INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

Volume-1, Issue-2 June-Aug :2011 *www.ijpaes.com* *ISSN 2231-4490*

IN SILICO IDENTIFICATION OF FUSARIUM STRAIN NFCCI 2157 ISOLATED FROM CUMIN WILT

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ABSTRACT: Fusarium, FUSARIUMID v. 1.0, a publicly available database of partial translation elongation factor 1-alpha (TEF) DNA sequences, Users can generate sequences using primers that are conserved across the genus, and use the sequence as a query to BLAST the database, which can be accessed at http://fusarium.cbio.psu.edu, or in a phylogenetic analysis. Correct identification of a known species in these groups often can be performed using this gene region alone. Fusarium strain NFCCI 2157 was identified as fusarium equiseti by using FUSARIUMID v.1.0 and NCBI-BLAST, which is isolated from wilted cumin plants.
Key words: Fusarium, Cumin wilt, Blast

INTRODUCTION

Fusarium species that is responsible for a range of plant diseases. Wilt is serious one. Most of crops are affected by fusarium genus. Cumin wilt is one of them. The conidiophores of Fusarium may persist in soil for many years in the absence of a susceptible host (Agrios, 1991). Traditional control measures of the Fusarium wilt disease include crop rotation, pre-sowing treatments for the seeds with certain chemical fungicides and management of cultural practices (Agrios, 1991) or soil fumigation (Larkin and Fravel, 1998). Fusarium, the single most important genus of toxigenic fungi, has had a confusing and unstable taxonomic history. A number of factors, including a lack of clear morphological characters separating species, leading to species concepts that are too broad, together with variation and mutation in culture, have conspired to create taxonomic systems that poorly reflect species diversity(David M. Geiser et al, 2004).

MATERIALS AND METHODS

The approach for identifying an isolate of Fusarium using the FUSARIUM-ID database is outlined in Figure 1. It involves obtaining a pure culture of an isolate, extracting genomic DNA, amplification of the TEF gene region, and sequencing. BLAST is then used to identify the closest matches between the unknown sequence and those contained in the FUSARIUM-ID sequence database and NCBI-BLAST.

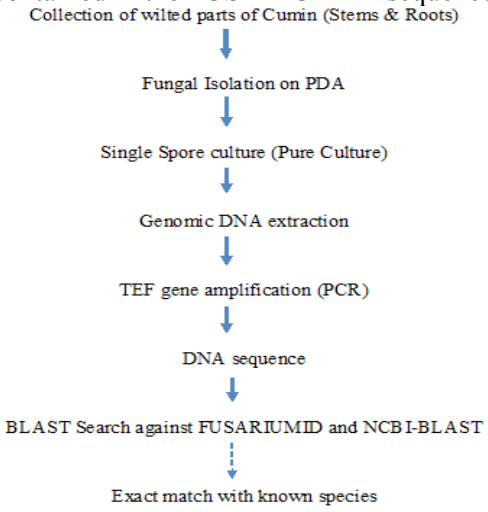

Fig-1, Flowchart describing the process of using TEF DNA sequences to identify Fusarium species.

Pure culture of *Fusarium* strain NFCCI 2157

Mixed cultures or cultures contaminated with bacteria and other fungi are a problem for all types of identification. Care should be taken to obtain a pure culture, particularly one derived from single conidia to ensure that the culture represents a clone. Methods for obtaining pure cultures are outlined by Summerell et al. (2003) and (Choi, Y.W et al. 1999).

DNA Extraction

Fusarium strain NFCCI 2157 was grown on PDA plates for 7 days and mycelia were harvested. Total DNA was extracted from ground mycelium of isolate (~100 mg wet weight) using a Genei Plant DNA extraction Kit (Genei, India) according to the manufacturer's instructions.

BLAST

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Identification by comparison with databases

The FUSARIUM-ID server at http://fusarium.cbio.psu.edu contains a BLAST search tool that Allow users to query unknown sequences against the database. Alternatively, the GenBank database is publicly available for identification purposes, and can be accessed via the Entrez website at the US National Center for Biotechnology Information (NCBI): http://www.ncbi.nlm.nih.gov/Entrez/. FUSARIUM-ID is strongly recommend because it contains vouchered and well-characterized sequences that correspond to publicly available cultures that can be used for confirmation. Of course, FUSARIUM-ID can be used in conjunction with GenBank (David M. Geiser et al, 2004). Once edited, the TEF sequence from an isolate of interest can be copied and pasted into a web browser connected to the BLAST server and used as a query for comparison to the database. Nucleotide BLAST will retrieve the closest matches to the query sequence, and present the matches as a series of DNA alignments with corresponding percentage match information.

RESULT AND DISSCUSION

The culture was deposited under accession number NFCCI 2157 (National Fungal Culture Collection of India) at Mycology and Plant Pathology Group Agharkar Research Institute, Pune. It was showed 99% sequence similarity with genus *Fusarium* Link (1809) species *F. equiseti* (Corda) Sacc. (1886) **(**NCBI Accession HM130559.1**)** by run NCBI-BLAST (Geiser et al., 2004 summerbell et al., 2006) (table-1&fig-2) ITS 1&2 marker was also used to confirmed *Fusarium* species at a local BLAST (Basic Local Alignment Search Tool) server, in FUSARIUM-ID v. 1.0, which can be accessed at http://fusarium.cbio.psu.edu. Query sequence of NFCCI 2157 was aligned with the best score of 466/467 with 99.78% similarity (table-2). DNA sequence-based identifications and species-specific PCR assays are usually needed to accurately identify species. Thus, we used species-specific PCR, ITS 1&2 marker and sequence analysis to confirm identify pathogen isolate.

Fusarium equiseti strain Fe1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Length=505
Score = 863 bits (467), Expect = 0.0
Identities = 472/474 (99%), Gaps = 2/474 (0%)
Strand=Plus/Plus
Fusarium equiseti strain Fe3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Length=509
Score = 861 bits (466), Expect = 0.0
Identities = 474/477 (99%), Gaps = 3/477 (1%)
Strand=Plus/Plus

Fusarium equiseti strain EUF2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence Length=500
Score = 861 bits (466), Expect = 0.0
Identities = 473/476 (99%), Gaps = 2/476 (0%)
Strand=Plus/Plus
Fusarium equiseti isolate PCO.33 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Length=546
Score = 861 bits (466), Expect = 0.0
Identities = 474/477 (99%), Gaps = 3/477 (1%)
Strand=Plus/Plus
Fusarium equiseti strain AGR12 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence Length=540
Score = 861 bits (466), Expect = 0.0
Identities = 473/476 (99%), Gaps = 2/476 (0%)
Strand=Plus/Plus

Align organisms	BLAST-score	Similarity %
Fusarium equiseti strain Fe1	863 bits (467)	99%
Fusarium equiseti strain EUF2	861 bits (466)	99%
Fusarium equiseti strain AGR12	861 bits (466)	99%

Table-1 Best similar alignment organisms against Fusarium NFCCI 2157 at NCBI database.

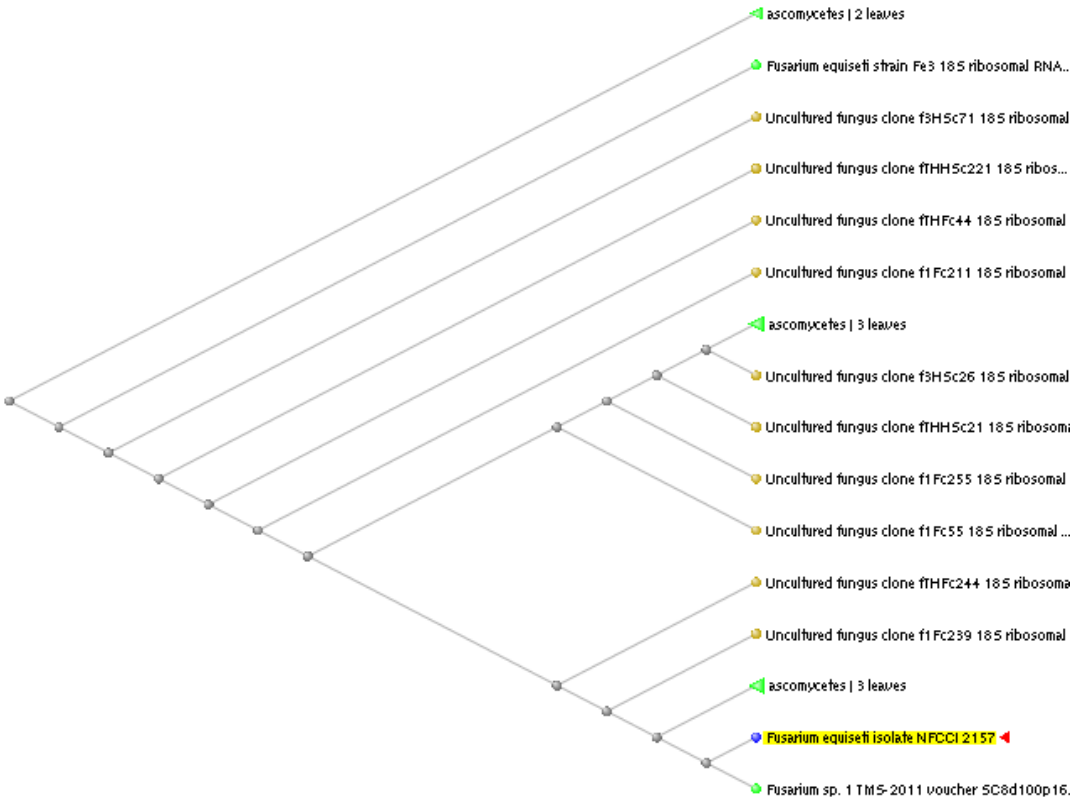

Fig-2 phylogenetic tree

Align organisms	BLAST-score	Similarity %
F. incarnatum-equiseti species complex isolate NRRL 43619, MLST15-a	466 / 467	99.78%
F. incarnatum-equiseti species complex isolate NRRL 43297, MLST24-b	466 / 467	99.78%
F. incarnatum-equiseti species complex isolate NRRL 36548, MLST17-b	466 / 467	99.78%
F. incarnatum-equiseti species complex isolate NRRL 13379, MLST23-b	466 / 467	99.78%

Table-2 Best similar alignment organisms against Fusarium NFCCI 2157 used ITS 1&2 markers at FUSARIUMID v.1.0 database.

ACKNOWLEDGEMENTS

The authors are grateful to **David M. Geiser et al.** for developed FUSARIUMID v.1.0 to identify fusarium species.

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