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EFFECT OF UV RADIATION ON THE GROWTH AND PETROLEUM HYDROCARBON DEGRADATION ABILITY OF BACTERIA

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Abstract: Accidental leakages of oil during the transportation and other anthropogenic activities results, pollution in environment. Petroleum hydrocarbons are highly toxic to plants animals and humans and they have carcinogenic and mutagenic properties. Bioremediation is as an efficient, economic, versatile and environment friendly technique. The present study is focused to enhance oil degrading ability of bacteria by inducing random mutation using UV radiation. Bacterial strain was isolated from petroleum oil contaminated soil samples. Forty nine oil degrading bacteria were isolated and among them four bacterial isolates (ALK-14, ALK-16, ALK-23 and ALK-35) have shown maximum potential to degrade petroleum hydrocarbon and identified as *Alcaligenes* species, *Bacillus* species, *Enterobacter* species and *Corynebacterium* species respectively. The pure cultures of these bacterial isolates were subjected to induced mutation at two distance 20cm and 40cm from UV lamp (15W) for different times intervals 20, 40, 60, 80, 100, and 120 sec and it was observed that number of colonies decreased as compare to control depending on exposure time and distance from UV source. The present study mainly focused on petroleum hydrocarbon degradation ability of mutant strains as compared to their wild strains. Mutant strains *Alcaligenes* species M2 and *Corynebacterium* species M1 exhibited higher degradation than their wild strains. This study reflects that gene specific mutationenhanced the degradation ability of bacteria and can be employed in bioremediation of petroleum hydrocarbons in soil and water.
Keywords: *Alcaligenes; Corynebacterium,* Petroleum hydrocarbon, UV Radiation.
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INTRODUCTION

Ecosystems have been affected by the pollution due to large scale industrialization. Petroleum hydrocarbons are major source of energy for industrial and domestic use. Petroleum contamination has spread to almost all components of environment. Extensive damage has been caused by contamination of petroleum hydrocarbons to water and soil (Ajisebutu, *et al.,* 2003). Petroleum consists of mixture of hydrocarbons and other organic compounds such as organo-metallic compounds. Petroleum consists of aliphatic, aromatics hydrocarbon and resins (Overton *et.al.,* 1994). Researchers have shown that the massive and extensive petroleum hydrocarbon pollution of the environment constitutes socio economic and public health hazard. Some of the hydrocarbons and related materials are known to be highly toxic to plants, animals and humans. Crude oil aromatic fractions is lethal at concentration of a few ppm (parts per million) and sub-lethal at ppb levels (parts per billion) (Odiete, 1999). During the last three decades, various bioremediation approaches have been used in the degradation of petroleum hydrocarbons, among them bioremediation is considered to be an environmental friendly and safe method. To date many bioremediation methods of pollutants have been attempted and few were successful (Bishnoi *et al.,* 2006; Potentini and Rodriguez Malaver, 2006; Demir *et al.,* 2007; Singh *et al.,* 2007). Since the late 1930s, mutation has been employed as

technique of strain improvement in many research fields and it is an essential and often most direct and least expensive means of improving microorganisms for specific application (Rowlands, 1984). Balz (1986) reported that mutagenesis improved the performance of organism and enhanced the product quality with fewer unwanted properties. Improved bacterial and fungal strains have been extensively developed by mutation by using physical and chemical agents (Hopwoods, 1970). There are varieties of mutagenic agents; however selection of mutagenic agents is of prime significance. Mutagenic agents are highly toxigenic and their criteria of selection are based on safety from the mutagen and simplicity of technique and availability of the mutagen (Okafor, 1987). Mutants play a vital role in increasing the desirable potential for the degradation of petroleum products and could thus be exploited in the bioremediation of petroleum or its products.The UV radiation is commonly used to generate mutant strains of microorganisms. It is less energetic than X–and gamma rays (ionizing radiation), but its wavelengths were preferentially absorbed by nucleotides of DNA and by aromatic amino acids of proteins, so it has important biological and genetic effects. This research is thus aimed to isolate petroleum oil degrading bacteria from oil-contaminated soil, initiating mutation using ultra violet (UV) radiation, determining the mutant(s) with higher petroleum degrading potentials which could be employed in the bioremediation of petroleum pollutants.

EXPERIMENTAL

Sample collection and enrichment cultures: The bacteria used in this study were isolates from petroleum oil contaminated soil collected from vicinity of Gwalior, M.P. India. Soil samples were collected in the sterilized polythene bags from depth of 5-10 cm and stored at 4°C.All bacterial enrichment and isolations were performed in Bushnell Haas (BH) media. Its composition as following (g/L): $MgSO_4$ 0.2, $CaCl_2.6H_2O$ 0.02, KH_2PO_4 1.0 $K_2HPO_4$1.0 NH_4NO_3 1.0 and $FeCl_3$ 0.05. The pH of the medium was adjusted to 7.0 and 3% (v/v) petrol was added as sole carbon source to autoclaved media.

Isolation of Bacteria: Ten gm soil sample was suspended in 100 mL sterilized distilled water and homogenized for 5 minutes and suspension was left to settle down soil particles and then 0.5ml suspension was spread on enriched Bushnell Hass agar medium and incubated at 37°C for 48 hrs in incubator and morphologically different bacteria were selected for further study.

Screening of Potential Bacterial Isolates: The screening of potent oil degrading bacteria was based on maximum growth on BH broth and zone of degradation method.

i. **Maximum Growth:** Screening of isolated bacterial cultures was done on the basis of maximum growth in terms of optical density. BH broth containing petrol as a carbon source were inoculated with each isolate and incubated at 120 rpm, 37°C in incubator shaker. The growth was measured after every 24 hrs in term of optical density at 600nm by using UV-Vis spectrophotometer (Celik *et al*, 2008).

ii. **Zone of degradation:**The screening of petroleum hydrocarbon degrading isolates were done on the basis of clearing zones formed around the bacterial colonies on BH agar medium by spot inoculation (Zhang *et al.*, 2004).

Identification and Characterization of Screened Isolates: Identification and characterization of selected bacteria were done by cell morphology, cell physiology and biochemical characteristics; IMViC test, starch hydrolysis, casein hydrolysis, gelatin hydrolysis, TSIA test, catalase test, H_2S test, carbohydrate fermentation and nitrate reductase test (Cappicuno and Sherman, 2005).

Mutagenesis of Selected Isolates using UV Irradiation: Nutrient agar plates with 100µl of overnight grown bacterial isolates were exposed to the UV lamp (15W) at distance of 20 cm and 40cm from lamp for different time intervals 20,40,60,80,100 and 120 sec. and UV exposed plates were incubated at 37°C for 24 hrs. The number of colonies and morphology of each plate were observed and recorded and

experiment was performed in triplicate and mean values were taken and three mutated bacterial colonies were selected for further studies.

Determination of Petroleum Hydrocarbon degradation by Selected Mutant strains: BH broth containing petrol as a carbon source were inoculated with each wild isolate and its mutant strains and incubated at 120 rpm, 37°C in incubator shaker. The growth was measured after regular time interval (24hrs) at 600nm by using UV-Vis spectrophotometer and experiment was performed in triplicate and mean values was taken.

Experimental Control: In all experiments control containing petrol, but no bacterial cells, was used to monitor a biotic losses of organic compound.

RESULTS AND DISCUSSION

Isolation and Screening of Petroleum oil Degrading Bacteria

Total 49 petroleum degrading bacteria (ALK-1 to ALK-49) were isolated from different petroleum contaminated soil samples. Out of 49 isolates, four bacterial isolates (ALK-14, ALK-16, ALK-23, and ALK-35) were selected on bases of maximum growth and zone of degradation ability. These isolates have higher potential to degrade petroleum hydrocarbons (Figure 1).

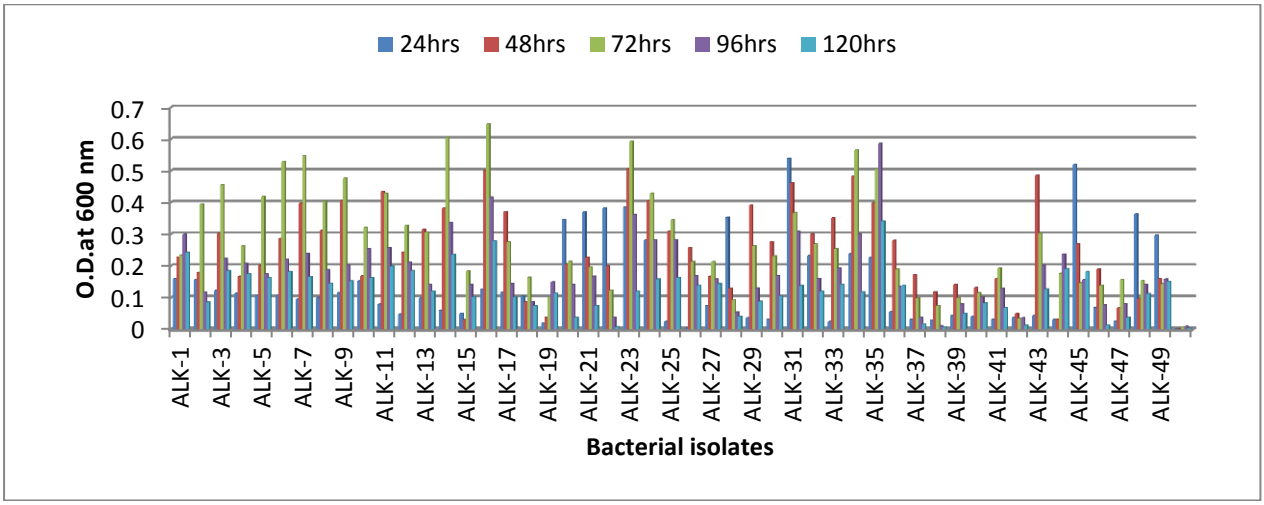

Figure 1. Screening of Petroleum Hydrocarbon degrading bacteria on the basis of maximum growth

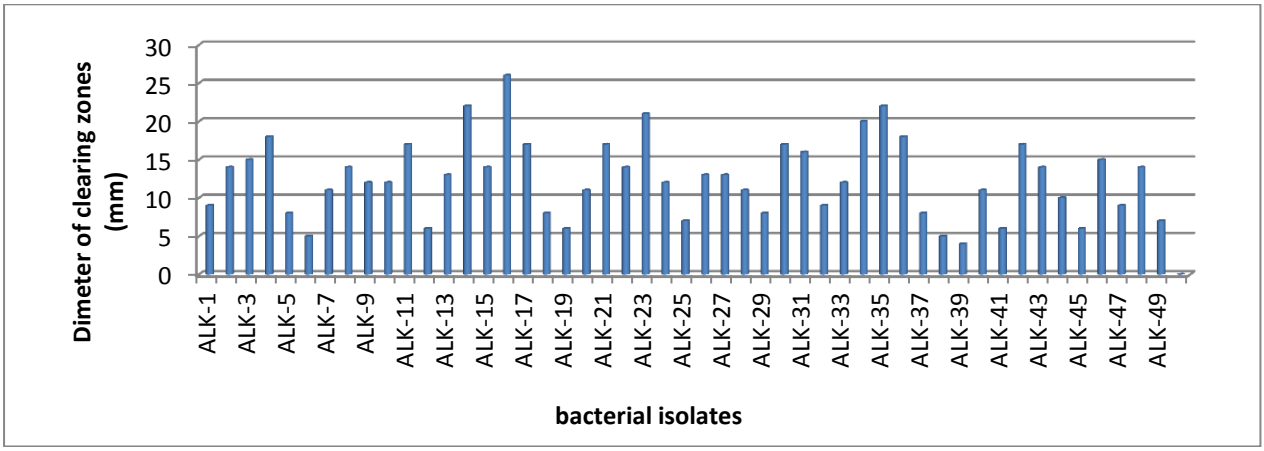

Figure 2. Diameters clearing zones (mm) of hydrocarbon degradation activity by bacterial isolates

Identification and Characterization of Screened Isolates

According to the morphological, biochemical characterization, selected bacterial isolates ALK-14, ALK-16, ALK-23 and ALK-35 were identified as *Alcaligenes* species, *Bacillus* species, *Enterobacter* species and

Corynebacterium species respectively (Figure 2).

Mutagenesis of Selected isolates through UV irradiation

In the treatment of UV radiation, number of bacterial colonies decreased as compared to control with different time of exposure and distance from UV source. In *Alcaligenes* species the number of colonies decreased with distance of UV source and time of exposure. UV exposure at a distance of 20 cm from UV lamp for 20 seconds and 120 seconds, the number of colonies were 152 and 5 respectively, while UV exposure at distance of 40 cm, for 20 seconds and 120 seconds, the number of colonies were 237 and 8 respectively (Figure 3). In *Bacillus species*, the number of colonies decreased with distance of UV source and time of exposure. UV exposure at a distance of 20 cm from UV lamp for 20 seconds and 120 seconds, the number of colonies were 251 and 13 respectively, while UV exposure at distance of 40 cm, for 20 seconds and 120 seconds, the number of colonies were uncountable and 21 respectively (Figure 4). In *Enterobacter* species, the number of colonies decreased with distance of UV source and time of exposure. UV exposure at a distance of 20 cm from UV lamp for 20 seconds and 120 seconds, the number of colonies were 168 and 4 respectively, while UV exposure at distance of 40 cm, for 20 seconds and 120 seconds, the number of colonies were 257 and 9 respectively (Figure 5). In *Corynebacterium* species, the number of colonies decreased with distance of UV source and time of exposure. UV exposure at a distance of 20 cm from UV lamp for 20 seconds and 120 seconds, the number of colonies were 172 and 9 respectively, while UV exposure at distance of 40 cm, for 20 seconds and 120 seconds, the number of colonies were 207 and 16 respectively (Figure 6).

Determination of petroleum degradation by selected mutant strains

Alcaligenes species M2 and *Corynebacterium* species M1 exhibited higher degradation than their wild strains while *Bacillus species* and *Enterobacter* species exhibited higher degradation than their mutants. Idise *et al.,* (2010) reported that mutant strains through the nitrous acid enhance the ability to degrade petroleum hydrocarbon than their wild strain and also reported that mutants by the x-rays lacked the beneficial ability to degrade the petroleum hydrocarbon than the parent. Hanna *et al.,* 2009 reported that mutant strains of *Pseudomonas aerogenosa* and *Pseudomonas putita* through UV rays showed 20x time and 30 X time, highest activities to enhance petroleum hydrocarbon degradation ability as compared to their wild strains respectively. Mutant *Alcaligenes* species M2 and *Corynebacterium* species M1 showed the higher growth in comparison to *their* wild strain.In case of *Bacillus species* and *Enterobacter* species, Wildstrains showed the higher growth in comparison to their mutant strains (Figure 7).

Table 1. Characterization of isolates ALK-14, ALK-16, ALK-23 and ALK-35

Characteristics	*Alcaligenes* species	*Bacillus* species	*Enterobacter* species	*Corynebacterium* species
Morphology	Coccal rod	Rod	Rod	Rod
Diameter (µm)	0.6×1.3	0.5×1.7	0.6×1.7	0.6× 0.8
Gram staining	_	+	_	_
Indole production	_	+	_	+
Methyl red	_	+	_	+
Vogesproskauer	+	+	+	-
Citrate utilization	+	+	+	+
Phenylalanine diaminase test	_	+	+	-
Carbohydrate fermentation	+	+	+	+
Glucose	_	_	_	_
Lactose	_	_	_	_
Sucrose				
Nitrate reductase	+	+	_	+

Casein hydrolysis	_	_	_	-
Starch hydrolysis	+	+	+	+
Gelatin hydrolysis	_	_	_	+
Catalase	+	+	+	+
Urease	+	+	+	+
H_2S production	_	_	+	+
Lipid hydrolysis	+	+	+	+
Oxidase	+	_	_	+
Tripal sugar iron agar test	-	-	Glucose	-

Key: + = Positive, = Negative

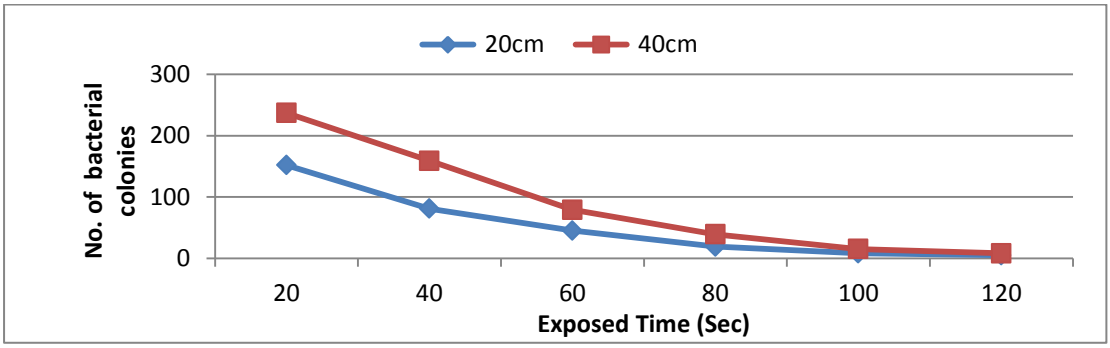

Figure 3. Effect of UV radiation on the growth of *Alcaligenes* species placed at 20 cm and 40cm distance from the UV lamp

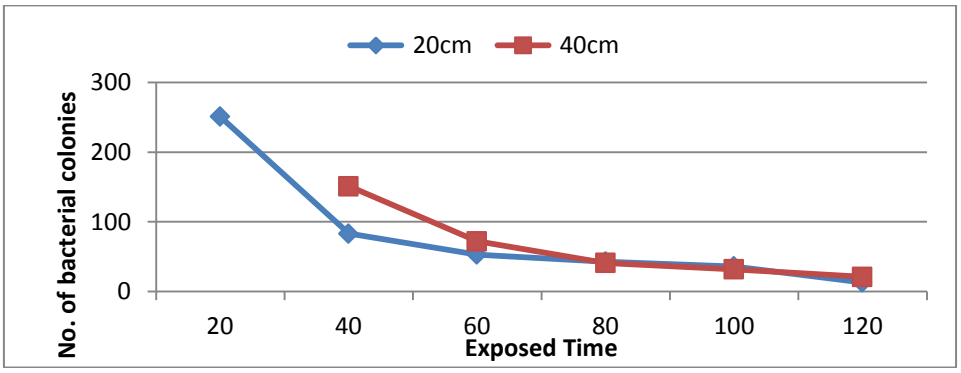

Figure 4. Effect of UV radiation on the growth of *Bacillus species*placed at 20 cm and 40cmistance from the UV lamp

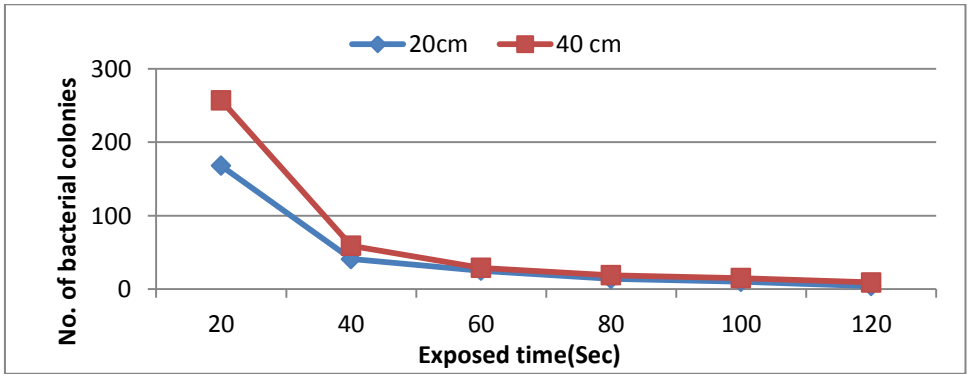

Figure 5. Effect of UV radiation on the growth of *Enterobacter* species placed at 20 cm and 40 cm distance from the UV lamp

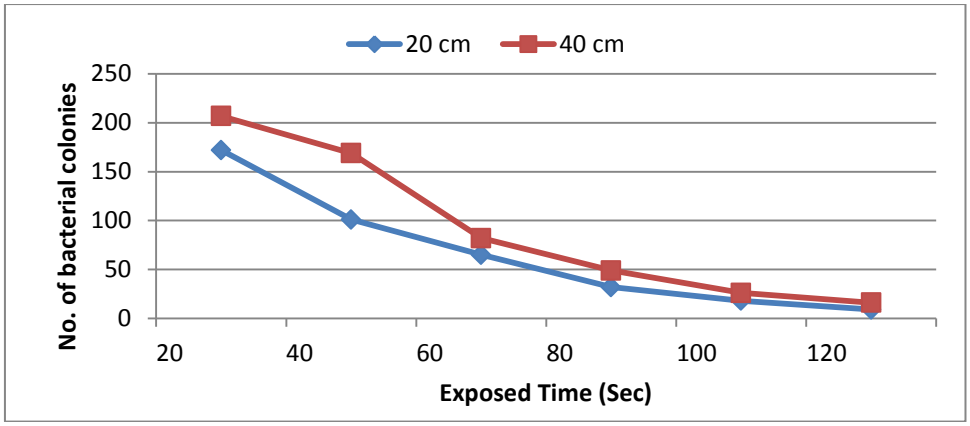

Figure 6. Effect of UV radiation on the growth of *Corynebacterium* species placed at 20 cm distance from the UV lamp

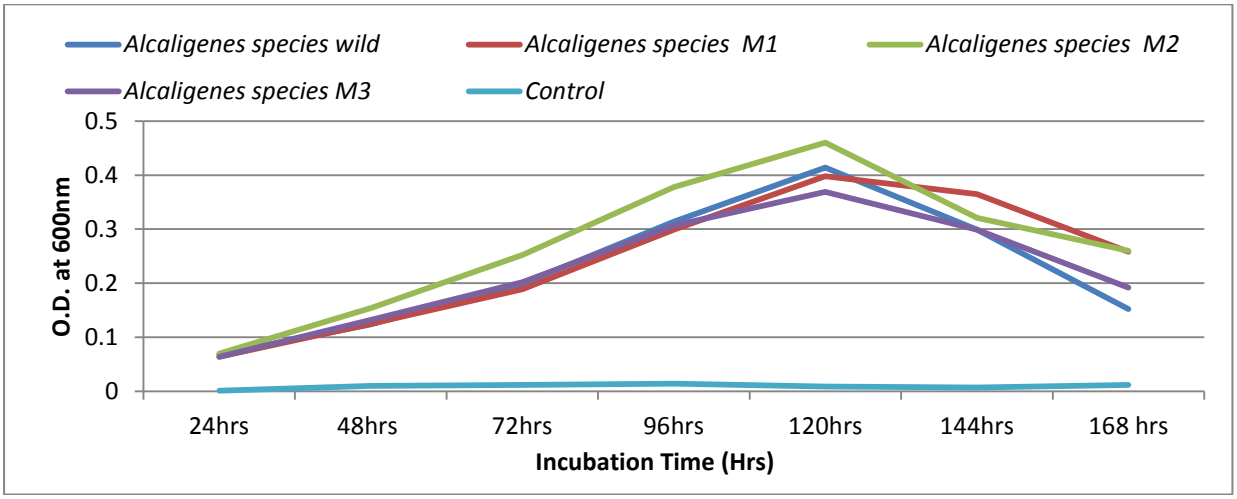

Figure 7. Degradation of petroleum by *Alcaligenes* speciesand their mutant strains

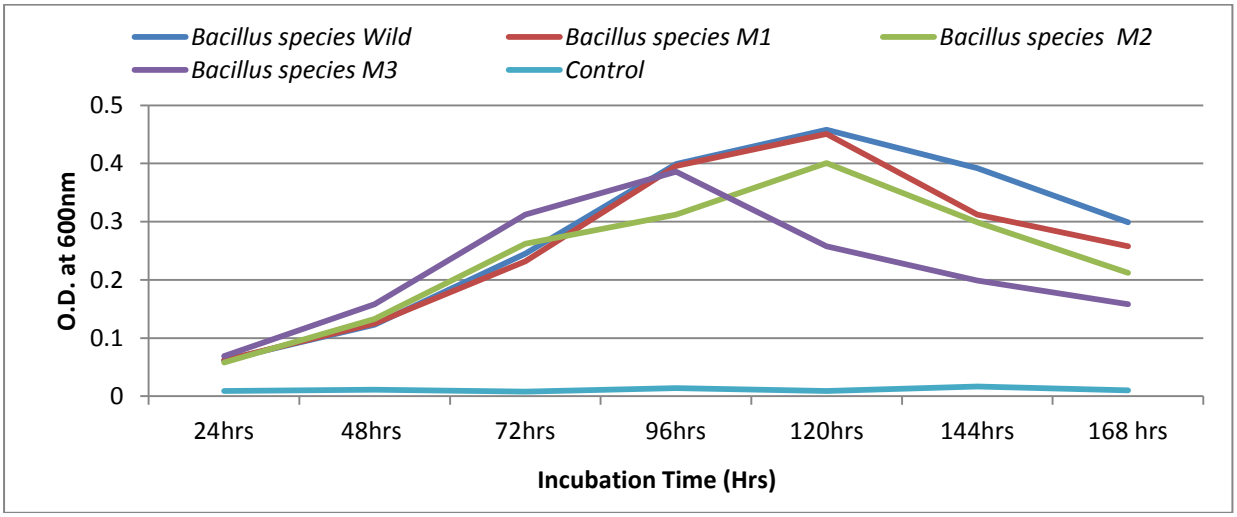

Figure 8. Degradation of petroleum by *Bacillus methylotrophicus* and their mutant strains

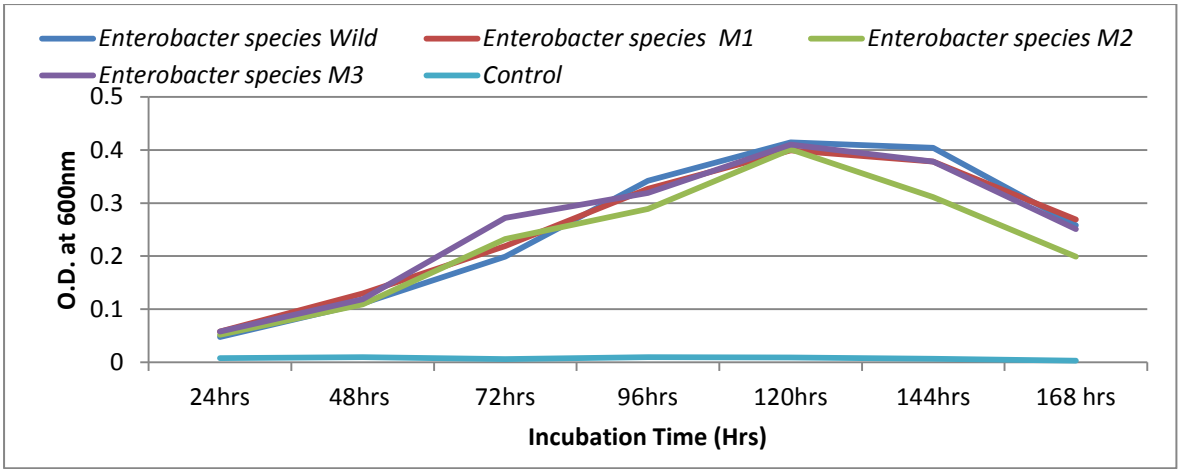

Figure 9. Degradation of petroleum by *Enterobacter* speciesand their mutant strains

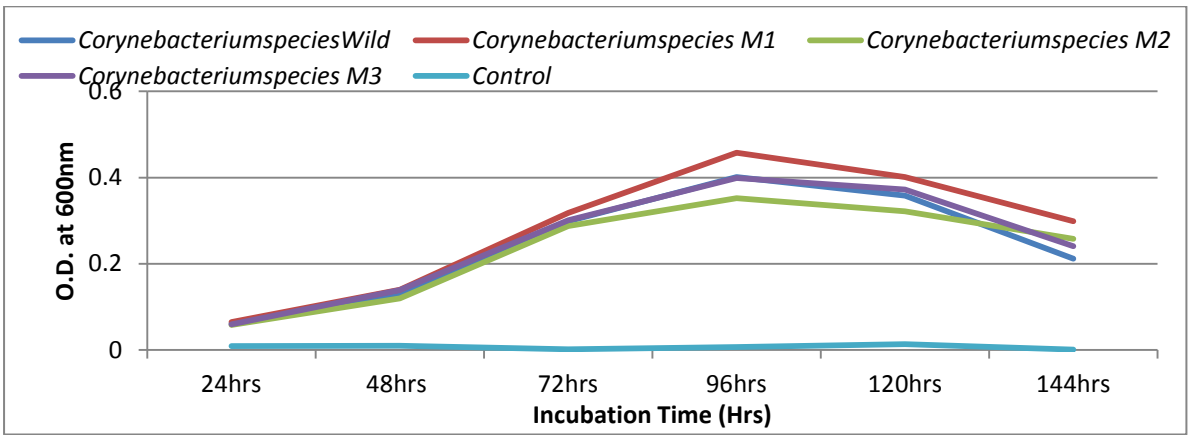

Figure 10. Degradation of petroleum by *Corynebacterium* species and their mutant strains

Mandalaywala and Trivedi (2016) reported that petroleum degradation ability is enhanced by UV radiation mediated mediated five mutant strain of *Pseudimonas aeruginosa*. *Pseudimonas aeruginosa* strain JQ-41 has highest potential to degrade petroleum products. Alsulami *et al,* 2014 mutated four bacterial species *Aeromonas hydrophila, Bacillus subtilis, Pseudimonas aeruginosa* and *Pseudimonas fluorescens* by exposing bacteria to millard reaction products. These mutants showed enhanced biodegradability potential of crude oil. *Bacillus subtilis* mutants increased biodegradation from 60.6 to 92.5% and increase in degradation of crude oil by other three mutants species ranged from 37 to 72.3%. Chaudhari and Fulekar (2013) reported that UV mutated *Pseudimonas pseudoalcaligenes* MHF ENV mutant enhanced degradation of dibutyl phosphate from 30% to 90% over a period of 6 days. Bacillus amyloliqueficieus was exposed to doses of gamma radiation with increase in radiation dose, the viable count of these bacteria decreased. Mutant did not show increase growth on naphthalene than the parent strain, but showed enhanced growth on phenanthracene, anthracene, pyrene and benzo-a-anthracene (Parila 2013). Chean *et al.,* (2011) observed that in the soil contaminated with viscous oil the microbial consortium degraded oil by about 49.22 % within a week, however UV mutant single strain enhanced degradation of viscous oil from 41.83 to 52.42% over a period of one week. Naveen kumar *et al* 2010 reported that mutagenesis of bacteria inducted an increase in petroleum degradation activity in three bacteria, *Micrococcus* species, *Staphylococcus* species and *Pseudomonas* species mutant. Most promising for petroleum oil degrading activity was *Pseudomonas* species mutant.

CONCLUSION

The present study showed that mutagenesis of bacteria by UV radiation increase the

degradation ability of native bacterial isolates. In this study, Mutant strains *Alcaligenes* species M2 and *Corynebacterium* species M1 exhibited higher degradation than their wild strains. This study reflects that gene specific mutation enhanced the degradation ability of bacteria and can be used to the development of novel remediation strategies.

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