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Fungi and bacteria as potent hydrocarbon degraders

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Abstract- The most dangerous pollution in the environment are the unwanted hydrocarbon in form of oil and petroleum which is the result of leak from the coastal oil refiners. Which leads several researchers to investigate its distribution and effects in the environment and impact on human. This contamination effect almost every type of ecosystem (marine, fresh and terrestrial). Current study focus to evaluate the bacterial and fungal species as a hydrocarbon degrader. From the biodegradation test in case of fungi, it was revealed that on day 6th Aspergillus tubingensis (1.63×109) degrade high amount of the hydrocarbon as compared followed by the Aspergillums niger (1.35×108). Lowest consumption was recorded for the Alternaria tenuissima (1.30×103). On the other hands in case of bacteria high degradation was recorded for the strain of the Pseudomonas spp (1.65×109), followed by the Pseudomonas aeruginosa (1.60×108). Potent strains of the bacteria and fungi were further identified and confirm by molecular techniques. From the current study it was concluded that introduction of these portent bacteria and fungi can greatly reduce the petroleum pollution in the environment. Future study should be encouraged to dig out novel strains of bacteria, fungi and other microbes to control these pollutions.

Keywords: Petroleum pollution, Biodegradation of oil pollutant, Bacteria, Fungi

I. INTRODUCTION

From the last decade several ecosystem has been changed due to anthropogenic activities. Many of that change is effecting human life in negative ways, so a need has been emerged to protect this ecosystem in order to sustain better life. Pollution in the environment are the major problem emerged due to human activity and has to be controlled. The most dangerous pollution in the environment are the unwanted hydrocarbon in form of oil and petroleum which is the result of leak from the coastal oil refiners. Which leads several researchers to investigate its distribution and effects in the environment and impact on human. This contamination effect almost every ecosystem (marine, fresh and terrestrial). Annually 5millions tons of crude oil comes from the human activity into the environment (Hinchee and Kitte, 2001). The main source of this pollution are the spills from the tankers, vessels (pipelines) and barges. These spills got attention of many researchers including chemists, engineers, biotechnologists and environmentalists (Braddock et al., 2000; Tazaki et al., 2004). It is important to aware the public and exact legal restrictions on the releasing of contaminants in different localities which pollute the environment, it is significant to invent affordable and effective technologies to deals with the oil of industries contamination. One of the important is biological method through which microorganisms are participating and depends on the metabolic potentials to remove the pollutants (Megharaj et al., 2011). This biological approach (techniques of bioremediation) is flexible, ecofriendly and economically well (Obi et al., 2016). Due to this view the bioremediation is achieving very large significance due to remediation of contaminated areas. Many studies described the catabolic capabilities of native microorganism including the bacteria, algae and fungi to reduce the hydrocarbons (Wang et al., 2011). That type of microbes is adjusted to the pollutants environment are well equipped of definite enzymes system that make them able to utilize the hydrocarbons as single source of carbon. Several microbes are involved in the degradation of hydrocarbons including archaea and bacteria which are been reported in the polluted environment comprising of hydrocarbons (Fowler et al., 2016; Hazen et al., 2013). Current study aimed to evaluate bacterial and fungal species as hydrocarbon degraders.

II. METHODOLOGY

Bacteria and fungi were kindly provided by the microbiology laboratory of Kohat University of Science and Technology. Bacteria and fungi were subjected to hydrocarbon degradation test for this Bushnell Hass broth of 100ml was prepared into 4 separate conical flasks. Flask were supplemented with 1ml of spent engine oil. Further bacteria and fungi were inoculated into the flask, which were then placed in the shaker. Further, absorbance of the culture was measured using spectrophotometer.

Serial dilution of the plate was done in the conical flask after each two days to enumerate the number of colonies that thrived and/or can survive the Bushnell Hass broth-engine oil mixture. Those microbes that survive were identified using molecular techniques for this, DNA was extracted by using Phenol Chloroform method and were identified using 16sRibosomal RNA.

III. RESULTS

From the biodegradation test in case of fungi, it was revealed that on day 6th Aspergillus tubingensis (1.63×109) degrade high amount of the hydrocarbon as compared followed by the Aspergillums niger (1.35×108). Lowest consumption was recorded for the Alternaria tenuissima (1.30×103). on the other hands in case of bacteria high degradation was recorded for the strain of the Pseudomonas spp (1.65×109), followed by the Pseudomonas aeruginosa (1.60×108). table 1 shows the per detail count of the degradation test while table 2 shows the absorbance at 520nm.

Table 1: Total count during biodegradation test

Fungal strain	Day 2	Day 4	Day 6
Aspergillums niger	1.12×10^5	1.25×10^7	1.35×10^8
Penicillium citrinum	1.15×10^4	1.25×10^5	1.30×10^8
Alternaria tenuissima	1.56×10^2	1.2×10^3	1.30×10^3
Aspergillus tubingensis	1.64×10^5	1.54×10^8	1.63×10^9
Bacterial strain			
Pseudomonas spp	1.13×10^8	1.25×10^8	1.65×10^9
Bacillus cereus	1.25×10^3	1.26×10^5	1.40×10^7
Staphylococcus aureus	1.66×10^3	1.60×10^4	1.50×107
Streptococcus pneumoniae	1.64×10^5	1.54×10^8	1.33×10^7
E. coli	1.32×10^4	1.45×10^7	1.55×10^7
Pseudomonas aeruginosa	1.25×10^5	1.23×10^6	1.60×10^8
Acinetobacter sp.	1.46×10^2	1.40×10^4	1.30×10^7

Table 2: Absorbance at 520nm

Fungal strain	Day 2	Day 4	Day 6
Aspergillums niger	0.22	0.33	0.45
Penicillium citrinum	0.24	0.32	0.54
Alternaria tenuissima	0.23	0.28	0.51
Aspergillus tubingensis	0.24	0.35	0.53
Bacterial strain			
Pseudomonas spp	0.24	0.35	0.45
Bacillus cereus	0.21	0.27	0.40
Staphylococcus aureus	0.23	0.32	0.41
Streptococcus pneumoniae	0.24	0.36	0.54
E. coli	0.25	0.45	0.56
Pseudomonas aeruginosa	0.23	0.41	0.52
Acinetobacter sp.	0.32	0.42	0.53

Molecular identification of the potent hydrocarbon degrading bacteria and fungi

After extraction of DNA and PCR, the amplified sequence was confirmed using gel electrophoresis. After sequencing the phylogenetic tree was constructed for two bacterial strain that were potent and two fungal strain that were powerful degrading. The phylogenetic tree is given in the figure 1 for bacteria and figure 2 for fungi.

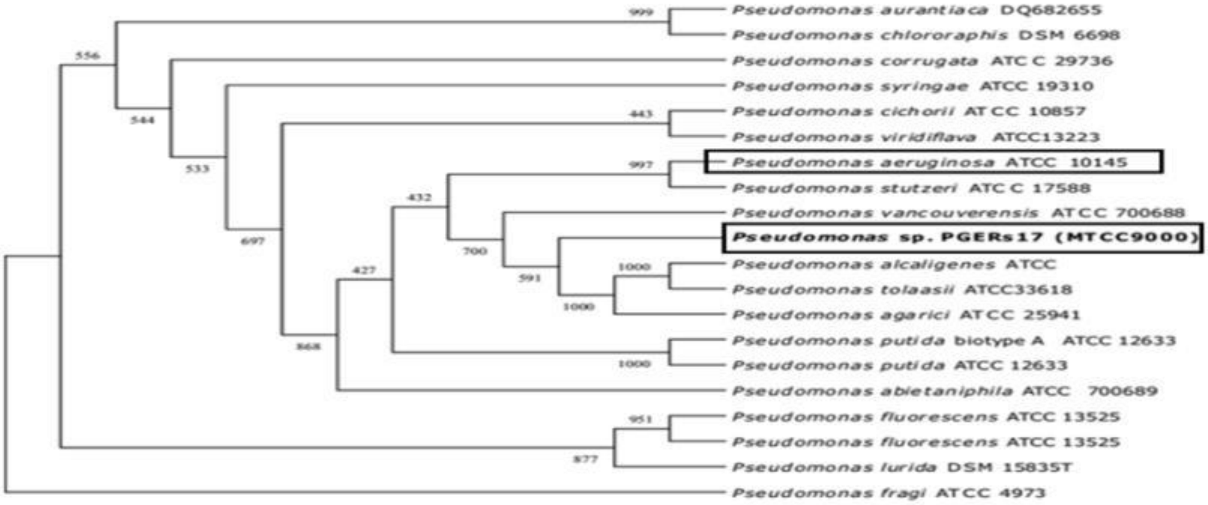

Figure 1: Cladogram of bacterial species

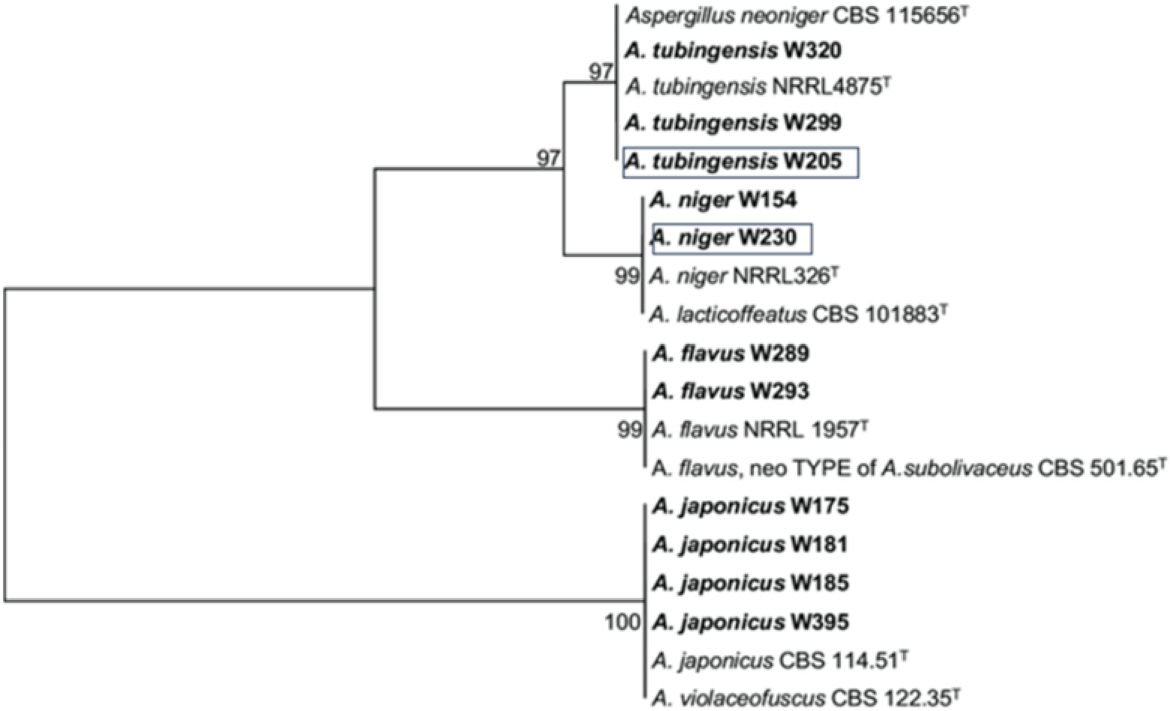

Figure 2: Cladogram of fungal species

IV. DISCUSSION

Discharge of hydrocarbons into the environment either naturally or due to anthropogenic activity is considered as the main source of pollution of both aquatic and terrestrial environment (Holliger et al., 2001). These pollutants can accumulate in the organism (both plant and animal) tissues and can affect other organism through food chain or even cause death or mutation in the organism in which they are accumulated (Alvarez and Vogel., 2005). Serval methods and technology have been used in the remediation of the hydrocarbon in these ecosystems such as, evaporation, burying, mechanical, washing and dispersion. But unfortunately these techniques/technology are very expensive or unavailable in most of the cases and besides this it also leads to incomplete decomposition or breakdown of the hydrocarbon (contaminant) of the environment. So alternative method known as bioremediation are used which can be defined as 'use of microorganisms to detoxify or remove pollutants'. Due to their capacity or capabilities of their metabolic functions. This technique is one of the most evolving procedure to remove or detoxify the pollutant in the environment even includes petroleum industries products (Jones et al., 2011). Furthermore, this technique is also better in term of cost and non-invasive (April et al., 2000). Current study aimed to evaluate the bacterial and fungal species for hydrocarbon degradation test. Result revealed that, fungi, it was revealed that on day 6th Aspergillus tubingensis (1.63×109) degrade high amount of the hydrocarbon as compared followed by the Aspergillums niger (1.35×108). Lowest consumption was recorded for the Alternaria tenuissima (1.30×103). On the other hands in case of bacteria high degradation was recorded for the strain of the Pseudomonas spp. (1.65×109), followed by the Pseudomonas aeruginosa (1.60×108). and then potent/powerful strains were subjected to phylogenetic analysis.

Those organisms which are capable to degrade the hydrocarbon are distributed widely such as in Soil, fresh and marine water ecosystem. and isolation and identification of the organism from oil pollutant soil or other sample is considered as the evidence that these are actively involved in the degradation of the oil in the ecosystem. Furthermore, identification and isolation of these organism from the soil, indicate strategic evolution which help them to adjust and adopt to the oil contaminated environment and are also capable of using these substances as source of energy. Among isolated bacteria presence of the Bacillus can be endorsed to their capacity of forming spores which enable them to survive in harsh environment such as oil pollutant environment (Ghazali et al., 2004). Furthermore, different fungal strains are also reported by several researcher as potential degrading strains of hydrocarbon such as Chaillan et al., (2004) reported Amorphoteca, Talaromyces, Neosartorya and Candida from the soil sample contaminated with the oil and proved as perfect bio degrader of the hydrocarbon. Singh, (2006) reported Pencillium, Aspergillus and Cephalo sporium and were tested against the hydrocarbon degradation test and proved as potential degrader of the pollutant. Besides this several yeast species are also reported by the Bogusławska and Browski (2001) such as Rhodotorula Mucilaginosa, Geotrichum sp, Trichosporon mucoides and Candida lipolytica and were capable of degrade pollutant in the soil.

V. CONCLUSION

From the current study it was evident that hydrocarbon degrading microorganism can be easily isolated from the oil contaminated soil samples and can be used for the bioremediation for the reducing hydrocarbon pollutant soil. These pollutants are one of the most dangerous with high toxicity. And bioremediation of these pollutant through the microorganism is considered as very efficient and eco-friendly method.

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