INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
© VISHWASHANTI MULTIPURPOSE SOCIETY (Global Peace Multipurpose Society) R. No. MH-659/13(N)
www.vmsindia.org

MOLECULAR CHARACTERIZATION OF SOIL MICROBES USED FOR BIORECLAMATION OF DEGRADED AGRICULTURAL LAND BY FLY ASH AS AN AMENDMENT MATERIAL

S. S. Wankar[1] and V. S. Wadhai[2]
[1],Janata Mahavidyalaya, Chandrapur.
[2],Sardar Patel Mahavidyalaya, Chandrapur.*
supriyawankar2012@gmail.com

Abstract:
Soil microbial flora viz. PGPR and PSB play a vital role in improvement of soils physio-chemical properties and also helps plants by providing essential nutrients. Amendments of soil with fly ash increased the contents of minerals and essential trace elements except N_2[1]. Microorganisms like PGPR helps to increase N_2 content for crop plants. Molecular characterization of this soil microorganisms have been done by 16S r RNA gene sequencing method. The rRNA genes are the allied component of DNA of all organisms and 16rRNA is being used as biological maker of organisms [2]. 16Sr RNA gene sequencing has been used for phylogenetic study and to study the sequence similarity % with closely related species by using some data base like Blast Analysis and sequence alignment program CLUSTAL.
Keywords: PGPR, bio-reclamation, 16s rRNA, gene sequencing.

Introduction:

Molecular characterization of microorganisms is useful in phylogenetic classification and identification of new genera. Variety of techniques are available to study the diversity and similarity % among the isolated soil microorganisms including mole % G+C, DNA-DNA hybridization, DNA micro array, 16s r DNA sequencing and amplified r DNA restriction analysis, etc. Analysis of 16S r RNA genes provides molecular data which is important in identifying similarity between organisms[3].

For bio-reclamation of degraded agricultural soil with fly ash as an amendment material, the microorganisms viz. PGPR, PSB play a vital role. Therefore in present study, two microbial cultures viz**. JR1**and **P1** isolated from soil were showing PGPR properties were phylogenetically classified by 16s rRNA and 18sRNA gene sequencing which were amplified using polymerase chain reaction (PCR).

Methods and materials:

The microbial cultures isolated from soil viz. JR1 and P1 were identified as Gram negative bacterium and fungus respectively. For the nomenclature, this two isolates were identified using standard method of 16sRNA gene sequencing. Genomic DNA of each isolate was extracted by N-cetyl-N,N,N-trimethyl-ammonium bromide(CTAB)method. The quantification of isolated DNA was checked in UV-visible spectrophotometer (VivaspecBiophptpmeter, Germany). From stock solution, 1ul DNA was mixed with 49 ul sterile distilled water to get 50 times dilution. The A260/A260ratio was recorded to check the purity of DNA preparation. PCR amplification of primer was done in a 20ul of reaction mixture containing PCR buffer,1x (kappa, SA) and by mixing 50ng of template DNA with 2.5 mM concentration of each deoxynucleotide triphosphate(dNTP),Taq polymerase,0.05U and primer for isolate JR1 16sFP(5'-3' AGA, GTT, TGA, TCC, TGG, CTC, AG) and 16s RP (5'-3' AAG, GAG, GTG, ATC, CAG, CA)and for fungus isolate primer ITS4(5'-3'TCCTCCGCTTATTGATATG)and ITS6(5'-3'GACACTCAAACAGGTGTACC).

PCR temperature profile was carried out with initial denaturation at 94^{0}C (2 min). Primer annealing at 48^{0}C for 30s and primer extension at 72^{0}C for 1 min 30s and finally extension at 72^{0}C for 6min in a 30 thermo cycles.

Amplified PCR product of 16s and 18sRNA gene were separated on 2% agarose gel in TAE buffer. 500bp, 1000bp and 1500bp DAN marker was used as a reference. Gel were stained with Ethidium bromide (20mg/ml) and visualized under UV light. Gene sequences of JR1 and P1 was obtained by BLAST analysis.

Phylogenetic Analysis:

The purified PCR Product samples were sequenced using 16s FP and RP and 18s ITS4 and ITS6 gene sequencing primers. All the sequences were aligned using multiple sequence alignment program CLUSTAL[4]. The analysis of alignment and homology of the partial nucleotide sequences of Rhizobium and fungus was carried out by the basic tool alignment search tool (BLAST analysis)[5].

The pair wise distance were computed. The multiple distance matrix obtained was then

used to construct phylogram (phylogenetic tree) using Neighbors joining method.

Sequences of all related species were retrieved to get the exact phylogenetic relationship of the bacterial isolate JR1 and fungus isolate P1. DNA accession number of each isolate was obtained from DNA Data bank.

Gel Data

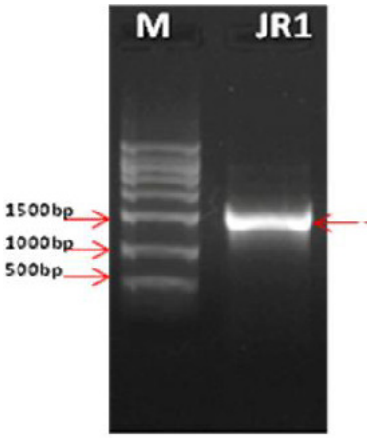

Figure 1: PCR amplification for JR1

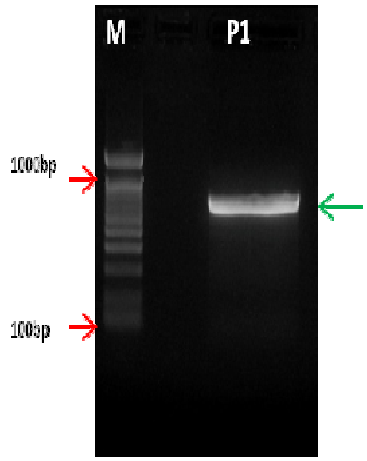

Figure 2: PCR amplification for P1

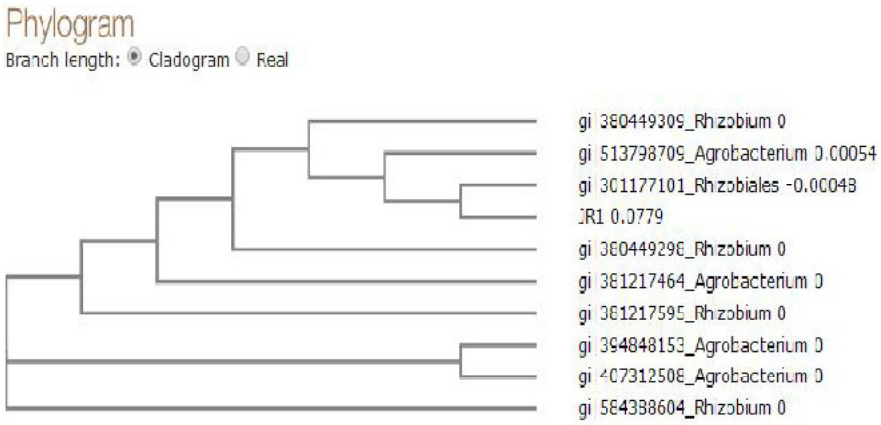

Figure 3: Phylogenetic tree showing the relationship of the isolate JR1 to closely related Rhizobacteria.

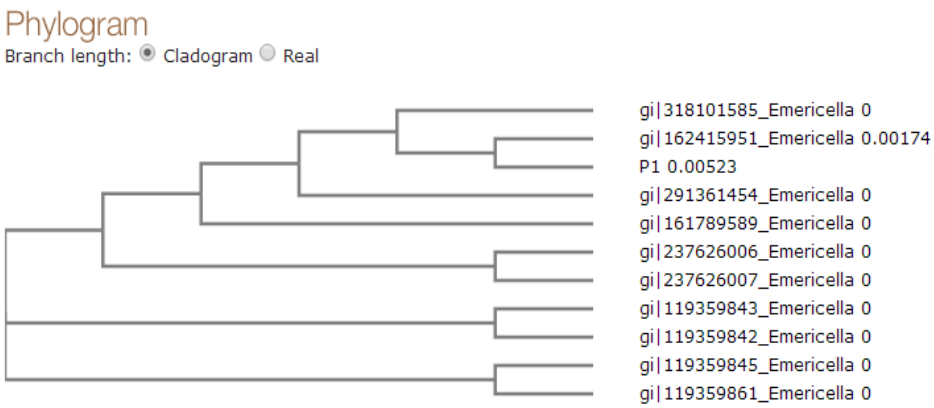

Figure 4: Phylogenetic tree showing the relationship of the isolate P1 to closely related fungi.

Result and Discussion:

Genomic DNA of microbial isolate was isolated by by CTBA method. 16s and 18s gene sequences of the isolates JR1 and P1 was PCR amplified by using specific primers .An amplification product of1500bp was obtainted for JR1 and 500-600bp for the isolate P1. Phylogenetic analysis was assessed by using Neighbors joining method. A partial 16s rRNA and 18s rRNA sequences carried out in the

present study for rhizobial spp. JR1 and fungus isolate P1 respectively. The sequences found in the clone library represented 90% sequence identity to other data base entries of Rhizobials bacterium and JR1 was identified as *Rhizobium spp*. And the sequences found in the clone library showed 99% sequence identity to other data base entries of fungus Isolate P1 was identified as *Emericella nidulance*.

Conclusion:

Phylogenetic anaylysis on the basis of 16srRNA sequences provided better understanding in similarity % of *Rhizobia(*JR1) and fungus (p1) isolated from soil. By using some computer software like BLAST analysis, CLUSTAL and from the other data base entries of organism from Gene data bank, the isolated strain JR1 has been found to show 90% similarity with Rhizobium spp. And isolate P1has been found 99% similarity with *Emericella nidulance* which might have PGPR and PSB properties and will prove to be beneficial in bioreclamation of degraded agricultural soil with fly ash as an amendment.

References:

1. **Siddiqui Z.A. and Singh L.P.(2005):** *Effects of fly ash and soil micro-organisms on plant growth, photosynthetic pigments and leaf blight of wheat.* Journal of Plant Diseases and Protection. 112(2), 146-155, ISSN0340-8159.

2. **Rifat Hayat and et al (2013):** *Molecular characterization of soil bacteria for improving crop yield in Pakistan.* Pakistan Journal of Botany. 45(3): 1045-1055

3. **Edward,U. ,Rogall, T. , Blocker, H.(1989) :** *Isolation and direct complete nucleotide determination of entire genes characterization for 16s rRNA.* Nucleic acid research, 17(19), 7843-7853.

4. **D.G.Higgins, A.J.Bleasly and R. Fuchs (1992):** *"CLUSTAL V: improved software for multiple sequence aligment", computer application in the biosciences*, 8(2), 189-191

5. **Balayogan Sivasankari and Marimuthu Anandharaj (2014*):*** *Isolation and molecular characterization of potential plant growth promoting B. cereus and Ps. Spp GGBSTDS from vermi source*. Advance in Agriculture. Vol. 2014, Article ID 248591