AC_1	G+ 0.5x2.0	0.5x3.0	0.4x5.0	0.4x5.0	2.5 yellow

B. Isolated on Methane and Cultured on Plain Nutrient Agar Medium.

Pseudomonas	V1&3C_1	No Growth				
Pseudomonas	V1DC_1	No Growth				
Pseudomonas	V2AC_1	No Growth				
Pseudomonas	V5BC_1	G- 0.5x1.0	0.5x1.5			2 green-white

Table XV. (Continued)

Organism	Code	Gram Reaction Size in µm.	Wet Mount-Size in µm. C_1	C_4	C_8	Colony Size (mm)& Color
Corynebacterium	$V4BC_1$	G+ 0.8x1.5-5.0	1.0x2.0-6.0			2 pink
Corynebacterium	$V5AC_1$	G+ 0.8x2.0	0.5-0.8x2.0-5.0			2 yellow

TABLE XVI.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM LULING SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Methane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Pseudomonas	$L8EC_1$	G- 0.8x1.0	1.5x2.0			1.5 white
Corynebacterium	$L1C_1$	G+ 0.5x1.5	0.8x1.5	0.8 by 1.0-4.0	0.8 by 1.0-3.0	2 yellow
Corynebacterium	$L7C_1$	G+ 0.3x1.0	0.8 by 1.0-3.0	0.8 by 1.0-3.0	0.8 by 1.0-3.0	2 yellow
Streptomyces	$L2AC_1$					2 white

B. Isolated on Methane and Cultured on Plain Nutrient Agar Medium.

Pseudomonas	$L8EC_1$	No Growth				
Corynebacterium	$L1C_1$	G+ 0.8 by 1.0-2.0	0.8x1.0-3.0			2 yellow
Corynebacterium	$L7C_1$	G+ 0.3x1.0	0.8x2.0-3.0			3 yellow
Streptomyces	$L2AC_1$					3 white

TABLE XVII.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM WEST TEXAS SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Methane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Corynebacterium	W1,2,3, 6,11,12G_1	G+ 0.5x1.5	0.5-0.8 by 0.5x1.5 1.0-3.0		0.5-1.0 by 1.0-3.0	2 yellow
Corynebacterium	W7&L2C_1	G+ 0.8x1.5	0.8x2.0	1.5-2.0	1.0x3.0	3 pink-orange

B. Isolated on Methane and Cultured on Plain Nutrient Agar Medium.

Corynebacterium	W1,2,3, 6,11,12C_1	G+ 0.5x1.0	0.5x1.0-1.5			1 yellow
Corynebacterium	W7&L2C_1	G+ 0.8x1.0	1.0x1.5			1 pink

TABLE XVIII.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM OKLAHOMA SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Methane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm)	& Color
Pseudomonas	$P9C_1$	G- 0.5x1.0	0.8x1.0	1.0x2.5	0.8x2.0	1	white-green
Mycobacterium	$P10C_1$	G+ 1.0x1.5	2.0x3.0	1.5x3.0	1.5x2.5-7.0	2	yellow-orange
Corynebacterium	$P1AC_1$	G+ 0.8-1.0 by 1.5-2.0	1.0x2.5	1.5x3.0-5.0	1.0-1.5 by 3.0-6.0	2	yellow
Corynebacterium	$P1BC_1$	G+ 0.8x1.0	1.2x1.2	2.0x5.0	1.5x4.0	2	cream-white
Corynebacterium	$P4AC_1$	G+ 0.5x0.8	1.5 by 1.5-2.5	1.5 by 1.5-2.5	1.0x1.5	2	white

B. Isolated on Methane and Cultured on Plain Nutrient Agar Medium.

Pseudomonas	$P9C_1$	G- 0.5x5.0-10.0	0.8x5.0-10.0			2	yellow-brown
Mycobacterium	$P10C_1$	G+ 1.0x1.5-3.0	1.5x1.5			2	pink

Table XVIII.(Continued)

Organism	Code	Gram Reaction Size in µm.		Colony Size (mm) & Color	
Corynebacterium	$P1AC_1$	G+ 0.8x1.0	0.8-1.0x1.0-2.0	1	yellow
Corynebacterium	$P1BC_1$	G+ 0.5x0.5-1.0	1.0-1.5x1.0-1.5	1	white
Corynebacterium	$P4AC_1$	G+ 0.5x0.8	1.0-1.5x1.0-1.5	2	white

TABLE XX.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM LULING SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Butane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Pseudomonas	$L1BC_4$	G- 0.5 by 1.0-2.0		1.0 by 2.0-4.0		1 white
Pseudomonas	$L12AC_4$	G- 0.3 by 0.8-1.0		0.5 by 2.0-3.0	0.5 by 1.0-2.5	3 white
Corynebacterium	$L2AC_4$	G+ 0.5 by 1.0-2.0		0.5x1.5	0.5 by 2.0-3.0	3 pink
Corynebacterium	$L2C\&7C_4$	G+ 0.8x2.0		0.8 by 1.0-3.0	0.8 by 1.0-3.0	3 yellow
Corynebacterium	$L3BC_4$	G+ 0.5x1.5		0.5=1.0 by 1.5-4.0	0.5 by 1.5-3.0	2 yellow
Corynebacterium	$L5C_4$	G+ 2.0x4.0		2.0 by 4.0-6.0	1.0-2.0 by 2.0-7.0	3 pink
Streptomyces	$L3AC_4$					2 white

Table XX. (Continued)

B. Isolated on Butane and Cultured on Plain Nutrient Agar Medium.

Organism	Code	Size in μm.		Colony Size (mm) & Color
Pseudomonas	$L1BC_4$	G- 0.8x2.0	1.0x3.0	2 white
Pseudomonas	$L12AC_4$	G- 0.3 by 0.8-1.0	0.4x2.0	2 beige
Corynebacterium	$L2AC_4$	G+ 0.5x1.5	0.5x1.0	1 white-pink
Corynebacterium	$L2C\&7C_4$	G+ 0.8x2.0	0.8x2.5	1 yellow
Corynebacterium	$L3BC_4$	G+ 0.5x1.5	0.8-1.0x2.0	1 yellow
Corynebacterium	$L5BC_4$	G+ 0.5 by 0.8-1.0	0.8-1.0 by 1.0-2.0	2 pink-red
Streptomyces	$L3AC_4$			2 white

TABLE XXI.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM WEST TEXAS SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Butane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Pseudomonas	W10,12BC$_4$	G- 0.5x1.0		0.4x1.0	0.5x1.5	1 clear
Mycobacterium	W7A&10CC$_4$	G+ 0.2x1.0-4.0		0.5x2.0	0.5x1.0-4.0	3 white
Mycobacterium	W7BC$_4$	G+ 0.8 by 1.5-2.5		0.5x1.5	0.8 by 1.0-3.0	2.5 yellow
Mycobacterium	W7,10 12,13C$_4$	G+ 0.8x2.0		0.8x1.5	0.8 by 1.0-3.0	2.5 yellow
Mycobacterium	W11AC$_4$	G+ 0.8 by 1.5-3.0		0.4 by 3.0-6.0	0.5 by 2.0-8.0	3 white
Corynebacterium	W2,4,5C$_4$	G+ 0.5x1.5		0.5x3.0	0.8x1.5	3 yellow
Corynebacterium	W6AC$_4$	G+ 0.5x1.5	0.5x1.5	0.8x3.0	0.8x3.0	2.5 yellow
Corynebacterium	W6BC$_4$	G+ 1.0x1.0-3.0		1.0-2.0 by 1.5-7.0	1.0-2.0 by 2.0-7.0	3 yellow
Corynebacterium	W6CC$_4$	G+ 0.8x3.0	1.5x2.0	3.0x5.0	3.0x10.0	1 pink-white

Table XXI. (Continued)

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Corynebacterium	$W9BC_4$	G+ 0.8x1.5	0.8x1.0	0.8x2.0	0.8x2.0	1.5 white-pink
Corynebacterium	$W11BC_4$	G+ 0.5x1.0		0.5x2.0	0.8x2.5	2.5 white-yellow
Streptomyces	$W9AC_4$					3 white
B. Isolated on Butane and Cultured on Plain Nutrient Agar Medium.						
Pseudomonas	$W10\&12C_4$	G- 0.3x0.8	0.4x1.0			1 clear
Mycobacterium	$W7\&10C_4$	G+ 0.5x0.8-2.0	0.8x1.0-4.0			2 pink
Mycobacterium	$W7BC_4$	G+ 0.5x1.5	0.5-0.8x2.0			1 clear
Mycobacterium	$W7,10,12,13C_4$	G+ 0.5-0.8 by 1.0-2.0	0.8x1.0-3.0			1 yellow
Mycobacterium	$W11AC_4$	G+ 0.5x1.0-2.0	0.8x1.5-3.0			2 white
Corynebacterium	$W2,4,5C_4$	G+ 0.5x1.5	0.8x1.0-3.0			1 yellow
Corynebacterium	$W6AC_4$	G+ 0.5x1.5	0.5x1.5			1 yellow
Corynebacterium	$W6BC_4$	G+ 0.8-1.0x1.0	1.5x1.5			3 orange

Table XXI. (Continued)

Organism	Code	Gram Reaction Size in μm.		Colony Size (mm) & Color
Corynebacterium	$W6CC_4$	G+ 1.0x1.0	1.5-2.0x1.5-2.0	2.5 pink
Corynebacterium	$W9BC_4$	G+ 0.8x1.0	1.0-1.5x1.0-1.5	4 white
Corynebacterium	$W11BC_4$	G+ 1.5x1.5	0.8x1.0-1.5	1 yellow
Streptomyces	$W9AC_4$			3 white

TABLE XXII.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM OKLAHOMA SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Butane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Mycobacterium	$P4C_4$	G+ 0.5-0.8 by 1.0-3.0	0.8 by 2.0-7.0	0.8 by 2.0-7.0	0.8 by 2.0-7.0	3 white
Corynebacterium	$P1AC_4$	G+ 0.8x1.5	0.8x1.5	1.0x3.0	1.0x2.5	1 white
Corynebacterium	$P1BC_4$	G+ 1.0x2.0	1.0x2.5	1.0x3.5	1.0x4.0	2 yellow
Corynebacterium	$P1CC_4$	G+ 0.5-0.8 by 2.5-4.0	0.5-1.0 by 2.0-4.5	0.8x2.5	0.8-1.0 by 2.5-3.5	2 white
Corynebacterium	$P2AC_4$	G+ 0.8x1.0-3.0	0.8x2.0	1.0x6.0	1.0x4.8	1 white
Corynebacterium	$P2BC_4$	G+ 1.5x3.0	2.0x7.2	1.5x2.5	2.0x4.5	1 orange
Corynebacterium	$P3AC_4$	G+ 0.8x2.0	0.8x2.0	0.8x3.0	1.0x6.0	1 yellow
Corynebacterium	$P5AC_4$	G+ 0.8-1.0 by 1.0-2.0	1.5x2.0	1.5x2.0	1.0x3.0	2 yellow-orange
Corynebacterium	$P9C_4$	G+ 1.0x3.0	0.5x2.5	1.0x4.0	0.8x2.0	2 white
Streptomyces	$P3BC_4$					1 white
Streptomyces	$P5BC_4$					3 white-gold

Table XXII.(Continued)

B. Isolated on Butane and Cultured on Plain Nutrient Agar Medium

Organism	Code	Gram Reaction Size in μm.		Colony Size (mm) & Color
Mycobacterium	$P4C_4$	G+ 0.5x1.0-2.0	1.0x1.0-4.0	4 white
Corynebacterium	$P1AC_4$	G+ 0.8x1.0	0.8-1.0x1.5-2.5	1.5 yellow
Corynebacterium	$P1BC_4$	G+ 0.5x0.8	1.0x2.0-3.0	1.5 yellow
Corynebacterium	$P1CC_4$	G+ 0.8x1.0-2.0	1.0x1.0-2.0	2 white
Corynebacterium	$P2AC_4$	G+ 0.8x0.8-1.5	1.0x1.0-3.0	2 yellow-gold
Corynebacterium	$P2BC_4$	G+ 1.0x1.0-3.0	1.5-2.0x1.5-4.0	2 pink
Corynebacterium	$P3AC_4$	G+ 0.5-2.0 by 2.0-3.5	0.8x1.0-2.0	1 white
Corynebacterium	$P5AC_4$	G+ 1.0x1.0	1.5-2.0x1.5-4.0	1 pink
Corynebacterium	$P9C_4$	G+ 0.8x0.8-1.0	0.8x1.0-3.0	2 yellow
Streptomyces	$P3BC_4$			1.5 beige
Streptomyces	$P5BC_4$			2 pink

TABLE XXIII.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM VICTORIA SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Octane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in µm.	Wet Mount-Size in µm. C_1	C_4	C_8	Colony Size (mm) & color	
Pseudomonas	$V4AC_8$	G- 0.3x2.0		0.8 by 2.0-8.0	0.8-1.0 by 3.0-8.0	2	white
Pseudomonas	$V4CC_8$	G- 0.3x3.5			0.4x5.0	3	white-clear
Pseudomonas	$V5AC_8$	G- 0.5x2.0		0.5x1.5	0.5x2.0	2	white
Pseudomonas	$V5BC_8$	G- 0.3x1.5			0.4x1.5	3	white-clear
Pseudomonas	$V6BC_8$	G- 0.5x1.5			0.5x2.0	3	white
Mycobacterium	$VS1\&3C_8$	G+ 0.5x2.0		1.0x3.0	0.8x2.0	2	beige
Mycobacterium	$VS1CC_8$	G+ 0.5x1.5		1.0x2.5	0.8x3.0	2.5	yellow
Corynebacterium	$V1AC_8$	G+ 0.4x2.0		0.8x1.0-6.0	1.0x2.0-7.0	3	orange
Corynebacterium	$V5CC_8$	G+ 0.5x2.0		1.0x3.0	1.0x3.0	3	beige
Corynebacterium	$V6AC_8$	G+ 0.5x2.5			0.8x3.0	3	pink
Corynebacterium	$V7AC_8$	G+ 0.8 by 1.0-3.0		0.8 by 2.0-6.0	1.0 by 4.0-6.0	3	orange-pink

Table XXIII (Continued)

Organism	Code	Gram Reaction Size in µm.	Wet Mount-Size in µm. C_1	C_4	C_8	Colony Size (mm) & Color
Streptomyces	$VS3CC_8$					3 grey
B. Isolated on Octane and Cultured on Plain Nutrient Agar Medium.						
Pseudomonas	$V4AC_8$	G- 0.5x1.5		0.8x1.5		3 white-green
Pseudomonas	$V4CC_8$	G-0.5x2.5		0.5-0.8x1.0-5.0		0.5 clear
Pseudomonas	$V5AC_8$	G- 0.5x2.0		0.8x1.5		0.5 clear
Pseudomonas	$V5BC_8$	G- 0.5x1.0		0.5x1.5		2 white
Pseudomonas	$V6BC_8$	G- 0.5x1.0		0.5-0.8x1.5		2.5 beige
Mycobacterium	$VS1\&3C_8$	G+ 0.5x1.5		0.8-1.0x1.0-2.0		1 yellow-orange
Mycobacterium	$VS1CC_8$	G+ 0.5-1.0 by 1.0-4.0		0.5-0.8x1.5		1 yellow
Corynebacterium	$V1AC_8$	G+ 0.5-0.8 by 0.8-1.5		1.0-1.5x1.5-2.0		1 pink
Corynebacterium	$V5CC_8$	G+ 0.5x1.5		0.5-1.0x0.5-1.5		3 cream
Corynebacterium	$V6AC_8$	G+ 0.8x1.0-1.5		1.0x2.0		2 pink

Table XXIII.(Continued)

Organism	Code	Gram Reaction Size in µm.	Wet Mount-Size in µm. C_1	C_4	C_8	Colony Size (mm) & Color
Corynebacterium	$V7AC_8$	G+ 0.4x1.0-2.0	1.0x2.0-3.0			1 pink
Streptomyces	$VS3CC_8$					4 white-gray

TABLE XXIV.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM LULING SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Octane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm)	& Color
Pseudomonas	LNF4C_8	G- 0.5 by 1.0-2.0		0.5-0.8 by 1.0-2.0	1.0 by 3.0-8.0	3	yellow
Pseudomonas	LNF5C_8	G- 0.4 by 3.0-5.0		0.5 by 1.0-2.0	0.8-1.0 by 3.0-5.0	3	orange
Pseudomonas	L2DC_8	G- 0.3x1.0		0.8 by 1.0-2.0	0.5-0.8 by 1.0-3.0	3	white
Pseudomonas	L3B&5AC_8	G- 0.4 by 1.0-2.0		0.8 by 3.0-4.0	0.5 by 3.0-4.0	3	green-white
Pseudomonas	L7BC_8	G- 0.5x1.0		0.5x2.0	0.5x2.0	3	white-beige
Pseudomonas	L7CC_8	G- 0.4x0.8			0.5 by 1.0-3.0	3	white
Pseudomonas	L8AC_8	G- 0.4x1.0			0.3x1.0	4	pink-beige
Pseudomonas	L12C_8	G- 0.4 by 1.0-3.0		0.8-1.0 by 1.0-3.0	0.5 by 1.0-4.0	3	white

Table XXIV.(Continued)

Organism	Code	Gram Reaction Size in µm.	Wet Mount-Size in µm. C_1	C_4	C_8	Colony Size (mm) & Color
Pseudomonas	$L13C_8$	G- 0.5x1.5		0.5 by 1.0-4.0	0.5x2.0	2 yellow
Mycobacterium	$L9DC_8$	G+ 0.4 by 1.0-4.0		0.5 by 2.0-3.0	0.5 by 3.0-6.0	3 white
Mycobacterium	$L11BC_8$	G+ 0.5-0.8 by 1.0-3.0		0.4 by 1.0-3.0	0.4 by 1.0-3.0	3 yellow
Mycobacterium	$L11CC_8$	G+ 0.4x1.0		0.4x2.0	0.4x1.5	3 white
Corynebacterium	$L2CC_8$	G+ 1.0x2.5	0.8x2.5	1.0 by 2.0-4.0	1.0x2.5	3 white
Corynebacterium	$L7AC_8$	G+ 0.4 by 1.0-3.0		0.5-0.8 by 1.0-2.0	1.5-1.0 by 2.0-4.0	3 orange
Corynebacterium	$L7DC_8$	G+ 0.5 by 1.0-3.0		0.8x2.0	0.8 by 3.0-4.0	3 yellow
Corynebacterium	$L7EC_8$	G+ 0.3 by 1.0-3.0		0.5x1.0	1.0 by 2.0-6.0	3 pink
Corynebacterium	$L8BC_8$	G+ 0.2x1.0	0.8x1.0	1.0x3.0	0.8x2.0	2 white
Corynebacterium	$L8CC_8$	G+ 0.5x1.5		1.0x1.5	1.0x2.0	2 beige
Corynebacterium	$L9CC_8$	G+ 0.5x1.0			0.8x2.0	3 pink

Table XXIV.(Continued)

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Corynebacterium	$L10AC_8$	G+ 0.4 by 1.0-4.0		0.5x1.0	0.5 by 2.0-4.0	3 orange-pink
Corynebacterium	$L10EC_8$	G+ 0.5-0.8 by 1.0-2.5			0.5x2.0	1 white
Corynebacterium	$L11DC_8$	G+ 0.4x1.0		0.5x2.0	0.5x1.5	1 white
B. Isolated on Octane and Cultured on Plain Nutrient Agar Medium.						
Pseudomonas	$LNF4C_8$	G- 0.8x1.0	1.0x3.0			3 yellow
Pseudomonas	$LNF5C_8$	G- 0.4x1.0	0.8x2.0			3 orange
Pseudomonas	$L2DC_8$	G- 0.5x2.5	0.5-1.0x2.0-5.0			3 beige
Pseudomonas	$L3B\&5AC_8$	G- 0.5-1.0 by 2.0-3.0	0.8-1.0x3.0-6.0			2 grey-white
Pseudomonas	$L7BC_8$	G- 0.5x2.0	0.5-0.8x2.0-4.0			3 beige
Pseudomonas	$L7CC_8$	G- 0.8 by 1.0-3.0	0.8-1.0x1.5-3.0			3 beige
Pseudomonas	$L8AC_8$	G- 0.4x2.0	0.8x2.0-6.0			1 yellow
Pseudomonas	$L12C_8$	G- 0.8x2.0	0.8-1.0x2.0			0.5 clear
Pseudomonas	$L13C_8$	G- 0.5x2.0	1.0x2.0			1 yellow

Table XXIV.(Continued)

Organism	Code	Gram Reaction Size in μm.		Colony Size (mm) & Color	
Mycobacterium	$L9DC_8$	G+ 0.5x0.8-2.0	1.0x2.0	3	white
Mycobacterium	$L11BC_8$	G+ 0.5-0.8 by 1.0-2.0	0.8-1.0x0.8-2.0	2	yellow
Mycobacterium	$L11CC_8$	G+ 0.5x1.0	0.5x1.0-1.5	3	white
Corynebacterium	$L2CC_8$	G+ 0.8x1.0-4.0	1.0-2.0x3.0-7.0	3	cream
Corynebacterium	$L7AC_8$	G+ 0.5-1.0 by 0.5-1.0	1.0x2.0	2	pink
Corynebacterium	$L7DC_8$	G+ 1.0x1.0-2.0	1.0x1.0-2.0	2	yellow
Corynebacterium	$L7EC_8$	G+ 0.4x0.8	0.4x1.0	2	orange-beige
Corynebacterium	$L8BC_8$	G+ 1.0x2.0-5.0	1.0-2.0x2.0-5.0	1	white
Corynebacterium	$L8CC_8$	G+ 0.8x1.0	1.5x1.5	3	white
Corynebacterium	$L9CC_8$	G+ 0.5x0.8-1.0	0.5x1.5	0.5	pink
Corynebacterium	$L10AC_8$	G+ 1.0x1.0-3.0	1.0x2.0-3.0	2	pink-orange
Corynebacterium	$L10EC_8$	G+ 0.5-0.8 by 1.0-2.0	1.0x2.0-4.0	1	white
Corynebacterium	$L11DC_8$	G+ 0.5x1.5-2.0	0.3x1.0-1.5	1	white

TABLE XXV.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM WEST TEXAS SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Octane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm C_1	C_4	C_8	Colony Size (mm) & Color
Pseudomonas	W1AC	G- 0.4x1.0			0.4x1.5	3 green-white
Pseudomonas	W1BC	G- 0.4x0.8		0.5 by 4.0-7.0	0.8x1.5	1 white
Pseudomonas	W2C1C	G- 0.5x1.5		0.5x3.0	0.5x2.0	2.5 white-grey
Pseudomonas	W5AC	G- 0.3x1.0			0.5x1.5	2 white
Pseudomonas	W5CC	G- 0.3x1.0		0.5x1.5	0.5x1.5	2 white
Pseudomonas	W7&11C	G- 0.4x1.5		0.3x2.0	0.3x2.0	3 charcoal
Pseudomonas	W7CC	G- 0.4x2.0		0.8x3.0	0.8x3.0	3 beige-white
Pseudomonas	W8AC	G- 0.4x1.5		0.5x3.0	0.4x2.0	3 yellow-green
Pseudomonas	W9A&10DC	G- 0.3x2.0		0.5x3.0	0.5 by 3.0-10.0	3 white-beige
Pseudomonas	W9&10C	G- 0.5x1.5		0.5x3.0	0.5x2.0	3 white-beige
Pseudomonas	W9BC	G- 0.5x1.0	0.5x1.5	0.5x3.0	0.4x2.0	1 white

Table XXV. (Continued)

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Pseudomonas	W10EC	G- 0.5x1.5		0.4x3.0	1.5x4.0	3 white
Pseudomonas	W12EC	G- 0.5x0.8		0.4x1.0	0.8x2.0	2.5 green-white
Pseudomonas	WS13CC	G- 0.3x1.0			0.5x3.0	3 green-white
Mycobacterium	W4BC$_8$	G+ 0.8 by 2.0-6.0		0.4 by 2.0-5.0	0.5 by 2.0-7.0	3 white
Mycobacterium	W10&S13C$_8$	G+ 0.5 by 1.5-5.0		0.8x1.5	0.5 by 3.0-5.0	3 yellow
Mycobacterium	W11EC$_8$	G+ 0.4x2.0		0.4x1.5	0.4x2.0	2 white-beige
Corynebacterium	W2C2C$_8$	G+ 0.8x1.5		1.0x2.0	0.8x2.0	w white
Corynebacterium	W4AC$_8$	G+ 0.5x1.5		0.5x1.0	0.5x2.5	3 red-orange
Corynebacterium	W4CC$_8$	G+ 0.4 by 0.8-2.0		0.8 by 1.5-3.0	0.4 by 1.0-3.0	3 pink
Corynebacterium	W5BC$_8$	G+ 0.5x1.5			0.8x2.0	3 pink
Corynebacterium	W6BC$_8$	G+ 0.5x1.5		0.8x1.5	0.8x3.0	3 red-orange
Corynebacterium	W12AC$_8$	G+ 0.5x1.5		0.8x1.0	0.8x3.0	3 pink-red
Corynebacterium	WS13B3C$_8$	G+ 0.3x1.0		0.8x1.5	0.8x2.5	3 pink

Table XXV. (Continued)

Organism	Code	Gram Reaction Size in µm.	Wet Mount-Size in µm. C_1	C_4	C_8	Colony Size (mm) & Color
Corynebacterium	$WS13JC_8$	G+ 0.5 by 1.5-2.5			0.8 by 1.4-2.5	2 pink
Streptomyces	$W10B1C_8$					3 white-gold
B. Isolated on Octane and Cultured on Plain Nutrient Agar Medium.						
Pseudomonas	$W1AC_8$	G- 0.3x1.5	0.8x3.0			3 green-white
Pseudomonas	$W1BC_8$	G- 0.3x1.5	0.8x1.0-3.0			0.5 clear
Pseudomonas	$W2C1C_8$	G- 0.4x1.5	0.8x2.0			2 green-white
Pseudomonas	$W5AC_8$	G- 0.3x1.5	0.8x2.0-3.0			1 clear
Pseudomonas	$W5CC_8$	G- 0.3x1.0	0.5x2.0			3 beige
Pseudomonas	$W7\&11C_8$	G- 0.5x0.8	0.5x1.0			1 white
Pseudomonas	$W7CC_8$	G- 0.3x1.5	0.5x2.0			3 charcoal
Pseudomonas	$W8AC_8$	G- 0.3-0.7 by 1.0-3.0	0.8x2.0			2 white
Pseudomonas	$W9A\&10DC_3$	G- 0.5x1.0	0.8x1.0-2.0			3 white
Pseudomonas	$W9\&10C_8$	G- 0.3x0.8	0.8x1.5			1.5 white
Pseudomonas	$W9BC_8$	G- 0.4x1.0	0.5x1.5			3 white

Table XXV. (Continued)

Organism	Code	Gram Reaction	Size in μm.		Colony Size (mm)	& Color
Pseudomonas	$W10EC_8$	G-	0.4x1.0	0.5x1.5	2	white
Pseudomonas	$W12EC_8$	G-	0.3x1.5	0.5x2.0	1	white
Pseudomonas	$WS13CC_8$	G-	0.4x1.5	0.5x2.0	3	clear
Mycobacterium	$W4BC_8$	G+	0.5x1.5	0.5x2.0	1	white
Mycobacterium	$W10\&S13C_8$	G+	0.5x0.8	0.8x1.0	1	yellow
Mycobacterium	$W11EC_8$	G+	0.5x2.0	0.5x3.0	1	white
Corynebacterium	$W2C2C_8$	G+	1.0x1.0-4.0	1.5x2.0	3	white
Corynebacterium	$W4AC_8$	G+	0.4x1.0	0.4x2.0	1	white-pink
Corynebacterium	$W4CC_8$	G+	0.8x1.0	0.8x1.5	3	red-pink
Corynebacterium	$W5BC_8$	G+	0.5x0.8	0.5x1.0	1	pink
Corynebacterium	$W6BC_8$	G+	0.5x0.8	0.5x1.0	1	red
Corynebacterium	$W12AC_8$	G+	0.4x1.0	0.8x1.0-4.0	1	red
Corynebacterium	$WS13B3C_8$	G+	0.5x1.5	0.8x2.0	1	pink
Corynebacterium	$WS13JC_8$	G+	0.8x1.0	1.0x2.0	1	white
Streptomyces	$W10B1C_8$				3	white-gold

TABLE XXVI.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM OKLAHOMA SOIL SAMPLES AND CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Octane and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Pseudomonas	$P1DC_8$	G- 0.5x1.5			0.8x1.3	3 pink-gold
Pseudomonas	$P1EC_8$	G- 0.8x1.2			0.8x1.0	2 orange-gold
Pseudomonas	$P3A1C_8$	G- 0.4x1.3		0.3x1.3	0.8x1.0	2 white
Pseudomonas	$P4C_8$	G- 0.3x1.5			0.8x2.0	3 yellow
Mycobacterium	$P2BC_8$	G+ 0.5-0.8 by 1.0-2.5		0.3x1.5	0.3-0.5 by 1.0-3.0	2 white
Mycobacterium	$P5BC_8$	G+ 0.8x2.0			0.5x2.5	2 red-orange
Mycobacterium	$P5DC_8$	G+ 0.8x2.5			0.6-1.0 by 2.5-5.0	3 orange
Mycobacterium	$P9AC_8$	G+ 0.5-0.8 by 1.0-.25		0.8x1.5	0.8-1.0 by 2.0-5.0	2 red-pink
Mycobacterium	$P9BC_8$	G+ 0.4x1.5		0.5x2.0	0.5x2.5	1 white
Mycobacterium	$P10AC_8$	G+ 0.5x0.8			0.8x1.5	2 yellow

Table XXVI.(Continued)

Organism	Code	Gram Reaction Size in µm.	Wet Mount-Size in µm. C_1	C_4	C_8	Colony Size (mm) & Color	
Corynebacterium	$P2AC_8$	G+ 0.8x3.0	0.5x4.5	0.5x4.5	0.6x5.0	2	white
Corynebacterium	$P3A2C_8$	G+ 1.5x1.5	1.5x1.5	0.8x1.0	2.0x2.0	3	orange-pink
Corynebacterium	$P5EC_8$	G+ 0.8x2.5		0.6x1.0	0.6x3.5	3	orange-red
Streptomyces	$P1AC_8$					3	white-grey
B. Isolated on Octane and Cultured on Plain Nutrient Agar Medium.							
Pseudomonas	$P1DC_8$	G- 0.8x1.5	1.0x2.0-4.0			3	gold-orange
Pseudomonas	$P1EC_8$	G- 0.8x1.5	0.8x1.5			2	yellow
Pseudomonas	$P3A1C_8$	G- 0.5x1.5	1.0x3.0-4.0			3	green-white
Pseudomonas	$P4C_8$	G- 0.3x1.0	0.5x1.0-2.0			3	yellow
Mycobacterium	$P2BC_8$	G+ 0.5-1.0x2.0	0.8x2.0			2	white
Mycobacterium	$P5BC_8$	G+ 1.0x2.0-5.0	1.0x2.0-12.0			1	pink
Mycobacterium	$P5DC_8$	G+ 0.8x2.0	0.8x1.0-3.0			1	pink
Mycobacterium	$P9AC_8$	G+ 0.4x3.0-4.0	0.4-0.8-3.0-5.0			1	red

Table XXVI.(Continued)

Organism	Code	Gram Reaction Size in μm.		Colony Size (mm) & Color
Mycobacterium	$P9BC_8$	G+ 0.8x3.0	0.8-1.0x3.0-5.0	1 white
Mycobacterium	$P10AC_8$	G+ 0.5x2.0	0.5-0.8x1.0-3.0	1 yellow-white
Corynebacterium	$P2AC_8$	G+ 0.8x1.0-3.0	1.0x2.5	2 white
Corynebacterium	$P3A2C_8$	G+ 1.0-1.0-2.0	1.5x1.5-2.5	1.5 white
Corynebacterium	$P5EC_8$	G+ 0.8-1.0 by 1.0-3.0	1.0x2.5-4.0	0.5 pink
Streptomyces	$P1AC_8$			2 white

TABLE XXVII. A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF BACTERIA ISOLATED FROM ANOMALOUS SOURCES

A. Isolated on Various Hydrocarbons and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Pseudomonas	$HSC1C_8$	G- 0.4x2.0			0.5x4.0	3 white
Pseudomonas	$HSC3C_8$	G- 0.5x1.0		0.5x2.0	0.5x1.5	2.5 white
Pseudomonas	$HSC3BC_8$	G- 0.4x1.5			0.5x2.0	4 white
Pseudomonas	$HSC5C_8$	G- 0.4x2.0		0.4x1.5	0.5x1.5	2.5 white
Mycobacterium	$VIRG1C_8$	G+ 0.8x2.0-7.0		0.5x1.5	0.5 by 2.0-4.0	1.5 white
Mycobacterium	$PHIL2C_3$	G+ 0.5x1.0-2.0	0.5x1.0	0.5x1.0	1.0x3.0	2 yellow
Mycobacterium	$LT1BC_3$	G+ 0.5x1.0-4.0		1.0x3.0	0.5 by 1.0-5.0	2.5 red
Mycobacterium	L-22	G+ 1.0-1.7 by 3.0-5.0		0.8x2.8	0.8x3.0	2 orange-pink
Streptomyces	$RAM101C_3$					4 yellow

Table XXVII.(Continued)

B. Isolated on Various Hydrocarbons and Cultured on Plain Nutrient Agar Medium.

Organism	Code	Gram Reaction Size in μm.		Colony Size (mm) & Color
Pseudomonas	$HSC1C_8$	G- 0.5x2.5	0.5x3.0-5.0	3 clear
Pseudomonas	$HSC3C_8$	G- 0.5x1.0	0.5x2.0	2 clear
Pseudomonas	$HSC3BC_8$	G- 0.5x1.5	0.5x1.0-3.0	2 clear
Pseudomonas	$HSC5C_8$	G- 0.3x1.0	0.8x1.0	2 clear
Mycobacterium	$PHIL2C_3$	G+ 0.5x1.0	0.5-0.8x1.0-3.0	2 yellow
Mycobacterium	$LT1BC_3$	G+ 0.3x5.0	0.5x3.0-7.0	1 red
Mycobacterium	$VIRG1C_8$	G+ 0.5x1.0	0.8x1.5	0.5 white
Mycobacterium	L-22	G+ 1.5-3.0	1.5x3.0	1 pink
Streptomyces	$RAM101C_3$			3 white

TABLE XXVIII.

A COMPARISON OF THE MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF LABORATORY STOCK CULTURES CULTURED ON VARIOUS HYDROCARBONS

A. Isolated on Various Hydrocarbons and Cultured on Methane, Butane, and Octane.

Organism	Code	Gram Reaction Size in μm.	Wet Mount-Size in μm. C_1	C_4	C_8	Colony Size (mm) & Color
Mycobacterium	A107C_{16}	G+ 1.0-1.5 by 2.0-5.0		0.8 by 3.5-5.5	1.8 by 3.5-5.5	2 red-pink
Mycobacterium	7S1C_3	G+ 1.0-1.5 by 3.0-4.0	0.9x1.8	0.9x1.8	1.1x1.8	3 white
Mycobacterium	758AC_3	G+ 1.5-2.0 by 2.5-4.5		1.8x5.4		1 orange-pink
Mycobacterium	0S13C_3	G+ 1.2x3.5		1.6x3.6	1.6x4.5	2 orange
Mycobacterium	0C2ARC_3	G+ 2.5x6.0		1.8x7.2	1.8x7.2	3 pink-orange
Mycobacterium	0C2BC_3	G+ 1.0-2.0 by 3.0-5.5	1.2-1.8 by 2.0-7.0	1.6 by 3.6-5.4	1.6x7.2	2 yellow
Mycobacterium	0C2AC_3	G+ 1.5-3.0 by	2.0x7.2	1.8x7.2	2.7x7.2	3 orange
Mycobacterium	A-33C_{16}	G+ 1.0-2.0 by 2.0-5.0		0.5 by 0.5-1.0	0.5-1.0 by 1.0-3.5	3 white
M. album	7E4AC_3	G+ 1.5 by 3.0-5.2	1.6x7.4	1.6x7.4	1.2x8.2	3 white

Table XXVIII. (Continued)

Organism	Code	Gram Reaction Size in µm.	Wet Mount-Size in µm. C_1	C_4	C_8	Colony Size (mm) & Color	
M. album	$7E4BC_3$	G+ 1.0x3.5	0.8x2.2	0.8x2.2	0.9x2.0	3	white
M. album	$7E1B1WC_3$	G+ 1.0x5.0	1.4x3.6	1.6x5.4	1.4x3.6	3	white
M. rhodochrous	$7E1CC_3$	G+ 1.0 by 4.0-7.0		1.2x3.6	1.6x5.4	2	pink-orange
M. rhodochrous	$7E1CRRRC_3$	G+ 1.2x5.0		1.0 by 3.0-7.0	1.8x5.4	2	orange
M. rhodochrous	$0FSC_3$	G+ 1.5-2.5 by 3.0-7.0	1.4x5.0	1.8-5.0		2	orange
M. rhodochrous	$A\text{-}78C_{16}$	G+ 1.0-2.0 by 4.0-6.0	1.0 by 3.6-7.2	1.8 by 3.6-7.2	2.0x7.2	3	white
M. vaccae	$J0B5C_8$	G+ 1.0x4.0-6.0		1.2x3.6	1.2x3.6	2	yellow
Corynebacterium	$0SAE1C_3$	G+ 1.5x2.0	0.5x1.8	0.9x3.6	0.9x3.6	3	yellow-orange
Corynebacterium	$0S1AC_3$	G+ 0.8-1.5 by 1.0-5.0		1.0-1.5 by 1.5-3.0	1.0 by 1.0-5.0	2	red
Corynebacterium	$0S1BC_3$	G+ 1.0 by 2.0-3.0		1.0-1.5 by 1.0-2.0	1.0-1.5 by 1.0-5.0	2	red

Table XXVIII. (Continued)

B. Isolated on Various Hydrocarbons and Cultured on Plain Nutrient Agar Medium.

Organism	Code	Gram Reaction Size in μm.		Colony Size (mm) & Color	
Mycobacterium	A107C_{16}	G+ 1.0x4.0	0.8-1.0x3.0-5.0	1	pink
Mycobacterium	7S1C_3	G+ 1.0x2.0-3.0	0.8-1.0x2.0-4.0	1	white
Mycobacterium	758AC_3	G+ 1.5x2.0	2.0x2.0-4.0	1	pink
Mycobacterium	0S13C_3	G+ 1.0x2.5	1.0-1.5-1.5-3.0	1	pink-red
Mycobacterium	0C2ARC_3	G+ 2.0x7.0-12.0	2.0x7.0-27.0	1	white-pink
Mycobacterium	0C2BC_3	G+ 1.0x5.2	1.5x5.0-10.0	1	white
Mycobacterium	0C2AC_3	G+ 2.0x8.0	0.8-1.4x3.6-18.0	1	pink
Mycobacterium	A-33C_{16}	G+ 1.0x1.0	1.0x3.0	3	white
M. album	7E4AC_3	G+ 1.0x2.0	0.5x3.3	1	white
M. album	7E4BC_3	G+ 1.0x4.0	1.0-1.5x3.0-5.0	2	white
M. album	7E1B1WC_3	G+ 1.0x2.0	1.0x4.0	1	white
M. rhodochrous	7E1CC_3	G+ 1.0x2.5	1.0-3.2x2.0-7.2	1	red-pink

Table XXVIII. (Continued)

Organism	Code	Gram Reaction Size in μm.		Colony Size (mm) & Color	
M. rhodochrous	$7E1CRRRC_3$	G+ 1.0x2.5	1.8x5.4	1	red
M. rhodochrous	$OFSC_3$	G+ 1.5-2.0 by 4.0-7.0	1.8-2.0x5.0-9.0	1	pink
M. rhodochrous	$A\text{-}78C_{16}$	G+ 1.5x2.0-5.0	1.5-2.0x2.0-5.0	3	white
M. vaccae	$JOB5C_8$	G+ 1.0x3.0	0.8-1.0x1.5-3.0	1	yellow
Corynebacterium	$OSAE1C_3$	G+ 1.2x1.2	1.0-1.5x1.5-2.0	1	yellow
Corynebacterium	$OS1AC_3$	G+ 0.8x1.0	1.0x2.0	2	red
Corynebacterium	$OS1BC_3$	G+ 1.0x3.0	1.0-1.5x2.0-4.0	1	red-pink

DISCUSSION

A wide variety of hydrocarbons occur in nature. The fact that none of these accumulate on the surface of the earth is indicative of a continuous degradation process involving the microbial flora of the soil. Early studies on many of the hydrocarbon-utilizing organisms involved aerobic isolation and cultivation of organisms from soil samples obtained fron oil field spillage areas or other sources in nature. Various alkanes and other more complex hydrocarbons were used as carbon sources.

There is a paucity of information concerning the isolation of hydrocarbon-utilizing organisms and comparative studies regarding the ability of microbes to utilize a homologous series of hydrocarbons. In the present study 35% of the total hydrocarbon-utilizing organisms isolated were Pseudomonas spp. These organisms did not present a problem in identification. The different species were typical and followed the biochemical, morphological, and staining characteristics described in Bergey's Manual (1957). The water soluble pigment produced by these organisms was also a feature which assisted in identification.

The majority of the isolated organisms were of the genus Corynebacterium spp., and represented 41% of the total organisms isolated from the oil field soils.

Mycobacterium spp. were found less frequently, and represented only 19% of the total organisms isolated as hydrocarbon utilizers; while Streptomyces spp. represented only 5% of the total microorganisms recovered.

The only two molds isolated which were considered to be hydrocarbon-utilizers were the filamentous fungi, Phoma sp. and Cephalosporium sp. Other molds were isolated initially on different hydrocarbons, but these failed to grow after 8 or more transfers on alkanes. Yeasts were not recovered in the present study, although they are often considered a major microbial group concerned with the ecological breakdown of hydrocarbons (Fuhs, 1961; Jones and Edington, 1968; Jones, 1969; Lowery, Foster, Jurtshuk, 1969, and Sheda, 1966). The methods by which molds and yeasts have been tested for their ability to utilize hydrocarbons, in vitro, may produce results indicating that these two groups of organisms are important in hydrocarbon degradation. One method includes respirometric studies. Other methods include placing fresh soil in enrichment culture broths or sprinkling soil on the surfaces of agar plates containing salts media. Hydrocarbons are then tested for their ability to support growth. Soil particles themselves, provide sufficient amounts of nutrients to support growth. Therefore, experiments in which only a single growth test has been carried out, without

subculturing several times on hydrocarbons, tend to give invalid results. Molds are especially considered a major constituent of the soil microflora involved in hydrocarbon-utilization (Foster, 1962). In those studies in which molds have been reported as a dominant population, the fact that the organisms were not subjected several times to subculture on alkanes may also have produced inconclusive results. In some respiratory studies used to show that organisms were capable of metabolizing certain hydrocarbons, concomitant growth of cells has not been measured.

Studies were performed in which nitrogen-free medium and alkanes were used together as a substrate for the isolation of organisms. These organisms are capable of fixing not only nitrogen from the atmosphere, but can also utilize hydrocarbons as a source of carbon. In these experiments only two organisms were isolated from the soils used. The organisms, LNF4C_8 and LNF5C_8 from Luling soils, were capable of carrying out this physiological process, and were identified as Pseudomonas spp.

Evaluation studies of organisms as hydrocarbon-utilizers have been carried out on laboratory stock cultures of molds, yeasts, and bacteria. These organisms were isolated originally from different sources not specifically associated with petroleum products (Fuhs, 1961; Lowery, Foster, Jurtshuk, 1969). These stock cultures have been included

in lists of organisms capable of hydrocarbon utilization, although they were tested only once or twice on a few alkanes. It is not unusual for the broth medium, used to culture the organisms during the tests, to contain small quantities of yeast extracts or other metabolites as stimulants for growth. The possibility exists that these organisms are able to metabolize "building blocks," or utilize stored foods (endogenous) for growth during the length of time the tests were performed. Repeated transfer of organisms on hydrocarbons without the presence of metabolites is necessary before any organism is considered to be a hydrocarbon-utilizer. Loss of viability occurs with each transfer in cases where organisms are not "true" hydrocarbon-utilizers.

All organisms isolated from either methane, butane, or octane enrichment cultures were compared for their ability to grow on individual n-alkanes. Only those organisms capable of good to profuse growth (3+ to 4+) were considered. A summary of these experiments are shown in Table XXIX.

TABLE XXIX

SUMMARY OF ORGANISMS ISOLATED FROM METHANE, BUTANE, AND OCTANE, COMPARED FOR THEIR ABILITY TO GROW ON ALKANES.

	Isolated From		
	Methane Enrichment	Butane Enrichment	Octane Enrichment
Growth on			
C_1	69%	7%	5%
C_2	50	47	8
C_3	56	66	11
C_4	63	90	30
C_5	75	90	71
C_6	75	90	91
C_7	69	71	95
C_8	69	71	100
C_{10}	63	70	98
C_{12}	56	63	86
C_{18}	13	40	29

These data indicate that (1) of the total number of organisms isolated originally from methane enrichment culture, 69% continued to utilize methane as the sole carbon source after 8 or more transfers on the isolating hydrocarbon; (2) these same methane isolates utilized other

higher n-alkanes (C_2 - C_{12}) almost equally as well; and (3) octadecane could be used only by a limited few methane isolates. Certain organisms in this study were found to be obligate methane utilizers. Kester (1961) did not consider these isolates to be unusual even though only a relatively few organisms have ever been reported as "true" obligates. Kester indicated that methane gas obtained from commercial sources contains sufficient amounts of other higher n-alkanes to sustain growth of those organisms often considered to be "obligate methane utilizers." In this study, however, not any of the obligate methane organisms which were placed on the higher n-alkanes, were able to metabolize these hydrocarbons; nor were any of the complex peptone media, usually employed in the laboratory, capable of supporting growth. These results tend to confirm the fact that the obligate methane utilizers obtained in these experiments were "true" obligates.

In Table XXIX the total number of organisms isolated on butane enrichment is also listed. Ninety percent of the organisms could utilize butane as a substrate after 8 or more subsequent transfers to this hydrocarbon. Butane isolates preferred the higher alkanes (C_3 - C_{12}), and to a much less extent ethane and octadecane. It is significant that only 7% of all butane isolates could use methane as a carbon source. There were no obligate

butane utilizers observed in these studies, nor have any been previously reported.

All of the octane enrichment isolates continued to grow after the initial isolation of these from the soil and other source samples used in this study. Table XXIX shows that 100% of these organisms could use octane as a carbon source without loss of viability when subcultured on this hydrocarbon. It is apparent that the octane isolates could not use methane, ethane, or propane as efficiently for growth as they could the longer carbon chain alkanes. Butane and octadecane cannot be assimilated as well as other alkanes by these octane isolates.

In the primary isolation of hydrocarbon utilizers, as the number of carbons in the chain length of the test alkanes increases, there is also an accompanying decrease in the number of those organisms which are capable of utilizing the short chain alkanes.

The type of alkane used for the isolation of hydrocarbon-utilizers from soils is important. The longer chain alkanes were more satisfactory for this purpose. Of the total number of organisms recovered in this study, 16% were isolated on methane culture, 30% on butane, and 54% were isolated on octane culture.

The dominant hydrocarbon-utilizing organism obtained from different oil field soils varied in kinds and numbers.

Although soil samples from any one of these fields contained more or less the same types of organisms peculiar to that field, the dominant organisms differed from one field to another.

Hydrocarbons of the alkane series were used almost exclusively for the isolation and cultivation of hydrocarbon utilizers. However, cyclohexane was introduced as a representative of a cyclic hydrocarbon in order to determine whether organisms, usually capable of attacking straight chain alkanes, were also able to utilize ring structures for energy and growth. Various soils from different environments were placed in contact with this hydrocarbon for 6 - 8 weeks or longer. No organisms were isolated which were considered capable of metabolizing cyclohexanes.

During the primary isolations and subsequent purification procedures performed when selective hydrocarbons were used for the separation of mixed populations, there often occurred an almost complete disappearance of bacterial growth. This phenomenon was observed in broth cultures as well as on agar surfaces of streak plates. Microscopic examination revealed that several different types of protozoa and nematodes were actively ingesting the entire bacterial population. The large supply of bacteria actively stimulated the growth of the protozoan

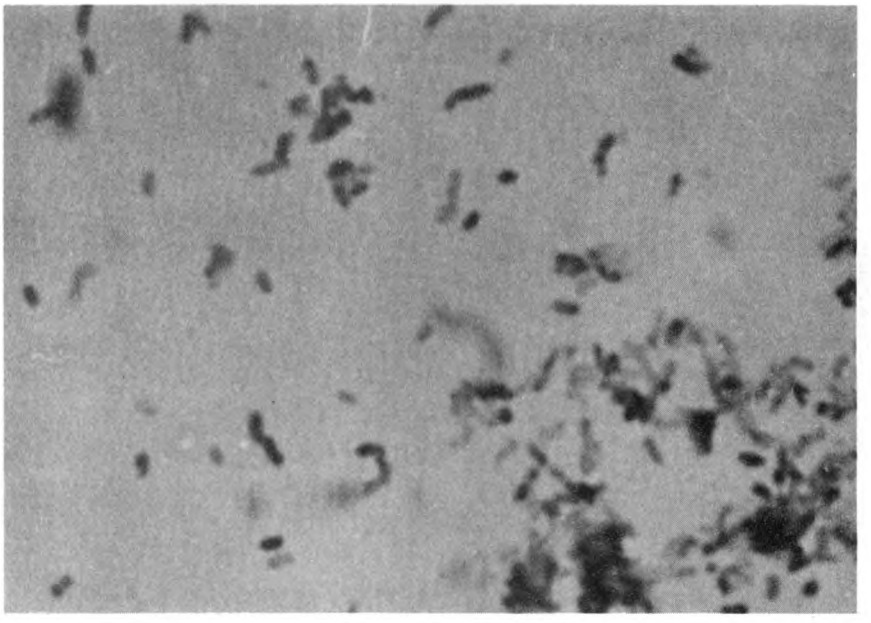
component of the mixed culture. Although hydrocarbons saturated the environment of the growth chambers, their presence did not interfere with or inhibit the growth of the otherwise fastidious protozoa. As soon as bacterial numbers decreased, the protozoa encysted, and these cysts were then carried along with new inocula when subcultures were made. It was very difficult in many of these isolations to separate the cysts from the bacterial cells because of the unusually large amounts of capsular material present on the bacteria.

This unique situation, where large numbers of protozoa exist, can also occur in nature; and this may, in turn, account for increased soil fertility where petroleum products have been used in fields for this purpose.

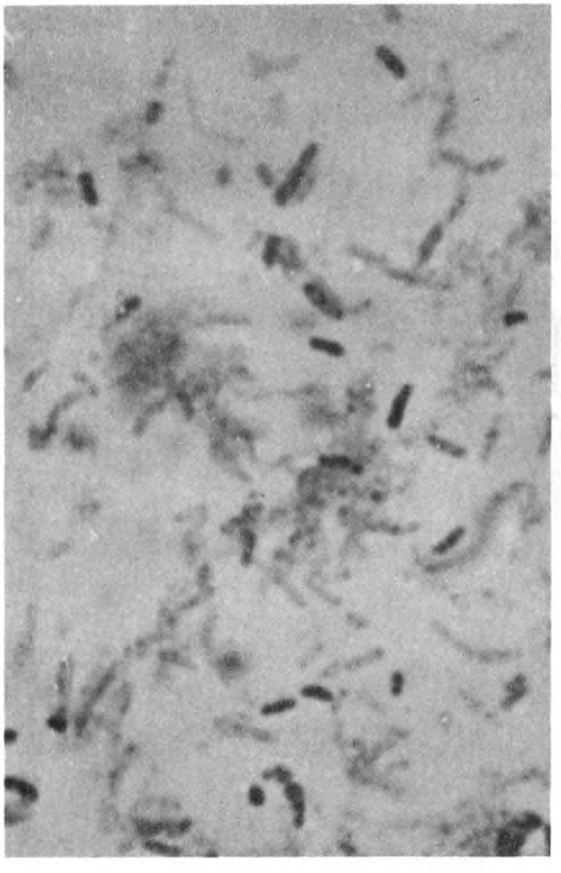
The identification of the different bacteria isolated in these studies remains a complex problem. The similar morphological characteristics of species of Corynebacterium, Mycobacterium, and Nocardia present difficulties in separation of the genera. The close relationship of these organisms to each other and the criteria used to identify and describe the species accurately have not been satisfactorily determined. The cultivation of organisms on different hydrocarbons often produces bizarre morphological variation not only in cell sizes and shapes, but also in their ability to stain.

Figure 1 illustrates the variation in the cell shape as well as cell size in Mycobacterium sp. (Virg.), when these bacteria are cultured on butane, octane, and plain nutrient agar. There is an obvious difference in the length of the cells when cultured on hydrocarbons in comparison to the cell shape when the organism is grown on plain nutrient agar. In Figure 2, Corynebacterium sp. (V_4BC_8), are rod-shaped when grown in octane, but appear as large micrococci or streptococci when placed on nutrient agar. In Figure 3, Mycobacterium sp. (V_5BC_4) appears as large cocco-bacilli when cultured on butane, but when cultured on plain nutrient medium the cells are more or less rod-shaped, and have polar staining. Figure 4 illustrates the staining quality of Corynebacterium sp. (W_5BC_8) when this bacterium is cultured on octane, in comparison to cultivation on plain nutrient agar. The polar bodies do not stain as deeply in octane grown cells as they do when grown on plain nutrient. This organism does not appear typical on octane; however, on close inspection the small cells are, in reality, in a palisade arrangement. During the staining procedure there was a slight displacement of the cells from the palisade orientation. On plain nutrient medium the cells grow as large cocco-bacilli, in comparison to the short rods usually seen on octane substrate.

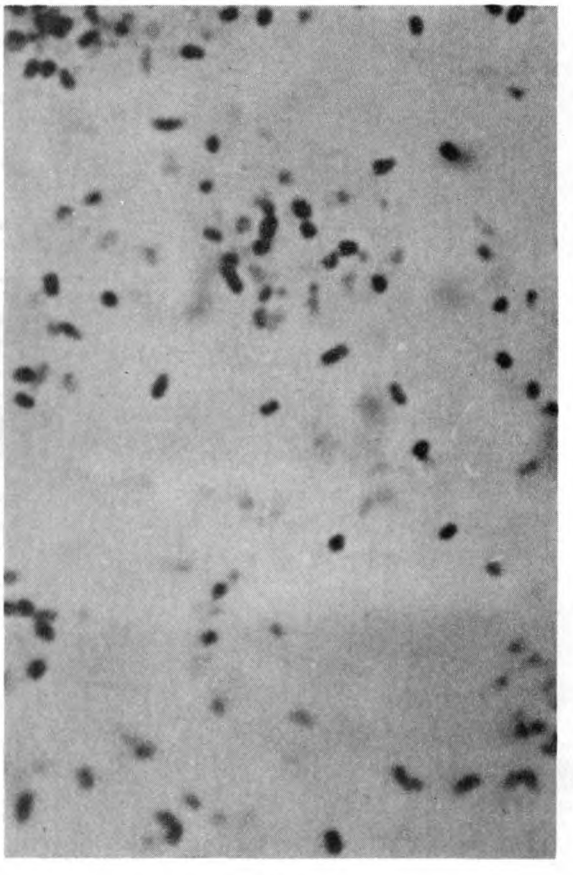
Figure 1. (970X) Mycobacterium sp. (Virg). Gram stain showing the variation in cell morphology when cultured on (a) butane (b) octane (c) plain nutrient agar.



a

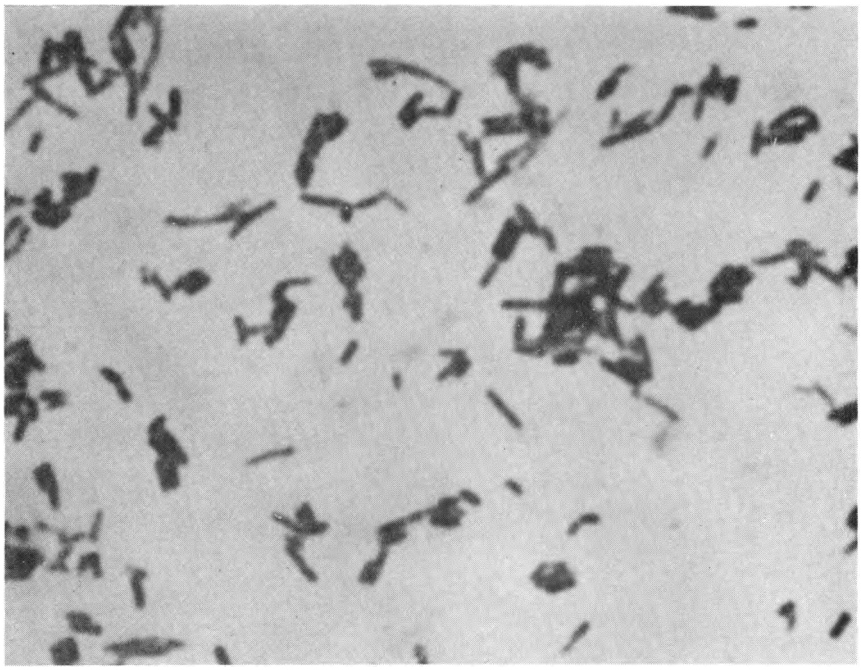


b

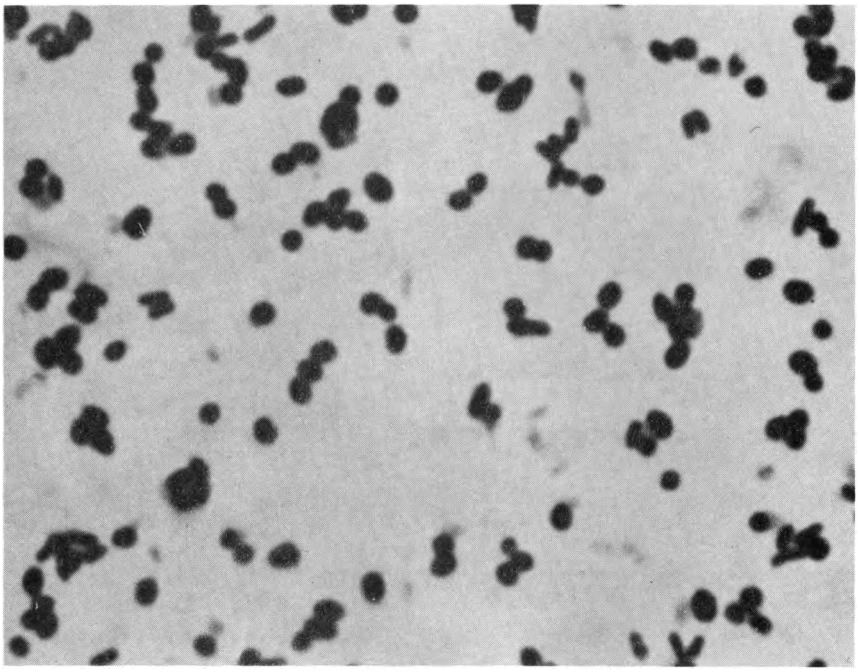


c

Figure 2. (970X) Corynebacterium sp. (V_4BC_8). Gram stain showing the variation in cell morphology when cultured on (a) octane (b) plain nutrient agar.

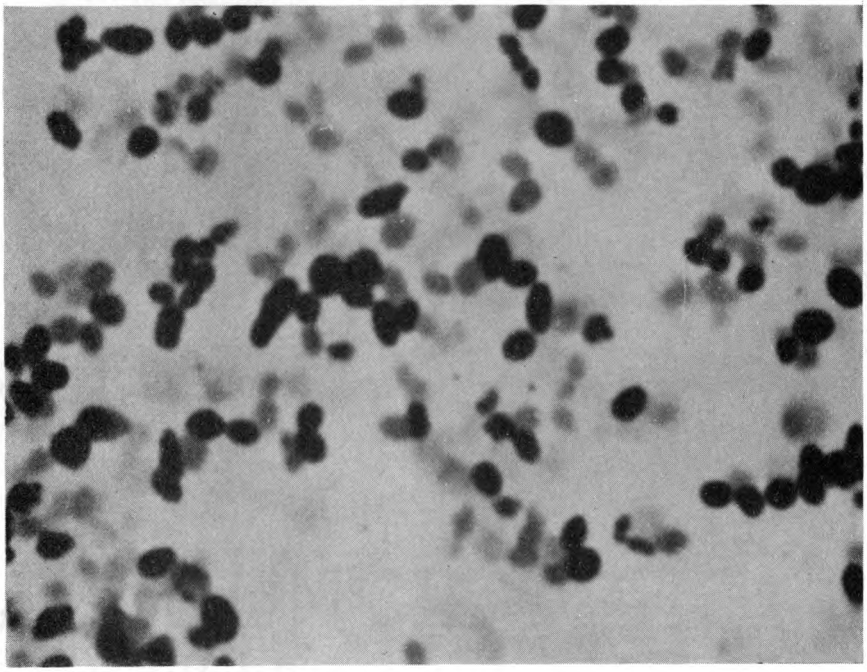


a

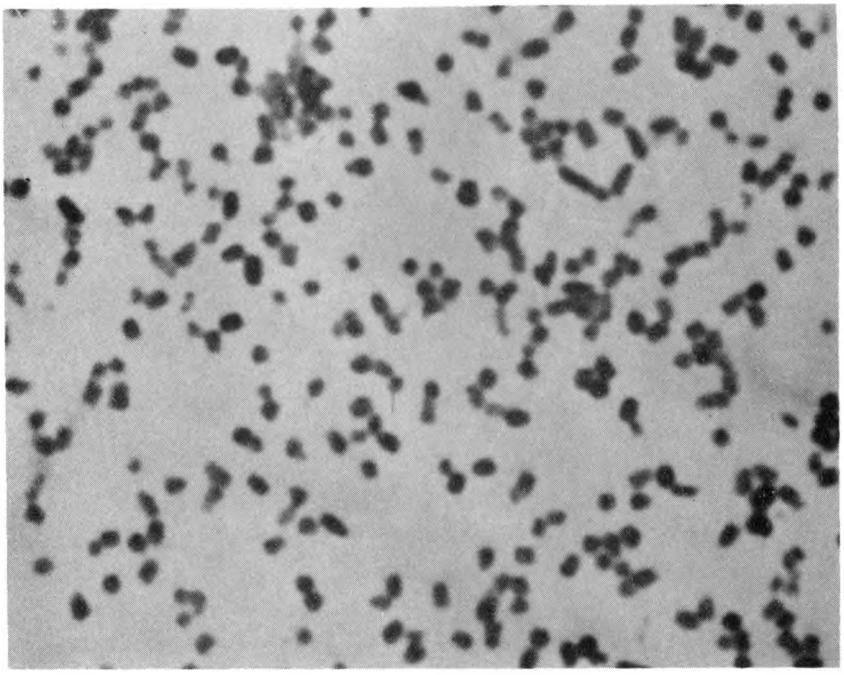


b

Figure 3. (970X) Mycobacterium sp. (V_5BC_4). Gram stain showing the variation in cell morphology when cultured on (a) butane (b) plain nutrient agar.

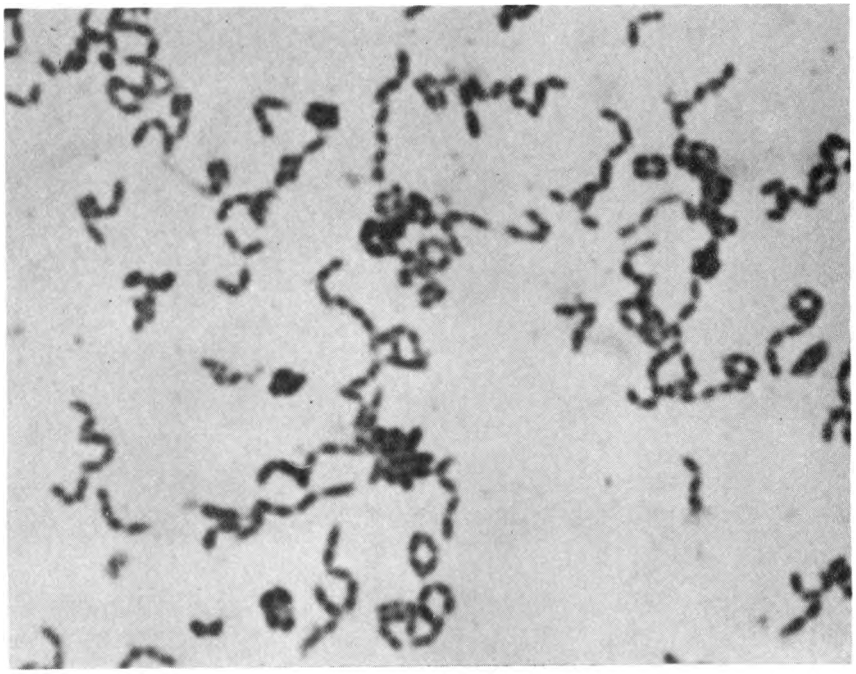


a

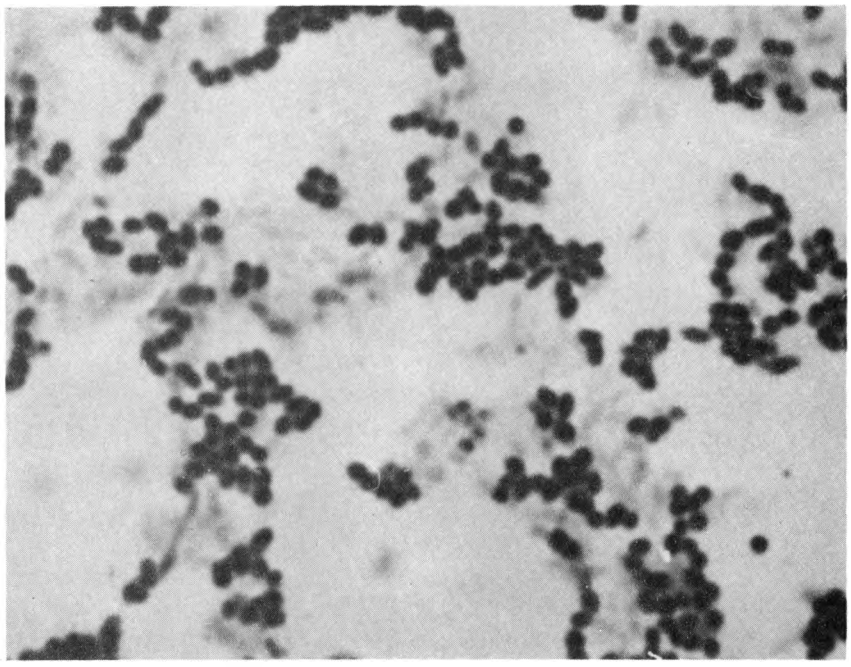


b

Figure 4. (970X) Corynebacterium sp. (W_5BC_8). Gram stain showing the variation in cell morphology when cultured on (a) octane (b) plain nutrient agar.



a



b

SUMMARY

Soil samples collected from oil fields and other sources in nature such as soil from the vicinity of a service station, virgin soil, and an oil-water mixture from a Houston ship channel, were studied in order to determine the presence of hydrocarbon-utilizing microorganisms. A total of 158 organisms were isolated and identified. These were represented by the bacterial genera Corynebacterium, Pseudomonas, Mycobacterium, Streptomyces, and also the molds Phoma and Cephalosporium.

Enrichment culture and "sprinkle-plate" methods were employed for the isolation of the organisms found in this study. For each sample studied, methane, butane, and octane were used individually as the sole source of carbon and energy for the primary isolations. The isolates were further tested and compared for their ability to utilize other hydrocarbons from C_1 - C_8, C_{10}, C_{12}, and C_{18}. Many of the organisms isolated were able to metabolize different n-alkanes, but each organism had its own substrate specificity range. The organisms were grown on various paraffines, and the differences were compared both culturally and morphologically. Several Pseudomonads were found to be obligate methane-utilizers, and were not able to grow on other hydrocarbons or complex media.

Attempts were made to isolate organisms on the cyclic hydrocarbon, cyclohexane; but no organism was isolated which had the ability to utilize this substrate as a carbon source.

The numbers of each species of organism isolated from a single soil sample depended on the particular hydrocarbon used for isolation, i.e., the hydrocarbon which a particular organism may use for growth was fairly selective.

Photomicrographs are presented showing some of the changes which occur in the morphological features of the cells when these organisms were grown on different hydrocarbons.

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