STUDY OF INTERNAL TRANSCRIBED SPACER REGION-II AND FULL *ITS* REGION IN DIFFERENT NATURAL STRAINS OF *SORDARIA FIMICOLA*

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ABSTRACT: *The present study was directed for the sequence analysis of the internal transcribed spacer region two (ITS2) and full ITS region of ribosomal DNA gene cluster in six strains of Sordaria fimicola. These regions were targeted for the authentication of the strains under study. This study revealed that these regions are effective in differentiation of different strains of S. fimicola and its other reported species. With the use of universal primer sets ITS3/ITS4B and IT1F/ITS4, PCR products of 336bp and 619 bp of ITS 2 and full ITS was obtained in all eight strains. The DNA sequence analysis of ribosomal regions showed a conserved tendency on both ITS regions for the six strains of S. fimicola, and variations with other reported species of Sordaria due to insertion, deletion and base substitution.*

Keywords-component; Sequence analysis, ITS, region 2, *Sordaria fimicola*, strains, authentication

INTRODUCTION

The current revolution in molecular biology has provided techniques to identify various fungi. Molecular techniques investigate important variations in DNA [1]. The utilization of molecular markers for identifications and investigation of differences in sequence of DNA is a standout amongst the most significant progress in the field of molecular genetics. A molecular marker can be characterized in many ways: (a) chromosomal point of reference or allele that takes into consideration finding an exact portion of DNA; (b) a piece of DNA with a known position on the genome or (c) a gene whose phenotypic expression is for the most part recognized, used to observe an individual or a cell that carries it, or as a probe to label a chromosomes, or locus [2]. A careful consideration is required while selecting a marker for genetic analyses because different markers have their own standards, strategies and applications. As indicated by the sort of study to be embraced, an analyst needs to pick a mixed variety of molecular markers and procedures, to achieve the targets [3].

The "Evolution Canyon" is a small scale environment with two inverse slopes that have different climatic conditions, supporting the advancement in real life creating sign of in numerous groups of fungi [9].

sympatric speciation, and of particular impact and interest [4]. The contrasting slopes of "Evolution Canyon" shows extreme physical and biotic differentiation at smaller scale. More noteworthy solar radiations (up to 300% more) on south-facing slope (SFS) makes it more profound, drier, more diverse and unstable than in the North-facing slope (NFS), despite the fact that the slopes are isolated by just 100 meter at the bottommost and 400 meter at the highest [5].The station-1, 2 and 3 were situated on the (SFS) with extreme conditions, for example, high temperature and UV radiations. The station-5, 6 and 7 fit in with north-facing slope [6]. The most sequenced genetic marker for tending to research enquiries at and below the fungal genus level is the ITS region [7]. The Internal Transcribed Spacer (ITS) region of ribosomal DNA (rDNA) is mutable and widely used to differentiate different species of fungi by PCR. It is necessary to use many primer sets to oblige the range of fungi under study, potentially making artificial divisions for fungal genomes that increase with more than one primer set [8]. The (ITS) region has turned into the major genetic marker for characterization and different species-level interests

Table 1 List of ITS primers used to characterize *Sordaria fimicola.*

Primer	Sequence (5′-3′)	Targeted Region
ITS3	5′-GCATCGATGAAGAACGCAGC-3′	ITS2 Region [22]
ITS4 B	5′-CTTGGTCATTTAGAGGAAGTAA-3′	
ITS1F	5′-CTTGGTCAT TTAGAGGAAGTAA-3′	ITS1, 5.8S, ITS2 [22]
ITS4	5′-TCCTCCGCTTATTGATATGC-3′	

Table 2: List of reported strains of *Sordaria fimicola* and Species of *Sordaria* used with gene identification number

Serial No.	Strains and Species of *Sordaria*	Gene Identification no.
1	*Sordaria fimicola* strain 1-HL3-1	gi|953835229|
2	*Sordaria fimicola* UOA/HCPF	gi|807045376|
3	*Sordaria fimicola* Reference sp.	gi|298356928|
4	*Sordaria fimicola* strain WTS10	gi|294847834|
5	*Sordaria fimicola* strain CM1S-R2A	gi|559101932|
6	*Sordaria fimicola* strain xsd08002	gi|195979294|
7	Sordaria sp.	gi|355528469|
8	*Sordaria alcina*	gi|261362579|
9	*Sordaria humana*	gi|195979295|
10	*Sordaria lappae*	gi|56411515|
11	*Sordaria sp.*	gi|942678512|
12	*Sordaria macrospora*	gi|7547010|

13	*Sordaria sp.*	gi|748806819|
14	*Sordaria sp.*	gi|748806817|
15	*Sordaria sp.*	gi|346654872|
16	*Sordaria tomento-alba*	gi|56411539|
17	*Sordaria sp.*	gi|748806807|

Table 3: Aligned sequences of ITS2 region of rDNA from different strains of *S. fimicola*

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6        AGCTCTGCTTGCGTTGGGGATCCGCGGCTGCCCGCGGTCCCTCAAAAACAGTGGCGGGCT
4        AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
5        AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
3        AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
2        AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
S2       AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
S3       AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
S1       AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
N5       AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
N6       AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
N7       AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
1        AGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCT
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6        CGCTAGTCACACCGAGCGTAGTAACTTTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
4        CGCTAGTCACACCGAGCGTAGTAACTCTGCATCGCTATGGTCGTGCGGCGGGTTCTTGCC
5        CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
3        CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
2        CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
S2       CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
S3       CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
S1       CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
N5       CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
N6       CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
N7       CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
1        CGCTAGTCACACCGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCC
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15       TGCGTTGGGGATCCGCGGCTGCCCGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
16       TGCGTTGGGGATCCGCGGCTGCCCGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
11       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
14       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
17       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
13       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
12       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
10       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
8        TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
S2       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
S3       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
S1       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
N5       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
N6       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
7        TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
N7       TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
9        TGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCA
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* sign= similarity, gap = base substitution and – sign = deletion of base

Table 4: Aligned sequences of full ITS region of rDNA from different strains of *S. fimicola* and reported species of *Sordaria*

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humana            GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
macrospora        GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
tomento-alba      GATCATTACAGAGTTGCAAAACTCCCACAAACCATCGCGAATCTTACCCGTACGGTTGCC
superba           GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
S2                GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
N5                GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
S3                GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
S1                GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
N7                GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
N6                GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
alcina            GATCATTACAGAGTTGCAAAACTCCCACAAACCATCGCGAATCTTACCCGTACGGTTGCC
fimicola          GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
lappae            GATCATTACAGAGTTGCAAAACTCCAACAAACCATCGCGAATCTTACCCGTACGGTTGCC
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humana            TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGCCCCCCGGATCCTCGGGTCTCC-CCTCGCG
macrospora        TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGCCCCCCGGATCCTCGGGTCTCCCGCTCGCG
tomento-alba      TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGACCCTCGGGTCTCCCGCTCGCG
superba           TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGCCCCCCGGATCCTCGGGTCTCCCGCTCGCG
S2                TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
N5                TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
S3                TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
S1                TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
N7                TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
N6                TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
alcina            TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
fimicola          TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
lappae            TCGGCGCTGGCGGTCCGGAAGGCCCTCGGGC-CCCCGGATCCTCGGGTCTCCCGCTCGCG
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humana            GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
macrospora        GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
tomento-alba      GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
superba           GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
S2                GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
N5                GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
S3                GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
S1                GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
N7                GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
N6                GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
alcina            GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTTTCTCTGAGTAA
fimicola          GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
lappae            GGAGGCTGCCCGCCGGAGTGCCGAAACCAAACTCTTGATATTTTATGTCTCTCTGAGTAA
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humana            TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
macrospora        TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
tomento-alba      TCAAGCTCTGCTTGCGTTGGGGATCCGCGGCTGCCCGCGGTCCCTCAAAAACAGTGGCGG
superba           TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
S2                TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
N5                TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
S3                TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
S1                TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
N7                TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
N6                TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
alcina            TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
fimicola          TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
lappae            TCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGA-CGCGGTCCCTCAAAAACAGTGGCGG
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* Sign= similarity, gap = base substitution and – sign = deletion of base

These spacer regions on ribosomal gene are fragments of non-functional RNA surrounding the structural ribosomal RNAs (rRNA) on transcript. Read from 5' to 3', encompasses the 5' external transcribed sequence (5' ETS), 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA and ultimately the 3' ETS [10].

Objectives

- To amplify the full internal transcribed spacer region and region-2 (ITS2) of six (S1, S2, S3, N5, N6 and N7) strains of *S. fimicola* by PCR.
- To detect whether the ITS sequences vary or conserved among the strains of same species situated on two contrasting slopes of Evolution Canyon and among different reported species of *Sordaria*.
- To generate phylogenetic tree based upon the rDNA sequences.

MATERIALS AND METHODS

Experimental Organism

Six strains of *S. fimicola* were used during this study. Stock cultures of these parental strains were originally isolated by Nevo's colleague from soil by dilution plating method from dung [11]. These stock cultures were sub cultured under sterile conditions and were maintained on solidified PDA media.

DNA Isolation and Quantification

DNA of all the six strains was extracted using an adaptation of Gardes and Bruns [12] method with slight modifications (without phenol). Isolated DNA was quantified on Agarose gel running 1Kb plus ladder DNA (Invitrogen).

ITS-PCR Analysis and Amplification

To amplify genomic DNA regions, DNA of all the strains were subjected to ITS-PCR on Thermo cycler (Peltier-Effect cycling PTC-06). Universal oligonucleotides primers were used in lyophilized forms which were stored at -20ºC. During PCR, conditions were optimized by adjusting different temperature and $MgCl_2$ concentrations. The standard reagents supplied by BIOLINE were used. PCR was carried out with 1µl of 100µM of each primer and 2µl of template DNA. An initial denaturation at 94°C was followed by 40 cycles of 1 min at 94°C, 1 min at annealing temperature for ITS3 (54.8), ITS4B (50.8) ITS1F (49.5) and ITS4 (50.2) respectively) and 1 min at 72°C. A final step of 5 min at 72°C for final elongation was done to complete the PCR amplifications of the targeted regions. The amplified products were then visualized by gel electrophoresis.

DNA Sequencing and Data Analysis

Six parental strains amplicons; amplified by the primer set 1 and 2 (Table 1) were sent for direct sequencing to Macrogen, Korea. Raw data was subjected to Sequencher 4.1 GeneCode software for sequence analysis and peak corrections; aligned by the Clustal Omega to generate the phylogenetic tree based upon UPGMA.

RESULTS AND DISCUSSION

The study have been detected the conserved and varied behavior of ITS region of rDNA among related strain of *S. fimicola* and other reported species by the use of ITS-PCR analysis. ITS primer pair ITS3/ITS4B amplified the same size 336bp product (ITS2) in all the strains while the length of full ITS region was found to be 619 bp amplified by primer pair ITS1F and ITS4 in all strains (Figure 1). Both of the sequences were compared for their homology with already published and available data of other species and strains of *Sordaria* given in GenBank of NCBI. Table 2 indicates the species and strains used for comparison and differences in the nucleotides in rDNA of ITS region are given in Table 3 and 4.

As the ITS region is located within conserved gene region, so can be widely used for recognizing closely related species and populations. It is very easy to amplify the ITS regions by PCR because there are multiple copies of these genes per nucleus [13]. Schocha et al [14] selected ITS region as the universal genetic barcode for fungi. In the sequence analysis of ITS region of understudy six strains of *S. fimicola* no nucleotide differences have been found. These findings are supported by the research work done by Estrada et al [15]. Similar results were found by Razaq et al [16] who amplified target region of rDNA (ITS1 5.8S ITS2) of *Tricholomopsis* sp. by using same universal fungus primers (as are used in the current study) showed 100% matching with *Tricholomopsis flammula* (Reported in GenBank) upon its alignment by performing BLAST.

Similarly Blaalid *et al* [17] studied ITS1 and ITS2 regions of known taxonomic affiliations and a third including ITS1 and ITS2 amplicon pyro sequencing information. Cluster analyses uncovered that a 97% comparability for both ITS1 and ITS2. ITS1 and ITS2 generally collect comparable results when utilized as DNA standardized identifications for organisms. Pandey *et al* [18] did not recognize any variety or length polymorphism when they examined different isolates of a foliar endophytic species of *Phyllosticta*, proposing that they all have a place with the same species, demonstrating that (ITS) region is profoundly conserved within the isolate of same species as portrayed in the present study.

Variability in the genetic sequence in an otherwise conserved region is a tool to be used in making related taxa identifiable. Among these detected variations are base substitution, SNPs, insertions or deletions and point mutation. In the current study certain variations were detected in nucleotide sequences among different reported strains of *S. fimicola* i.e. in strain xdos8002 (6) T is substituted by G at position 27, at position 31 A is substituted by C, at position 32 there is insertion of C, at position 87 C is substituted by T, and in strain WTS10 (4) G is substituted by A at position 89 (Table 3). As shown in Fig. 2A both these strains are separated into two different clades. According to Fig. 2B reported species and strains under discussion are separated into 5 major clusters on the basis of ITS sequences of rDNA. In first main group only two species 15 and 16 are present (*Sordaria* sp. P1OER and *S. tomento-alba*), while in cluster II there is only one sp. of *Sordaria* i.e. UASWS112 (14) and six

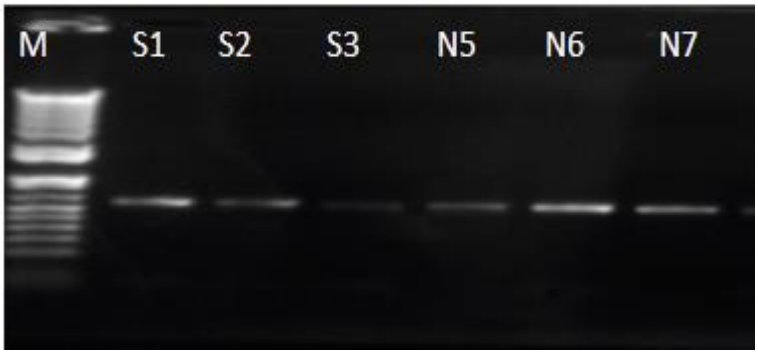

Figure 1: PCR amplification of Full ITS region in six strains of *S. fimicola by ITS1F and ITS4 primers*

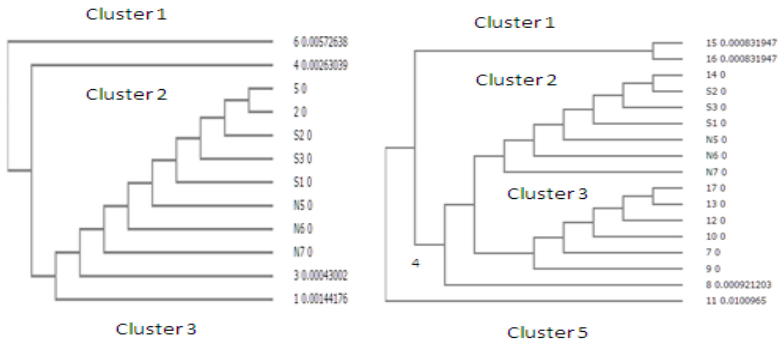

Figure 2: Phylogenetic tree of ITS-2 constructed by Clustal W2 of different strains of *S. fimicola* and species

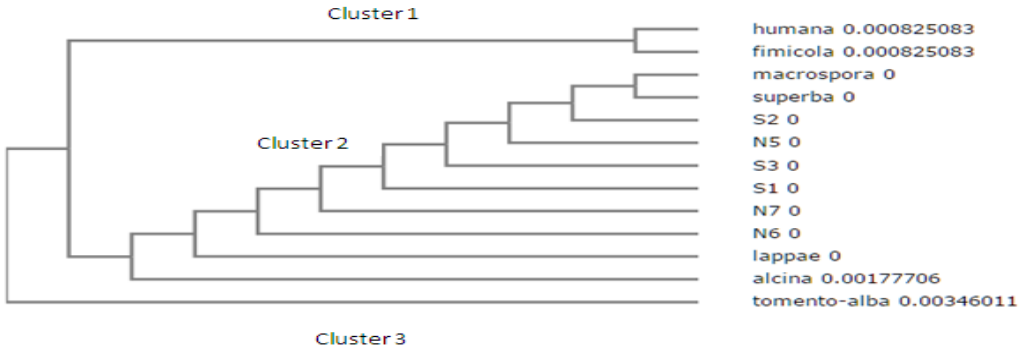

Figure 3: Phylogenetic tree of full ITS region constructed by Clustal W2 of different strains of *S. fimicola* and species of *Sordaria*

strains (S1, S2, S3, N5, N6 and N7). There are six species in cluster III while sp. 8 (*Sordaria* OT3-207) and 11 (*S. alcina*) separated into two different clusters (Cluster IV and V). At species level (Table 2) in species 15 and 16 there is a base substitution of G into T at position 18, at position 22 (A to C) and insertion of "C" at position 23. Similarly Fritz et al [19] amplified 363-369 bp in ITS2 region for population of the malaria vector *Anopheles nuneztovari*, their data demonstrate that types of *Anopheles nuneztovari* are recognized by exceptionally slight variations in DNA sequence of the ITS2 region. Hao et al [20] analyzed ITS region of *Angelica* from diverse living spaces to figure out whether there are variations in sequences. Angelicas from three living spaces were utilized as a part of this study, including Taiwan, Sichuan Province (China), and Gansu Province (China). The outcomes demonstrated that the similarity of ITS-1 and ITS-2 rDNA sequences in Angelicas produced in Gansu and Sichuan Provinces is up to 100%, and that created in Taiwan and Sichuan Province is 88% and 87%, individually. Their sequencing analyses recognize the same strains as there is 100% similarity in Angelicas produced in Gansu and Sichuan Provinces.

The phylogenetic UPGMA trees were constructed with online software ClustalW2 based on Complete ITS region including (ITS1, 5.8S and ITS2) and ITS region 2 of six strains of *S. fimicola* isolated from different environments and reported sequences of related species from Gene bank The UPGMA tree including sequenced ITS region of 12 different reported strains and data was clustered into three major clades. S1, S2, S3, N5, N7 and N6 were clustered in clade-III and were close to each other, while reference sequences were assorted in clade-I and II (Fig. 2A). Phylogenetic tree of ITS regions (ITS1, 5.8S and ITS2) of different species and strains (Fig. 3) shows that there are three major divisions. *S. tomento-alba* is

very different from other species, while rest of the species divided into three groups. In group one there are two species (*S. humana*, *S. fimicola*). In second group there are three species (*S. macrospora*, *S. superba* and *S. lappae*) and strains under study also present in this group. *S. alcina* get separated from group 2.

In our current investigation the highly conserved nature of (ITS-2 and ITS) region in six parental strains of *S. fimicola* indicates that highly conserved sequences are present between all strains of the opposite slopes. The conserved sequences can occur within nucleic acid sequences, protein structure or polymorphic carbohydrate across species or within different molecules produced by the same organism. The conserved nature of ITS-2 and ITS region in our strains implies that conserved genes of ITS regions are present. It demonstrates that ITS regions may have been kept up by evolution regardless of speciation. As the succession data is for the most part exchanged from parents to descendants by genes so we likewise discovered no polymorphism or arrangement variety inside of strains. As indicated by different investigators if transformation occur in "conserved regions" then there is a non-feasible life form, this life form is rejected through natural selection (Bejerano et al [21]. Sequence homology of ITS regions in different strains of *S. fimicola* can also be taken as structural and functional conservation; it also indicates evolutionary relationship between the sequences of different species belonged to same class or family. Highly conserved sequences are also present in active sites of enzymes and in the binding sites of proteins. In short it is concluded from the above studies that ITS markers are located within highly conserved region, can be used to differentiate, for authentication and to characterize closely related species, taxa and populations.

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