

Original Research

Isolation and Molecular Methods for the Identification of *Fusarium solani* from Solid Waste

Chetan DM^{1*}, Nataraja S², Krishnappa M³, Bharath BR⁴, Ravikumar Patil HS¹ and Raghavendra HL⁵

¹ Department of Biotechnology NMAM Institute of Technology- Nitte, Karnataka, India

² Department of Botany Sahyadri Science College, Kuvempu University-Shimoga, Karnataka, India

³ Department of studies and research in Applied Botany, Kuvempu University, Shankaraghatta, Karnataka, India

⁴ Department of Biotechnology and Bioinformatics, Jnanasahyadri, Kuvempu University, Shankaraghatta, Karnataka, India

⁵ College of Health and Medical Sciences, Wollega University, Post Box No: 395, Nekemte, Ethiopia

Abstract	Article Information
A major goal in microbial ecology is to link specific microbial populations to environmental processes. Solid waste degradation is an imperative aspect of environmental processes mediated by microorganisms individually or symbiotically. In the current study an attempt has been made to isolate and identify a microorganism	Article History: Received : 08-01-2013 Revised : 24-03-2013 Accepted : 25-03-2013
which can degrade the hazardous xenobiotic compound propaxur commonly known as baygon from solid waste by both <i>in vitro</i> and <i>in silico</i> approaches. 28s rDNA gene has indicated the organism as <i>Fusarium solani</i> . Further studies were extended through bioinformatics approaches like BLAST (Basic Local Alignment Search Tool) and MSA (Multiple Sequence Analysis) to determine the relative phylogeny of this organism. This	Keywords: 28s rDNA BLAST MSA Propoxur
approach has shed light on evolutionary relationship among the organisms taken in the study. By this approach we have identified the <i>Fusarium solani</i> .	*Corresponding Author: Chetan DM E-mail: chetanmurthy@gmail.com

INTRODUCTION

"Solid waste" means any garbage, refuse, sludge from a waste treatment plant, water supply treatment plant, or air pollution control facility and other discarded material, including solid, liquid, semisolid, or contained gaseous material resulting from industrial, commercial, mining, and agricultural operations, and from community activities. Total quantity of waste generated in the country (based on weighment exercise by local bodies) is not reported. However, in the year 2000 Ministry of Urban Development in its manual on solid waste management has estimated waste generation of 100,000 MT annually. Central Pollution Control Board (CPCB) with the assistance of National Environmental Engineering Research Institute (NEERI) has conducted survey of solid waste management in 59 cities (35 metro cities and 24 state capitals 2004-05). Quantities and waste generation rates in 59 cities are as under: In India, the amount of waste per capita generated is estimated to increase at a rate of 1-1.33% annually (Shekdar, 1999).

Organic waste is a major component of Municipal Solid Waste (MSW), MSW compost contains a significant amount of humic substances. Organic waste is produced wherever and whenever there is human habitation. The main forms of organic waste are household food

waste, agricultural waste, human and animal waste. In industrialized countries the amount of organic waste produced is increasing dramatically every year. Although many gardening enthusiasts compost some of their kitchen and garden waste, much of the household waste goes into landfill sites and is often the most hazardous waste. The organic waste component of landfill is broken down by micro-organisms to form a liquid 'leachate' which contains bacteria, rotting matter and maybe chemical contaminants from the landfill.

Micro organisms that dwell in solid wastes are grouped under Solid Waste Microflora (SWM). The most common organisms that are generally found in solid waste are bacteria and fungi. These microorganisms use the components of the waste as the substrate for their growth. They grow and multiply on these wastes by utilizing the various components that make up the solid waste. Further a wide variety of pathogenic microorganisms have been reported to be present in these organic wastes (Amalraj *et al.*, 2006).

MATERIALS AND METHODS

Study Area

Udupi (Kannada) is a temple town located in Udupi District near Mangalore, Karnataka state, India. As of the 2001 India census Udupi had a population of 1,13,039. The weather is fairly similar throughout the year, due to the nearby Arabian Sea. Temperature ranges from 30 to 35°C in day time and falls by 10 degrees during night and humidity is normally high round the clock with rainy season spanning between April to September.

Collection of Waste Sample

The Domestic waste about 400gms was collected in a clean plastic and air tight container of 500gm capacity. The waste was collected from different residential areas once in a week weekly. Many specific kinds of microorganisms can be allowed to grow from organic wastes by providing the specific optimum environmental conditions which may likely enhance the growth of desired microbes over undesired micro-organisms. Characteristics of the organisms which give them special advantages over other organisms are exploited in the formulation of culture media and the choice of incubation conditions. The collected materials are plated on plastic petri plate as (Standard Blotter Method) SBM and incubated at 28±2 °C. Observations are done every day under Stereobinocular microscope. The organisms were identified by using Barnett manual (Subramanian, 1983).

Sci. technol. arts Res. J., Jan-Mar 2013, 2(1): 42-49

Isolation of Fungi

A fungus was isolated from waste sample using pour plate method. This method is useful for quantifying micro organisms that grow on solid Potato Dextrose Agar (PDA) medium.

Maintenance of Pure Culture

Isolated fungus was sub cultured on plates and agar slants at regular interval of time to maintain viability and was successfully stored in refrigerators at 4 °C for 3-4 months until further studies.

Molecular Methods

The ~5kb rDNA fragment was amplified from isolated genomic DNA using high –fidelity PCR polymerase. The PCR product was sequenced bi-directionally using the forward and reverse primers. The sequence data was aligned and analyzed to identify the whether yeast or fusarium solani and members of its family and distant organisms.

Phylogentic tree builder uses sequences aligned with System Software aligner. A distance matrix is generated using the Jukes-Cantor corrected distance model. When generating the distance matrix alignment model positions are used ignoring the alignment inserts and the minimum comparable position is 200. The tree is created using Weighbor with alphabet size 4 and length size 1000.

Weighbor Tree: Weighbor is a weighted version of Neighbor Joining, that gives significantly less weight to the longer distances in the distance matrix. The weights are based on variances and covariances expected in a simple Jukes-Cantor model.

The phylogenetic tree was validated using Bootstrap method, this statistical method used for estimating the sampling distribution by resampling with replacement from the original sample. In making phylogenetic trees, the approach is to create a pseudo alignment by taking random positions of the original alignment. Some columns of the alignment could be selected more than once or not selected at all. The pseudo-alignment will be as long as the original alignment and will be used to create a distance matrix and a tree. The process is repeated 100 times and a majority of consensus tree is displayed showing the number (or percentage) of times a particular group was on each side of a branch without concerning the sub grouping.

In silico Methods

To substantiate the in vitro methods for the identification of unknown organism in silico sequence analysis was carried out. For sequence analysis the orthologs sequences for unknown query sequence were retrieved by performing Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against nonredundant databases. BLAST finds the regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

Sci. technol. arts Res. J., Jan-Mar 2013, 2(1): 42-49

The most similar ortholog sequences were retrieved in FASTA format as an input for MSA. ClustalW2 is a general purpose multiple sequence alignment program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen. Evolutionary relationships can be seen via viewing Cladograms or Phylograms, ClustalW2 was employed for MSA and phylogenetic analysis by setting the gap opening and gap extension parameters as 1 and 0.5 respectively. The tool Tree view V1.6.6 was used to visualise the tree given by ClustalW2 in .ph format.

RESULTS

Seq 1. The unknown sequence in FASTA format: (2956 bp)

>Unknown

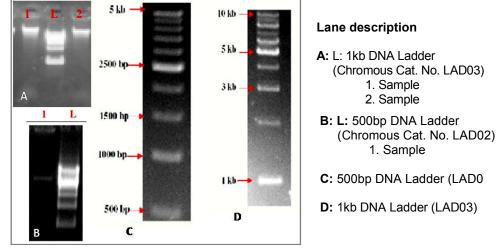
AGGGAGAAAGAACCAACAGGGATTGCCCTAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCTGGCTCTCGGGC CCGAGTTGTAATTTGTAGAGGATACTTTTGATGCGGTGCCTTCCGAGTTCCCTGGAACGGGACGCCATAGAGGGTGAGAGCCC CGTCTGGTTGGATGCCAAATCTCTGTAAAGTTCCTTCAACGAGTCGAGTAGTTTGGGAATGCTGCTCTAAATGGGAGGTATATG AAAAAGTACGTGAAATTGTTGAAAAGGGAAGCGTTTATGACCAGACTTGGGCTTGGTTAATCATCTGGGGTTCTCCCCCAGTGCAC TTTTCCAGTCCAGGCCAGCATCAGTTTTCCCCCGGGGGGATAAAGGCGGCGGGAATGTGGCTCTCTTCGGGGGAGTGTTATAGCC CACCGTGTAATACCCTGGGGGGGGGGCTGAGGTTCGCGCATCTGCAAGGATGCTGGCGTAATGGTCATCAACGACCCGTCTTGA AACACGGACCAAGGAGTCGTCTTCGTATGCGAGTGTTCGGGTGTCAAACCCCTACGCGTAATGAAAGTGAACGCAGGTGAGA TGGGCATGGGGGCGAAAGACTAATCGAACCTTCTAGTAGCTGGTTTCCGCCGAAGTTTCCCTCAGGATAGCAGTGTTGAACTC AGTTTTATGAGGTAAAGCGAATGATTAGGGACTCGGGGGCGCTATTTAGCCTTCATCCATTCTCAAACTTTAAATATGTAAGAAG CTCTTGTTGCTTAATTGAACGTGAGCATTCGAATGTATCAACACTAGTGGGCCATTTTTGGTAAGCAGAACTGGCGATGCGGGA TGAACCGAACGCGAGGTTAAGGTGCCAGAGTAGACGCTCATCAGACACCACAAAAGGTGTTAGTACATCTTGACAGCAGGACG AAGCGTCTCACCCATACCTCGCCCTCAGGGTAGAAACGATGCCCTGAGGAGTAGGCGGACGTGGAGGTCAGTGACGAAGCCT AGGGCGGAGCCCCGGTTGAACGGCCTCTAGTGCAGATCTTGGTGGTAGTAGCAAATACTTCAATGAGAACTTGAAGGACCGAA GTGGGGAAAGGTTCCATGTGAACAGCGGTTGGACATGGGTTAGTCGATCCTAAGCCATAGGGAAGTTCCGTTTCAAAGGCGCA CTATGCGCCGTCTGGCGAAAGGGGAGCCGGTCAATATTCCGGCACCTGGATGTGGGTTTTGCGCGGCAACGCAACTGAACGC GGTTTAATGGCTGGAAGAGCCCAGCACCTCTGCTGGGTCCGGTGCGCTCTCGACGTCCCTTGAAAATCCGCGGGAAGAAATA ATTCTCACGCCAGGTCGTACTCATAACCGCAGCAGGTCTCCCAAGGTGAACAGCCTCTGGTTGATAGAACAATGTAGATAAGGG AAGTCGGCAAAATAGATCCGTAACTTCGGGATAAGGATTGGCTCTAAGGGTTGGGCACGCAGGGCCTTGGGCGGACGCCATG GGGGCAGGCTGCTTCTAGCCGGGCAACCGGCCGGCGGCGGCCAGCACCCGTGCGCTGATGCCCTTGGCAGGCTTCGGCCG TCCGGCGTGCGGTTAACAACCAACTTAGAACTGGTACGGACAAGGGGAATCTGACTGTCTAATTAAAACATAGCATTGCGATG GCCAGAAAGTGGTGTTGACGCAATGTGATTTCTGCCCAGTGCTCTGAATGTCAAAGTGAAGTAATTCAACCAAGCGCGGGTAA ACGGCGGGAGTAACTATGACTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCATGAATGGATTAACGAGAT TCCCACTGTCCCTATCTACTATCTAGCGAAACCACAGCCAAGGGAACGGGCTTGGCAGAATCAGCGGGGAAAGAAGACCCTGT TGAGCTTGACTCTAGTTTGACATTGTGAAAAGACATAGGAGGTGTAGAATAGGTGGGAGCTTCGGCGCGGTGAAATACCACTA CTCCTATTGTTTTTTACTTATTCAATGAAGCGGGGCTGGATTTACGTCCAACTTCTGGTTTTAAGGTCGTTCGCGGGCCCGAGC CGGGTAGAAGACATTGTCAGGTGGGGAAGTTTGGCTGGGGCGGCACATCTGTTAAACCATAAGCAGGTGTCCTAAGGGGGGG CTCATGGAGAACAGAAATCTCCAGTAGAACAAAAGGGTAAAAGTCCCCTTGATTTTGATTTTCAGTGTGAATACAAACCATGAAA GTGTGGCCTATCGATCCTTTAGTCCCTAGACATTTGAGGCTAGAGGTGCCAGAAAAGTTACCACAGGGATAACTGGCTTGTGG CGGCCAAGCTTCATAGCGACGTCGCTTTTTGATCCTTCGATGTCGGCTATTCCTATCATACCGAAGCAGAATTCGGTAAGCGTT GGATTGTTCACCCACTAATAGGGAACGTGAGCTGGGTTTAGACCGTCGTGAGACAGGTTAGTTTTACCGTACTGATGACCTCA CCGCAATGGTAATTGAGCTTAGTTAAAAGGGCAATCGCTCTATTCTAAGAAT

Isolation and Identification

Microscopical observations of collected waste material, the organisms were identified according to the Barnett manual. The observation revealed that the growth of fungus on waste was comparatively more than other organisms. The isolation of the waste decomposing fungus was quantified from the solid medium. The isolated fungus was sub-cultured on PDA plates and slants.

It is difficult to identify an organism only by morphological studies, thus genomic DNA was isolated from the organism in pure culture using fungal Genomic DNA Isolation Kit (RKT13) and was subjected for electrophoresis with reference to 1kb DNA Ladder (Chromous Cat. No. LAD03) Sci. technol. arts Res. J., Jan-Mar 2013, 2(1): 42-49

(Figure 1A). The isolated DNA was used as a template for the PCR amplification of ~ 5kb rDNA fragment using high --fidelity PCR polymerase. The reaction mixture used for the PCR is shown in Table 1. The PCR product was sequenced bidirectionally using the forward and reverse primers as per the profile shown in Table 2. The polymerised sample was subjected for electrophoresis by taking 500bp DNA Ladder (Chromous Cat. No. LAD02) as reference (Figure 1B). The reference ladders for DNA isolation 1kb DNA Ladder and PCR amplification 500bp DNA Ladder are shown in Figure 1C and Figure 1D respectively. The sequence data was aligned and analyzed to identify the Yeast and its closest neighbours.



- Figure 1: Gel images A: Extraction of Genomic DNA from fungal sample using the Fungal Genomic DNA Isolation Kit (RKT13). B: PCR amplification of rDNA fragment from fungal sample. The size of PCR amplified product is ~500 bp. C: 500 bp ladder contains 10 DNA fragments of size 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 bp D: 1 kb ladder contains 10 DNA fragments of size1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 kb.
- Table 1: Sequences selected for MSA: Information of gene sequences taken for MSA, information contains the organism name from which the sequences were retrieved and the length of the sequences.

Sequence format	Pearson						
Sequence number	Organism	Length					
Sequence 1	Hypocrea-jecorina	5556 bp					
Sequence 2	Metrahizium-anisopliae	8118 bp					
Sequence 3	Tetracladium-marchalianum	5218 bp					
Sequence 4	Tetracladium-breve	5236 bp					
Sequence 5	Tetracladium-palmatum	5219 bp					
Sequence 6	Neurospora-crassa	8847 bp					
Sequence 7	Verticillium-dahliae	7216 bp					
Sequence 8	Magnaporthe-grisea	8412 bp					
Sequence 9	Fusarium-solani	3830 bp					
Sequence 10	Unknown	1349 bp					

NCBI Accession No.	Organism Name	Score
FJ345352	Fusarium solani	97
AF510497	Hypocrea jecorina	95
AF218207	Metarhizium anisopliae	95
FJ360521	Neurospora crassa	93
DQ493955	Magnaporthe grisea	92
AF104926	Verticillium dahliae	90
EU883418	Tetracladium breve	89
EU883423	Tetracladium marchalianum	89
EU883431	Tetracladium breve	89
EU883424	Tetracladium palmatum	89

 Table 2: Distance matrix with Sample sequence taken as reference sequence.

Score: Sequence Match Score obtained based on nucleotide alignment.

Identification by In silico Methods

In order to verify and to substantiate the identification of an unknown solid waste degrading organism as *Fusarium solani* the most powerful insilico tool i.e phylgogenetic analysis has been employed. Here the sequence obtained from the sequencing was taken as a query sequence. The orthologs sequences for query sequence were retrieved by performing BLAST against non-redundant databases. Phylogram was obtained by performing MSA of all the ten sequences with query sequence shown in Table 3 using the tool ClustalW. The pylogram obtained

by performing MAS is shown in Figure 2. Distance between all the orthologs with sample unknown sequence taken as reference sequence is given in Table 4. Later the alignment gave us information about the conservation level of unknown gene through genes from different organisms. The unknown sequence has shown very good conservation with all the 28s rDNA sequences from different organisms taken for the study as shown in Figure 3. The phylogenetic tree was validated by bootstraping; this analysis supported the identification of an unknown organism as *Fusarium solani*.

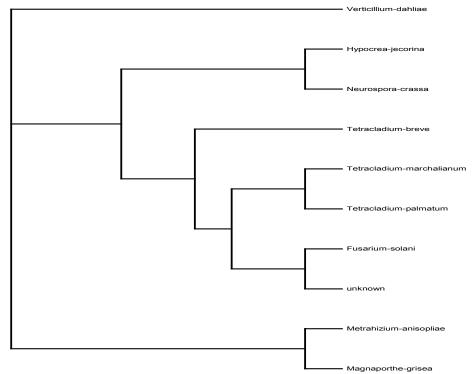


Figure 2: Phylogram obtained from ClustalW2 after performing MSA. Unknown and Fusarium solani were observed in a single clade.

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknow Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grises Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-grassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknow Clustal Consensus

Hypocrea-jecorina Metrahizium-anisoplia Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisoplia Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Sci. technol. arts Res. J., Jan-Mar 2013, 2(1): 42-49

4410 4420 4430 4440 4450 4460 4470 4480 4490 4500 ACCTCGAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGC ACCTCGGATTAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGC ACCTCGGATTAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGC ACCTCGGATTAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCTCAGTAACGGCGAGTGAAGCGGC ACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCTAGTAACGGCGAGTGAAGCGGC ACCTCGGATCAGGAATACCCCCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCTTAGTAACGGCAGGTGAAGCGGC ACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGC ACCTCGAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGC ~AG-GGAG--AAAGAA-CCAACAGGGATTGCCCTAGTAACGGCGAGTGAAGCGGC

	4510	4520	4530	4540	4550	4560	4570	4580	4590	4600
			
AACAGC	TCAAATTTG/	AATCTGGCC	CTTTC-GGGTC	CGAGTTGTA	ATTTGTAGAGG	ATGCTTTTG	-G-CAAGGCG	CCG-C-CC-G	AGTTCCCTGGA	ACGG
AACAGC	TCAAATTTG/	AATCTGGTC	CCCAGGGCC	CGAGTTGTA	ATTTGCAGAGG	ATGCTTTTG	-G-TGAGGTG	CCT-TCCG	AGTTCCCTGGA	ACGG
AAAAGC	TCAAATTTG/	AATCTGGCT	TTTTAGGGT	CGAGTTGTA	ATTTGTAGAAG	ATG-TTTCG	GGTGTGGCT	CCGGT-TT-A	AGTTCTTTGGA	ATAT
AAAAGC	TCAAATTTG/	AATCTGGCT	CTTTTAGGGTC	CGAGTTGTA	ATTTGTAGAAG	ATG-TTTCG	GGTGTGGCT	CCGGT-TT-A	AGTTCTTTGGA	ATAT
AAAAGC	TCAAATTTG/	AATCTGGCT	TTTTAGGGTC	CGAGTTGTA	ATTTGTAGAAG	ATG-TTTCG	GGTGTGGCT	CCGGT-TT-A	AGTTCTTTGGA	ATAT
AACAGC	TCAAATTTG/	AATCTGGCT	TCGGCC	CGAGTTGTA	ATTTGTAGAGG	AAGCTTTTG	G-TGAGGCAG	CT-TCTG	AGTCCCCTGGA	ACGG
AACAGC	TCAAATTTG/	AATCTGGCT	CCTTCGGGGGTC	CGAGTTGTA	ATTTGTAGAGG	ATG-CTTCG	AGTTATGGTT	CCT-TCCG	AGTTCCCTGGA	ACGG
AACAGC	TCAAATTTG/	AATCTGGCC	CCC-CGGCC	CGAGTTGTA	ATTTGCAGAGG	ATGCTTTTG	-G-TGAGGCAG	CCTACC-G	AGTCCCCTGGA	ATGG
AACAGC	TCAAATTTG/	AATCTGGCT	CTC-GGGCC	CGAGTTGTA	ATTTGTAGAGG	ATGCTTTTG	-G-TGAGGTG	CCT-TCCG	AGTTCCCTGGA	ACGG
AACAGC	TCAAATTTG/	AATCTGGCT	TC-GGGCC	CGAGTTGTA	ATTTGTAGAGG	ATACTTTTG	A-TGCGGTG	CCT-TCCG	AGTTCCCTGGA	ACGG
** ***	*******	******	** *	******	**** *** *	* ** *	** *	** :	*** * ****	**
	4610	4620	4630	4640	4650	4660	4670	4680	4690	4700

	1010	1020	1050	1010	1050	1000	10/0	1000	1050	1/00
								[]		
GACGCC	ACAGAGGG	TGAGAGCCC	CGTCTG-G-CT	GG-CCGCCG-	-A-GCCTC	TGTAAAGCTCC	TTCGACGAGT	GAGTAGTTTG	GGAATGCTGCTC	AAA
GACGCC	ATAGAGGG	TGAGAGCCC	CGTCTG-G-TT	GGAT ACCGA	~~~GCCTC-	TGTAAAGCTCC	TTCGACGAGT	GAGTAGTTTG	GGAATGCTGCTC	TAA
TACATO	ATAGAGGG	TGAGAATCC	CGTATGTGACC	GG-CAGCCT-	TCGCCTA	TGTGAAACTCT	TTCGACGAGT	GAGTTGTTTG	GGAATGCAGCT	AAA
TACATO	ATAGAGGG	TGAGAATCC	CGTATGTGACC	GG-CAGCCT-	TCGCCTA	TGTGAAACTCT	TTCGACGAGT	GAGTTGTTTC	GGAATGCAGCT	CAAA
TACATO	ATAGAGGG	TGAGAATCC	CGTATGTGACC	GG-CAGCCT-	TCGCCTA	TGTGAAACTCT	TTCGACGAGT	GAGTTGTTTC	GGAATGCAGCT	CAAA
GGCGCC	ATAGAGGG	TGAGAGCCC	CGTATA-G-TC	GGAT GCCGA	TCCAA	TGTAAAGCTCC	TTCGACGAGT	GAGTAGTTTC	GGAATGCTGCTC	AAA
GACGCC	ATAGAGGG	TGAGAGCCC	CGTCTG-G-TA	GA-AACCA-	-T-GC-TC/	ATGTGAAGCTCC	CTCGACGAGT	GAGTAGTTTC	GGAATGCTGCTC	TAA
GGCGCC	ATAGAGGG	TGAGAGCCC	CGTATG-G-TA	GAC-GCCGA	~~~ACCTC-	TGTAAAGCTCC	TTCGACGAGTO	GAGTAGTTC	GGAATGCTGCTC	TAA
GACGCC	ATAGAGGG	TGAGAGCCC	CGTCTG-G-TT	CAC-ACCA	T-CCTC	TGTAAAGCTTC	TTCGACGAGTO	GAGTAGTTC	GGAATGCTGCTC	TAA
									GGAATGCTGCTC	
		*****		** **	AL 0 10	*** ** *			********	

4710	4720	4730	4740	4750	4760	4770	4780	4790	4800
	1			.					I
ATGGGAGGTATATG	TCTTCTAAAGCT	AAATATTGGC	CAGAGACCGA	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGCACCTI	GAAAAGAGGG	TAA
ATGGGAGGTATATG	TCTTCTAAAGCT	AAATATTGGC	CAGAGACCGA	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGCACTTT	GAAAAGAGGG	TAA
ATGGGAGGTATATT	TCTTCTAAAGCT	AAATATTGGC	CAGAGACCGA	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGCACTTT	GGAAAGAGAG	TAA
ATGGGAGGTATATT	TCTTCTAAAGCT	AAATATTGGC	CAGAGACCGA	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGCACTTT	GGAAAGAGAG	TAA
ATGGGAGGTATATT	TCTTCTAAAGCT	AAATATTGGC	CAGAGACCG	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGCACTTI	GGAAAGAGAG	TAA
ATGGGAGGTAAATT	TCTTCTAAAGCT	AAATACCGGC	CAGAGACCGA	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGCACTTT	GAAAAGAGGG	TAA
ATGGGAGGTATACT	CCTTCCAAGGCT	AAATACCGGT	TAGAGACCGA	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGCACTTT	GAAAAGAGAG	CAA
ATGGGAGGTAAATT	TCTTCTAAAGCT	AAATACCGGC	CAGAGACCGA	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGCACTTI	GAAAAGAGGGT	TAA
ATGGGAGGTATATG	TCTTCTAAAGCT	AAATACCGGC	CAGAGACCG	TAGCGCACAA	TAGAGTGAT	GAAAGATGAA	AAGAACTTI	GAAAAGAGAG	TAA
ATGGGAGGTATATG									
********	**** ** ***	***** **	*******	*******	********	********	*** ** **	* ***** **	**
4810	4820	4830	4840	4850	4860	4870	4880	4890	4900
ATAGTACGTGAAAT									
ATAGTACGTGAAAT									
ACAGTACGTGAAAT	TGTTGAAAGGGA	AGCGTTTGCA	ACCAGACTTG	CACGCAG-TT-	GATCATCCG	G-TGTTC-T-	-CACCG-GG	GCACTCTGC	T-G
ACAGTACGTGAAAT	TGTTGAAAGGGA	AGCGTTTGCA	ACCAGACTTG	CACGCAG-TT-	GATCATCCG	G-TGTTC-T-	-CACCG-GG	GCACTCTGC	T-G
ACAGTACGTGAAAT	TGTTGAAAGGGA	AGCGCTTGCA	ACCAGACTTG	CACGCAG-TT-	GATCATCCG	G-TGTTC-T-	-CACCG-GG	GCACTCTGC	T-G
ATAGCACGTGAAAT	TGTTGAAAGGGA	AGCGTTTGTG	ACCAGACTTG	CGC-C-G-TTC	CGATCATCCG	G-TGTTC-T-	-CACCG-GT	GCACTCGGGAC	G
ACAGCACGTGAAAT	TGTTAAAAGGGA	AGCGCTCGCT	ACCAGACGTG	GGTTCGG-TG-	GTTCAACCA	GGTCCATG	AC-CTG-GG	GCACTCCGC	C-G
AAAGTACGTGAAAT	TGTTGAAAGGGA	AGCGCTTGTG	ACCAGACTTG	CGC-CGGGCG-	GATCATCCA	G-CGTTC-T-	-CGCTG-GT	GCACTCCGC	CCG
AAAGTACGTGAAAT	TGTTGAAAGGGA	AGCGCTTGTG	ACCAGACTTG	GGCTTGG-TT-	GATCATCCG	G-GGTTC-T-	-CCCCG-GT	GCACTCTTC	C-G
AAAGTACGTGAAAT	TGTTGAAAGGGA	AGCGTTTATG	ACCAGACTTG	GGCTTGG-TT-	AATCATCTG	G-GGTTC-T-	-CCCCA-GT	GCACTTTTC	C-A
* ** *******	**** ******	*** *	****** **	*	*** *	* ** *	* * *	**** *	

	4	910	4920	4930	4940	4950	4960	4970	4980	4990	5000
		1					1				
т	GTCCAGG	CCAGCAT	CAG-TTCGT	G-C-G-GG-	GGAAAAAGG	CTTCGGG-A	ACGTGGCTC-	CCCT~~~GGG	GTGTTATAGC	CCGTTGCA	TAATA
G	GGTTCAGG	CCAGCAT	CAG-TTCGC1	rc-c-g-gg-	GGATAAAGG	CTTTGGG-A	ATGTGGCTC-	CCTC~~~GGG	GTGTTATAGC	CCATTGCG	CAATA
c	GTTCAGG	CCAGCAT	GG-TTT	rgGrggr	TGGATAAAGG	CCTTGGG-A	ATGTGGCTT-	CCTTCG GGG	GTGTTATAGC	CC-TCGG-TG	CAATG
C	GTTCAGG	CCAGCAT	GG-TTT	rgGrggr	TGGATAAAGG	CCTTGGG-A	ATGTGGCTT-	CCTTCG-GGG/	GTGTTATAGC	CC-TCGG-TG	CAATG
C	GTTCAGG	CCAGCAT	CGG-TTT	rgGrggr	TGGATAAAGG	CCTTGGG-A	ATGTGGCTT-	CCTTCG-GGG/	GTGTTATAGC	CC-TCGG-TG	CAATG
	GCTCAGG	CCAGCAT	CGG-TTT	CGC-GGGG-	GGATAAAGG	TC-CGGGGA	ACGTAGCT	CCTCCGGG/	GTGTTATAGC	CCCGGGCG	TAATG
	-GCCCAGG	CCAGCAT	CAGCTTT-CO	G-TCG-GG-	-GG-CAAAGG	CGTCGGG-A	ATGTGGCTCI	CCTTCGGGGGG/	GTGTTATAGC	CCGTCGCG	TCATA
	-GTTCAGG	CCAGCAT	CGG-TTT-TC	CG-CCG-GG-	GGACAAAGG	CTTCGGG-A	ACGTGGCTC-	CTTTCG-GGG/	GTGTTATAGC	CCGTTGCG	TAATA
	-GCTCAGG	CCAGCAT	CAG-TTCGC	C-T-G-GG-	GGATAAAGG	CTTCGGG-A	ATGTGGCTC-	TCTCCG-GGG/	GTGTTATAGC	CCGCTGCG	TAATA
	-GTCCAGG	CCAGCAT	CAG-TTTTC	CCC-G-GG-GG-	GGATAAAGG	CGGCGGG-A	ATGTGGCTC-	TCTTCG-GGG/	GTGTTATAGC	CCACCGTG	TAATA
	* ****	******	* * **	* **	** *****	*** *	* ** ***	****	*******	** *	**
	5	010	5020	5030	5040	5050	5060	5070	5080	5090	5100
			.							.	1
С	CCT-G-	CGGTGGA	TGAGGAC-C	CGCGCATC-T	GCAAGGATGC	TGGCGTAAT	GGTCACCAGC	GACCCGTCTTG	AAACACGGAC	CAAGGAGTCG	rcr
С	CCT-G-	TGGCGGGG	CTGAGGTT-C	GCGCTTTAT	GCAAGGATGC	TGGCATAAT	GGTCATCAGT	GACCCGTCTTG	AAACACGGAC	CAAGGAGTCG	$\mathbf{r}\mathbf{c}\mathbf{T}$
С	AGCCT-A-	CCG-GGA	CCGAGGAC-C	CGCGCTTC-G	GCTAGGATGC	TGGCGTAAT	GGTTGTAAAC	GACCCGTCTTC	AAACACGGAC	CAAGGAGTC-	TAACA
С	AGCCT-A-	CCG-GGA	CCGAGGAC-C	CGCGCTTC-G	GCTAGGATGC	TGGCGTAAT	GGTTGTAAAC	GACCCGTCTTG	AAACACGGAC	CAAGGAGTC-	TAACA
C	AGCCT-A-	CCG-GGA	CCGAGGAC-C	CGCGCTTC-G	GCTAGGATGC	TGGCGTAAT	GGTTGTAAGC	GACCCGTCTTG	AAACACGGAC	CAAGGAGTC-	TAACA
С	CCTCG-	CCG-GGA	CGAGGTT	CGCGCATC-T	GCAAGGATGC	TGGCGTAAT	GGTCATCAAC	GACCCGTCTTG	AAACACGGAC	CAAGGAGTCA	AGGTT
С	CCT-T-C	CGGGGGGGG	TGAGG-TAC	CGCGC-TC-C	GCAAGGATGC	TGGCGTAAT	GGTAGCTAGT	GACCCGTCTTG	AAACACGGAC	CAAGGAGTCA	ACC
С	CCC-GG	CGG-GGA	CCGACGAC-C	CGCGCTTC-G	GCAAGGATGC	TGGCGTAAT	GGTCATCAGC	GACCCGTCTTG	AAACACGGAC	GAGGAGTCA	AGCA
С	CCT-G-	TGGCGGA	TGAGGTT-C	CGCGCATT-C	GCAAGGATGC	TGGCGTAAT	GGTCATCAGT	GACCCGTCTTG	AAACACGGAC	CAAGGAGTCG	$\mathbf{r}\mathbf{c}\mathbf{T}$
С	CCT-G-0	GGGGGGGA	TGAGGTT-C	CGCGCATC-T	GCAAGGATGC	TGGCGTAAT	GGTCATCAAC	GACCCGTCTTG	AAACACGGAC	CAAGGAGTCG	rcr
*	**	* ** *	* * * *	* * * *	** ******	**** ****	*** *	********	*********	* ******	

5110 5120 5130 5140 5150 TC-TA-TGCGAGTGTTTTGGGTGTTAAACCCATA-CGCGTAATGAAAGTGAACGGAGGTGAGAACCCTTAAGGGTGCATCATCGACCGGTCCTGATGTCTT TC-TA-TGCGAGTGTTTGGGTGTTAAACCCATA-CGCGTAATGAAAGTGAACGGAGGTGAGAACCCTTAAGGGTGCATCATCGACCGGTCCTGATGTCTT T-TG-CGCGAGTGTTTGGGTGTAAAACCCGCA-CGCGTAATGAAAGTGAACGTAGGTGAGAGC--TTC--GGCGCATCATCGACCGATCCTGATGTTAT TTATG - TGCGAGTGCCCGGGCGTAAAACCC-CAGCGCGGAATGAAAGTGAACGTAGGTGAGAGC--TTC--GGCGCATCACCGACCGATCCTGATGTCCT The Technology of technology

5160 5170 5180 5200 5190

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown

Hypocrea-jecorina Metrahizium-anisopliae Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

Hypocrea-jecorina Metrahizium-anisoplia Tetracladium-marchalianum Tetracladium-breve Tetracladium-palmatum Neurospora-crassa Verticillium-dahliae Magnaporthe-grisea Fusarium-solani unknown Clustal Consensus

CGGATGGATCTGAGTAAGAGCAT A GCTGT T GGGACCCGAAAGATGGTGAACTATGCGTGAAAGCGTGAAGCCAGAGGAAACTCTGGTGGAGGCC CGGATGGATTTGAGTAAGAGCGTTA A - GCCTTGG ACCCGAAAGATGGTGAACTATGCTTGGATGGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCC CGGACGGATTTGAGTGAGAGCAT-ATAGGGT-T-GG-ACCCGAAAGAAGATGAACTATGCGTGTGTAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTC CGGAAGGATTTGAGTAGGAGCATTA-AC-GC-TTGG-ACCCGAAAGATGGTGAACTATACTTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCCC

CGGATGGATTTGAGTAAGAGCAT - ACGGGGC - C - GG - ACCCCGAAAGAAGGTGAACTATGCCTGTGTAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGGCTC CGGATGGATTGAGTARGAGCAT ACGGGGC-C-GCACCCGAAGAGAGGTGAACTATGCCTGTATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTC 5340 5350 5360 5370 5380 5310 5320 5330 5390 5400 GCAGCGGTTCTGACGTGCAAATCGATCGTCAAATTTGCGCATAGGGGCGAAAGACTTATCGAACCATCTAGTAGCTGGTTCCTGCCGAAGTTTCCCTCAG GAGCGGTTCTGACGTCCAATCGATCGTCAAATCGACCATGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTACGGCGAAGTTTCCCTCAG GCAGCGGTTCTGACGTGCAATCGATCGTCAAAATGGACCATGGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTACGGCGAAGTTTCCCTCAG GCAGCGGTTCTGACGTGCAAATCGATCGACATGGGATATGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTTCGGCGACGATGTTCCCTCAG GCAGCGGTTCTGACGTGCAATCGATCGTCAAACTAGGGCCGAAGGACTAATCGAACCATCTAGTAGCTGGTTTCGGCGACGATTTCCCTCAG GCAGCGGTTCTGACGTGCAAATCGATCGTCAAATATGGGCATGGGGGGCGAAAGACTAATCGAACCTTCTAGTAGCTGGTTTCCGCCGAAGTTTCCCTCAG ** ************ ****** * *** ********

CGGATGGATCTGAGTAAGAGCAT-A-GCTGT-T-GGGACCCGAAAGATGGTGAACTATGCGTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCTC

CGGATGGATCTGAGTAAGAGCAT-A-GCTGT-T-GGGACCCGAAAGATGGTGAACTATGCGTGAATAGGGTGAAGCCAGAGGAAACTCTGGTGGAGGCCC

	5410	5420	5430	5440	5450	5460	5470	5480	5490	5500
						.				I
	GATAGCAGTGTTGAA	-CTCAGTTTTA	TGAGGTAAAG	CGAATGATT	AGGGACCC	GGGGGCG	CT-AT-ATT	GCCTTCATCC	ATTCTCAAAC	TTTA
	GATAGCAGTGTTGATT	TCTCAGTTTT#	TGAGGTAAAG	CGAATGATT	AGGGAC-CC	GGGGG <mark>C</mark> GG-	CTTATA	GCCTTCATCC	ATTCTCAAAC	TTTA
m	GATAGCAGTGTTGAA	-TTCAGTTTT	TGAGGTAAAG	CGAATGATT	AGAGGCCTT	GGGGTT-G	AA-ACA	ACCTTAACCT	ATTCTCAAAC	TTTA
	GATAGCAGTGTTGAA-	-TTCAGTTTT	TGAGGTAAAG	CGAATGATT	AGAGGCCTT	GGGGTTG	AA-ACA	ACCTTAACCT	ATTCTCAAAC	TTTA
	GATAGCAGTGTTGAA	-TTCAGTTTTI	TGAGGTAAAG	CGAATGATT	AGAGGCCTT	GGGGTT-G	AA-ACA	ACCTTAACCT	ATTCTCAAAC	TTTA
	GATAGCAGTGTTGTTC	-TTCAGTTTT	TGAGGTAAAG	CGAATGATT	AGGGAC-TC	GGGGG <mark>C</mark> G	CT-TTTTA	GCCTTCATCC	ATTCTCAAAC	TTTA
	GATAGCAGTGTTGAT	-CCCAGTTTT/	TGAGGTAAAG	CGAATGATT	AGGGAC-TC	GGGGGG <mark>CTCGC</mark> G	CT-ATTTA	GCCTTCATCC	ATTCTCAAAC	TTTA
	GATAGCAGTGTCGTC-	-TTCAGTTTT	TGAGGTAAAG	CGAATGATT	AGGGAC-TC	GGGGG <mark>C</mark> G	AT-TTTTA	GCCTTCATCC	ATTCTCAAAC	TTTA
	GATAGCAGTGTTGAA	-CTCAGTTTT/	TGAGGTAAAG	CGAATGATT	AGGGAC-TC	GGGGG <mark>C</mark> G	CT-ATTTA	GCCTTCATCC	ATTCTCAAAC	TTTA
	GATAGCAGTGTTGAA-	-CTCAGTTTT#	TGAGGTAAAG	CGAATGATT	AGGGAC-TC	GGGGG <mark>C</mark> G	CT-ATTTA	GCCTTCATCC	ATTCTCAAAC	TTTA
	********* *	*******	********	*******	** * *	**** *	*	**** * *	********	****

5510	55	520	5530	5540	5550	5560	5570	5580	5590	5600
AATATGTAAGAAGO	CCTTGI	TTGCTTA	ATTGAACG	FGGGCATTC	GAATGTATC	AACACTAGTGGG	CCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAGO	CCTTG	TGCTTA	GGTGAACG	FGGGCATTC	GAATGTATC	AACACTAGTGGG	CCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAGT	CCTTGT	TACTTA	ATTGAACG	FGGACATTC	GAATGTACC	AACACTAGTGGG	GCCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAGT	CCTTG	TACTTA	ATTGAACG	FGGACATTC	GAATGTACC	AACACTAGTGGG	CCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAG	CCTTGT	TACTTA	ATTGAACG	FGGACATTC	GAATGTACC	AACACTAGTGGG	GCCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAGO	CCTTG	TACTTA	ATTGAACG	FGGGCATTC	GAATGTACC	AACACTAGTGGG	CCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAG	CCCCGI	TTACTTT	ATTGAACG	GGACGCTG	GAATGAAAC	AACACTAGTGGG	GCCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAGO	CCTTGI	TACTTA	GTTGAACG	TGGGCCTTC	GAATGTACCO	GACACTAGTGGG	SCCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAG	CCTTGT	TGCTTA	ATTGAACG	FGGGCATTC	GAATGAATC	AACACTAGTGGG	CCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
AATATGTAAGAAGO	TCTTGT	TGCTTA	ATTGAACG	FGAGCATTC	GAATGTATC	AACACTAGTGGG	CCATTTTTGG	AAGCAGAACT	GGCGATGCGG	GATGA
********	* **	** ***	*****	* * *	**** * *	********	********	********	********	****
5610	50	620	5630	5640	5650	5660	5670	5680	5690	5700

ACCGAACGCGAGGTTAAAGGTGCCAGAGTAGACGCTCATCAGACACCACAAAAGGCGTTAGTACATCTTGACAGCAGGACGGTGGCCATGGAAGTCGGAA ACCGAACGCGAGGTTAAGGTGCCAGAGTAGACGCTCATCA-ACACCAACGGTGTTAGTACATCTTGACAGCAGGACGGTGGCCATGGAAGTCGGAAT ACCGAACGTGAAGTTAAGGTGCCGGAATATACGCTCATCAGACACCACAAAAGGTGTTAGTTCATCTAGACAGCAGGACGGTGGCCATGGAAGTCGGAAT ACCGAACGCGAGGTTAAGGTGCCCGGAGTGGACGCTCATCAGACACCACAAAAGGTGTTAGTACATCTTGACAGCAGGACGGTGGCCATGGAAGTCGGAAT ACCGAACGCGGGGTTAAGGTGCCGGAGTGGACGCTCATCAGACACCACAAAAGGCGTTAGTACATCTTGACAGCAGGACGGTGGCCATGGAAGTCGGAAG ACCGAACGCGAGGTTAAGGTGCCAGAGTAGACGCTCATCAGACACCACAAAAGGTGTTAGTACATCTTGACAGCAGGACGGTGGCCATGGAAGTCGGAA

	5710	5720	5730	5740	5750	5760	5770	5780	5790	5800
										1 1
	CCGCTAAGGACTGTG	TAACAACTCAC	CTGCCGAATG	TACTAGCCCT	GAAAATGGATG	GCGCTCAAG	CGTCTCACCO	ATACCTCGCC	-CTCGGG-	GTAGAA
	CCGCTAAGGACTGTG	таасаастсас	CTOCCOAATO	PACTAGCCCT	AAAATGGATG	GCGCTCAAG	COTCTCACCC	ATACCTCGCC	-CTCGGG-	GTAGGA
	CCGCTAAGGAATGTG									
1										
	CCGCTAAGGAATGTG									
	CCGCTAAGGAATGTG	TAACAACTCAC	CTGCCGAATG	AACTAGCCCT	GAAAATGGATG	GCGCTTAAG	CGTATTACCC	ATACTTCACO	GC-CAGG-	GTAGAA
	CCGCTAAGGACTGTG	TAACAACTCAC	CTGCCGAATG	TACTAGCCCT	JAAAATGGATG	GCGCTCAAG	CGTCCCACCC	ATACCCCGCC	CTCAGG-	GTAGAA
	CCGCTAAGGACTGTG	TAACAACTCAC	CTGCCGAATG	TACTAGCCCT	GAAAATGGATG	GCGCTCAAG	CGTCCCACCC	ATACCTC-CC	-T-C-GGC	GCGGGC
	CCGCTAAGGACTGTG	TAACAACTCAC	CTGCCGAATG	PACTAGCCCT	AAAATGGATG	GCGCTCAAG	CGTCCCACCC	ATACCCCGC	-CCCAGG-	GTAGAA
	CCGCTAAGGACTGTG	таасаастсас	CTGCCGAATG	PACTACCCCT	AAAATGGATG	CCCCTCAAG	COTOTOACCO	ATACCTCCCC	-CTCAGG-	GTAGAA
	CCGCTAAGGACTGTG									
	CCGCTAAGGACTGTG	TAACAACTCAC	CIGCCGAATG	TACTAGCCCTC	JAAAATGGATG	GCGCTCAAG	CGTCTCACCC	ATACCTCGCC	CTCAGG	GTAGAA
	5810	5820	5830	5840	5850	5860	5870	5880	5890	5900
			.			.				1 1
	ACGATGC-CCCGAG-	GAGTAGGCGG/	ACGTGGAGGTC	GTGACGAAG	CCT AGGGCGT	GAGCCCGGC	TCGAACGGC	TCTAGTGCA	ATCTTGGT	GGGTAG
	ACGATGC-CCCGAG-	GAGTAGGCGG	COTOGOGO	AGTGACGAAG	ccc-agggcg	CARCCCCCC	TCGAACGGCC	CCTAGTGCAC	ATCTTOGT	GGGTAG
	ACGATGC-CCTG-GC									
	ACGATGC-CCTG-GC									
	ACGATGC-CCTG-GC	GAGTAGGCAG	GCGTGGAGGTC	AGTGACGAAG	CCTTGGGG-G1	GACCCCGGG	TAGAACGGC	TCTAGTGCA	JATCTTGGT	G-GTAG
	ACGATGC-CCTGAG-	GAGTAGGCGGG	CGTGGAGGTC	AGTGACGAAG	CCT-AGGGCG1	GAGCCCGGC	TCGAACGGC	TCTAGTGCAC	ATCTTGGT	GGGTAG
	TCGGCGCGTC-GAG-	GAGTAGGCGG/	ACGTGGAGGTC	GGTGACGAAG	CCT-AGGACG1	CGAGTCCGGG	TGGAACGGC	TCTAGTGCA	ATCTTGGT	G-GTAG
	ACGATGC-CCTGGG-	GAGTAGGCTGA	ACGCGGGGGGT	AGCGACGAAG	GCT-AGGGCGT	GAGCCCGGC	TAGAGCTGCC	CCTGGTGCAC	ATCTCGGT	GAG-AG
	ACGATGC-CCTGAG-	GAGTAGGCGG	CGTGGAGGTC	AGTGACGAAG	CCT-AGGGCGT	CARCCCCCC	TTGAACGGCC	TCTAGTGCA	ATCTTGGT	GGTAG
	1001100 001010	01011000000	0010010010			010000000	11010100000	1017101007		0 0110

ACGATGC-CCTGAG-GAGTAGGCGGACGTGGAGGTCAGTGACGAAGCCT-AGGGCG-GAGCCCCGGTTGAAC ******* * * ****** * 5910 5920 5930 5940 5950 5960 5970 5980 5990 6000

 JS10
 JS20
 JS30
 JS40
 JS50
 JS60
 TAGCAAATACT-CAAATGAGAACTTTGA-GGACTGAAGTGGGGAAAGGTTCCATGTGAACAGCAGTTGGACATGGGTTAGTCGATCCTAAGCGATAGGGA TAGCAAATACT-CAAATGAGAACTTTGA-GGACTGAAGTGGGGAAAGGTTCCATGTGAACAGCAGTTGGACATGGGTTAGTCGATCCTAAGCGATAGGGA TAGCAAATACT-CAAATGAGAACTTTGA-GGACTGAAGTGGGGAAAGGTTCCATGTGAACAGCAGTTGGACATGGGTTAGTCGATCCTAAGCCATGGGA

TAGCAAATACTTCAA-TGAGAACTT-GAAGGACCGAAGTGGGGAAAGGTTCCATGTGAACAGCGGTTGGACATGGGTTAGTCGATCCTAAGCCATAGGGA

Figure 3: Result of MSA showing the conservation of unkown gene sequence (from 4447th nucleic acid to 5782nd nucleic acid through the sequences of all the organisms taken for study.

Sci. technol. arts Res. J., Jan-Mar 2013, 2(1): 42-49 5290 ··· 5300

DISCUSSION

Solid waste was selected for the study to understand diversity of micro flora on solid waste. The most common organisms that are generally found in solid waste are bacteria and fungi. These microorganisms use the components of the waste as the substrate for their growth. So we inoculated the solid waste to the medium suitable for fungal growth. A wide variety of microorganisms have been reported to be present in these organic wastes (Amalraj *et al.*, 2006). Accordingly wide variety of micro flora was observed on the culture medium. An attempt has been made to obtain the pure culture.

By the daily microscopical observations of collected waste material the organisms were identified according to the Barnett manual. It is not easy to identify the microorganisms only by the microscopical observation so the 28s rDNA sequencing method was employed for the identification of the organism on solid waste. The 28s rDNA sequencing was carried out by the isolation and polymerization methods. Genomic DNA was isolated from the organism in pure culture using fungal Genomic DNA Isolation Kit (RKT13) and was subjected for electroporesis with reference to 1kb DNA Ladder (Chromous Cat. No. LAD03) (Figure 1A).

The isolated DNA was used as a template for the PCR amplification of ~5kb rDNA fragment using high –fidelity PCR polymerase. The PCR product was sequenced bi-directionally using the forward and reverse primers. The polymerised sample was subjected for electrophoresis by taking 500bp DNA Ladder (Chromous Cat.No.LAD02) as reference.

The molecular method of identification was followed by the *in silico* methods. The orthologs sequences for query sequence were retrieved by performing BLAST against non-redundant databases. Phylogram was obtained by performing MSA of all the ten sequences with query sequence. Both *Fusarium solani* and unknown sequences were observed on a same Sci. technol. arts Res. J., Jan-Mar 2013, 2(1): 42-49

clade of phylogram as shown in Figure 2. From the phylogram analysis it was confirmed that the unknown sequence is from *Fusarium solani*. Later the alignment gave us information about the conservation level of unknown gene through genes from different organisms. The unknown sequence has shown very good conservation from 4447th base to 5872nd base with all the 28s rDNA sequences from different organisms taken for the study as shown in Figure 3. The phylogenetic tree was validated by bootstraping; this analysis supported the identification of an unknown organism as *Fusarium solani*.

CONCLUSION

The results of the present study reveals that, the differences in *Fusarium solani* identification by phenotypic and 28s rDNA sequencing method have shown that an application of a molecular analysis is essential to complement classic biochemical methodology. Our results, together with the review of literature, suggest that 28s rDNA sequencing method can be helpful in resolving ambiguous results, for characterizing uncommon strains, and for epidemiological investigations.

The phylogenetic tree was validated by bootstraping; this analysis supported the identification of organism as *Fusarium solani* through *in-silico* method. It provides information to facilitate researchers to comprehend the basic views on gene homology and its various applications in the identification of novel strains in microorganism.

REFERENCES

- Amalraj, S. (2006). Introduction to Environamental Science and Technology. *Laxmi publication*, 61-66.
- Shekdar. (1999). Municipal solid waste management. *The Indian perspective Journal of Indian Association for Environmental Management*, 26, 100-108.
- Subramanian CV (1983). Thypomycetes taxonomy and biology. Academic press London, 2.