International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue 5, May 2015, PP 171-178
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Comparative Alignment of Ribosomal Sequence of the Floral Waste Degrading Fungi: A Way to Establish the Evolutionary Correlation

Shobha Shouche*, Zeemi Nema, Sachin Rahangdale

Govt. Madhav Science College, Ujjain (M.P.) India
**shobha.shouche@gmail.com*

Praveesh Bhati

Advance College of Commerce and Science, Ujjain (M.P.) India

Abstract: *This experiment was done to recognize the phylogenetic relationships among vermicomposting fungi by comparing the sequences of internal transcribed spacer regions (ITS) and 18S ribosomal DNA (rDNA) repeat unit. The ITS sequences of Aspergillus flavus, A.fumigatus, Alternaria alternata and A.terreus were amplified, determined and compared with each other.The sequence alignment was done to reveled the distance between the fungi. In the phylogenetic tree, the fungal strains generally divided into four groups. Result illustrate that fungal strain showed functional similarity which indicate their evolutionary closeness.*

Keywords: *Vermicomposting, fungi, evolutionary relationship, comparative analysis, MSA*

1. INTRODUCTION

The most of the decomposing organisms are belong to group fungi and have long been used for decomposition of several types of organic wastes (Suberkropp & Klug, 1976; Pope *et. al.* 1963;). Specially *Aspergillus, Rhizopus, Penicillium, Alternaria* species have been regarded as commercial decomposer for degrading municipal solid waste and agricultural waste. These fungi are known to secrete several types of enzymes (Abdullah & Taj-Aldeen, 1989; Zemek *et.al.,* 1985). Several investigators have been reported that enzymes of fungi are commercially used in detergent industry, medicinal industry and paper industry. In view of floral waste degradation, *Aspergillus* and *Alternaria* species play an important role to decompose it and turn into manure. The commercial use of these fungi required their correct identification. The taxonomy and accurate detection of the decomposing fungi is a typical task. Generally, the classification of these fungi is based on the morphology of the fruiting body and their mycelia structure. Besides morphological traits of fruiting bodies, other special characteristics have also been investigated for the systematics of the fungi. A various culture studies were conducted to classify the fungi. Recently, molecular techniques such as DNA/DNA hybridization, electrophoretic karyotyping, RFLP, and DNA sequencing have been used for phylogenetic analysis of various kinds of organisms (Sasamu *et al.*, 1998). PCR direct sequencing method reported by White *et al*. is an excellent method applicable to the fungi because it can be carried out using a small amount of starting material (White *et al.*, 1990). Hirata *et al*. improved the method to determine the rDNA sequences of fungi using tiny amounts of material (Hirata and Takamatsu, 1996). Eukaryotic rDNA is composed of tandemly repeated clusters of 18S, 5.8S, and 28S rRNA genes, which are transcribed as a precursor molecule by RNA polymerase I (Raue and Planta, 1995). The external and internal spacer molecules are then removed in nucleolus before escaping for cytoplasm. The nucleotide sequences of the conservative rRNA coding regions have been widely used for phylogenetic analysis among families or distantly related genera (Berbee and Taylor, 1993; White *et al.*, 1990). However, the variable ITS regions have an advantage of the phylogenetic analysis and identification of the closely related fungal species (Kim *et al.*, 1999).

In this work, we studied 16S rDNA and ITS regions to infer their applicability for the systematics of floral decomposing fungi. The objective is to construct the phylogenetic relationship among the regions of ITS1 and ITS2, and 16S ribosomal RNA gene of *Aspergillus* and *Alternaria* species to compare the evolutionary correlation regarding to their function. To address these aims, we amplified

and sequenced the 16S rDNA, and ITS .Our results could reveal the detailed phylogenetic relationship among the closely related *Aspergillus* and *Alternaria* and related taxa.

2. MATERIALS AND METHODS

2.1. Preparation of Fungal Isolates

In the present study, four isolates i.e. *Aspergillus flavus, A.fumigatus , Alternaria alternata* and *A.terreus* were obtained from floral waste vermicomposting process done in Ujjain (M.P.), India. The test strains were cultured by shaking flask method done in 100 ml medium of Czepex dox broth medium at 28°C for seven days. The fungal mycelia were harvested by filtration, and stored at low temperature until they were used.

2.2. Extraction of Fungal DNA

Fungal DNA was extracted from each sample according to the miniprep protocol described by Cenis (1992) and Abd-Elsalam (2003). Fungal mycelium were filtered and suspended with 500 µl Tris-EDTA buffer. The mat was then transfered in 300 µl of extraction buffer for few min. 150µl of 3 M sodium acetate (pH 5.2) was added, and the mixture was cooled to 20°C for 10 min. After the lyses of mycelium, fungal debris was pelleted by centrifugation at 10,000 rpm for 5 min. The supernatant was taken into a fresh tube, and an equal volume of ice-cold isopropanol was added. DNA was then pelleted by centrifugation at 10,000 rpm for 10 min. Excess salt was removed by washing with 70% ethanol, and DNA was resuspended in Tris-EDTA.

2.3. PCR Amplification of r DNA

The ITS and the inverting 5.8S coding rDNA were amplified by PCR using the primers ITS described by White *et al*. (1990). The PCR profile was prepared at 95°C for 2 min, followed by 30 cycles of 94°C for 1 min, 54°C for 30 s, and 72°C for 1 min. The bands of interest were separated on agarose gel by electrophoresis. The isolated DNA excised from agarose gels and re-amplified by PCR using the same primer pair that was used for generating the ITS bands (Fig.1).

2.4. Fungal rDNA Sequencing

The nuclear 18S rDNA region containing ITS gene was amplified by polymerase chain reaction from each strain. Primers were derived from the conserved region of 18S and 28S rDNA, respectively. PCR and sequencing was carried out on the behalf of BioAxis DNA research centre private limited.(MH.).The PCR products from the amplification were subjected to preparative electrophoresis in a agarose gel in buffer. All PCR products yielded only a single visible band. The PCR products were excised from the ethidium bromide-stained gel and purified using a gel elution kit. Direct sequencing of PCR products was done by a sequencer according to the standard protocol (Gyllensten, 1989; Hiraishi, 1992; Smith *et al.*, 1986). Four primers, were used for sequencing in both directions and the DNA sequences were edited and assembled with the help of program.

2.5. Molecular Phylogeny Analysis

Multiple sequence alignment was obtained by using Clustal- W 2.1 program. CLUSTAL W is desired to provide an adequate alignment of a large number of more closely related sequences and a reliable indication of the domain structure of those sequences. Clustal W also has options for adding one or more additional sequences with weights or an alignment to an existing alignment (Higgins et al. 1996).it was used to generate the phylogenetic tree. Molecular phylogeny analysis (MPA) was carried out using the data set of complete genome of *Aspergillus fumigates* strain ATCC42826 YLL034C, *Asprgillus flavus* strain AF12, *Alternaria alternata* sp.L2785 18s, *Aspergillus terrus* strain MF12 using neighbor joining (NJ), Maximum likelyhood (ML) and Maximum persimony (MP) method and trees are shown in fig. 2

3. RESULT AND DISCUSSION

The obtained product of rDNA by agarose gel electrophoresis and DNA sequencing of PCR product reveled that amplified DNA was pure expected rDNA (Fig.1). The alignment data of the DNA sequences of ITS, and 18S rDNA using CLUSTAL W were shown in Fig. 2. There is significant sequence variation in the ITS sequences and regions of the 18S rDNA. The sequence difference of different species in *Aspergillus* and *Alternaria* genus is supposed as the variation of cultural, geographical, environmental conditions, and gene variability. The sequence number 3with 4 showed

higher alignment score that was 62.31 while sequence 1 with 4 showed lowest score that was 39.77.By using Clustal W the following result were obtained in Fig.3and Fig.4.

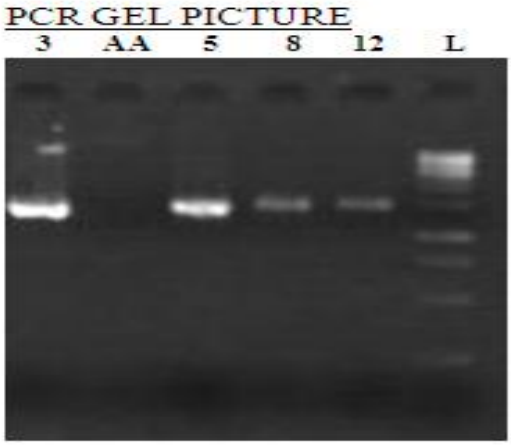

Fig1. *PCR amplified Gel Picture*

3.1. Fungal rDNA Sequencing

Sequence 1:

The given Fungal culture was identified Bacillus as Aspergillus fumigatus strain ATCC42826 YLL034C

Length: 615

Score: 1136 bits (615)

>ATATGAGGAGGCTCGCCGAAGAATGCGAAGGATTCAGCGGTGCGGATTTGGGAAGTTT
GCTACGCCGTGCCGGTTATTCTGCAATCAAAAGACGCGATCAGATCAGCTTTGAGGACTT
CGTTGCCGCTAAGGCTTTCATTCGGCCTAGTGTTACCGATCTCAAAAAGTATGAGAAGCT
CAGGAGAGAGTGGAGCGGCGGCGTGTTGTAGATATCCGACCAGTGTGGCATACAAAGTC
AACGAGAGCATTGTGTACGTACGGGATAATGGTCATATACCAAAAGCTTTGCATGTGCG
GATGCTGACAATCTCCTCGCTTGCTTGGATGTTTGTACTCAATGAGTCTACCAATCGTTCG
TTCTTCCACATAATACCTCCAAGAATTAGACTTGATATCGGGATGCCAGGTTGCTTGGAC
CATCAGACGAGGGCTTATTTTTAAGGAAACAGTTTCTATAAGGACTATAACTTTTATAAA
GCGGATATATCTCTTGACGAAAACATTGATGCACACCAAGACGCCTCTCATGTAGACATG
ACCTGATAAATTAACAGTTGGAAATCCCAATATGTACGCTTCGAATCGACGTGTGATTAC
ATCCAGAGA CGGCAAGTT

Sequence 2:

The given Fungal culture was identified as Apergillusflavus isolate AF13C

Length: 1216

Score: 2340 bits (1267)

>ATTCCTGAATTCCTTCCTCACCTCCACGATGGTTGACCATATCTCCCCCCGGGCATCTCC
CGGACCGATCCGTTCCTCCCAGACTCGCCGCGCCCGAAAGCTCCGGGATAGCTGTACGA
GTTGTGCCAGCTCAAAAGTGCGATGCACCAAGGAGAAACCGGCCTGTGCTCGGTGTATC
GAACGTGGTCTTGCCTGTCAATACATGGTCTCCAAGCGGATGGGCCGCAATCCGCGCGCT
CCCAGTCCCCTTGATTCAACTCGGCGACCATCAGAGAGTCTTCCTTCAGCCAGGTCGGAA
CAGGGACTTCCGGCGCATAACACGTACTCAACGCCTCATGCTCATACGCAGGCCCACACT
CATGCTCATTCTCATCCGCAACCGCATCCACAATCTCATCCTCAATCGAATCAACCACCA
CACGCTCTGCCCACCCCCAATGGTAGCAGTAGCGTCTCCGCCATCTTTTCTCATCAGAGT
CCGCCGCCACCCGTGGAGACCCAGGGCCTTGGAGGAGATCTGGCTGGTCAGGAGCAAAG
CACCCTGTCTTCCCTAACAGTCGATTCGGAATTCGGGGGCTCTTTGCAGTCAATGGAACA
CGGAAACCATGCCGATTTCTTGGCCGAGTCGACGGGGAGTCTTTTCGACGCGTTTTTGGA
AGTAGGGACCCCCATGATCGACCCGTTCCTCGAGTCGGCCCCACTACCACCGTTTCAGGC

GCGCTATTGCTGCTTTTCGCTAGCACTACAAACACTGACCCACCTCTTCCCCCACGCCCCG
CTGGGCTGTCAACTACGGCTGACGGACGGTGAGGACAGTTCGTGCAACCTGATGACGAC
TGATATGGTCATCTCGGGGAACAAGAGGGCTACCGATGCGGTCCGGAAGATCCTCGGGT
GTTCGTGCGCGCAGGATGGCTACTTGCTGAGCATGGTCGTCCTTATCGTTCTCAAGGTGC
TGGCATGGTATGCTGCGGCAGCAGGCACCCAGTGTACCTCAACGGCGGCGGGTGGAGAA
ACCAACAGTGGCAGCTGTAGCAACAGTCCCGCCACCGTGTCCAGTGGCTGTCTGACGGA
AGAGCGCGTGCTGCACCTCCCTAGTATGATGGGCGAGGATTGTGTGGATGAGGAAGACC
AGCCGCGAGTGGCGGCACAGCTTGTTCTGAGTGAACTGCACCGAGTCCAGTCGCTGGTG
AACCTATTGGCCAAGCGCCT GCAA

Sequence 3:

The given Fungal culture was identified as Alternaria sp. L2745 18S ribosomal RNA gene, partial sequence

Length: 573

Score: 736 bits (398)

>GCGTCAGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATC
GATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATC
GAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTC
ATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGAGACTC
GCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTC
TATCAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCAACTTTTGACCTCGGATCAGGTA
GGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGG

Sequence 4:

The given Fungal culture was identified as Aspergillus terreus strain MF12

Length: 535

Score: 976 bits (528)

>ATCTTTATGGCCACCTCCCACCCGTGACTATTGTACCTTGTTGCTTCGGCGGGCCCGCCA
GCGTTGCTGGCCGGCGGGGGGCGACTCGCCCCCGGGCCCGTGCCCGCCGGAGACCCCAA
CATGAACCCTGTTCTGAAAGCTTGCAGTCTGAGTGTGATTCTTTGCAATCAGTTAAAACT
TTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACT
AATGTGAATTGCAGAATCCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGG
TATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTT
GGGCCCTCGTCCCCCGGCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTC
CGGTCCTCGAGCGTATGGGGCTTCGTCTTCCGCTCCGTAGGCCCGGCCGGCGCCCGCCGA
CGCATTTATTTGCAACTTATTTATTCCAGGTGACCTCGGATCAGTAGGGAA

3.2. CLUSTAL 2.1 Multiple Sequence Alignment

CLUSTAL 2.1 multiple sequence alignment

```
Alternariaalternata     ------------------------------------------------------------
Aspergillusterreus      ------------------------------------------------------ATCTTT 6
Aspergillusflavus       ATTCCTGAATTCCTTCCTCACCTCCACGATGGTTGACCATATCTCCCCCCGGGCATCTCC 60
Aspergillusfumigates    ------------------------------------------------------ATATGA 6

Alternariaalternata     ------------------------------------------------------------
Aspergillusterreus      ATGGCCA-------CCTCCCA--------------------CCCGTGACTATTGTAC--- 36
Aspergillusflavus       CGGACCGATCCGTTCCTCCCAGACTCGCCGCGCCCGAAAGCTCCGGGATAGCTGTACGAG 120
Aspergillusfumigates    GGAGGC--------TCGCCGA----------------AGAATGCG-AAGGATTCAGCGG- 40

Alternariaalternata     ------------------------------------------------------------
Aspergillusterreus      ----------------------------------------------CTTGTTGCTTCG- 48
Aspergillusflavus       TTGTGCCAGCTCAAAAGTGCGATGCACCAAGGAGAAACCGGCCTGTGCTCGGTGTATCGA 180
Aspergillusfumigates    ----------------------------------------------TGCGGATTTG- 50
Alternariaalternata     ------------------------------GCG---------------------- 3
Aspergillusterreus      --GCGGGCCCGCC--------------------AGCG----------------------- 63
Aspergillusflavus       ACGTGGTCTTGCCTGTCAATACATGGTCTCCAAGCGGATGGGCCGCAATCCGCGCGCTCC 240
Aspergillusfumigates    --GGAAGTTTGCT--------------------ACG----------------------- 64
                                  **
Alternariaalternata     --------TCA------GT---------------------------------------- 8
Aspergillusterreus      --------TTG------CTGGCCGGCGGGGGGCGACTCGCCCCCGGGCCCGTGCCCGCCG 109
```

```
Aspergillusflavus       CAGTCCCCTTGATTCAACTCGGCGACCATCAGAGAGTCTTCCTTCAGCCAGGTCGGAACA 300
Aspergillusfumigates     --------CCG-------T-GCCGG-----------TTATTCTGCAATCAA--------- 88
                           *
Alternariaalternata     ----------AACA---------------------------------------- 12
Aspergillusterreus      GAGACCCC--------AACATGAACCCTGTTCTGAAAGCTTG----------CAGTCTGA 151
Aspergillusflavus       GGGACTTCCGGCGCATAACACGTACTCAACGCCTCATGCTCATACGCAGGCCCACACTCA 360
Aspergillusfumigates     ----------AAGACGCGATCAGATC----AGCTT----------TGA 112
                           ** *
Alternariaalternata     ----AATT---------AAT---AATTACAACTT-----TCAA----------------- 34
Aspergillusterreus      GTGTGATTCTT---TGCAATC--AGTTAAAACTT-----TCAA----------------- 184
Aspergillusflavus       TGCTCATTCTCATCCGCAACCGCATCCACAATCTCATCCTCAATCGAATCAACCACCACA 420
Aspergillusfumigates     G---GACTTC--------GTTGCCGCTAAGGCTT-----TCATTCGG------------ 143
                       * *             *    *    ***
Alternariaalternata     ----------CAACGG----------ATCTCTT--------GGTTC 52
Aspergillusterreus      ----------CAATGG----------ATCTCTT--------GGTTC 202
Aspergillusflavus       CGCTCTGCCCACCCCCAATGGTAGCAGTAGCGTCTCCGCCATCTTTTCTCATCAGAGTCC 480
Aspergillusfumigates     ----------CCTAGTGTTACCG----------ATCTCAA------AAAGTAT 170
                           * *            ****         **
Alternariaalternata     --TGGCATCGATGAAGAAC----------GCAGCGAAATGC 81
Aspergillusterreus      --CGGCATCGATGAAGAAC----------GCAGCGAAATGC 231
Aspergillusflavus       GCCGCCACCCGTGGAGACCCAGGGCCTTGGAGGAGATCTGGCTGGTCAGGAGCAAAGCAC 540
Aspergillusfumigates     GAGAAGCTCAGGAGAGAGTGG----------AGCGGCGGCGTGT 204
                       *    ***                  *  **
Alternariaalternata     ----------GATA----AGTAGTGTGAA----------TTGCAG--AATTCAGT 110
Aspergillusterreus      ----------GATA----ACTAATGTGAA----------TTGCAG--AATCCAGT 260
Aspergillusflavus       CCTGTCTTCCCTAACA----GTCGATTCGGAATTCGGGGGCTCTTTGCAGTCAATGGAAC 596
Aspergillusfumigates     TGTA--------GATATCCGACCAGTGTGGCA----------TACA---AAGTCAAC 240
                           * *       * *          * **  **  *
Alternariaalternata     G----AATCAT----------CGAATC----------TTTGAACGCACATT--- 137
Aspergillusterreus      G----AATCAT----------CGAGTC----------TTTGAACGCACATT--- 287
Aspergillusflavus       ACGGAAACCATGCCGATTTCTTGGCCGAGTCGACGGGGAGTCTTTTCGACGCGTTTTTGG 656
Aspergillusfumigates     G---AGAGCAT----------TGTGTACGTACG----- 260
                       * ***                  * *  ***
Alternariaalternata     ------GCGCCCTT-TGGT------ATTCC--AAA----------GGG 160
Aspergillusterreus      ------GCGCCCCC-TGGT------ATTCC--GGG----------GGG 310
Aspergillusflavus       AAGTAGGGACCCCCATGATCGACCCGTTCCTCGAGTCGGCCCCACTACCACCGTTTCAGG 716
Aspergillusfumigates     ------GGATAATGGTCAT------ATACC--AAA----------AGC 284
                       *     * *    * **                *
Alternariaalternata     CATGC--------CTGTTCG--AGCGT----------CATTTGTACCCTCAAGCTT 196
Aspergillusterreus      CATGC--------CTGTCCG--AGCGT----------CATTGCTGCCCTCAAGCCC 346
Aspergillusflavus       CGCGCTATTGCTGCTTTTCGCTAGCACTACAAACACTGACCCACCTCTTCCCCCACGCCC 776
Aspergillusfumigates     TTTGC--------ATGTGCG--GATGC----------TGACAATCTCCTCG---CT 317
                       **      * * **                * ** *
Alternariaalternata     TGCTTG-GTGTT----------GGGCGT--------CTTGTCT--CTA 223
Aspergillusterreus      GGCTTGTGTGTT----------GGGC-C--------CTCGTCC--CCC 373
Aspergillusflavus       CGCTGGGCTGTCAACTACGGCTGACGGACGGTGAGGACAG--------TTCGTGCAACCT 828
Aspergillusfumigates     TGCTTGGATGTT----------TGTACTCAATGAGTCTACCAATCGTTCGTTCTTCCA 365
                       *** *  ***              *          * **   *
Alternariaalternata     GCT----------TTGCTGGAGACT----------CGCCTTAAAG-- 248
Aspergillusterreus      GGC----------TCCCGGGGGACG----------GGCCCGAAAG-- 398
Aspergillusflavus       GATGACGACTGATATGGTCATCTCGGGGAACAAGAGGGCTACCGATGCGGTCCGGAAGAT 888
Aspergillusfumigates     CATAAT----------ACCTCCAAGAATTA----------GACTTGATAT-- 395
                               *    *               *
Alternariaalternata     ----------TAAT--TGGC---------AGC-CGG-CCTACTG-------- 269
Aspergillusterreus      ----------GCAG--CGGC---------GGCACCG-CGT-CCG-------- 419
Aspergillusflavus       CCTCGGGTGTTCGTGCGCGCAGGATGGCTACTTGCTGAGCATGGTCGTCCTTA------- 941
Aspergillusfumigates     ----------CGGGATGCCAGGTT-----GCTTGGACCATCAGACGAGGGC 431
                               * *       **  * *  *
Alternariaalternata     --GTTTCGGAGC-----GCAGCACAAGTCGCA--------CTCTCTAT---CAGCAAAG- 310
Aspergillusterreus      --GTCCTCGAGC-----GTATGGGGCTTCGT---------CTTCCGCT---CCGTAGGCC 460
Aspergillusflavus       TCGTTCTCAAGGTGCTGGCATGGTATGCTGCGGCAGCAGGCACCCAGTGTACCTCAACGG 1001
Aspergillusfumigates     TTATTTTTAAG------GAAACAGTTTCTATAA----GGACTATAACT--TTTATAAAGC 479
                       *    **    * *            *    *     *
Alternariaalternata     -GTC----------TAGCA----------TCCATTAA-GCCTTTTTT- 335
Aspergillusterreus      CGGC----------CGGCG----------CCCGCCGACGCATTTATT- 487
Aspergillusflavus       CGGCGGGTGGAGAAACCAACAGTGGCAGCTGTAGCAACAGTCCCGCCACCGTGTCCAGTG 1061
Aspergillusfumigates     GGATATATC----------TCTTGACGAAAACATTGATG--CACACCAAGACGCCTCTCA 527
                       *              *            *
Alternariaalternata     ----------CAACTT--TTGACC----------TCGGAT 353
Aspergillusterreus      ----------TGCAACTT-----ATT----------T----- 499
Aspergillusflavus       GCTGTCTGACGGAAGAGCGCGTGCTGCACCTC--CCTAGTATGATGGGCGAGGATTGTGT 1119
Aspergillusfumigates     TGTA----------GACATGACCTGATAAATT----------AA 551
                               *    *
Alternariaalternata     CAGGTAGGGATACCCG----------CTGAACT--------- 376
Aspergillusterreus      ---------ATTCCAG----------GTGACCT--------- 513
Aspergillusflavus       GGATGAGGAAGACCAGCCGCGAGTGGCGGCACAGCTTGTTCTGAGTGAACTGCACCGAGT 1179
Aspergillusfumigates     CAGTTGGAAATCCCAA----------TATGTACGCTTCG------ 580
                       *  **                  *  **
```

```
Alternariaalternata     ----------TAAGCATATCAAT-AAGCG-----GAGG- 398
Aspergillusterreus      ----------CGG----ATCAGT-AGG-G-----AA--- 528
Aspergillusflavus       CCAGTCGCTGGTGAACCTATTGGCCAAGCGCCTGCAA--- 1216
Aspergillusfumigates    -AATCGACGTGTGATTACATCCAG-AGACG---GCAAGTT 615
                          **   *  *    *
```

Fig2. *The alignment data of ITS, and 18S rDNA region sequences using CLUSTAL W. A hyphen represents a gap and four characters, '#', '*', ':', and '.', indicate positions that are 80~100%, 60~80%, 40~60% and 20~40% identical, respectively.*

3.3. Show Multiple Sequence Alignments

```
Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: Alternariaalternata      398 bp
Sequence 2: Aspergillusterreus       528 bp
Sequence 3: Aspergillusflavus       1216 bp
Sequence 4: Aspergillusfumigates     615 bp
Start of Pairwise alignments
Aligning...

Sequences (1:2) Aligned. Score: 71.36
Sequences (1:3) Aligned. Score: 71.86
Sequences (1:4) Aligned. Score: 59.80
Sequences (2:3) Aligned. Score: 68.75
Sequences (2:4) Aligned. Score: 47.35
Sequences (3:4) Aligned. Score: 62.93
Guide tree file created:   [clustalw2-I20150415-073811-0437-26135157-oy.dnd]

There are 3 groups
Start of Multiple Alignment

Aligning...
Group 1: Sequences:   2     Score: 5498
Group 2: Sequences:   3     Score: 5178
Group 3: Sequences:   4     Score: 4472
Alignment Score 7341

CLUSTAL-Alignment file created [clustalw2-I20150415-073811-0437-26135157-oy.aln]
```

Fig3. *CLUSTAL 2.1 multiple sequence alignment*

3.4. Phylogenetic Tree

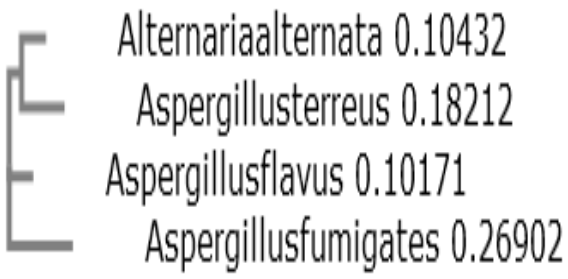

Fig4. *This is a Neighbour-joining tree with Branch length*

The rRNA genes, act as most conservative sequence of the genome hence it is commonly used in identification and taxonomic studies. There were several researchers carried out study on different aspect to carry out study about evolutionary relations ship among similar functional group. Nazar *et al*. (1991), specifically worked within species of plant pathogens. O`Donnell (1992) found a surprising level of divergence for ITS sequences within the species of *F. sambucinum*. In our study we amplified the ITS primers and 18S rDNA gene. The amplified DNA was sequenced with sequencing primers to develop a genus/species specific PCR assay for the rapid identification of *Aspergillus* and *Alternaria* genuses isolated from floral vermicomposting wastes and also find out alignment score.

4. CONCLUSION

The nucleotide sequence analysis of rDNA region has been widely accepted to have phylogenetic significance, and is therefore useful in taxonomy and the study of phylogenetic relationships (Hibbett, 1992). This approach, designing primers from the rDNA region has far superior reliability compared to the use of random non-defined probes or primers. After using Clustal-W for multiple sequence alignment of fungal sequences we come to know that there were good correlations among all the four fungi because if alignment score is 40 or more then 40 then this score is called significant to each other and in our result the pair wise alignment score is more creditable. We got 7341 score in multiple sequence alignment which is a good result which means there is a characterization similarity between the species, it also shows good relationship among them and the evolutionary distance between them is also very less. Good MSA score also indicates that there are higher numbers of conserved regions which means less evolutionary distance between them and if number of conserved regions are very less than the evolutionary distance will increase.

ACKNOWLEDGEMENT

- The authors are very thankful to MP- CST, Bhopal (India) to provide initial grants for genetically identification of floral waste degrading fungi.
- We are also thankful to our principal Dr. Usha Shrivastava for giving her help and support.

REFERENCES

Clustal W and Clustal X version 2.1. (2007 Nov 01) Bioinformatics (Oxford, England) 23 (21):2947-8 PMID: 17846036

Analysis Tool Web Services from the EMBL-EBI**.** (2013 Jul) Nucleic acids research 41 (Web Server issue): W597-600 PMID: 23671338

BioAxis DNA Research Centre Private Limited**.** (Report Reference: 13/FNG/SEQ/RREL-08/902)(Samflople ID: DMB/08/13/902)

Bryan GT, Daniels MJ, Osbourn AE (1995). Comparison of fungi within the Gaeumannomyces-Phialophora complex by analysis of ribosomal DNA sequence. Appl. Environ. Microbiol. 61: 681-689.

O´Donnell K (1992). Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete Fusarium sambucinum (Gibberella pulicaris). Curr. Genet. 22: 213-220.

Nazar RN, Hu X, Schmidt J, Culham D, Robb J (1991). Potential use of PCR-amplified ribosomal intergenic sequences in the detection and differentiation of Verticillium wilt pathogens. Physiol. Mol. Plant Pathol. 39: 1-11.

Cenis JL (1992). Rapid extraction of fungal DNA for PCR amplification. Nucl. Acids Res. 20: 2380

Abd-Elsalam, K.A.; Aly,I.N.; Abdel-Satar, M.A.; Khalil, M.S. and Verreet, J.A.(2003). PCR identification of Fusarium genus based on nuclear ribosomal-DNA sequence data. African Journal of Biotechnology. 2 (4): pp. 82-85

Berbee, M. L. and Taylor, J. W. 1993. Dating the evolutionary radiations of the true fungi. Can. J. Bot. **71**: 1114-1127.

Gyllensten, U. B. 1989. Direct sequencing of enzymatically amplified DNA. pp. 45-60. In Erlich, H. A., ed. PCR technology.

Principles and Applications for DNA Amplification. Stockton Press, New York.

Hiraishi, A. 1992. Direct automated sequencing of 16S rDNA by polymerase chain reaction from bacterial cultures without DNA purification. Lett. Appl. Mcirobiol. **15**: 210-213.

Hirata. T. and Takamatsu, S. 1996. Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia

and cleistothecia of several powdery mildew fungi. Mycoscience **37**: 265-270.

Kim, G. Y., Ha, M. G., Lee, T. H. and Lee, J. D. 1999. Chemosystematics and molecular phylogeny of a new bioflocculantproducing Aspergillus stain lsolated from Korean soil. J. Microbiol. Biotechnol. **9**: 870-872.

Smith, L. M., Sanders, J. Z., Kaiser, R. J., Hughes, P., Dodd, C.,Conner, C. R., Heiner, C., Kent, S. B. H. and Hood, L. E.

1986. Fluorescence detection in automated DNA sequence analysis. Nature **321**: 674-679.

Sasamu T., Hirata, T. and Sato, Y. 1998. Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (Erysiphaceae), Mycoscience **39**: 441-453.

White, T., Burns, J., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 482. In Innis, M. A. D. H.

Gelfand, J. J. Sinsky, and T. J. White. (eds.), PCR protocols: a guide to methods and applications. Academic Press, San Diego, California.

Abdullah, S. K. and Taj-Aldeen, S. J.(1989). Extracellular enzymatic activity of aquatic and aero-aquatic conidial fungi. Hydrobiologia .174: 217-223.

Pope, S., H. Knaust, and K. Knaust, (1963). Production of compost by thermophilic fungi. Mushroom Sci. 5:123-126.

Suberkropp, K. and Klug, M. J. (1976). Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology .57:707-719.

Zemek, J.; Marvanova, L.; Kniak, L. and Kadlecikova,B. (1985). Hydrolytic enzymes in aquatic hyphomycetes. Folia Microbiol. 30:363-372.

AUTHOR'S BIOGRAPHY

Dr. Shobha Shouche, M.Sc. M.Phil., Ph.D. FASEA, FMANU, FVMS

Currently working as an Assistant Professor (Zoology), co-ordinator of Bioinformatics Govt. Madhav Science P.G. College, Ujjian (M.P.), having 30 year of teaching experience. She has received National and C.S.I.R fellowship. More than 25 research papers have been published in reputed national and international Journals. The field of research is Toxicology, Environmental Microbiology and Entomology. She has also attended the National and International conferences at Nepal, Bangkok, Mauritius and also received best paper presentation award in ICSTS(international conferences on Science and Technology for society) 2014 held at Bangkok. Recently she has completed her major Project Entitled "Comparative study of fungal DNA isolated from floral vermicomposting using DNA sequencing methods" Funded by MPCOST Bhopal.