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Bacterial Community Structure Change Induced by Gamma Irradiation in Hydrocarbon Contaminated and Uncontaminated Soils Revealed by PCR-Denaturing Gradient Gel Electrophoresis

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Abstract: The effect of gamma irradiation on bacterial community structure in garden clay, uncultivated clay and hydrocarbon contaminated soils were investigated by exposing soils to various doses of ionizing radiation using a Co-60 source. Bacterial community structure in irradiated soils was examined 30 days after irradiation at 1, 2, 3, 4, 5 and 10 kGy doses. Gamma irradiation was found to have a selective impact on the microbial community structure in certain soils. Sensitivity to irradiation varies among microbial species and is affected by the properties of the soil. Denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA gene fragments amplified from the three soils were performed. Bacterial population in garden clay soil was stimulated by low irradiation doses and sharply decreased at higher doses. Phylogenetic analysis revealed predominance of only cloroflexi bacterium at elevated doses of irradiation in garden clay soil. In uncultivated clay soil, much of the bacterial populations were affected by irradiation treatment, however, two representatives of gammaproteobacteria, *Enterobacter* sp. and *Pseudomonas* sp. showed some resistance to irradiation and were present at all irradiation doses. DGGE profile suggested that bacteria in hydrocarbon contaminated soil were more resistant to irradiation than garden and/or clay soils. The bacterial diversity in polluted soil remained intact throughout all radiation treatments. Phylogenetic analysis showed that members belonging to three taxonomic groups, alphaproteobacteria, gammaproteobacteria and actinobacteria were present in hydrocarbon polluted soil that were not affected by all radiation treatments. Gamma irradiation had a greater effect on bacterial community in both garden and uncultivated clay soils.

Key words: Gamma irradiation, soil bacteria, contaminated soil, DGGE

INTRODUCTION

Gamma irradiation has been widely used for sterilizing medical products and other products such as wool, food and other sterilization purposes (Kainer *et al*., 2004; Mendonca *et al*., 2004; Osterholm and Norgan, 2004). Gamma radiation as an ionizing radiation is considered as a powerful mutagenic agent (Alabastro *et al*., 1978) and can be used for changing the metabolic activities of living cells. Gamma irradiation induces many changes to the cell including changes in the nucleus and molecular structure. It affects microorganisms via ionizing damage of cell DNA and double strand DNA breaks, radiolysis of cellular water and formation of active oxygen species and free radicals (Romanovskaya *et al*., 1999; Hall and Giaccia, 2006). The main biological effect of gamma radiation is free radicals production by ionization, which have a toxic effect on the cell (Scalo and Wheeler, 2002).

Gamma irradiation was also used to sterilize soil to eliminate biological activities for subsequent ecological research experiments (Degrange *et al*., 1997). While different sterilizing methods for the soil can be used, gamma irradiation still represents the ideal sterilizing method that achieve complete elimination of organisms without much affecting soil properties (Trevors, 1996). The response of soil microorganisms to radiation is dependent upon many factors such as type of radiation, kind of microorganisms, soil physico-chemical properties, vegetation and constituent substrates (Gholz *et al*., 2000).

The community response of soil microbes exposed to gamma irradiation showed that bacteria are usually more resistant than fungi which are usually eliminated at lower irradiation doses. Its generally accepted that gamma irradiation at 10 kGy will eliminate actinomycetes, fungi and invertebrates in most soils while bacteria are mostly eliminated by 20 to 25 kGy (McNamara *et al*., 2003, 2007).

Although several studies have investigated the effect of gamma irradiation on the viability of micro-organisms (Trampuz *et al*., 2006), little information is available

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regarding its effect on microbial structure in soils polluted with several major pollutants like hydrocarbons derived from petroleum oil pollution.

Soil contamination with petroleum oil and its derivatives is of a great concern for both industry and regulators. Hydrocarbon contaminated soil contains monocyclic and polycyclic aromatic hydrocarbons. Presence of these compounds in soil imposes different characteristics for the soil which determines a special microbial profile. Bacterial community structure in soil is generally affected by the stress imposed by the presence of such compounds (MacNaughton *et al*., 1999).

Response of soil microbes to gamma irradiation was recently studied (McNamara *et al*., 2007), however, there is no information available on the effect of gamma irradiation on soil microbial communities in contaminated soils and whether presence of contaminants in soil would impose some resistance to gamma irradiation.

This study focused on the effect of gamma irradiation on bacterial community structure in hydrocarbon contaminated soil compared to regular uncontaminated clay soils using PCR-DGGE method as a molecular technique to address such effects.

MATERIALS AND METHODS

Soil samples: Three soil samples were collected from different locations with special characteristics. A garden clay soil sample was collected from Cairo, Egypt. Regular uncultivated clay soil was collected near Helwan Industrialized city, Egypt. Hydrocarbon polluted soil was collected from petroleum oil station, Helwan, Egypt. About 10 g of soil was collected from 10 cm depth using sterile spatula in sterile polyethylene bag and transported to irradiation facility at Cairo, Egypt.

Soil irradiation: One gram of each soil sample in polyethylene bag was exposed to gamma irradiation (Co-60) in the Egypt's Industrial Mega-Gamma Irradiator in the Indian gamma cell (GC 40000A) at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo. The dose rate at the time of experiments was 6.66 kGy h^{-1}. The doses tried were 1, 2, 3, 4.5 and 10 kGy.

DNA extraction: Community DNA from soil samples was extracted with the Ultra Clean Soil DNA purification kit (Mo Bio Laboratories, Solana Beach, Calif.). About 0.2 g soil was transferred into bead beating tube. Samples were homogenized and DNA was solubilized by bead beating with a FastPrep instrument (Bio 101) for 30 sec at level 5.5. The supernatant was transferred and the DNA was precipitated and purified according to instruction manual. Obtained DNA was further precipitated by sodium acetate and absolute ethanol and finally, washed with 70% ethanol, dried up and resuspended in TE buffer for further study.

PCR-DGGE for 16S rRNA genes: Bacterial 16S rRNA genes were amplified for DGGE analysis using DGGE primers GC-341F(5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3') and 517R (5'-ATTACCGCGGCTGCTGG-3') (Muyzer *et al*., 1993). PCR for 16S rDNA was performed with bacterial primers 341F (5'-CCTACGGGAGGCAGCAG-3') and 517R. Amplification was performed as follows: Initial denaturation step at 94°C for 4 min, followed by 5 cycles of 95°C (40 sec), 65°C (40 sec) and 72°C (40 sec); 25 cycles of 95°C (40 sec), 60°C (40 sec) and 72°C (40 sec) and finally at 72°C for 5 min.

For DGGE analysis, the Dcode system for DGGE (Bio-Rad Laboratories Ltd., Hertfordshire, United Kingdom) was used. PCR products were electrophoresed on a plolyacrylamide gel containing 25 to 50% denaturing gradient of formamide and urea. Electrophoresis was performed in 0.5x TAE buffer at 60°C for 5 h at 200 V. After termination of electrophoresis corse, gels were removed and stained with SYBR Green I Nucleic acid gel stain (Cambrex Bio Science Rockland, USA).

Selected DNA bands were excised, soaked in TE buffer, boiled for 5 min, used as template for subsequent PCR amplification. PCR for excised DNA fragments were performed using same bacterial primers without GC clamp using the following program: Initial denaturation step at 94°C for 5 min, followed by 25 cycles of 95°C (40 sec), 56°C (40 sec) and 72°C (40 sec) and finally at 72°C for 5 min.

Cloning and sequencing of amplified fragments: PCR products were ligated to pGEM-T Easy vector (Preomega, USA) and used to transform *E. coli* JM 109 (Takara, Japan) according to manufacturer's recommendations. Recombinant plasmid DNA was extracted from produced clones by Wizard Plus SV Minipreps DNA purification system (Promega, USA) and used for sequencing. Sequence analysis for cloned fragments was performed by automated florescent dye terminator sequencing (Sanger *et al*., 1977) with ABI 310 genetic analyzer (Applied Biosystems, CA, USA).

Obtained sequences were analyzed by Genetyx-Win MFC application software version 4.0. The reference 16S rRNA gene sequences were obtained from the GenBank database (National Center for Biotechnology Information, National Library of Medicine, USA). Multisequence alignments were performed by ClustalX and Phylogenetic trees were constructed with the molecular evolutionary genetics analysis package (MEGA

version 3) (Kumar *et al*., 2001) with neighborjoining phylogeny and tested by bootstrap analysis (Saitou and Nei, 1987).

Related sequences were identified using BLAST search program (National Center for Biotechnology Information, National Library of Medicine, USA) and the closest match of known phylogenetic affiliation was used to assign the bands to taxonomic groups.

RESULTS AND DISCUSSION

Assessment of DGGE technique for profiling soil bacteria: The effect gamma irradiation on elimination of amplifiable DNA is considered as an issue of relevance to molecular diagnostic approaches (Trampuz *et al*., 2006). However, culture-dependent approaches would be inappropriate in this investigation as recovery and re-establishment of bacterial populations in soil after radiation treatment dose not require any additional nutrients or cultivation techniques to figure out natural nutrient availability, competence and culture independent changes in the treated soil. Consequently and as a replacement of classical cultivation-dependant methods, DGGE was used as a culture-independent molecular tool to monitor effects of gamma irradiation on bacterial community structure in soil.

DGGE analysis of irradiated soils: It has been stated that the community diversity with nucleic acid-based methods is more appropriate than those based on culturing organisms (Ovrea's and Torsvik, 1998). Various molecular techniques are now available for analyzing microbial community structure in a variety of environments. DGGE (denaturing gradient gel electrophoresis) technique (Muyzer *et al*., 1993) has been recently used as a molecular phylogenetic survey method to survey the dynamics of the microbial population from one place to another or to study effect of certain stress factors on a microbial population. In this study, DGGE was used as a molecular method to compare the bacterial community structure change revealed by 16S rRNA gene amplicons obtained from bacterial isolates from different soil samples in response to different doses of gamma irradiation.

The response of bacterial community to gamma irradiation was different among soil samples. In garden clay soil, gamma irradiation was found to be stimulatory as new bands appeared over the control with increasing irradiation doses up to 4 kGy at 30 days post-irradiation (Fig. 1). Increasing irradiation dose above 4 kGy resulted in a sharp decrease in bacterial population. A single band was detected at irradiation dose of 5 and 10 kGy which revealed a single radiation resistant bacterium at this soil.

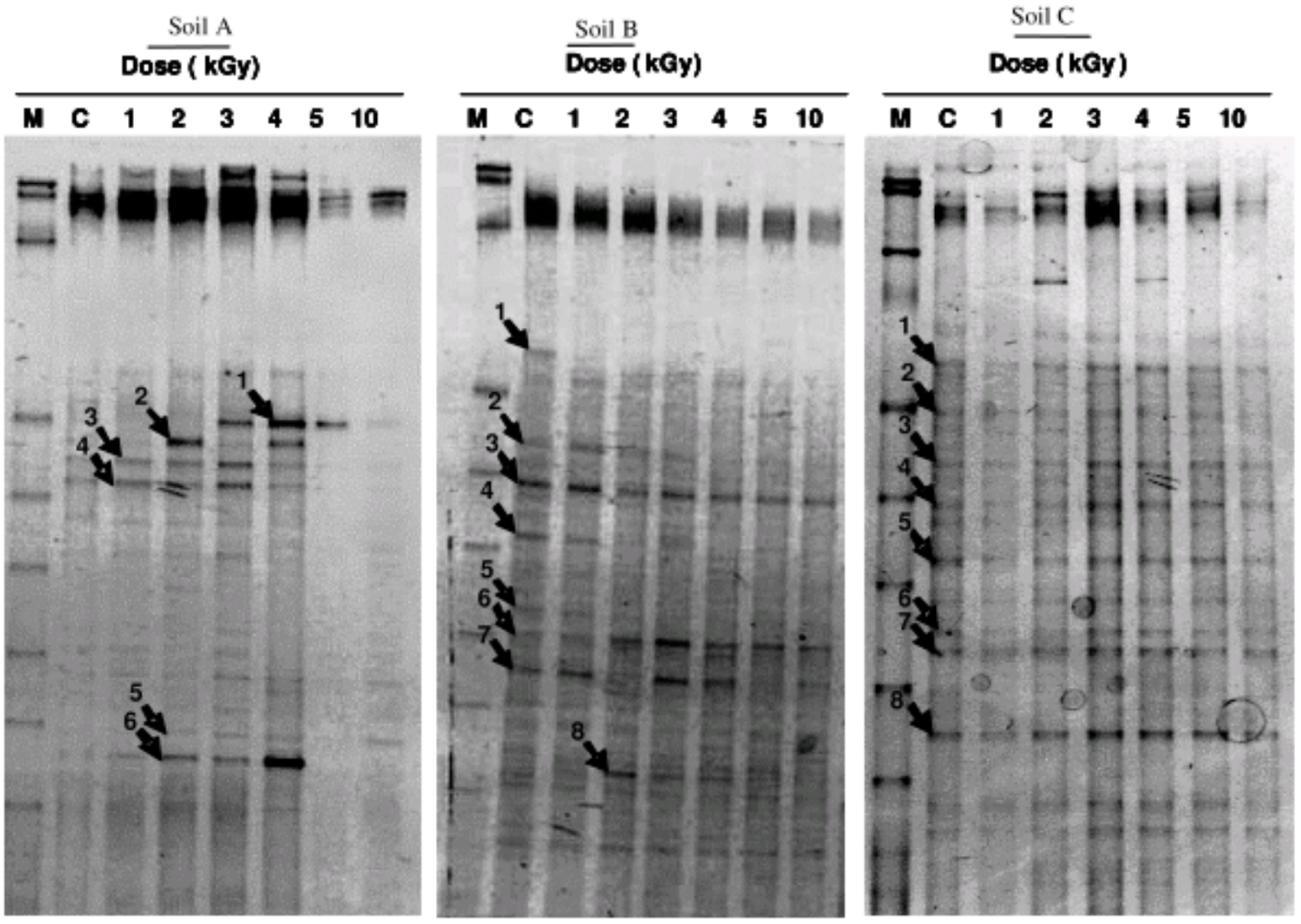

Fig. 1: DGGE pattern of 16S rRNA gene sequences from garden clay soil (A), uncultivated clay soil (B) and hydrocarbon contaminated soil (C) irradiated at 1, 2, 3, 4, 5, and 10 kGy doses. Lane M represents a DGGE marker. Lane C represents control of untreated soil sample. Lanes 1, 2, 3, 4, 5 and 10 represent irradiation doses. Numbers given on the gel represents bands that were excised for sequence analysis

It has been documented that, any change in the competition between soil bacteria and fungi or change in nutrient availability may lead to re-establishment of bacterial populations (Johnson and Osborne, 1964). MacNamara *et al*. (2007) stated that irradiated soil bacteria show a decrease in their populations immediately after irradiation, however, there was a rapid recovery in these populations with time. It was therefore concluded that, higher bacterial populations at higher irradiation doses may be due to elimination of most soil fungi at low irradiation doses that allows bacteria to thrive and flourish without competition. It may also be due to nutrient flushes after irradiation which are well documented and occur from the death and lyses of cells (Bowen and Cawse, 1964). In uncultivated clay soil, it was found that DGGE pattern of the control sample had the higher bacterial species which decreased with increasing irradiation doses. Only two bands were recovered at irradiation doses of 5 and 10 kGy at 30 days post-irradiation.

DGGE banding pattern of bacterial populations in hydrocarbon contaminated soil in consequence to gamma irradiation was completely different from previously mentioned regular soils. It was found that almost all bands were present at all doses indicating that much of the diversity remain intact and no change in community as a result of radiation treatment. There was some degree of protection against irradiation treatments including even heavy doses in contaminated soil. Such protection may be attributed to the unique physico-chemical properties of this soil due to pollution with different hydrocarbon derivatives that may embrace some properties acting as a protective shield to bacterial population living within such polluted soils.

Analysis of 16S rDNA sequences: To figure out the identity of bacterial species in each soil, DGGE bands were excised and sequenced. Obtained sequences were analyzed and closely related matches were determined using BLAST search program (NCBI, USA). Table 1 represents sequence based identification DGGE bands derived from bacterial 16S rDNA from the three different soil types.

Bacterial population in garden clay soil was identified and included the genera; Chloroflexi, *Flavobacterium*, *Sphingomonas*, *Bacillus,* *Mycobacterium* and *Azospirillum.*

A single band (A1) recovered at irradiation of 5 and 10 kGy was identified as cloroflexi bacterium, a green non-sulfur bacterium that showed some resistance to irradiation perhaps due to the presence of pigments. There is evidence that pigmentation provides some degree of protection to bacterial DNA (Rokitko *et al*., 2003).

Bacterial population in uncultivated clay soil was identified and included the genera; *Sphingomonas*, *Enterobacter*, *Acinetobacter*, *Arthrobacter*, *Pseudomonas* and *Azospirillum.* Among all bands detected, band B3 and B7 were present at all radiation treatments. At higher irradiation doses of 5 and 10 kGy only these two bacteria were dominant. They were identified as species of the genera *Enterobacter* and *Pseudomonas*, known members of gammaproteobacteria which are known to thrive in many different soils. The

Table 1: Sequence based identification and taxonomic affiliation of bands excised from DGGE gels derived from bacterial 16S rDNA from three different soil types

Bands[a]	Similarity (%)	Closest match[b]	Accession No.	Taxonomic group
Soil A				
A1	98	Uncultured Chloroflexi bacterium	DQ236250	Chloroflexi
A2	100	Uncultured *Flavobacterium* sp. TD1	AY313919	Bacteroidetes
A3	98	*Sphingomonas* sp. EM0300	EU448285	Alphaproteobacteria
A4	98	*Bacillus* sp. Mali-19	AY211113	Firmicutes
A5	100	*Azospirillum* sp. AP-500	AM743175	Alphaproteobacteria
A6	98	*Mycobacterium* sp. CNF	AB329633	Actinobacteria
Soil B				
B1	99	Uncultured gamma proteobacterium	DQ501353	Gammaproteobacteria
B2	100	*Sphingomonas* sp. LP7A	AB434710	Alphaproteobacteria
B3	98	*Enterobacter* sp. DAP21	EU302846	Gammaproteobacteria
B4	97	*Acinetobacter* sp. BSA 47	DQ517908	Gammaproteobacteria
B5	94	*Arthrobacter cumminsii*	EU086804	Actinobacteria
B6	99	*Pseudomonas stutzeri*	AY436612	Gammaproteobacteria
B7	98	*Acinetobacter* sp. L.	DQ189256	Gammaproteobacteria
B8	98	*Azospirillum brasilense*	EF634031	Alphaproteobacteria
Soil C				
C1	96	*Lysobacter spongiicola*	AB299978	Gammaproteobacteria
C2	100	*Sphingomonas* sp. AeL06	EU741013	Alphaproteobacteria
C3	100	*Sphingomonas* sp. YC6722	EU707560	Alphaproteobacteria
C4	99	*Arthrobacter* sp. YIM C733	EU135685	Actinobacteria
C5	98	*Rhodococcus* sp. FLN-1	EU718228	Actinobacteria
C6	100	*Erythrobacter* sp. JL1033	DQ985037	Alphaproteobacteria
C7	100	*Arthrobacter aurescens*	EU729736	Actinobacteria
C8	98	*Acinetobacter* sp. L.	DQ189256	Gammaproteobacteria

[a]Band numbers are the same as in Fig. 1, [b]Closest match was obtained from BLAST search (NCBI, USA)

Table 2: Effect of sub-sterilizing doses of gamma irradiation on bacterial communities in polluted and unpolluted soils

	Soil bacteria		
Dose (kGy)	Soil A	Soil B	Soil C
0	Uncultured *Flavobacterium* sp. TD1 *Sphingomonas* sp. EM0300 *Bacillus* sp. Mali-19	Uncultured gamma proteobacterium *Sphingomonas* sp. LP7A *Enterobacter* sp. DAP21 *Acinetobacter* sp. BSA 47 *Arthrobacter cumminsii* *Pseudomonas stutzeri* *Acinetobacter* sp. L	*Lysobacter spongiicola* *Sphingomonas* sp. AeL06 *Sphingomonas* sp. YC6722 *Arthrobacter* sp. YIM C733 *Rhodococcus* sp. FLN-1 *Erythrobacter* sp. JL1033 *Arthrobacter aurescens* *Acinetobacter* sp. L
1	*Sphingomonas* sp. EM0300 *Bacillus* sp. Mali-19 *Mycobacterium* sp. CNF	*Sphingomonas* sp. LP7A *Enterobacter* sp. DAP21 *Acinetobacter* sp. BSA 47 *Pseudomonas stutzeri* *Acinetobacter* sp. L	*Lysobacter spongiicola* *Sphingomonas* sp. AeL06 *Sphingomonas* sp. YC6722 *Arthrobacter* sp. YIM C733 *Rhodococcus* sp. FLN-1 *Erythrobacter* sp. JL1033 *Arthrobacter aurescens* *Acinetobacter* sp. L
2	Uncultured *Flavobacterium* sp. TD1 *Sphingomonas* sp. EM0300 *Bacillus* sp. Mali-19 *Azospirillum* sp. AP-500 *Mycobacterium* sp. CNF	*Sphingomonas* sp. LP7A *Enterobacter* sp. DAP21 *Pseudomonas stutzeri* *Acinetobacter* sp. L *Azospirillum brasilense*	*Lysobacter spongiicola* *Sphingomonas* sp. AeL06 *Sphingomonas* sp. YC6722 *Arthrobacter* sp. YIM C733 *Rhodococcus* sp. FLN-1 *Erythrobacter* sp. JL1033 *Arthrobacter aurescens* *Acinetobacter* sp. L
3	Uncultured Chloroflexi bacterium Uncultured *Flavobacterium* sp. TD1 *Sphingomonas* sp. EM0300 *Bacillus* sp. Mali-19 *Azospirillum* sp. AP-500 *Mycobacterium* sp. CNF	*Sphingomonas* sp. LP7A *Enterobacter* sp. DAP21 *Pseudomonas stutzeri* *Acinetobacter* sp. L	*Lysobacter spongiicola* *Sphingomonas* sp. AeL06 *Sphingomonas* sp. YC6722 *Arthrobacter* sp. YIM C733 *Rhodococcus* sp. FLN-1 *Erythrobacter* sp. JL1033 *Arthrobacter aurescens* *Acinetobacter* sp. L
4	Uncultured Chloroflexi bacterium Uncultured *Flavobacterium* sp. TD1 *Sphingomonas* sp. EM0300 *Bacillus* sp. Mali-19 *Mycobacterium* sp. CNF	*Enterobacter* sp. DAP21 *Pseudomonas stutzeri* *Acinetobacter* sp. L	*Lysobacter spongiicola* *Sphingomonas* sp. AeL06 *Sphingomonas* sp. YC6722 *Arthrobacter* sp. YIM C733 *Rhodococcus* sp. FLN-1 *Erythrobacter* sp. JL1033 *Arthrobacter aurescens* *Acinetobacter* sp. L
5	Uncultured Chloroflexi bacterium	*Enterobacter* sp. DAP21 *Pseudomonas stutzeri*	*Lysobacter spongiicola* *Sphingomonas* sp. AeL06 *Sphingomonas* sp. YC6722 *Arthrobacter* sp. YIM C733 *Rhodococcus* sp. FLN-1 *Erythrobacter* sp. JL1033 *Arthrobacter aurescens* *Acinetobacter* sp. L
10	Uncultured Chloroflexi bacterium	*Enterobacter* sp. DAP21 *Pseudomonas stutzeri*	*Lysobacter spongiicola* *Sphingomonas* sp. AeL06 *Sphingomonas* sp. YC6722 *Arthrobacter* sp. YIM C733 *Rhodococcus* sp. FLN-1 *Erythrobacter* sp. JL1033 *Arthrobacter aurescens* *Acinetobacter* sp. L

re-establishment and dominance of representatives of gammaproteobacteria at higher irradiation doses is relevant with their properties as fast growing and competitive species.

Bacterial populations in hydrocarbon polluted soil were also identified. Such contaminated soil was found to be predominated by different species known for their biodegradation potentials including the genera; *Sphingomonas*, *Arthrobacter*, *Rhodococcus* and *Acinetobacter*. Diversity of these bacteria was not affected by all irradiation doses and all species were present through all treatments. Table 2 shows the effect of sub-sterilizing doses of gamma irradiation on bacterial communities in polluted and unpolluted soils.

Phylogenetic analysis: Phylogenetic analysis was performed on the basis of the partial 16S rRNA gene sequences (~ 200 nucleotides) obtained directly from

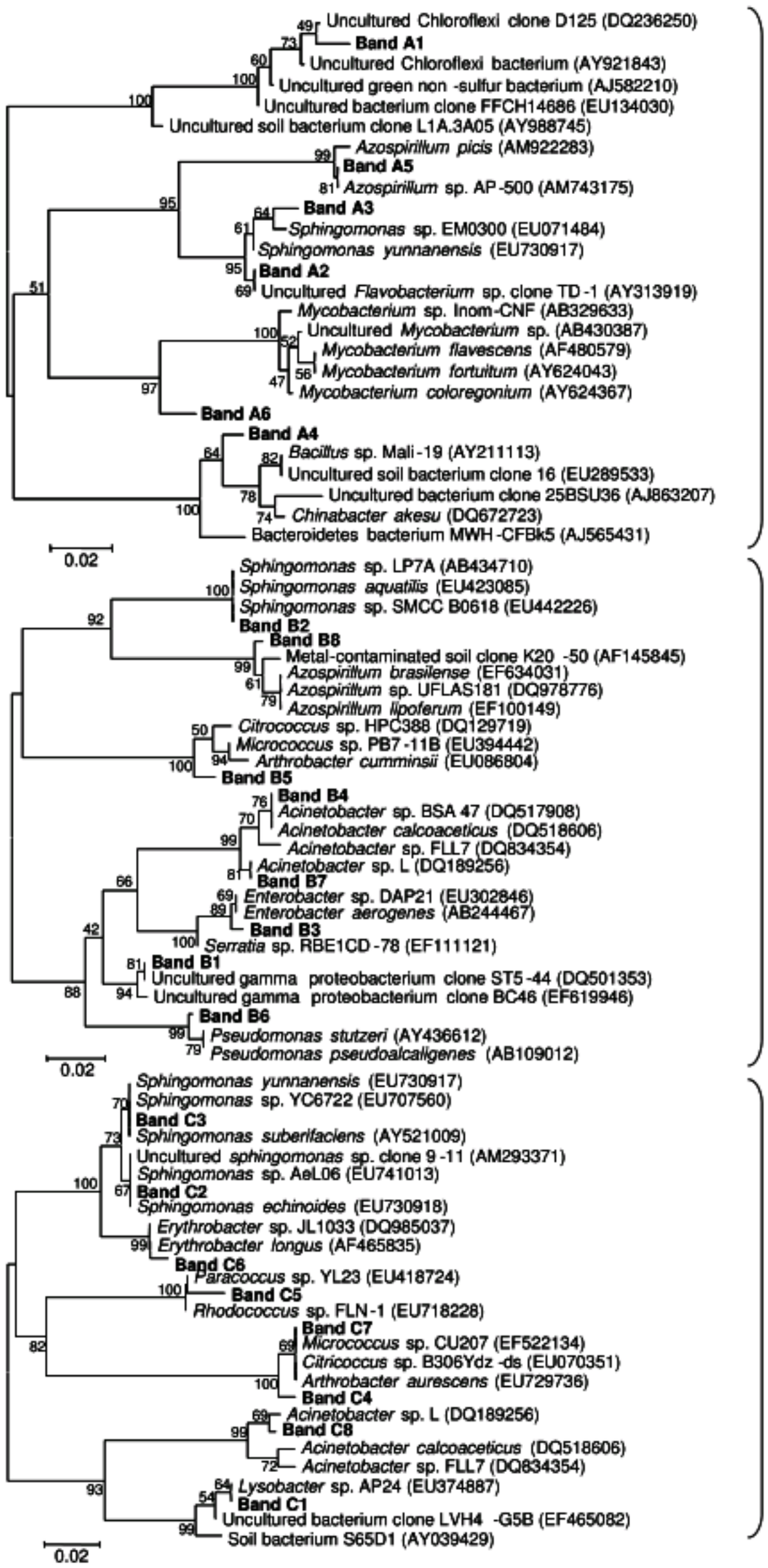

Fig. 2: Neighbor-joining trees representing the phylogenetic relationship of dominant 16S rDNA sequences from three soil types (A, B and C) with closely related sequences from BLAST search database. Bootstrap values are shown on the tree. Numbers in parentheses indicate the GenBank accession number. Bar indicates 2% sequence divergence

three different soil types (Fig. 2). Identification of endogenous soil bacteria revealed presence of members of chloroflexi, bacteroidetes, firmicutes, alphaproteobacteria, gammaproteobacteria and actinobacteria bacteria in regular uncontaminated clay soils, while contaminated soil was dominated by only members of alphaproteobacteria, gammaproteobacteria, and actinobacteria. The prevalence of different species of *Sphingomonas*, *Arthrobacter*, *Rhodococcus* and *Acinetobacter* in polluted soil was relevant with their known ability for bioremediation of many hydrocarbons.

The diversity of bacteria in hydrocarbon contaminated soil was not affected by different irradiation doses. It was found that bacterial members of the three taxonomic groups, alphaproteobacteria, gammaproteobacteria and actinobacteria were present and remain unaffected through all radiation treatments. These data suggested that hydrocarbons and perhaps other organic pollutants in the soil alter soil physico-chemical properties and provide some sort of protection for bacterial populations against gamma irradiation.

In contrast to polluted soil, there was a clear shift in bacterial community in unpolluted soils in response to irradiation. Most of the bacterial population disappeared upon increasing irradiation does and only representatives of gammaproteobacteria and chloroflexi remain dominant at elevated levels of irradiation. Shielding effect was discussed in the context of sample size and density. It was found that the higher the sample density the more doses of irradiation should be delivered due to sample shielding of irradiation (Yardin *et al*., 2000). It has been also stated that soil physico-chemical properties may alter the response of its microbial communities to gamma irradiation (Gholz *et al*., 2000).

In conclusion, present investigations showed that pollution of soil with hydrocarbon may lead to profound changes in its physicochemical properties which consequently impose some degree of protection (shielding effect) for the endogenous bacterial community from external effects like gamma irradiation or perhaps others.

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REFERENCES

Alabastro, F.E., A. Pineda, A. Pangan and M. Delvalle, 1978. Irradiation of fresh cavendish bananas (Musa cavendish) and Mangoes mongifera. International Symposium on Food Preservation by Irradiation, Nov. 21-21, International Atomic Energy Agency, pp: 282-303.

Bowen, H.J.M. and P.A. Cawse, 1964. Some effects of gamma radiation on the composition of the soil solution and soil organic matter. Soil Sci., 98: 358-361.

Degrange, V., R. Lensi and R. Bardin, 1997. Activity, size and structure of a Nitrobacter community as affected by organic carbon and nitrite in sterile soil. FEMS Microbiol. Ecol., 24: 173-180.

Gholz, H.L., D.A. Wedin, S.M. Smitherman, M.E. Harmon and W.J. Parton, 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Global Change Biol., 6: 751-765.

Hall, E.J. and A.J. Giaccia, 2006. Radiobiology for the Radiologist. 6th Edn., Lippincott Williams and Wilkins, Philadelphia, PA.

Johnson, L.F. and T.S. Osborne, 1964. Survival of fungi in soil exposed to gamma radiation. Can. J. Bot., 42: 105-113.

Kainer, M.A., J.V. Linden, D.N. Whaley, H.T. Holmes and W.R. Jarvis *et al*., 2004. *Clostridium* infections associated with musculoskeletal-tissue allografts. N. Engl. J. Med., 350: 2564-2571.

Kumar, S., K. Tamura, I.B. Jakobsen and N. Masatoshi, 2001. MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics, 17: 1244-1245.

MacNaughton, S.J., J.R. Stephen, A.D. Venosa, G.A. Davis, Y.J. Chang and D.C. White, 1999. Microbial population changes during bioremediation of an experimental oil spill. Applied Environ. Microbiol., 65: 3566-3574.

McNamara, N.P., H.I.J. Black, N.A. Beresford and N.R. Parekh, 2003. Effect of gamma irradiation on chemical, physical and biological properties of soils. Applied Soil Ecol., 24: 117-132.

McNamara, N.P., R.I. Griffiths, A. Tabouret, N.A. Beresford, M.J. Bailey and A.S. Whiteley, 2007. The sensitivity of a forest soil microbial community to acute gamma-irradiation. Applied Soil Ecol., 37: 1-9.

Mendonca, A.F., M.G. Romero, M.A. Lihono, R. Nannapaneni and M.G. Johnson, 2004. Radiation resistance and virulence of Listeria monocytogenes Scott A following starvation in physiological saline. J. Food Protect., 67: 470-474.

Muyzer, G., E.C. de Waal and A.G. Uitterlinden, 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified gene coding for 16S rRNA. Applied Environ. Microbiol., 59: 695-700.

Osterholm, M.T. and A.P. Norgan, 2004. The role of irradiation in food safety. N. Engl. J. Med., 350: 1898-1901.

Ovrea's, L. and V. Torsvik, 1998. Microbial diversity and community structure in two different agricultural soil communities. Microb. Ecol., 36: 303-315.

Rokitko, P.V., V.A. Romanovskaya, Y.R. Malashenko, N.A. Chernaya, N.I. Gushcha and A.N. Mikheev, 2003. Soil drying as a model for the action of stress factors on natural bacterial populations. Mikrobiologiia, 72: 756-761.

Romanovskaya, V.A., P.V. Rokitko, Y.R. Malashenko, T.P. Krishtab and N.A. Chernaya, 1999. Sensitivity of soil bacteria isolated from the alienated zone around the chernobyl nuclear power plant to various stress factors. Mikrobiologiia, 68: 465-469.

Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Sanger, F., S. Nicklen and A.R. Coulson, 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA., 74: 5463-5467.

Scalo, J. and J.C. Wheeler, 2002. Astrophysical and astrobiological implications of gamma-ray burst properties. Astrophys. J., 566: 723-737.

Trampuz, A., K.E. Piper, J.M. Steckelberg and R. Patel, 2006. Effect of gamma irradiation on viability and DNA of *Staphylococcus epidermidis* and *Escherichia coli*. J. Med. Microbiol., 55: 1271-1275.

Trevors, J.T., 1996. Sterilization and inhibition of microbial activity in soil. J. Microbiol. Meth., 26: 53-59.

Yardin, M.R., I.R. Kennedy and J.E. Thies, 2000. Development of high quality carrier materials for field delivery of key microorganisms used as bio-fertilisers and bio-pesticides. Radiat. Phys. Chem., 57: 565-568.