Isolation, Molecular and Biochemical Characterization of Oil Degrading Bacteria from Contaminated Soil at the Jordanian Oil Refinery in Zarqa

By: –

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Bacterial enumeration and identification were carried out on 40 oil contaminated soil samples collected from Jordanian Oil Refinery at Zarqa, Jordan. Bacterial enumeration ranged between $5.0 \times 10^6$ and $3.0 \times 10^7$ CFU/gm indicating high bacterial counts in aged contaminated soils. Bacterial colonies were plated on mineral salt media supplemented with 0.075% crude oil (three times) to confirm its ability to grow on crude oil hydrocarbons as a sole carbon and energy source. Thirty four (34) bacterial isolates were picked and plated on nutrient agar and characterized morphologically. Then molecular identification was carried using three primes specific to the 16S rDNA gene sequences: universal to all bacteria, to the genus *Pseudomonas*, and to the genus *Acinetobacter*, respectively. All bacterial isolates showed positive results with the universal primer pair indicating that all isolates were bacteria and represented by the amplification product 1500 bp. Eighteen (18) of the bacterial isolates showed a positive result for the genus *Pseudomonas* with its specific primer and represented by the amplification product 150 bp and three (3) bacterial isolates showed a positive result for the *Acinetobacter* with its specific primer and represented by the amplification product 1500 bp. Biochemical and physiological identification was performed to all isolates which indicated the presence of the following bacterial genera and species: *Pseudomonas Acidovorans, P. aeruginosa, P. alcaligenes, P. fluorescens,*
P. cepacia, P. mallei, P. maltophilia, P. oleovorans, P. putida, P. stutzeri
P. vesicularis, Acinetobacter calcoaceticus, Acinetobacter lwoffi,
Micrococcus luteus, M. varians, M. lylae, M. roseus, Alcaligenes
denitrificans, Bacillus megaterium, Comamonas sp., Moraxilla sp.,
Bordetella sp.

Detection of some genes that codes for certain enzymes was carried
out using two primer pairs for the catechol 2,3 dioxygenase gene sequence
and for the alkane monoxygenase gene sequence respectively,
Pseudomonas aeruginosa, P. putida, P. stutzeri, and P. mallei showed
positive results with the primer specific for the catechol 2,3 dioxygenase
gene with an amplification product of the size 238 bp. Pseudomonas
oleovorans showed a positive result with the primer specific for the alkane
monoxygenase gene with an amplification product 348 bp. All bacterial
isolates were tested to grow on 0.1% diesel and monitored during time
intervals. Sixteen (16) bacterial isolates showed a positive biodegradation
on diesel, they were: Pseudomonas aeruginosa, Bacillus megaterium,
Pseudomonas mallei TDJ4, Acinetobacter calcoaceticus, Pseudomonas
putida, Pseudomonas maltophilia, Moraxilla TDJ9 sp., Comamonas sp.,
Micrococcus luteus, Pseudomonas mallei TDJ12, Pseudomonas oleovorans
TDJ13, Micrococcus roseus, Pseudomonas cepacia, Pseudomonas
vesicularis, Pseudomonas mallei TDJ29, Pseudomonas oleovorans TDJ34
while the rest of the bacterial genera and species showed no effect.