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Study of protein expression in *Pseudomonas putida* and *Pseudomonas aeruginosa* on degarding hydrocarbon compounds (Diesel)

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ABSTRACT

Bioremediation is the process of using agents to convert or degrade organic compounds such as crude oil and gasoline in to non-toxic substances such as carbon dioxide and water. Oil spill in the environment is a problem for which bio remediation by micro organisms is a natural, viable and economic solution. When an oil spill occurs often due to a ruptured oil tanker or leaking oilrig, people around the world are awed by the damage to the environment. However, there are also naturally occurring oil spills such as sea page from oil deposits beneath ocean floor. Regardless of their origins oil spills have an impact on the environment. Bacteria capable of degrading oil includes Gram negative rods such as *Pseudomonas, Acetobacter, FlavoBacteria, Cornibacterium and Alcaligenes*. The hydrocarbon (Diesel) degrading strains are separated from the nutrient broth medium and proteins are extracted and compared with the proteins extracted from the strains grown without adding hydrocarbon compound. The total protein concentration in the treated *Pseudomonas putida* and *Pseudomonas aeruginosa* was 55µg and 35µg respectively whereas the concentration in the normal *Pseudomonas putida* and *Pseudomonas aeruginosa* was found to be 30µg and 25µg.

KEYWORDS

Bioremediation;
Hydrocarbon;
Pseudomonas putida;
Pseudomonas aeruginosa;
Biodegradation.

INTRODUCTION

Petroleum hydrocarbons are the most widespread contaminants in the environment. Biodegradation of many components of petroleum hydrocarbons at low temperatures in Arctic, Alpine and Antarctic soils has been reported and is a result of the degradation capacity of indigenous cold-adapted microorganisms. Cold adapted psychrophilic and psychrotrophic microorganisms are able to grow at temperatures around 0°C and have adapted their metabolism to function optimally at low temperatures. Psychrophiles have an optimum growth temperature of 15°C and do not grow above 20°C, whereas psychrotrophs (or psychrotolerant or-

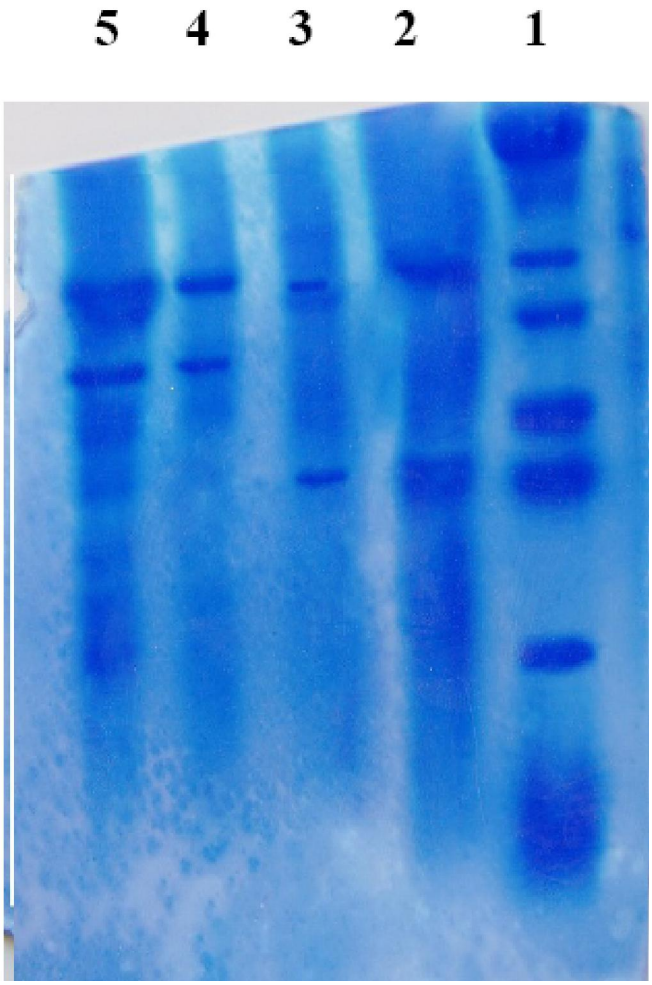

Figure 1 : SDS-PAGE; Protein expression of test and control of *Pseudomonas aeroginosa and Pseudomonas putida.*
1-Marker, 2-Test (*Pseudomonas aeroginosa*),3-Control (*Pseudomonas aeroginosa*),4-Control (*Pseudomonas putida*),5-Test (*Pseudomonas putida*)

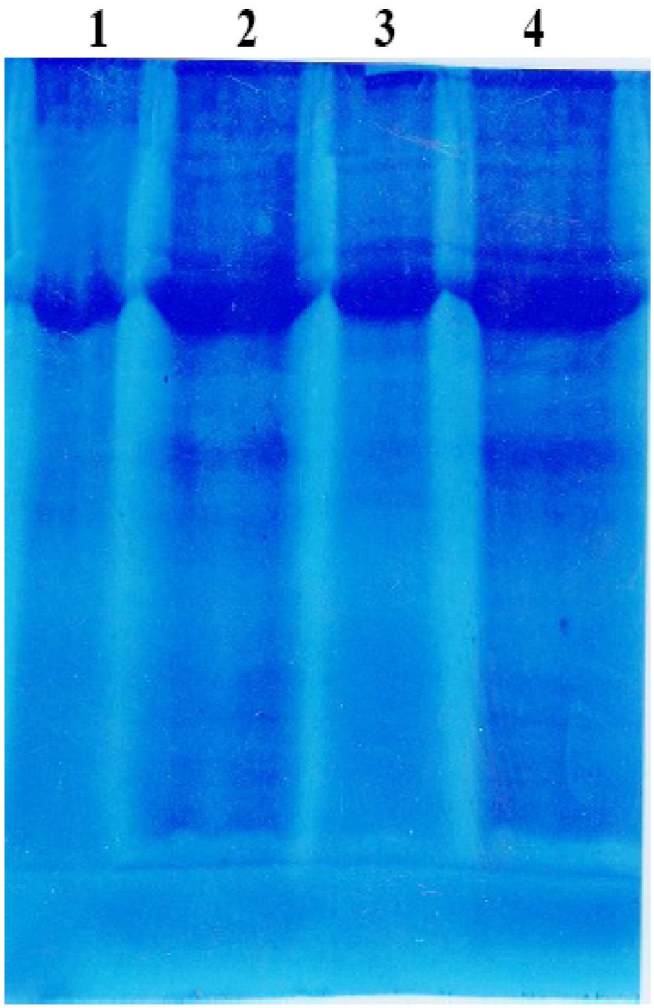

Figure 2 : NATIVE GEL
1 & 3CONTROL -*Pseudomonas aeruginosa* & *Pseudomonas putida*. 2&4 Protein expression of *Pseudomonas aeruginosa* & *Pseudomonas putida*

ganisms) have optimum and maximum growth temperatures above 15 and 20°C, respectively[2].

Various methods are used to characterize hydrocarbon-degrading populations in soil. Soil biological investigations, such as measurements of soil respiration, enzyme activities, and microbial counts, can give information about the presence of viable microorganisms and on the impact of the effects of environmental stresses, such as hydrocarbon contamination, on the metabolic activity of soil. Direct, non-cultivation-based molecular techniques for detecting microbial pollutant-degrading genes in environmental samples are also powerful tools for studying the structure and functions of complex microbial communities[4].

Pseudomonas and this relative occupy a prominent position in nature for their active participation in the carbon cycle. *Pseudomonas* metabolism has been the subject of intensive biochemical research and many of the unique catabolic pathways. The degradation of natural and artificially synthesized compounds by pseudomonas has been exploited in approaches to solution of environmental pollution problems[5].

The present study was conducted to assess the protein expression in *pseudomonas putida* and *pseudomonas aeruginosa* on degrading hydrocarbon.

MATERIALS AND METHODS

Isolation of microorganisms

The bacterial cultures utilized in this study were *Pseudomonas putida* and *Pseudomonas aeruginosa*,

obtained from culture collection. The microorganisms were maintained on 10 ml slopes of nutrient agar (storage culture) at 4°C and regularly sub cultured and monitored for purity.

Inoculum production

The inoculum was prepared using bacterial cells transferred from the storage culture to a test tube containing 10 ml of nutrient broth. After incubation at 30°C for 14 h, the inoculum was propagated to a 500 ml flask containing 100 ml of the same medium, and incubated with shaking (150rpm) to mid-log phase at 30°C. According to dry weight curve plotted for all species, the inoculum volume was calculated to obtain approximately 106 cells per gram of soil. All bacterial cells used were grown separately.

Sample preparation

The Hydrocarbon (Diesel) along with nutrient broth was serially diluted with equal volumes of the sample buffer. Heating of the sample was avoided in the case, as the native gel is non-denaturing. Then it was suspended to centrifuge to obtain pellet. The protein obtained in the pellet, is dissolved in 200ml of 0.5% Sodium carbonate.

The protein profile was developed by running SDS-PAGE.

RESULT AND DISCUSION

The hydrocarbon compound degraded by *Pseudomonas putida* and *Pseudomonas aeruginosa*, and the protein expression during degradation was observed. Both strain was allowed to grow in nutrient broth medium by adding a hydrocarbon compound (diesel) and kept it in a controlled condition for seven days. The hydrocarbon (diesel) which was added with nutrient medium are degraded by both *Pseudomonas putida* and Pseudomonas *aeruginosa* in seven days.

Protein expression in degraded hydrocarbon strain

The proteins extracted from the hydrocarbon degraded strains and the normal strains in crude form were analysed. The concentration in the treated *Pseudomonas putida* and *Pseudomonas aeruginosa* was 55µg and 35µg respectively where as the concentration in the normal *Pseudomonas putida* and *Pseudomonas aeruginosa* was 30µg and 25µg respectively. This shows that the concentration of protein was about 95% and 75% over the control.

The bands of different molecular weight were identified in the protein profile. The band formed in the test sample (Hydrocarbon) was compared with the band formed on normal. The band thickness of the test sample was observed to be higher than that of the normal. The sample was also observed in the native gel. This confirms that the quantity of protein was higher in hydrocarbon degrading strain.

The molecular weight of the proteins in the bands of both the test and the control were found to be more or less the same ranging from 116000 to 6000 Daltons. When comparing the band thickness between the normal and the test sample it was observed the thickness of the bands in *Pseudomonas putida* and *Pseudomonas aeruginosa* was found at the position of molecular marker 29kilo Dalton. When compared with *Pseudomonas aeruginosa*, the band found was thicker than normal. This shows that 27 kilo Dalton gene in the test sample was over expressed when compared to the control. This indicates that *Xcp* gene in *Pseudomonas aeruginosa* was expressed and produced exoprotein (Xcp protein).

The band at the position of molecular marker 116KD in *Pseudomonas putida* is thicker than normal. This shows that 125KD gene in the test sample was over expressed when compared to the control. This indicates alk gene in *Pseudomonas putida* was expressed which synthesized protein, and degrades the hydrocarbon[1].

According to Ridgeway *et al.* (2004), among 121 bacterial strains isolated from groundwater, that are able to use gasoline as carbon and energy source, 18.4% were *Pseudomonas putida*, 1.6% as *Pseudomonas alcaligenes* and 0.41% *Pseudomonas cepacia*.

Wacket. and Gibson (1989) concludes that Micro array genotyping of 18 diverse strains in the *C.elegans* model; shows 58 PA 14 gene cluster did not correlate with these strains virulence.

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