

Assessing *Phytophthora* Database as a valuable global resources for *Phytophthora* diseases: A review



ENVIRONMENTAL SCIENCE

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ABSTRACT

The *Phytophthora* database (PD) has emerged as an important application of bioinformatics science to enhance rapid detection and diagnosis of *Phytophthora* spp., a highly virulent and globally distributed plant pathogen. The database (<http://www.phytophthoradb.org>) consists of tools for searching, analyzing, archiving, browsing, storage, and submission of data related to the pathogen. BLAST search, ClustalW analysis and Virtual RFLP are the important functional tools that help in searching and analyzing the data. In general it aimed to generate the Global *Phytophthora* Network (GPN), detailed global distribution of its species, molecular diagnostic and informatics tools supporting the use of archived data for detection and identification, a Geographic Information Systems (GIS) platform for visualizing the distribution and change of the pathogen in environmental and geospatial contexts for monitoring and managing the diseases. Moreover, the database is also designed with an option to make changes in genotype as per amount and type of data. This review highlights the important features and examines the potentiality of the database in regard to study the existence, diversity, monitoring and managing the pathogen at global scale.

Introduction

The global agricultural biosecurity requires a user-friendly informatics platform that supports the integration and use of available data and cooperation within and between countries in plant disease monitoring and management. The science of bioinformatics through various datasets and databases has emerged as a significant resource for global plant disease information. The integration of disparate data sets, ranging from pathogen ecology to geospatial, agronomic and environmental contexts helps to identify the cause(s) of disease outbreaks, forecasting their spread, and implementing appropriate disease management strategies.

Phytophthora, an oomycete plant pathogen, belonging to the kingdom stramenopila (Baldauf et al. 2000, Kamoun et al (2003) are devastating plant pathogens in both agricultural and natural environments and there has been increasing interest in *Phytophthora* genetics and genomics, culminating in the recent release of three complete genome sequences (*P. ramorum*, *P. sojae*, and *P. infestans*) (Blair et al. 2008). In the last decade, the number of recognized species has nearly doubled and new species are added almost on a monthly basis (Kroon et al. 2012). Approximately 124 sps, has been described for this pathogen, thus the morphological characterization for species identification is a great challenge (Martin, 2013). Given the rapid discovery of novel species, an increase in the number of species of regulatory concern, and the prevalence of encountering *Phytophthora* spp. in diagnostic labs, a contemporary tool for identification of *Phytophthora* isolates to a species level is urgently needed (Grünwald et al. 2011).

Phytophthora Database (PD)

Phytophthora Database (PD) (<http://www.phytophthoradb.org>) project initiated by United States Department of Agriculture(USDA) in 2005 under the program Agricultural Research Service(ARS) aimed to enhance rapid detection and diagnosis of *Phytophthora* spp. by archiving genotypic and phenotypic diversity of the genus that can be easily accessed and utilized by the global community of plant health professionals. The database provides applied and molecular information on *Phytophthora* species along with a suite of data analysis tools, and has more utility than GenBank (Ivors et al. 2007). Currently the database includes nearly 123 sps., 23 provisional sps., 2623 isolates and 6315 sequences of the pathogen.

Database mainly comprises of four functional components database, search and analysis, molecular diagnostics and community platform. Database components comprises of five sections species, genetic markers, hosts, appendix and sequence download. The species page contained an array of information, such

as a genus wide phylogenetic tree, nomenclature, morphological and growth characteristics, hosts and disease symptoms, life cycle, and selected references, to help users quickly learn about key characteristics of each species (Park et al. 2013). There are at present 123 formally described species with *P. infestans* representing maximum number of isolates (330). The tendency towards a more phylogenetic species concept based on DNA sequences is most obvious in genetic markers page in which isolates that form a distinct cluster are considered as representing new species. (Kroon et al. 2012). PCR reaction conditions for amplifying marker loci, including the sequences and positions of primers used, can be found in genetic markers section. A total of 14 genetic markers along with their comments and sequences are described in this section. The host browser page helps to add newly discovered host for the pathogen at global level. The appendix section describes a detailed information, distribution, substrate, host and grouping of each species. Sequence download page categorizes the species under ten clades grouped under a common ancestor and all the descendants of that ancestor. The total number of haplotypes for each species is described in this section.

How to search the database

Search and analysis segment comprises of five functional keys- search by sequence, search by multi-locus, search by SSR loci, search by keyword and virtual RFLP. Sequence based search can be conducted by three functional keys BLAST search, ClustalW analysis and Virtual RFLP. The PD supports the identification of an unknown isolate by querying the sequence database using one or more of the marker sequences. The BLAST tool containing sequence data helps in sequencing the loci of selected isolates that represent most of the known species to establish a genus-wide phylogenetic framework. It uses sequences of the internal transcribed spacer (ITS) regions of ribosomal RNA-encoding genes which helps to identify unknown isolate at or near the species level. SSR marker-based search helps in monitoring and visualization of population dynamics within species which is searched through their SSR genotype or any of the information associated with the isolates or both. Isolate map browser helps to locate the existence and geological distribution of the isolate in the world map. The fast-evolving genetic markers can be used to examine the evolutionary processes acting on recently introduced pathogens and to infer their putative migration patterns, thus showing promise for the application of forensics to plant pathogens (Goss et al. 2009). Multi-locus section helps to simultaneously perform BLAST analysis using up to four loci and SSR marker-based search helps to search by SSR loci.

Tools used in working of *Phytophthora* database

Microsatellite markers database

To ensure the accuracy of species identification with future sequence deposits, prior to uploading the new data they will be compared against a sequence database of standard isolates representing all species in the genus. Any sequences with a questionable species classification will be re-checked against sequences from all isolates of the indicated species to assess if differences are due to intraspecific variation or if the isolate needs to be rechecked to correct the species classification. Recently, the database for microsatellite markers was designed to archive data from other species which help researchers to deposit their results.

Geographic information system (GIS) tools

The GIS tools are based on ortho-imagery from the National Aeronautics and Space Administration's Geospatial Interoperability Program and the USDA Farm Service Agency. The map with a zoom function can be overlaid with reference data layers, including political boundaries, rivers, roads, and railroads. These tools can be incorporated into a number of places (e.g., the species page, BLAST results, and microsatellite search) and allow users to visualize the distribution of chosen isolates.

Molecular Diagnostics

The "Molecular Diagnostics" section provides an overview of the following molecular diagnostics techniques used with *Phytophthora*: (i) DNA sequencing, (ii) gel-based identification of species, (iii) genus-specific diagnostic markers, (iv) species-specific diagnostic markers (v) identification of subpopulations, and (vi) macro/micro-arrays. A brief description of each method and its applications with key references are also provided.

Community Platform

This tool provides educational materials and helps PD users in contribution of new materials. It includes four sections: (i) *Phytophthora* Blog, (ii) Report a Problem (iii) Slide Presentations, and (iv) Publications. *Phytophthora* Database contains a sequence download page in which sequence data from up to nine loci have been generated from more than 2,000 isolates from known and novel species (94 in total) and deposited in PD so that these loci can be used for species identification (Blair et al., 2008; Park et al., 2008). "Report a Problem" function is used to make a suggestion for improvement. The "Slide Presentations" section currently holds presentations given at two international workshops on *Phytophthora* to make the information available to a broader audience. The "Publications" section provides a list of all *Phytophthora* related papers archived in PubMed.

Detection of the pathogen in asymptomatic tissue is a major challenge in preventing the spread of *Phytophthora*. With the increased volume of plant germplasm being transported across national boundaries each year it is obviously impossible for a country to test every plant that crosses its borders so a more strategic approach needs to be adopted. It is crucial that quarantine operators have access to the latest information on outbreaks of *Phytophthora* diseases in different parts of the world and in this regard initiatives such as *Phytophthora* database can play a very important role (O'Brien et al. 2009). Therefore *Phytophthora* database proves an effective progressive bioinformatics tool with chances for improvement and contribution of data by the global *Phytophthora* research community. The long-term sustainability and growth of this database depends heavily on contributions from the researcher community. There is a need to discover and describe novel species. The curated phylogenetic data in this database will help to facilitate the development of sequence-based detection and identification methods and the recognition of historical errors in strain identification and questionable species boundaries.

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