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Screening of Hydrocarbon Degrading Bacteria and Fungi from Waste Motor Engine Oil Contaminated Soil, their Distribution Frequency and Hydrocarbon Utilization Potentials

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Abstract: The screening of hydrocarbon degrading bacteria and fungi from spent motor engine oil contaminated soil from mechanic village, Uyo, Akwa Ibom State, their distribution frequency and hydrocarbon utilization potentials were examined using standard methods. The results of the screening revealed that *Bacillus mycoides, Staphylococcus aureus, Listeria murrayi, Actinomycete viscosus, Clostridium sporogenes, Bacillus licheniformis, Corynebacterium ulcerans* and *Clostridium histolyticum* were bacteria identified. The fungi were *Penicillium notatum, Aspergillus fumigatus, Rhizopus stolonifer, Rhizopus oryzae, Fusarium oxysporum, Cryptococcus terreus* and *Rhodotorula mulcilaginosa.* Their distribution frequency were *B. mycoides* (27.9%), *S. aureus* (8.1%), *L. murrayi* (9.9%), *A. viscosus* (11.7%), *C. sporogenes* (16.2%), *B. licheniformis* (7.2%), *C. ulcerans* (12.6%) and *C. histolyticum* (6.3%) for bacteria. For the fungi *P. notatum* (20.4%), *A. fumigatus* (11.2%), *R. stolonifer* (10.2%) *R. oryzae* (24.5%), *F. oxysporum* (15.3%), *C. terreus* (4.1%) and *R. mulcilaginosa* (14.3%). The ability of the microbial isolates to utilize spent motor engine oil from petrol and diesel driven vehicles as their sole source of energy were examined and noticed to vary among the microorganisms. *C. sporogenes* was the most utilizer of spent oil from petrol driven vehicle whereas *S. aureus* was the least. *C. histolyticum* utilized spent oil from diesel vehicle the most. Among the fungi, *R. mulciginosa* showed most utilization potential of spent oil from petrol driven vehicle, while *C. terreus* was the least. The spent oil from diesel vehicle, *A. fumigatus* exhibits highest utilization capacity, whereas *R. oryzae* was the least. The results imply that consortium of the isolates could be effective for remediation of hydrocarbon contaminated soil.

1. Introduction

One of the major environmental problems today is due to the petroleum hydrocarbon contamination of the environmental arising from exploration and exploitation of crude oil by oil companies and the activities of oil and allied industries (Onwurah, 2000; Ikpe *et al*., 2018). Disposal of waste oil and accidental releases of petroleum products on the environment are of Particular concern to the environmental scientists (Wang *et al*, 2012; Ekanem *et al*., 2019). According to Itah and Essien. (2001), hydrocarbon contamination of land and water environment has been a problem since the discovery of oil as fuel service particularly in the developing countries like Nigeria. The primary contaminants in the petroleum - contaminated soil are toxic and hazardous aliphatic, cycloaliphatic, and aromatic and hydrocarbons (Zhang *et al*., 2019; Wang *et al*., 2019; Shafqat, 2021; Li *et al.,* 2023). They decrease the diversity of plants and microbes in the soil, deplete soil fertility, disrupt soil ecological balance, and even put human health at risk (Varjanic *et al*., 2016; (Alabi *et al*., 2021; Oloruntoba and Ogunbunmi, 2020; Ajeh *et al*., 2022). Crop germination is delayed, the chlorophyll content is poor and some crops perish when grown in high petroleum contaminated soil (Li *et al.*, 2020). Microorganism are reported to be widely distributed on biosphere because of their metabolic capability which are very impressive and they can easily grow in a wide range of environmental conditions (Veler *et al*., 2020; Viera *et al*., 2020). The nutritional versatility of microorganism can be exploited for decontamination of pollutants. This kind of process is termed as bioremediation (Ambust *et al*., 2021). There is however, reliable evidence that autochthonous (indigenous) microorganisms have some advantages over allochthonous microbes in the degradation of hydrocarbons as these organisms are able to develop naturally over the years and are well adapted for survival and proliferation in such environment (Adegbola *et al*., 2014). These microbes can convert, modify and utilize toxic pollutants in order to obtain energy and biomass production in process (Enim, 2013; Su *et al*., 2023).

Microbial remediation is inexpensive and completely mineralize organic pollutants into carbon dioxide, water, inorganic compounds and cell proteins or convert organic pollutants into other simpler organic compounds (Barua *et al*., 2011; Das *et al*., 2012, Wang *et al*., 2019). However, for the microbes to be used for bioremediation of organic pollutants research, they must first be isolated from their natural ecosystem, characterized and identified base on their peculiar morphological and biochemical features using standard analytical techniques (Senko *et al*., 2014; Woke *et al*., 2017; Kufan *et al*., 2021).

2.Methodology

2.1 *Sample Collection*

The waste motor engine oil contaminated soil sample used in this study was collected aseptically at 0-20cm deep with the aid of a sterile soil augar from the waste oil dump site in the mechanic village, Uyo, Akwa Ibom State, Nigeria unto sterile polythene bag for microbiological analysis.

2.2 Microbiological Analysis

2.2.1. Isolation and Identification of Hydrocarbon Degrading Microbial isolates

Hydrocarbon utilizing bacteria (HUB) and fungi (HUF) were isolated from the soil sample by pour plate technique. Culturable bacteria were obtained in a nutrient agar medium plate supplemented with 0.5 mg Nystation per a litre of the medium to inhibit fungi contaminants. The hydrocarbon utilizing bacteria plates were cultured and subcultured onto nutrient agar and incubated at 28°C for 24hours. Distinct colonies of bacteria were further subcultured onto slant nutrient agar in Bijou bottles and incubated at 28°C for 24hours. The slant cultures were stored in a refrigerator at 4°C and served as pure stock culture for subsequent characterization and identification of the isolates for the hydrocarbon utilizing fungi, culturable isolates were obtained in a potato dextrose agar medium plate supplemented with 0.5mg streptomycin per litre of the medium to inhibit bacteria growth. The hydrocarbon degrading fungi were subculture onto potato dextrose agar and incubated at room temperature for 5 days. Pure colonies were each picked onto potato dextrose agar medium in McCartney's bottles and incubated at room temperature for identification of the isolates.

2.3 Enumeration of Microorganisms

Total viable count of bacteria and fungi were enumerated after incubation of the microbes by direct plate count technique using a colony counter. Colonies on each plate were counted and mean number of viable colonies on triplicated plates were obtained before purification of the microorganisms for characterization and identification.

2.4 Characterization and Identification of Isolates

Bacterial isolates were characterized by morphological and biochemical characteristics. The biochemical tests carried out on each of the bacterial isolates were; gram staining, spore staining, motility, oxidation fermentation tests, catalase, oxidase, indole, methyl red (MR), Voges Proskauer (VP), citrate, sugar hydrolysis, urease, coagulase, nitrate reductase and sugar fermentation tests. Sugars fermented were manitol, lactose, glucose, maltose, xylose, sucrose, arabinose, mannose, dextrose, galactose and fructose. Identification was done using the identification manuals of Holt *et al.* (1994); Barrow and Feltham (2003). Fungi isolates were characterized by morphological and cultural characterization and microscopic examination (Cheesbrough, 2006). Identification was done using identification schemes of Barnet and Hunter (1987) and Samson *et al.* (1984).

2.5 Determination of Hydrocarbon degrading potential of isolates

The capacity of the isolates to degrades spent motor engine oil from petrol and diesel driven vehicles respectively were investigated using the method of Okpokwasili and Okorie (1998). This method has previously been adopted by Ijah and Ukpe (1992), Itah and Essien (2001) and Essien *et al.* (2003) using mineral salt medium of Zajic and Supplison (1996). Three milliliters (3.0 ml) of filtered (0.5µm pose size filter, Millipore Corporation England) sterilized spent motor engine oil from petrol and diesel driven vehicles respectively were transformed into 15 sterile test tubes based on the number of microbial isolates (bacteria and fungi) identified for use in this work. The same was applicable for their respective control test tubes. The test tubes contain 0.5ml each of the sterile mineral salt broth. Furthermore, the spent engine oil supplemented mineral salt medium was then inoculated with 0.1ml volume of 24 hours nutrient both culture of each bacterial isolates and 5 days potato dextrose broth culture for fungi and each isolate fifteen uninoculated test-tubes containing only mineral salt and spent engine oil from petrol and diesel driven vehicles respectively were included as control. Monitoring of degradation was done 48 hourly, and the amount of oil left in each test tubes was determined by gravimetric method (Itah, 1999; Itah and Essien (2002); Nwakanma and Obih (2015). Percentage weight loss of the soil was determined as follows;

$$\% \text{ Weight loss} = \frac{\text{wt of spent oil (control)} - \text{wt of spent oil (degraded)} \text{X } 100}{wt\ of\ spent\ oil\ control}$$

The degree of weight loss of the medium in the test tubes was used as the index of spent motor engine oil utilization by microbial isolates after 24 days of incubation.

3. Results and Discussion

The present study has identified *Bacillus mycoides, Staphylococcus aureus, Listeria murrayi, Actinomycete viscosus, Clostridium sporogenes, Bacillus licheniformis, Corynebacterium ulcerans* and *Clostridium histolyticum* as hydrocarbonoclastic bacteria isolated from spent motor engine oil contaminated soil from Mechanic Village Uyo, Akwa Ibom State (Table 1). Table 2 and 3 revealed seven fungi (molds and yeasts) isolates identified from the same soil sample.

Table 1: **Morphological and biochemical characteristics of bacteria isolated and identified in soil impacted by spent motor engine oil from the mechanic village, Uyo, Akwa Ibom State**

Study location	S/C	Morphological Characteristics	Gram. Reaction	Spore.	Motility	O-F Test	Catalase	Oxidase	Indole	MR	VP	Citrate	Sugar	Urease	Coagulase	Nitrrate	Sugar Fermentation											Probable Organism
																	Manitol	Lactose	Glucose	Maltose	Xylose	Sucrose	Arabin	Mannos	Dextros	Galacto	Fructos	
MECHANIC VILLAGE, UYO, AKWA IBOM STATE	AA$_1$	Expansive white hairy colonies with characteristic swirls	Rod +	+	-	+	+	+	-	+	+	+	+	+	-	+	AG	AG	AG	AG	OG	AG	AO	AG	OG	AG	AG	*Bacillus mycoides*
	BB$_2$	Round golden yellow colonies with grapelike clusters	Cocci cluster +	-	-	+	+	-	-	+	+	+	+	+	+	+	AG	AG	AG	AG	OG	AO	AO	AG	AG	AG	AG	*Staphylococcus aureus*
	CC$_3$	Small translucent, grayish colonies	Short rod +	-	+	+	+	-	-	+	+	+	+	+	-	+	AG	AG	AG	OO	OG	OO	AO	AO	OO	OO	OO	*Listeria murrayi*
	DD$_4$	Gray and white colonies form fungus – like branched networks of hyphae	Rod +	-	-	+	+	-	-	+	-	-	-	+	-	+	AG	AG	AG	AO	OG	AG	AO	AO	AO	AG	AG	*Actinomycete viscosus*

EE$_5$	**Small to medium side rhizoidal colonies with raised yellow gray centre and flattened periphery**	**Rod +**	+	+	-	-	+	-	+	-	+	+	+	-	-	**AG**	**OO**	**AG**	**AO**	**AG**	**OO**	**AO**	**AO**	**AG**	**AO**	**OO**	***Clostridium sporogenes***
FF$_6$	**Colonies with rough wrinkled surface, with "licheniform" or hair-like growths with opaque to white colour**	**Cocci bacilli** +	+	+	-	+	+	-	+	+	+	+	+	-	+	**AG**	**AG**	**AG**	**AO**	**AG**	**AG**	**AO**	**AO**	**AG**	**AG**	**AO**	***Bacillus licheniformis***
GG$_7$	**Small, grayish colonies with a granular appearance, mostly translucent, but opaque centre**	**Rod +**	-	-	+	+	-	-	+	-	+	+	+	-	-	**OG**	**OG**	**AO**	**AG**	**AO**	**AG**	**AO**	**AG**	**AG**	**AG**	**AG**	***Corynebacterium ulcerans***
HH$_8$	**Clumping in pairs or short chains and rods. Cells richly flagellate and motile**	**Rod +**	+	+	+	+	-	-	-	-	+	-	+	-	-	**AO**	**OO**	**OO**	**OO**	**AG**	**OO**	**AO**	**AO**	**AO**	**AG**	**OO**	***Clostridium histolyticum***

Table 2: Morphological and biochemical characteristics of molds isolated and identified in soil impacted by spent motor engine oil from mechanic village, Uyo, Akwa Ibom State

Study location	S/C	Surface Colour	Reverse Colour	Appearance	Nature of Hyphae	Nature of Conidium/Sporangium	Vegetative Reproductive Cell	Structure of Sexual Spore	Probable Organism
MECHANIC VILLAGE, UYO, AKWA IBOM STATE	PP_1	Blue – Green	Yellow	Velvety	Septate and branched hyphae	Conidia are numerous and closely packed brush-like structure	Reproduced through asexual conidiospore	Round and rough chains of spores from brush-shaped coniodiospore	*Penicillium chrysogenum (notatum)*
	QQ_2	Smoky-gray green with whiter bording lines	Slightly yellow	Woolly to cottony	Septate hyphae and hyaline	Conidial heads are strongly columnar	Reproduced through asexual conidiosphores	Conidia are round and smooth-walled	*Aspergillus fumigatus*
	RR_3	Black	Milky	Fluffy mass	Aseptate and unbranched hyphae	Bigger sporangia are globose with liberated sporangiosphores	Reproduced asexually by sporangiospores	Sporangium at the tip of the sporangiosphore is rounded	*Rhizopus stolonifer*
	SS_4	White to brown	Milky	Cottony	Aseptate and unbranched hyphae	Smaller sporangia are globose with air-dispersed sporangiospores	Reproduced asexually by sporangiospores	Sporangiosphores are elliptical	*Rhizopus oryzae*
	TT_5	White	Pale violet	Cottony	Septate and branched hyphae	Chlamydospores are multicellular, with cells connected by pores in the septae	Reproduced asexually by fragmentation of chlamydospores	Fusiform, slightly curved, pointed at the tip with seption	*Fusarium oxysporum*

Table 3: **Morphological and biochemical characteristics of yeasts isolated from soil impacted by spent motor engine oil from the mechanic village, Uyo, Akwa Ibom State**

Study location	S/C	Surface colour	Reverse colour	Appearance	Nature of Hyphae	Vegetative reproductive structure	Sugar Fermentation						Sugar Assimilation						Probable organism
							Glucose	Lactose	Mannose	Sucrose	Galactose	Arabinose	Glucose	Lactose	Mannose	Sucrose	Galatose	Arrabinose	
MECHANIC VILLAGE, UYO, AKWA IBOM STATE	PP_1	Milky	Milky	Oval, mucoid appearance	No true hyphae	Reproduced asexually by budding	OO	OO	OO	OO	OO	OO	+	+	+	-	+	+	*Cryptococcus*
	QQ_2	Orange to pink	Orange	Soft, smooth, moist and spherical appearance	Absent of hyphae	Reproduced asexually by multilateral budding	OO	OO	OO	OO	OO	OO	+	-	-	+	+	+	*Rhodotorula mulcilaginosa*

Key: OO =non-fermentative += Positive- = Negative

They include; *Penicillium notatum, Aspergillus fumigatus, Rhizopus stolonifer, Rhizopus oryzae, Fusarium oxysporum, Cryptococcusterreus* and *Rhodotorula mulcilaginosa.* These results showing variable bacteria and fungi isolates identified from oil polluted soil agrees with studies of Jobson *et al.* (2002) that hydrocarbon degraders are usually present in large numbers in many soils polluted soils and water compared with uncontaminated environment. It also corroborates Ekundayo *et al.* (2012) that the present of microbes in sites impacted with petroleum products suggest their ability to utilize the hydrocarbons as their sole carbon source for growth.

Table 4 revealed the distribution frequency of bacteria isolated from the oil contaminated soil. *B. mycoides* showed the highest percentage occurrence of 27.9 % while *C. histolyticum* was the lowest with 6.3% percentage occurrence. Again, *P. notatum* exhibits the highest percentage occurrence 20.4% *among the fungi isolated whereas C. terreus* was the lowest with 4.1%percentage occurrence. Meanwhile, it is noticed in this study the relatively low bacterial and fungal population dynamics which might be so as a result of toxic impacts of the spent motor engine oil in the soil. This is in accordance with report by Jensen (1975) suggesting a toxic or inimical impact of the waste oil on the heterotrophic microbial counts in hydrocarbon impacted environment.

Table 4: Distribution frequency of bacteria isolated and identified in soil impacted by spent motor engine oil collected in mechanic village Uyo, Akwa Ibom State

Probable orgasms	Distribution frequency (n=111)	Percentage occurrence (%)
Bacillis mycoides	31	27.9
Staphylococcus aureus	9	8.1
Listeria murrayi	11	9.9
Actinomycete viscosus	13	11.7
Clostridium sporogenes	18	16.2
Bacillus licheniformis	8	7.2
Corynebacterium ulcerans	14	12.6
Clostridium histolyticum	7	6.3

Table 5: Distribution frequency of fungi isolated and identified from soil impacted by spent motor engine oil collected in mechanic village, Uyo, Akwa Ibom State

Probable orgasms	Distribution frequency (n=98)	Percentage occurrence (%)
Penicilluim notatum	20	20.4
Aspergillus fumigatus	11	11.2
Rhizopus stolonifer	10	10.2
Rhizopus oryzae	24	24.5
Fusarium oxysporum	15	15.3
Cryptococcus terreus	4	4.1
Rhodotorula mulcilaginosa	14	14.3

Furthermore, the microbes demonstrated variable potentials to utilize hydrocarbon in the spent oil from petrol and diesel driven vehicles respectively. For instance, *C. sporogenes* exhibited the highest hydrocarbon utilization efficiency in spent oil from petrol driven vehicle with 38.09% degradation on the 24th day of incubation in the oil. The least ability to utilize the oil (19.28%) was shown by *S. aureus*. This in agreement with Pinholt *et al.* (1999), that the efficiency of bioremediation of crude oil by bacteria in soil ranges from 0.13% to 50%. But, Cameota and Singh (2008) reported 90% degradation efficiency of hydrocarbons in 6 weeks in liquid culture by a consortium of *Pseudomonasaeruginosa* and *Rhodococcuserythropholis*. In this work, *C. histolyticum* exhibits the highest ability to degrade spent engine oil from diesel driven vehicle (35.00%) after 24 days incubation in the oil (**Table 6**). The results reveal that *Clostridium species* were bacteria with the best degrading potential for spent oil. *C. ulcerans* shows least hydrocarbon utilization efficiency with 17.31% degradation on the 24th day of incubation in spent oil from diesel driven vehicle.

Table 6: Spent engine oil utilization by monoculture of bacterial and fungal isolate

	Microorganism	Incubation Period (Days)											
		2	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**
	Bacteria Monoculture	%	%	%	%	%	%	%	%	%	%	%	%
Petrol Driven Engine	*Bacillus mycoides*	1.98	3.96	4.95	5.94	8.74	9.90	10.89	12.87	13.86	15.84	17.82	20.79
	Staphylococcus aureus	1.43	4.29	5.00	7.14	9.29	10.71	12.14	14.29	15.71	16.43	17.86	19.28*
	Listeria murrayi	2.94	5.88	7.84	9.80	11.76	10.80	15.69	17.65	20.58	23.53	25.49	27.45
	Actinomycete viscosus	2.56	5.13	7.69	9.40	10.26	11.97	13.68	15.38	17.09	18.80	19.66	21.37
	Clostridium sporogenes	11.43	13.33	14.29	15.24	18.09	21.90	23.81	25.71	27.62	30.48	33.33	38.09*
	Bacillus licheniformis	9.28	11.34	12.37	14.43	15.46	17.53	19.59	23.71	26.80	29.89	32.99	35.05
	Corynebacterium ulcerans	3.03	4.14	6.06	9.50	11.41	12.12	14.20	16.18	18.23	20.18	22.22	24.26
	Clostridium histolyticum	4.08	6.12	7.14	10.20	12.24	14.29	15.31	18.39	19.38	21.43	22.45	24.49
Diesel Driven Engine	*Bacillus mycoides*	1.94	3.88	5.83	8.74	8.91	12.62	14.56	18.45	20.39	23.30	25.24	27.18
	Staphylococcus aureus	2.02	3.03	4.10	8.18	11.11	12.10	13.17	16.16	19.20	22.12	24.24	27.18
	Listeria murrayi	1.09	1.09	3.26	5.43	6.52	8.69	9.78	12.04	15.22	18.48	21.74	22.83
	Actinomycete viscosus	2.41	2.87	3.61	8.43	9.64	12.05	15.66	18.07	21.69	25.30	27.71	30.12
	Clostridium sporogenes	2.68	5.36	7.14	9.82	12.50	14.29	16.96	18.75	21.43	24.11	26.79	28.57
	Bacillus licheniformis	1.94	4.85	5.83	7.77	9.71	11.65	12.62	14.56	16.50	18.45	20.39	23.30
	Corynebacterium ulcerans	0.96	0.96	2.88	5.77	6.73	7.69	9.62	10.58	13.46	15.38	16.35	17.31*
	Clostridium histolyticum	8.67	17.50	19.17	20.83	22.50	24.17	25.83	27.50	29.17	30.83	32.50	35.00*
	Fungi Monoculture												
Petrol Driven Engine	*Penicillium notatum*	1.94	4.85	6.79	9.71	10.68	11.65	13.59	15.53	16.50	18.48	20.39	22.33
	Aspergillus fumigatus	1.04	2.08	4.17	6.25	7.29	9.38	11.56	13.54	15.63	18.75	19.79	21.88
	Rhizopus stolonifer	4.76	6.67	9.52	12.38	14.29	16.19	17.14	18.09	20.00	21.90	21.81	26.67
	Rhizopus oryzae	2.04	5.10	7.14	10.20	12.24	14.29	15.31	18.37	20.41	20.98	23.47	25.51
	Fusarium oxysporum	1.89	3.26	5.43	6.52	7.61	9.78	13.04	15.22	17.39	18.48	19.56	21.74
	Cryptococcus terreus	3.16	5.26	7.37	9.47	10.53	11.58	13.68	13.79	14.74	15.79	18.95	20.00*
	Rhodotorula malcilaginosa	3.03	4.55	9.09	10.61	13.64	16.67	19.69	22.73	27.27	28.79	31.82	34.85*

Diesel Driven Engine	*Penicillium notatum*	1.19	2.38	4.76	7.14	9.52	10.71	11.90	13.09	14.28	16.67	19.05	22.62
	Aspergillus fumigatus	2.70	5.41	6.76	9.46	10.81	12.16	14.86	18.92	21.62	24.32	24.68	29.73*
	Rhizopus stolonifer	1.08	2.15	4.30	7.53	9.68	11.83	13.98	16.13	19.35	22.58	24.73	27.96
	Rhizopus oryzae	1.98	3.96	4.95	5.94	6.93	7.92	10.89	12.87	14.85	15.84	17.82	20.76*
	Fusarium oxysporum	1.25	2.50	6.25	7.50	10.00	12.50	13.75	16.25	18.75	21.25	23.75	27.50
	Cryptococcus terreus	1.01	5.26	6.06	8.08	9.19	11.12	13.15	14.12	15.20	17.17	21.50	24.18
	Rhodotorula malcilaginosa	4.04	4.55	7.07	10.10	12.13	14.20	15.15	16.30	19.15	21.10	24.24	25.30

* Lowest and highest percentage hydrocarbon degradation potentials

This is in agreement with the report by Itah and Essien (2005) that *Corynebacterium* sp. and *Cryptococcus albidus* showed very weak tarball degradation. *Rhodotorula mulcilaginosa* showed 35.89% utilization efficiency of hydrocarbons in spent oil from petrol driven vehicle among the fungi used in this work. This agrees with Bhatt *et al.* (2002), that *Rhodotorula sp*. contributed effective degradation of low molecular weight PAHs and other hydrocarbon components in crude oil. *R. mulcilaginosa* also demonstrates highest ability to utilize 25.3% hydrocarbons in the spent oil from diesel driven vehicle. But *A. fumigatus* utilizes 29.73% the variability in the capacity of the microbial isolates to degrade hydrocarbons in the spent engine oil from the two sources agrees with Margesin *et al.* (2013); Nie *et al.* (2014) who suggested that the isolates secreted various catalytic enzymes responsible for the degradation of different fractions in the petroleum oil.

Conclusion

In conclusion, from the findings of this study, it suggests that *Clostridium* species were bacteria with the highest hydrocarbon utilization potentials whereas *R. mulcilagenosa* and *A. fumigatus* were fungi highest ability to degrade hydrocarbon, so could be employed in hydrocarbon contaminated site remediation.

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