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In vitro study on the effect of heavy metals on PGPR microbes from two different soils and their growth efficiency on *Oryza sativa* (L.)

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ABSTRACT

This study investigates the impact of heavy metals on culturable rhizobacterial *Pseudomonas* from hydrocarbon contaminated and ordinary soil. It deals with the isolation and characterization of culturable rhizobacterial from HC contaminated and normal soil also analyzed the influence of growth promoting efficiency of rice plant by *Pseudomonas*, Rhizobium. The heavy metal content of both soil samples were analyzed by AAS. Among the six heavy metals Calcium and Magnesium were found to be higher on both soils. Rhizobacterial strains isolated from hydrocarbon contaminated soil were more tolerant to heavy metals; the protein content was also higher when compared with rhizobacteria from normal soil. The *Pseudomonas* sp. from Hydrocarbon contaminated soil exhibited better resistance to all the metals (Zn, Pb, Mn) than the normal soil, while the *Rhizobium* sp., showed moderate resistance to the metal used on both soils. The representative PGPR strains of *Pseudomonas, Rhizobium* from both soils are tested for plant growth promoting activities and heavy metal tolerance pattern. The study revealed the following criterion of the observation such as when the rhizobacterial strains were isolated from hydrocarbon contaminated soil showed enhanced plant growth promoting activity also increased protein content compared with normal soil isolated rhizobacteria. Futhermore, *Pseudomonas* species from the normal soil proved the appearance of sharp phosphate solublization zone indicating this isolate was an efficient phosphate solubilizer than the normal soil. The results established that the hydrocarbon contaminated soil extracted Pseudomonas sp., bacterium possessed a major role for stimulation of root and shoot growth as well as enhances the rice yield production from hydrocarbon contaminated soil.

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INTRODUCTION

Different bacterial genera are vital components of soil and the plant growth promotion by PGPR (Plant Growth Promoting Rhizosphere Microbes) can be demonstrated in the absence of plant pathogens or other rhizosphere micro organisms, while indirect mechanisms involve the ability of PGPR to reduce the harmful effects of plant pathogens on crop yield (Kloepper, 1993). Without bacteria soil would not be fertile and organic matter such as straw or leaves would accumulate within a short time. Pollution of the biosphere by heavy metals due to industrial, agricultural and domestic activities has created a serious problem for the safe and rational utilization of soil (Srivasthava *et al.,* 2005). The pollution of the ecosystem by heavy metal is a real threat to the environment because metal cannot be naturally degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain so that metal toxicity may affect all forms of life in the world (Igwe *et al.,* 2005) and the high level of heavy metals influencing both microbial population and metabolic processes (Ahamed *et al*., 2004). Plants Growth Promoting Bacteria (PGPB) are associated with plant and are commonly present in the environment (Bashan and Holguin, 1998). The widely studied groups are Plant Growth Promoting Rhizobacteria (PGPR) colonizing the root surfaces and closely adhering soil interface, the rhizosphere (Kloepper *et al.,* 1999). The entophytic colonization of host plant organs and tissues reflects the ability of bacteria to selectively adapt to these specific ecological niches so that the intimate association between bacteria and host plants are formed without harming the plant (Grey and Smith, 2005). In the last few decades a large array of bacteria including species of *Pseudomonas, Azospirillum, Arthrobacter, Klebsiella,*

Enterobacter, Bacillus and *Serratia* have been reported to enhance plant growth. The direct growth promotion by PGPR entails either providing the plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment.

Microorganism has developed the mechanisms to cope with a variety of toxic metals for their survival in the environment (Burd *et al*., 2004). PGPR also help in reducing the toxicity of heavy metals to plants in polluted environments (Marques *et al.,* 2013). The selection of microorganisms both metal tolerant and efficient in producing PGPR compounds can be use full to speed up the recolonization of the plant rhizosphere in the polluted soil. Also the use of PGPR as inoculants is an efficient approach to replace chemical fertilizer (Kannahi and Kowsalya, 2013). However, very few work has done in the similar kind of research. Hence the present work has been designed with following objectives such as to isolate and collect indigenous plant growth promoting rhizosphere microbes from hydrocarbon contaminated and normal rhizosphere soil sample and to characterize their performance against different abiotic stresses including salinity, heavy metal effects and plant growth promoting traits. Estimation of protein and molecular characterization of protein by SDS-PAGE was also performed for each isolates. The microbe which showed high resistance to heavy metals, having multiple PGP traits can be used as a strong candidate for development as seed inoculants to enhance yields.

MATERIALS AND METHODS

The soil samples were collected from rhizosphere region of Hydrocarbon contaminated paddy field near Parassala and normal rhizosphere soil from paddy field near Kaliakkavilai. The collected sample were placed in sterile container and brought to laboratory for analyss. The soil samples were analyzed for physicochemical parameters like pH, conductivity, total organic carbon, organic matter, total nitrogen content, total phosphorous content, total potassium content etc using standard procedures (Rajan and Selvi Christy, 2010). Soil samples were oxidized by wet digestion method to determine the heavy metal content, followed by elemental analysis using Atomic Absorption Spectrophotometer (AAS) (Allen *et al.*, 1974).

Rhizobacteria

The soil samples were serially diluted in sterile distilled water and plated on appropriate medium for isolating different Rhizobacteria Vis: Nutrient agar for *Bacillus* sp., Cetremide agar for *Pseudomonas* sp. (Ahamed *et al*., 2008) and Yeast Extract Mannitol Agar (YEMA) for *Rhizobium* sp. After incubation at 28-30°C for 2-3 days, bacterial colonies were counted and representative colonies were selected based on distinct types and observed according to the morphological characteristic such as pigments; colony form elevation and margin; texture and opacity (Simbert and Krieg, 1981). Selected isolates of *Bacillus*, *Pseudomonas*, and *Rhizobium*, were biochemically characterized by Grams reaction, carbohydrate fermentation, oxidase test, O-F test, Hydrogen sulphide production, IMViC tests, and Starch and gelatin hydrolysis as per the standard methods (Cappuccino and Sherman, 1992).

Salt and heavy metal tolerance of Rhizobacteria

Resistance of isolates against different concentration of NaCl was determined on minimum salt medium containing with NaCl at various concentration ranging from 1, 2, 3 and 4 % (W/V). The plates containing 25 mL of medium and a loop full of each isolate was streaked on Petri dishes. The plates were incubated for 72 hrs at 28°C and the susceptibility to NaCl was recorded as positive or negative result (Rajan and Selvi Christy, 2010). These isolated bacterial strains were tested for their resistance to heavy metals by agar dilution method. Freshly prepared agar plates were amended with various soluble heavy metal salts namely Zn, Cu, Pb, Hg, and Ni at various concentration ranging from 25 to 200 µg mL^{1} were inoculated with overnight grown cultures. Heavy metal tolerance was determined by appearance of bacterial growth after incubating the plates at room temperature for 24-48 hours. The supernatant were collected from culture extract and the protein concentration of sample was determined by Lowry's method. The protein profiles of the

Rhizobacterial isolates were characterized by Sodium Dodecyl Sulphate - Polyacrylamide gel electrophoresis.

Characterization of Rhizobacteria for PGP traits

All the bacterial isolates were tested for the production of Plant Growth Promoting substances such as Indole Acetic Acid (Loper and Schroth., 1986), Ammonia and Catalase (Cappuccino and Sherman, 1992), Hydrogen Cyanide (Castric, 1975) and Phosphatase (Nautiyal, 2001).

Seed Germination Test

The bacterial culture having high PGP traits was taken for seed germination test. Rice seeds (*Oryza sativa*) were soaked in sulphuric acid for five minutes and washed with sterile water three times to remove sulphuric acid. The seeds were treated with bacterial strain for 30 minute. Treated seeds were placed on agar (2٪w/v). Treated and untreated seeds were kept for germination in dark for two days in an incubator at 27ºC. After seventh day of incubation seedlings were taken out for various studies like shoot and root length and the data were recorded. Seeds without coating the bacterial cultures were maintained as control. Bacterial inoculants that increases plant growth and germination rate, improved seedling emergence were used as soil inoculants to enhance crop yields where untreated sewage water is used in irrigation.

Statistical Analysis

To assess the similarity co-efficient of protein present in Rhizobacterial culture extract with that of marker protein the statistical analysis was done by Dendrogram UPGMA Analysis followed by SDS-PAGE.

RESULTS AND DISCUSSION

Soils showed different physico chemical properties. pH value varied significantly i.e. 5.1 strongly acidic for normal soil and 6.6 slightly acidic for hydrocarbon contaminated soil. Soil showed the variance in other properties also. The physico chemical properties of soils are shown in Table 1(A). The heavy metal content of soil samples were analyzed by Atomic Absorption Spectrophotometer and the results were shown in Table 1(B). Hydrocarbon contaminated soil had higher concentration of heavy metals than that of normal soil sample.

Table 1. Physicochemical analysis of soil samples

Physicochemical Properties		
Physicochemical Properties	Hydrocarbon Contaminated Soil	Normal Soil
pH	6.6	5.1
Electrical Conductivity (m)	0.2	0.2
Organic Carbon (%)	2.64	0.84
Available N (kg/ha)	200.7	553.93
Available P(kg/ha)	116.16	3.24
Available K(kg/ha)	247.5	4.95
Heavy metals content (ppm)		
Heavy metals (ppm)	Hydrocarbon Contaminated Soil	Normal Soil
Copper (Cu)	12.99	3.36
Zinc (Zn)	11.47	5.08
Manganese (Mn)	13.60	19.74
Iron (Fe)	224.61	80.66
Magnesium (Mg)	137.38	102.38
Calcium (ca)	1284.75	380.0

Rhizobacteria

Three bacterial isolates from normal soil and five bacterial isolates from Hydrocarbon contaminated soil were successfully isolated. They were designated as N_1, N_2, NP_1, NR_1, SP_1, SR_1 and S_1. The isolated bacterial strain from hydrocarbon contaminated soil designated as S_1, $SP_1$and SR_1 were characterized as *Bacillus* sp., *Pseudomonas* sp. and *Rhizobium* sp. respectively, whereas the bacterial isolates from normal soil designated as N_1, N_2, NP_1, NR_1 and NA_1 were characterized as *Shigella* sp., *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp. and *Agrobacterium* sp. respectively.

Salt Tolerance of Rhizobacteria

Differences in NaCl tolerance were observed among the isolates. *Pseudomonas* sp., *Rhizobium* sp. from hydrocarbon contaminated soil showed high resistance to NaCl. Growth of Rhizobium sp. from normal soil was also observed all concentration (1% - 4%) of NaCl whereas; *Pseudomonas spp.* from

normal soil has declined growth in 3 and 4% of NaCl.

Characterization of Rhizobacteria for PGPs

Table 3- showed that the bacterial isolates from both Hydrocarbons contaminated and normal soil sample produced plant growth promoting hormones. The *Pseudomonas* sp. from Hydrocarbon contaminated soil produced high IAA (110.0µg/mL) (Fig 1). When compared with normal isolate (90.0µg/mL). *Rhizobium* sp. from hydrocarbon contaminated and normal soil produced 50.0µg/mL and 60.0µg/mL of IAA respectively indicating isolate from Normal soil is a high IAA producer. All the Rhizobacterial species were also able to produce Ammonia. Among this *Pseudomonas* sp. from Hydrocarbon contaminated soil was found as efficient producer. *Pseudomonas* sp. from Hydrocarbon contaminate soil was found to efficient producers of HCN (Fig 2a) and catalase, whereas *Pseudomonas* sp. from normal soil failed to produce. Both *Rhizobium* sp. were poor in HCN and catalase production. All the isolates showed positive for phosphate solubilization in Pikovskaya's agar.

Table 2. Plant growth promoting characteristics

Organism	PGP Characteristics			
	Phosphate Solubilization	Ammonia production	HCN production	Catalase production
SP1	+++	+++	++++	+++
SR1	+	+++	++	+
NP1	+++	+++	++	+
NR1	+	++	+	+

++++; Excellent, +++; Good, ++; Moderate, +; low, - Negative

Investigation of heavy metal tolerance

The *Pseudomonas* sp. from normal soil showed development of sharp phosphate solubilization zone whereas *Pseudomonas* sp. from Hydrocarbon contaminated soil showed the development of hazy zone indicating normal isolate as efficient phosphate solubilizer (Fig 2b). The *Pseudomonas* sp. from Hydrocarbon contaminated soil exhibited high resistance to all the metals (Zn, Pb, Mn), whereas *Pseudomonas* sp. from normal soil was less sensitive.

Fig 2. Efficiency of HCN production (a) and Phosphate solublization (b) production by *Pseudomonas* sp. from experimental soil(s).

(a) **(b)**

The *Rhizobium* sp. from both the soils showed moderate resistance to the metal used. The result was represented in Table 4. On statistical analysis *Rhizobium* sp. from normal soil in the presence of zinc (50µg/mL) was found as highly significant where as in 200µg/ml was found as significant when compared with the control. *Rhizobium* sp. from hydrocarbon contaminated soil showed significant variability with zinc (200µg/mL), lead (100µg/mL and 200µg/mL). Further the result revealed that the *Pseudomonas* sp. from Hydrocarbon contaminated soil was found as significant in the presence of Manganese (50µg/mL, 100µg/mL) but was in significant at 250µg/mL when compared with the control.

Protein

The protein content in *Pseudomonas* and *Rhizobial* culture extract of hydrocarbon contaminate soil was found to be 4.6 mg/mL, 1.22 mg/mL where as protein content in *Pseudomonas* and *Rhizobial* culture extract of normal soil was found to be 4.3 mg/mL, 1.22 mg/mL respectively. SDS profile depicted that *Rhizobium* sp. from hydrocarbon contaminated soil having totally five bands range between 180 KDa to 330 KDa. This clearly indicates that high molecular weighed (330 KDa) bands appeared only in this organism followed by 310, 260 and 180 KDa. Among the polypeptides bands appeared, two lower molecular to optimum molecular weighed bands were thick intensity in nature and other three bands faded in nature. While other three tested organisms *Pseudomonas* sp., *Rhizobium* sp. from normal soil and *Pseudomonas* sp. from hydrocarbon contaminated soil possessed similar range of banding efficiency (180 KDa).

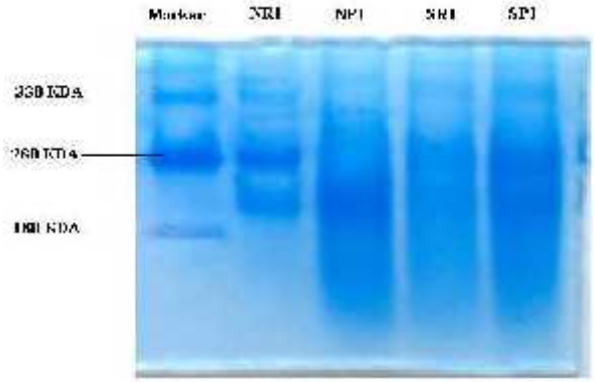
Table 3. Heavy metal tolerance of rhizobacterial isolates.

Metals	Isolates	Number of colonies					
		50µg/mL	100µg/mL	200µg/mL	250µg/mL	300µg/mL	control
Zinc	SP1	180±0.8	120±0.8	83±0.4	79±0.8	51±1.4	209±0.8
	SR1	62±0.8	21±0.8	18±0.4*	9±0.8	6±0.4	182±0.8
	NP1	21±0.4	4±0.8	2±0.9	1±1.4	_	18±0.8
	NR1	48±0.9**	34±0.4	25±1.4*	16±0.8	8±0.8	188±0.4
Lead	SP1	144±0.7	96±1.2	63±0.9	53±1.2	26±0.7	298±0.4
	SR1	36±0.9 ^{is}	24±0.4*	16±0.8*	6±0.4	3±0.4	144±0.8
	NP1	18±0.4	12±0.8	8±0.4	_	_	85±0.4
	NR1	30±0.4	18±0.8	10±0.8	2±0.4	_	137±0.8
Manganese	SP1	160±0.8*	130±0.8*	84±0.8	33±0.4^{is}	23±0.4	178±0.4
	SR1	97±0.4	63±0.9	18±0.4	13±0.8	9±0.4	129±0.8
	NP1	30±0.8	17±0.9	4±0.4	_	_	70±0.4
	NR1	71±0.4	62±0.8	26±0.8	21±0.4	13±0.8	102±04

** Highly significant, * Significant, is Insignificant

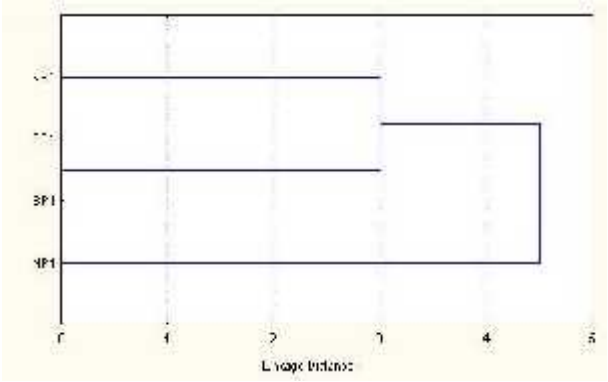
Moreover, *Rhizobium* sp. from normal soil showed all the total three bands of fairly low intensity in nature.

Fig 3. SDS Protein profile of hydrocarbon contaminated and normal PGPR microbes



Similarity coefficient for the SDS profile, the Dendrogram UPGMA analysis clearly indicated that the highest range of similarity coefficient was shared between *Pseudomonas* sp. from normal soil sample and *Pseudomonas* sp. from hydrocarbon contaminated soil. However consistent similarity was observed between *Rhizobium* sp. from normal soil sample and *Rhizobium* sp. from hydrocarbon contaminated soil. *Pseudomonas* sp. from normal soil and *Rhizobium* sp. from hydrocarbon contaminated soil also showed consistent similarity because from the original to optimum range 15% of similarity co-efficient was monitored in this analysis (Fig 5)

Fig 4. Similarity co-efficient for SDS profile test (UPGMA).

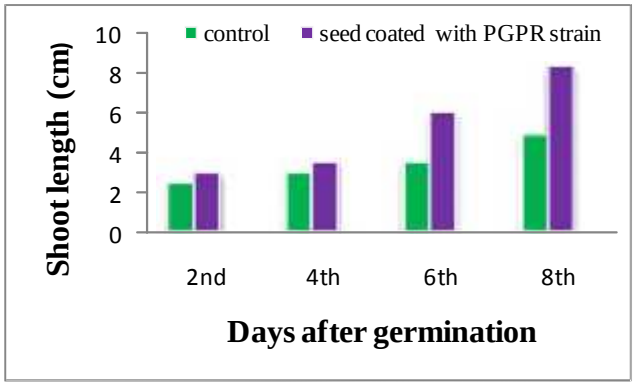


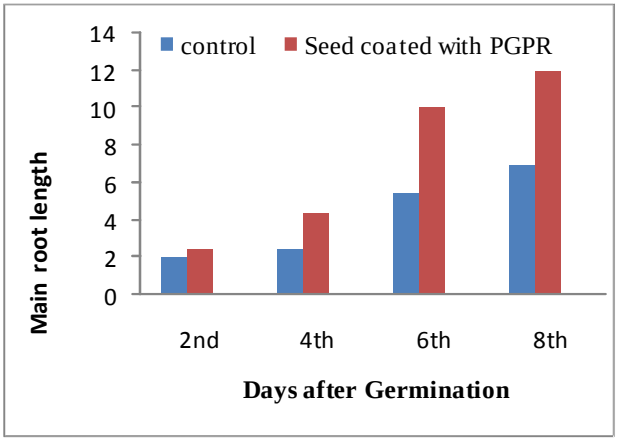
SEED GERMINATION TEST

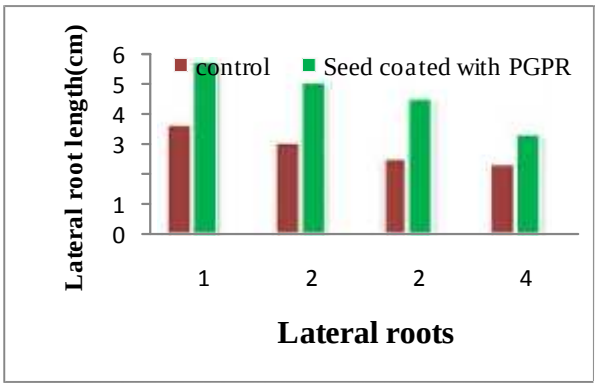
It is apparent that the bacterium *Pseudomonas* sp. from Hydro carbon contaminated soil had significant impact of stimulation of root and shoot growth (Fig.6 a, b and c). Roots and shoot from seeds treated with bacterial culture were longer than the roots from untreated control seeds after eight days. It was noticed that the level of total organic carbon and organic matter in hhydrocarbon contaminated

rhizosphere soil is higher when compared to normal soil. (Table 1 A).

Fig 5. Effect of *Pseudomonas* sp. on shoot growth (top), main root growth (middle) and lateral root growth (bottom) of rice.







Organic carbon content of Hydrocarbon contaminated soil is 2.64% which is greater than the normal soil (0.84%). This may be due to the exudates, sloughing of root tissues during root death and senescence (Graham and Haynose, 2006). The presence of high organic content in the rhizosphere promotes microbial proliferation. Quantification of heavy metal content revealed a strong contamination of soil in proportion to the amount of waste water applied in Hydrocarbon contaminated soil (Table 1 B) when compared with normal. The contamination of the environment with toxic metals has become biomass and fertility contributing to accumulation in the food chain. Although, organic content in sewage increases soil fertility, it also contributes to soil contamination with heavy metals (Muller *et al*., 2001). Rhizobacterial isolates of *Pseudomonas* sp., *Rhizobium* sp. which are predominant in both the soils were selected for present study.

Five bacterial isolates from normal soil and three bacterial isolates from sewage treated soil were successfully isolated and they were characterized a *Pseudomonas* sp, *Rhizobium* sp, *Agrobacterium* sp., *Shigella* sp., *Bacillus* sp. and *Pseudomonas* sp., *Rhizobium* sp., *Bacillus* sp. respectively. The isolates in Hydrocarbon contaminated soil indicates that microbial biomass is unaffected by waste treatment. This kind of similar study already have been documented that soil contamination results in the reduction of bacterial diversity, biomass and metabolic activity (Ellis *et al*., 2003). It is known that heavy metal pollution causes selection and /or development of tolerant microorganisms. However, contradictory results are also available on effect of waste water on soil microbial biomass unaffected by waste water application or increased after amendment with sewage sludge (Martin- Laurent *et al*., 2004).

Rhizobacterial isolates from Hydrocarbon contaminated and normal soil sample exhibited a couple of PGP traits (Table 3). PGPR increase plant growth by decreasing heavy metal toxicity (Burd *et al*., 2004). Rhizobacteria which establish positive interactions with plant roots, PGPR play a key role in agricultural environments and are promising for their potential use in sustainable agriculture (De Fago *et al*., 1994). It is clear that waste water treatment did not have profound inhibitory influence on PGP characteristics of rhizobacteria. The *Pseudomonas* sp. from sewage treated soil produced high IAA (110.0 µg/mL) when compared with normal isolate (90.0 µg/mL). *Rhizobium* sp. from sewage treated and normal soil produced 50.0µg/mL and 60.0 µg/mL of IAA respectively indicating isolate from normal soil is high IAA producer. (Fig-1) IAA plays a major role in promotion of root

elongation when a bacterium is associated with its host plant. IAA secreted by bacterium may promote root growth directly by stimulating plant cell elongation or cell division or indirectly influencing bacterial ACC deaminase activity (Glick *et al*., 1995). Production of Ammonia was found high frequent in *Pseudomonas* sp. from Hydrocarbon contaminated soil as compared with other rhizobacteria. Higher number of isolates of *Rhizobium* sp. and *Pseudomonas* sp. from sewage irrigated rhizosphere of *Pisum sativum* showed significant production of ammonia as compared to their counter parts from normal soil (Pramod *et al*., 2012).

Pseudomonas sp. from Hydrocarbon contaminated soil was found as efficient producers of HCN and catalase where as *Pseudomonas* sp. from normal failed to produce (Fig 2a). Most of the *Pseudomonas* sp. isolated from soils of wheat and pigeon pea are produced HCN as potent antifungal agent (Yogendra *et al.*, 2013). Bacterial strain showing catalase activity must be highly resistant to environmental, mechanical and chemical stress. Both *Rhizobium spp*. were found to be poor in HCN and catalase production (Kloepper *et al*., 2004).

All the isolates showed positive for phosphate solubilization in Pikovskaya's agar. The *Pseudomonas* sp. from normal soil showed development of sharp phosphate solubilization zone whereas *Pseudomonas* sp. from hydrocarbon contaminated soil showed the development of hazy zone indicating normal isolate as efficient phosphate solubilizer (Fig 2b). It has been reported that higher concentration of phosphate solubilizing bacteria are commonly found in polluted rhizosphere soil as compared to normal rhizospheric soil (Reyes *et al*., 2006). When the concentration of metals increased the colony number of the bacterium were declined slightly though, heavy metals at higher concentration are toxic to cells and may cause cell death by interacting with nucleic acids and enzymes active site. Surprisingly *Pseudomonas* sp. from normal soil was found sensitive to higher concentration of metals used (300 µg/mL -Zn, 250 µg/mL and 300 µg/mL - Pb, 250 µg/mL, and 300 µg/mL of Mn *Rhizobium* sp. from both the soil is found moderately tolerant to heavy metals. Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment with such metal (Burd *et al*., 1998).

The Rhizobacterial culture extract of *Pseudomonas* sp. from Hydrocarbon contaminated soil contained 4.6 mg/mL of protein while the *Rhizobial* sp., contained 1.2mg/mL. The *Pseudomonas* sp. and *Rhizobium* sp. from normal soil contained 4.3 mg/mL, 1.2mg/mL of protein respectively (Fig 3). From the result it was found that protein concentration in *Pseudomonas* sp. from Hydrocarbon contaminated soil was higher when compared to others. This study was similar to the finding of Saxena *et al*. (1996) that the tolerance mechanism of Rhizobacteria mainly depends upon the concentration of protein.

SDS profile depicted that *Rhizobium* sp. from hydrocarbon contaminated soil having totally five bands range between 180 KDa to 330 Da. This clearly indicates that high molecular weighed (330 KDa) bands appeared only in this organism followed by 310, 260 and 180 K Da. Among the Polypeptides bands appeared, two lower molecular to optimum molecular `weighed bands were thick intensity in nature and other three bands were faded in nature. While other three tested organisms *Pseudomonas* sp., *Rhizobium* sp. from normal soil and *Pseudomonas* sp. from hydrocarbon contaminated soil possessed similar range of banding efficiency (180 KDa). Moreover, *Rhizobium* sp. from normal soil showed all the totally three bands of fairly low intensity in nature (Fig 4). Dendrogram UPGMA analysis clearly indicated that the highest range of similarity coefficient was shared between *Pseudomonas* sp. from normal soil sample and *Pseudomonas* sp. from hydrocarbon contaminated soil. However consistent similarity was observed between *Rhizobium* sp. from normal soil sample and *Rhizobium* sp. from hydrocarbon contaminated soil. *Pseudomonas* sp. from normal soil and *Rhizobium* sp. from hydrocarbon contaminated soil were also showed consistent similarity because from the original to optimum range 15% of similarity co-efficient was monitored in this analysis (Fig 6). Protein electrophoresis has been of great value for delineation of numerous bacterial taxa (Costas, 1992). Each of the different electrophoretic techniques has its own discrimination level and field of application. It is also widely acknowledged that the electrophoretic separation of cellular proteins followed by Dendrogram – UPGMA Analysis is a sensitive technique which mainly provides

information on the similarity of the rhizobacterial strains. In addition, it is also generally accepted that the objective comparison of electrophoretic protein patterns provides a reliable measure of relationship between the rhizobacterial isolates (Woese, 1987). In the present study the *Pseudomonas* sp. from Hydrocarbon contaminated soil, one of the potential bacterial isolate (SP1) tolerant to heavy metals, produced multiple plant growth as well as both metal tolerant and efficient in producing PGP compounds depicted by Carlot *et al*. (2002) and plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plant from disease (Lugtenberg *et al*., 2002).

The present study clearly indicates that the rhizobacterial population from hydrocarbon contaminated soil was not affected by heavy metal contamination, possessed one or more PGP traits when compared with isolates from normal soil. The representative strain of *Pseudomonas* sp. from hydrocarbon contaminated soil was outstanding for heavy metal tolerance and PGP potential. The knowledge of plant associated bacteria of rhizosphere is not only for outstanding their ecological role and the interaction with the plant but also for future biotechnological application. From this study it was concluded that the PGP microbes are more efficient in the enhancement of plant growth and such organisms can be used for crop management which gives more yield.

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