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The need for culture collections to support plant pathogen diagnostic networks

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Abstract

Plant-pathogenic microorganisms, by virtue of their size, similarity in disease symptoms and closely related morphologies, are notoriously difficult to diagnose and detect. Diagnosis gives proof as to the causal agent of disease and is important for developing appropriate control measures. Detection shows the presence of a microorganism and is of importance for safeguarding national and international trade. Live reference collections are required to characterize the taxonomy and function of microorganisms as a prerequisite to development of tools for diagnosis and detection. Two case studies will be presented in this paper to demonstrate the importance of microorganism collections for facilitating knowledge sharing and the development of identification methods. Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* and sharka disease of stone fruits caused by plum pox virus (PPV) are considered. Both diseases consist of different races/strains with different host specificities, but Fusarium wilt poses a threat to food security, while PPV poses a threat to trade due to its classification as a quarantine pest, since there is no anti-virus treatment available to control sharka disease in orchards. It is only through comprehensive collections of correctly identified and well-maintained strains representing the genetic diversity of a target organism that robust, specific, reliable and efficient diagnostic and detection tools can be developed.

Keywords: Fungi; Viruses; Microorganisms; *Fusarium oxysporum*; Plum pox virus; Sharka disease

1. Introduction

Plant diseases are caused by microscopic pathogens belonging to a wide range of taxonomic groups including nematodes, viruses, viroids, phytoplasmas, bacteria, and fungi. Pathogenic microorganisms are difficult to diagnose and detect as a consequence of their small size and thus rely on technologically advanced methodologies for recognition of chemicals, proteins or molecular fragments specific to different organisms. Similarly, morphological identification of species and variants and, further characterization of their function, requires a wealth of experience. Advanced and traditional methods must be combined, as genetic codes do not necessarily link to function due to differences in e.g. phenotypic expression. Further, traditional methods such as morphological identifications or systematics (taxonomy) is essential to the study and communication of plant pathology, diagnostics and disease control (Rosman and Palm-Hernández, 2008). However expertise in systematics is globally rapidly decreasing and provides further evidence of the need to centralize such capacity at centers of excellence such as collections (House of Lords, 2008).

The risk of a plant disease epidemic arises as a consequence of interactions between different factors such as environmental conditions, plant variety, pathogen type and human practices such as agronomic and land use management techniques and trade patterns. Moreover, increased human movement, globalization of trade, and propagation material

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and seed supply systems can influence the introduction and proliferation of diseases across the world. One such example is plum pox virus (PPV), the causal agent of sharka disease of stone fruits, which following its identification in Bulgaria in 1943 spread to several other European countries, Asia, North and South America, following the commercialization and global movement of infected germplasm.

Developing countries, which have much greater populations than developed countries, produce comparatively less food and suffer much greater losses due to plant diseases. This is because of a lack of technical capacity and infrastructural linkages between institutions so as to diagnose and react to plant disease. The increased emphasis on agricultural trade as a means of poverty reduction in developing countries has drawn attention to the need for international help in establishing and coordinating diagnostic services. The responsibility for safeguarding plants against invasive pathogens is officially held by national plant protection organizations (NPPOs) under the guidance of the International Plant Protection Convention (IPPC). Constraints and opportunities to better organize diagnosis of plant disease through links of networks, in both developed and developing countries, is addressed by the review of Miller et al. (2009). An evaluation of risk is required in order to prioritize steps towards practical intervention. If a disease is diagnosed, but is found to pose no risk for the quantity or quality of agricultural production based on experience from other countries, then interventions are not justified. In other words, diagnostic systems are needed which clarify current and future risks when developing and implementing adaptation strategies. Furthermore, particularly for Africa, routine use of rapid and robust diagnostics would facilitate the shift from current curative control of epidemics to a preventive approach. This requires development of diagnostic methods that encompass the diversity of each target pathogen species. In turn, this requires knowledge of, and access to, the genetic diversity of pathogen populations for each species. This can be most easily facilitated through collections of microorganisms that are recovered and maintained because of the threat they pose to the production or quality of each crop and products that are derived from them. The greater the linkages between collections, the better the knowledge of variance of particular pathogens and thus the more likely it is that diagnostic tools developed using these as raw materials will have global applications. A system for designating isolates for each pathogen species as reference strains, in terms of unique plant host or unique geographical origin from which they were recovered, is required to enable collections to communicate with one another. Collections of live cultures act as repositories that can be characterized in terms of evolutionary similarity to serve as a living reference collection for further research to determine functional differences or for the development of diagnostic tools.

Many organizations have been set up to manage and promote the use of culture collections, and a series of guidelines to optimize preservation and access are available (OECD – http://www.oecd.org/dataoecd/7/13/38777417.pdf; UKNCC – http://www.ukncc.co.uk; CABRI – http://www.cabri.org). Furthermore, the increasing availability of online databases, in principle, makes access to these collections much easier. Fungi, bacteria and virus accessions are available from various collections distributed throughout the world, but there is no centralized collection fully dedicated to storage of plant pathogens or a knowledge database dedicated to linking information on these accessions held across different collections. In fact, often each collection has a crop- or pathogen-type focus linked to research objectives or commercial activities rather than providing a service to store, characterize and distribute plant pathogens.

Most collections have a specific focus, and long-term preservation of a diversity of phytopathogenic microbes is an area generally overlooked. Genetic resources of plant pathogens have been reduced over the years and this process is continuing. Collections have often been generated and maintained by individual researchers, and they might no longer be available when the researcher changes his/her field of interest or retires. Retirement is a key feature in the drastic reduction of expertise in morphological identification of organisms (Summerell et al., 2003).

The most extensive living culture collection in the United States is the American Type Culture Collection (ATCC), which contains both type strains and representatives of patented strains. It is also a global bioresource center, as defined by the Organization for Economic Cooperation and Development (OECD). There are several other collections that include plant pathogens, namely the Central Bureau of Schimmelcultures (CBS) in the Netherlands, Collections of Microorganisms and Cell Cultures [Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)] in Germany, Belgian Coordinated Collections of Microorganisms (BCCM), the Collection Francaise des Bacteries Phytopathogenes (CFBP) in France, the International Collection of Microorganisms from Plants (ICMP) in New Zealand and the Plant Virus GenBank (PVGB) in South Korea.

Globally, the culture collection community is represented by the World Federation of Culture Collections (WFCC). The WFCC web-site (http://wdcm.nig.ac.jp/wfcc/) has become a clearing house for information on resources around the world. In the framework of the WFCC, the European Culture Collection Organization (ECCO), established in 1981, comprises about 60 members from about 22 European countries. Presently, it groups together 57 collections that hold over 350,000 strains of different organisms including fungi, bacteria and viruses. Among the European culture collections, the Italian National Collection COLMIA (WDCM945) groups together nine scientific institutions all belonging to the Agricultural Research Council (CRA) and each specialized in a specific group and/or related functional field of agromicrobes, including plant pathogens and entomopathogenic microorganisms. The http://www.collezionedimicrorganismi.com/site has been created in order to consult and eventually require strains present in the COLMIA collections.

A plant pathogen collection is commonly preserved to capture the variability of a species, in terms of pathogenicity, virulence or metabolite and/or toxin production. The service provided by a comprehensive plant pathogen collection for maintaining plant health management can be summarized by