



Internal transcribed spacer sequence database of plant fungal pathogens: PFP-ITSS database



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ABSTRACT

The nurseries and plantations of medicinally and economically important plants are facing challenge on health front as they are being rapidly exposed to fungal pathogens thereby affecting their health and productivity adversely. The plant pathogenic fungi significantly damage and reduce the flora of subcontinent. Lack of knowledge and trouble in identification of fungal pathogens, are imposing a peril to both the flora and human health all over the world. Albeit routine pathological techniques can be used for the identification of plant fungal pathogens but these methods are time consuming and often need sound knowledge in mycotaxonomy. Recently, molecular (DNA sequence) data has emerged with the nuclear ribosomal internal transcribed spacer (ITS) region (DNA barcoding) as crucial biomarker to disclose the necessary information for the taxonomic identification of plant pathogenic fungi. However, development of nucleotide sequence database of plant pathogenic fungi will enable authentic pathogen identification easy and quickly even by a person not trained in fungal taxonomy. This study presents the development of a new plant fungal pathogen -Internal Transcribed Spacer Sequence database (PFP- ITSS Database) holding 1215 ITS sequences collected from various sources. It represents 1215 plant fungal pathogens (PFP) in relation to their respective economical and medicinal plants; and is available at <http://shaktisahislab.com/include/ITSdb>. PFP-ITSS Database will provide useful information for better understanding of plant pathogenic fungi, which cause disease in both economical and medicinal plants.

1. Introduction

Pathogens are a group of species that infect and disrupt the normal physiology of the hosts to complete their life cycle. Plant pathogens include fungi, nematodes, bacteria, and viruses which can cause diseases or damages in the plants [1]. Amongst these pathogens, fungi are known to cause maximum yield loss in numerous economically important crops [2]. In developing country like India, the yield of crop production is very low due to infection by pathogenic fungi [3]. Additionally, recent reports on emerging plant fungal pathogens and their cross-kingdom infections to animals and immune compromised persons emphasize the need for correct and quick identification [4,5]. However, the identification of the PFP using the traditional taxonomy

methods at species level are complicated due to lack of an adequate information on morphological characteristics or different phases of the life cycle [6,7]. Also, it has been reported that different PFP species have evolved a particular mode to infect and cause diseases in the plants [8]. There is a need for a novel, fast and accurate technique for identification of the PFP at species or strain level in the environment to carry out disease surveillance and implementation of a disease management strategy. Recently, the genomic data of the organisms have been widely explored to collect and scrutinize the relevant detailed information such as identification of PFP irrespective of their morphological characteristics, degree of cultivability and different phases of life cycle [9]. These studies have contributed towards a huge collection of taxonomically and technically conciliated DNA sequences, further

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pooled and shared by the international nucleotide sequence databases. Albeit the availability of these molecular databases can be used as reference data for the identification of unknown species; but this type of identification raises the problems, because novel sequence data produced may not be in a locus to evaluate whether a suggested taxonomic affiliation is reliable. For instance, recent studies reveal that the reliability of the produced molecular data in certain groups of organisms have been severely compromised without the adequate means to differentiate a significant data from the insignificant. As a consequence, these errors and inconsistencies are regularly included into the data and used by the research communities over the time. It eventually results in the misidentification of the species names and ecological properties from sequence resemblance searches.

Molecular identification through DNA sequences have promptly advanced the understanding of species frontiers and interactions in several important plant pathogenic genera, revealing numerous enigmatic species. In this concern, fungus identification usually relies on the sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region and has been proposed as the formal universal fungal barcode. The largest database designed for fungal ITS sequences is UNITE. UNITE mirrors and curates the International Nucleotide Sequence Database Collaboration INSDC: GenBank, ENA, and DDBJ for fungal ITS sequences and deals with broad propensities for the sequence analysis and third-party annotation of sequences to its users. Though the molecular databases have been gradually cited and recorded with fungal taxon in dated publications, but as our familiarity and survey of disease associated with fungi increases, the checklists and databases have become more and more inaccurate. For example, [10] studies concluded that the frequent occurrence of the fruit rot disease in tropics is not caused by *Colletotrichum gloeosporioides*. It is, therefore, required to group these pathogens in Plant-pathogen species complexes based on the modern molecular data protocols to present them as reference databases for the identification of unknown PFP. We report here the collection and identification of plant pathogens associated with medicinal and economical plants. The present database readily contains the large sets of plant pathogenic fungi information and internal transcribed spacer sequences. As fungal internal transcribed spacer region of ribosomal RNA gene sequence are available in plant, it will be updated into the database regularly. The output results are highly valued and easy to process, although it still needs to be updated regularly. The database is the first platform concerning fungal pathogen of economic and medicinal plant. It will assist users in related fields by providing comprehensive information (fungus name to reference column) on database. The database resource will be freely available for use in public.

2. Methodology

2.1. ITS sequence data collection

Total 1215 barcoding sequences (18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28 S ribosomal RNA gene, partial sequence) of 1215 species were retrieved as shown in [Supplementary A](#). The PFP-ITSS data were retrieved from various sources.

3. Development of PFP- ITSS database

3.1. ITS database architecture and web user interface

This Database was assembled and configured upon platform JavaScript, AJAX, MySQL, PHP and HTML. Java script language was used for flash designing as well as control development. The ITS Database architecture and web user interface cycle is shown in [Fig. 1](#). Java script was mainly implemented as part of a web browser in

order to create enhanced user interface and dynamic website. AJAX is a technique for creating fast and dynamic web pages. Since, AJAX allows web pages to be updated asynchronously by exchanging small amounts of data with the server at the back end, therefore, we employed AJAX for building the database web pages. This means that it is possible to update parts of a web page, without reloading the whole page. MySQL was used for back end designing as well as ITS Database deposition. For designing of the database, PHP- Hypertext Preprocessor v5.5.0 Alpha1 version was used to perform the control designing and back end connectivity. Additionally, Cascading Style Sheets (CSS) were used for front end designing of ITS Database. CSS is a simple mechanism for adding style like fonts, colors, spacing to Web documents.

3.2. Approaches

3.2.1. Wrapper

The interesting feature of ITS Database is usage of wrappers; which explicitly optimizes the performance criterion and verifies database by comparing the results with FILTER approach FCBF method, where if the data about the Fungus name, Region, Fungus Features, Affected part of the Host, Name of the Host Plant, Disease, Impact on Plant, ITS Sequence are not available in ITS Database then it will search the data in other similar databases using wrapper method and it will search out complete query unless and until the user is not satisfied.

3.3. Data integrity

Data integrity is the best features of ITS Database. It has been incorporated to make the ITS database more accurate and consistent, though data warehousing require this accuracy to stand against the errors occurring either due to human error, software or hardware. Federator Data warehouse approach has been highly envisaged in inter operating ability of the medical care and its research.

3.4. Data mining and data warehousing

Enterprise data warehouse was used for extracting the ITS data into data mart as it provided the clean data. The issues pertaining to data consolidation were addressed during maintenance procedure. ITS data was retrieved from the various databases.

3.5. Normalization

Normalization was done to validate the data. The data was arranged data in the database by creating tables and establishing relationships between tables thereby eliminating redundancy and inconsistent dependency. The data deposition was done using natural language processing (NLP) methods. ITS generated output can identify and classify different values like fungus name, region, fungus features, affected parts of the host plant, name of host plant, disease, impact on plant, ITS sequence and references of the sequence available in the literature.

3.6. Development of homology searching tool

Homology searching is a method used to find out the identical or similar homologs of an organism which is useful in the identification of particular species at molecular level. The homology search algorithms [11,12] were used for designing homology searching tool for PFP- ITSS Database.

3.7. Development of primer designing tool

ITS PRIMER Tool, a new primer designing program for finding primers from ITS sequences was developed. This tool is a very powerful and efficient PCR primer design program, which allows the user to have

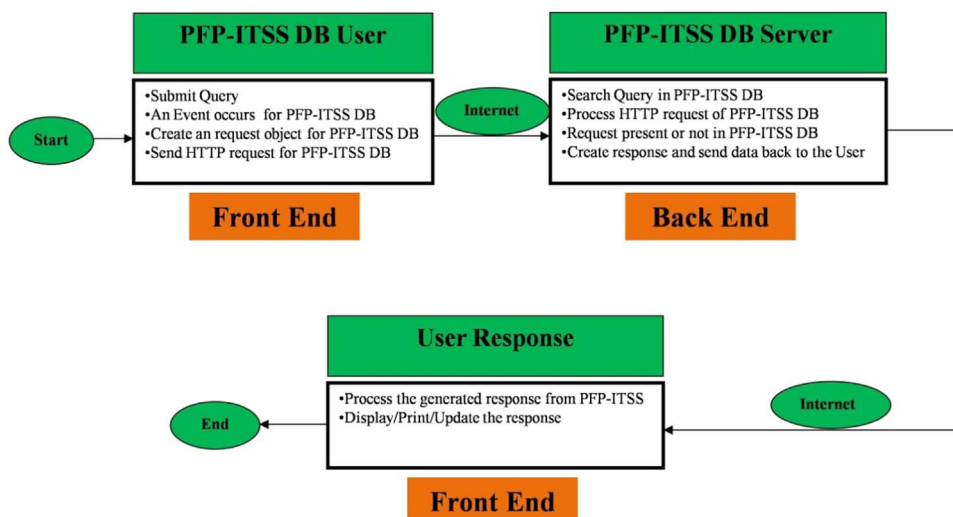


Fig. 1. Flow chart depicting methodology of PFP-ITSS Database, where DFD (Data Flow Diagram) represents the front end and back end response from PFP-ITSS Database.

considerable control over the nature of the primers, including size of the designed ITS primers, primer size, temperature range and presence and absence of 3'-GC clamp. ITS PRIMER Tool algorithm is accurate and based on the prime designing algorithms [13,14].

4. Results and discussion

4.1. PFP- ITSS database development and data accessing

A Database of plant pathogenic fungi, named “Internal Transcribed Spacer Sequence Database of Plant Fungal Pathogens: PFP- ITSS Database” containing 1215 ITS sequences representing 1215 plant pathogenic fungal species from various regions has been developed as shown in Fig. 2. Both automated search and manual curation were performed for storing candidate plant pathogenic fungus information from various regions. We have broadly included PFP generation, including basic fungal species characterization, myco taxonomic, internal transcribed spacer assignment, as well as information of host–pathogen complex. A BLAST for similarity search of internal transcribed spacer was also created. A user-friendly web interface and

regular updates make the database valuable to mycologist, biotechnologist and related fields. More than 1200 entry of economical and medicinal plant pathogen fungal species were stored in database. The database store more than 1200 fungal internal transcribed spacer (ITS) sequence of different fungal species collected from the various sources with research articles, web pages, books and other databases. The database stores information of pathogenic fungal species in different fields i.e. Fungus name, region, fungus features including taxonomic function, host from which species was found around (e.g. leaf, stem, seed, fruit and root), from which, name of the host plant, name of the disease, signs and symptoms, internal transcribed spacer (ITS) sequences of fungus and lastly the information table containing information references. Phenotypic and ITS information's were obtained by manual curation of the closely reviewed literature. A MySQL table store of nine entities information in nine blocks information format, permits comparisons and data analysis across the taxonomic space of fungal species. Internal transcribed spacer was mapped via their associated sequence information to reference genomes available in GenBank. The ITS sequence data of a plant pathogenic fungi can be obtained from PFP- ITSS Database using organism name/ GenBank ID/ PFP- ITSS



Fig. 2. PFP-ITSS sequence database for plant fungal pathogens database home page view.

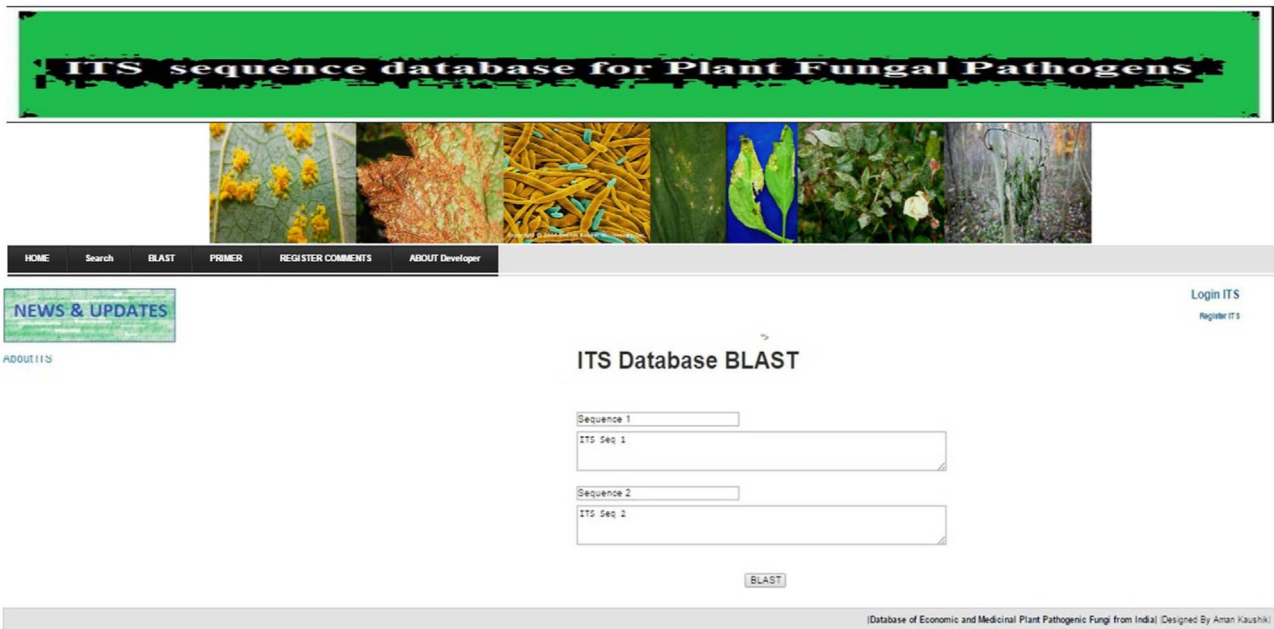


Fig. 3. Homology search tool of ITS sequence database for plant fungal pathogens.

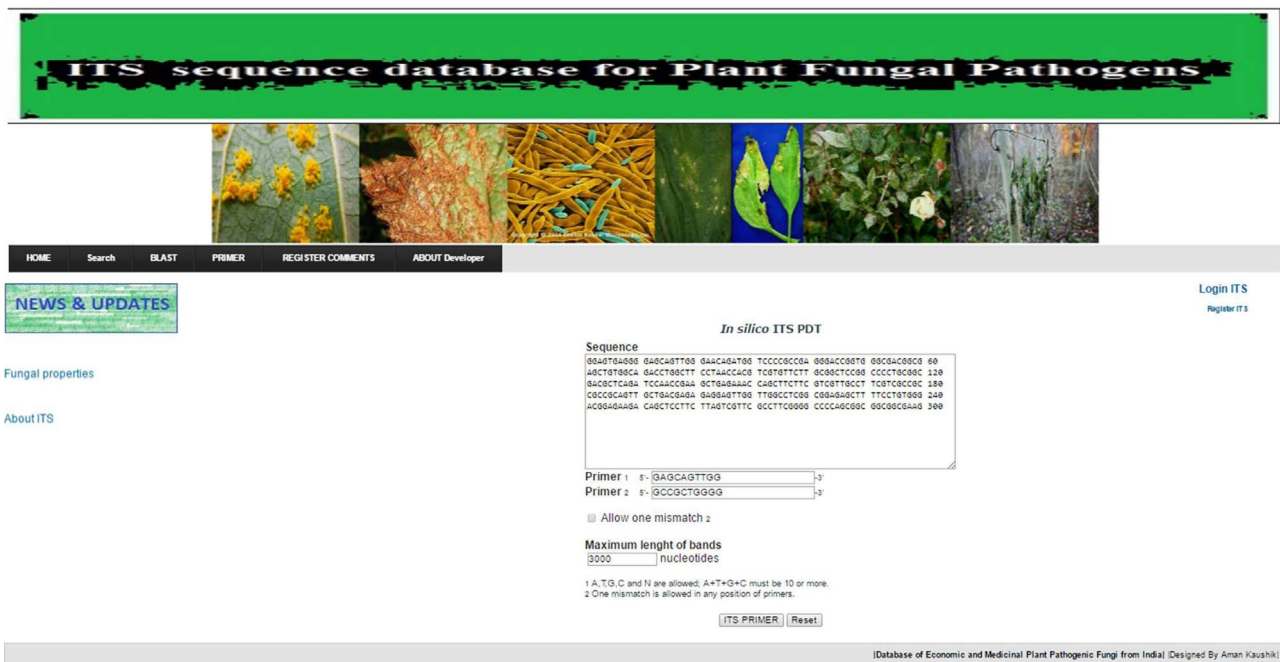


Fig. 4. ITS PRIMER tool of ITS sequence database for plant fungal pathogens database.

Database sequence ID.

4.2. Homology searching tool for PFP- ITSS database

For correct and quick identification of the plant pathogenic sequences, homology search algorithms were used to develop the homology searching tool for PFP- ITSS Database. This tool provides the related sequence similarity with the database sequences. The Homology searching tool shown in Fig. 3.

4.3. ITS sequence primer designing tool

Primers are specific short sequences used to amplify the specific regions in the DNA of organisms. The primer designing tool of PFP-

ITSS Database will be useful in designing species specific primers for the fungal pathogen species. After virtual validation, primers can be validated in wet lab. The primer designing program is shown in Fig. 4.

Biological sequence databases are the repositories of biological information about the macromolecular (DNA/ RNA/ Proteins) sequence data, collected from scientific experiments [15]. The DNA barcode sequence databases of pathogenic species play a significant role in the identification of a particular species at molecular level using homology search method. In plant pathology, due to the absence of disease warning / forecasting methodology timely action for disease management is difficult task. Dearth of trained fungal taxonomists further worsens the situation. The ITS sequences of plant fungal pathogens have emerged as a crucial biomarker to disclose the necessary information for the taxonomic identification. In this study,

we have developed a new database (PFP- ITSS Database) containing 1215 ITS sequences representing 1215 plant pathogenic fungi from various region which will provide useful information for better understanding of plant pathogenic fungi, which cause disease in economical and medicinal plant.

5. Conclusion

ITS Sequence database of plant pathogenic fungi various region will be useful for economical and medicinal plant has been developed. ITS db provides 1215 free access of plant pathogenic fungi sequence and their information. ITS db also provide links for validated ITS sequence submission. Information about current information of ITS as well as topics related to plant pathogenic fungi are also included. Two tools are also introduced for ITS sequence, Homology searching tool and primer designing tool. PFP- ITSS Database links over with advanced search and, users can explore query limit.

Conflict of interest

This article does not contain any studies with human participants performed by any of the authors. This article does not contain any studies with animals performed by any of the authors. Author A declares that she has no conflict of interest. Author B declares that he has no conflict of interest. I certify that ALL of the following statements are correct. The manuscript represents valid work; neither this manuscript nor one with substantially similar content under my authorship has been published or is being considered for publication elsewhere (except as described in the manuscript submission); and copies of any closely related manuscripts are enclosed in the manuscript submission. Funding Information is not available.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.imu.2017.02.006](https://doi.org/10.1016/j.imu.2017.02.006).

References

- [1] Boland GJ. Plant pathology, GN Agrios. Burlington, MA: Elsevier Academic Press; 2005. p. 922, [ISBN: 0-12-044565-4 2007].
- [2] Fletcher J, Bender C, Budowle B, Cobb WT, Gold SE, Ishimaru CA, Seem RC. Plant pathogen forensics: capabilities, needs, and recommendations. *Microbiol Mol Biol Rev* 2006;70(2):450–71.
- [3] Gauthier GM, Keller NP. Crossover fungal pathogens: the biology and pathogenesis of fungi capable of crossing kingdoms to infect plants and humans. *Fungal Genet Biol* 2013;61:146–57.
- [4] Kang S, Mansfield MA, Park B, Geiser DM, Ivors KL, Coffey MD, Blair JE. The promise and pitfalls of sequence-based identification of plant-pathogenic fungi and oomycetes. *Phytopathology* 2010;100(8):732–7.
- [5] Montesinos E. Development, registration and commercialization of microbial pesticides for plant protection. *Int Microbiol* 2003;6(4):245–52.
- [6] Otten L *Biochemistry and Molecular Biology of Plants*, Edited by Bob B. Buchanan, Wilhelm Gruissem and Russel L. Jones, American Society of Plant Physiologists, PO Box 753, Waldorf, MD 20604-0753, USA, (Drake International Services, Market House, Market Place, Deddington, Oxford OX15 0SE, UK). Hardback ISBN-0943088-37-2; £. 135.00, Paperback ISBN-0943088-39-9; £. 75.002001.
- [7] Samerpitak K, Van der Linde E, Choi HJ, van den Ende AG, Machouart M, Gueidan C, De Hoog GS. Taxonomy of *Ochroconis*, genus including opportunistic pathogens on humans and animals. *Fungal Divers* 2014;65(1):89–126.
- [8] Shenoy BD, Jeewon R, Hyde KD. Impact of DNA sequence-data on the taxonomy of anamorphic fungi. *Fungal Divers* 2007;26(1):1–54.
- [9] Udayanga D, Liu X, Crous PW, McKenzie EH, Chukeatirote E, Hyde KD. A multi-locus phylogenetic evaluation of *Diaporthe* (Phomopsis). *Fungal Divers* 2012;56(1):157–71.
- [10] Attwood TK, Gisel A, Bongcam-Rudloff E, Eriksson NE. Concepts, historical milestones and the central place of bioinformatics in modern biology: a European perspective. INTECH Open Access Publisher; 2011.
- [11] Bailey TL, Gribskov M. Combining evidence using p-values: application to sequence homology searches. *Bioinformatics* 1998;14(1):48–54.
- [12] Eddy SR. A new generation of homology search tools based on probabilistic inference. *Genome Inf* 2009;23(1):205–11.
- [13] Kämpke T, Kieninger M, Mecklenburg M. Efficient primer design algorithms. *Bioinformatics* 2001;17(3):214–25.
- [14] Evans P, Wareham HT. Practical algorithms for universal DNA primer design: an exercise in algorithm engineering. *Curr Comput Mol Biol* 2001:25–6.
- [15] Phoulivong S, Cai L, Chen H, McKenzie EH, Abdelsalam K, Chukeatirote E, Hyde KD. *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. *Fungal Divers* 2010;44(1):33–43.