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Short communication

The database of PCR primers for phytopathogenic fungi

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Abstract

We present the first on-line searchable database of primer sets useful for the detection of plant pathogenic fungi. This web resource is implemented entirely with open-source software (PHP, MySQL). Primer set details can be retrieved by organism name, primer name, nucleotide sequence comparison, target DNA, PCR technique, author name, journal and year of publication. Each record is linked directly to other reference databases to allow easy access to the correct nomenclature, taxonomical position and anamorph/teleomorph connections of the pathogen, GenBank-deposited source sequences of the primer sets and reference contents. The database is open to user contributions and can be consulted at http://www.sppadbase.com. While currently devoted to fungal organisms, in a future perspective the database may host primer sets specific to other phytopathogens, such as viruses or bacteria, including microbial herbicides and biological agents relevant in agroterrorism.

The precise identification of microbials affecting economically important plants or plant products is an essential step for obtaining high-quality and safe production. Ecologically-friendly practices of modern agriculture require the adoption of diagnostic techniques able to detect minimum inoculum levels of pathogens in soil, seeds, transplants or crops, to limit epidemics and to address the adoption of rational and efficient control means. Moreover, there is an increasing public and official awareness of the potential threat of bioterrorism directed against food and agriculture (Monke, 2004). Rapid detection techniques for bioweapon agents are a critical need for the first-responder community.

Among the nucleic acid-based diagnostic techniques, those involving the Polymerase Chain Reaction (PCR; Mullis and Faloona, 1987) are most suited for early detection of phytopathogenic fungi, due to their high sensitivity and the potential for automation. There are many ways to select sequence data that could be used for primer design to detect a pathogen. These may include, for instance, Random Amplified Polymorphic DNAs (Welsh and McClelland, 1990; Williams et al., 1990), internal transcribed spacer (ITS) regions of the ribosomal RNA genes (White et al., 1990) or other specific gene sequences. Primer sets can be designed to target specificity at the genus, species, or physiological race levels, to distinguish a particular pathogen from closely related organisms.

One of the most tedious tasks for researchers and technicians is to find bibliographic references of published and validated specific primer sets for a given pathogen. Today, this can be performed by searching through the internet, abstract collections

and monthly journal tables of contents. Very few examples of specific primer set collections for phytopathogenic agents are available (Louws et al., 1999) while, among a number of molecular biology databases (Galperin, 2005), no on-line repository of primer sets of this kind is accessible. To overcome this lack of information, here we present the first on-line searchable database of primer sets useful for the detection and identification of plant pathogenic fungi.

The Database of PCR Primers for Phytopathogenic Fungi can be accessed at http://www.sppadbase.com. SPPADBASE is an acronym that stands for Specific Primers for Phytopathogenic Agents Data Base and is a logical choice to host primer sets specific to other phytopathogens, such as viruses or bacteria, including microbial herbicides and biological agents relevant in agroterrorism.

Primer sets can be searched through a quick or an advanced search input field. In the first stage, users can search for primers typing the genus or species name of the target organism, the forward or the reverse primer name, the template DNA, the PCR technique, an author name or the year of publication; the use of word parts is allowed. In the second stage, users can focus their searches by typing or selecting an appropriate search term; with this interface it is possible to retrieve primer sets using as the search term the database ID (known from a previous search), the primer sequence, the GenBank accession numbers and the reference journal.

The database records matching the query criteria are tabled in a search results page. For each set, the forward and the reverse primer couple, the target DNA, the PCR technique (if the data are available), and the bibliographic reference are displayed. Clicking on the reference, a pop-up window will open, showing reference details in brief; if a Pubmed ID is available, a link will open the corresponding page at NCBI. Clicking on the database ID of a selected set, the complete details will be shown.

The primer set detail pages are organized into the following sections:

1. *Internet resources*: This section allows queries with external search engines (Google, Altavista, Alltheweb) and other reference databases: the NCBI Taxonomy Browser shows the taxonomical lineage of the organism of interest, as it appears in the GenBank database, and provides direct access to the Entrez-deposited sequences obtained from each organism; the CABI Bioscience Index Fungorum provides the correct organism nomenclature based on a database of fungal names containing over 350,000 names of fungi at species level and below, derived from a number of published lists; the Centraal Bureau vor Schimmelcultures database provides the anamorph/teleomorph connections of the organism.
2. *Primer set*: This section shows the name and the sequences of the forward/reverse primers of the selected set, the amplicon size (if available), and a tool to perform BLAST similarity searches with the selected sequence. If the primer set was designed for real-time PCR experiments based on TaqMan™ chemistry, the probe name and sequence are also displayed.
3. *Source Sequence*: If the GenBank accession number of the primer's source sequence is available, it is displayed and linked directly to the NCBI GenBank flat file.
4. *Reference*: Complete reference details are shown here. If available, the article abstract and the link to the corresponding Pubmed ID are displayed.
5. *Submitter*: Users are invited to submit basic data (authors, reference) of missing primer sets. Submitter details will appear in this section.

Only published primer sets, that do not require further amplification product analyses for the identification of the target organism, have been considered for the construction of the live database. Primer set reference collection has been performed scanning the tables of contents of the most widely distributed phytopathology-related journals from the early 1990s to date. More than 25 reference sources are now monitored to maintain the reference database. A bimonthly updating schedule is planned.

The accuracy of the organism nomenclature, the taxonomical position and anamorph/teleomorph connection, as well as GenBank accession numbers reported can be checked querying external databases accessible from the primer set details page. However, we encourage users to send us notification

regarding missing data. Users can submit a missing primer set through an appropriate interface providing detailed literature reference information and/or uploading an electronic copy of the published article. Submitters also are asked to provide personal details that will appear linked to the newly added primer set, following the system administration review, in the live database.

The Database of PCR Primers for Phytopathogenic Fungi is a Web service implemented with PHP (http://www.php.net/), an open-source server-side scripting language, designed for generating HTML contents. MySQL (http://www.mysql.com/), an open-source database, is used as the data-management system.

The application has been optimized for the Microsoft Internet Explorer 5 browser or higher and it is best viewed at 1024×768 dpi screen resolution.

Application functionality also has been tested with other widely used internet browsers such as the Gecko codebase class-based browsers (e.g. Netscape, Mozilla), Firefox and Opera, running in Windows, Linux or Mac environment.

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