Bulletin of Insectology ***64*** *(Supplement): S289-S290, 2011*
ISSN 1721-8861

Sharing information and collections on phytoplasmas: from QBOL to QBANK

Assunta BERTACCINI[1], Samanta PALTRINIERI[1], Olga MAKAROVA[2], Nicoletta CONTALDO[1], Mogens NICOLAISEN[2]
[1]*Dipartimento di Scienze e Tecnologie Agroambientali, Patologia vegetale,* Alma Mater Studiorum-*University of Bologna, Bologna, Italy*
[2]*Aarhus University, Department of Integrated Pest Management, Slagelse, Denmark*

Abstract

A total of 154 phytoplasma strains from 15 ribosomal groups were employed for barcode sequences production. Besides strains in periwinkle, 36 strains in natural infected plants such as napier grass, grapevine, plum, jujube, apple, pear, spartium, pine tree, hibiscus and erigeron were employed. Barcode sequences were produced for 16SrDNA, tuf and SecA gene for 36 phytoplasma strains, while for 54 strains 16SrDNA sequences were obtained, and for 118 and 89 strains respectively the tuf and secA barcode. All obtained sequences and protocol for extraction and PCR amplification will be available in Qbank.

Key words: phytoplasmas, collection, barcode, detection, quarantine.

Introduction

The increased international trade of plant material is increasing also exchanges of possible quarantine pathogens and a quick and reliable system for their identification is of the utmost importance.

DNA barcoding is a generic diagnostic method that uses short standardised genetic markers to aid species identification. The first genetic marker to be described as a "barcode" was the mitochondrial cytochrome *c* oxidase I (COI) gene which is used for species identification in the animal kingdom (Herbert *et al.* 2003). Among quality standards requirements for "barcodes" sequences are that the sequence data must be obtained from fully identified and vouchered specimens from a known origin with a unique identifier; sequence data must be at least 500 bp long and must be associated with trace files for the forward and reverse sequencing runs, and of forward and reverse primers used; primer sequences must be trimmed from the barcode sequence data (Hanner, 2009).

DNA barcoding protocols are under development and validation within the Quarantine organisms Barcoding of Life (QBOL) project financed by 7th framework program of the European Union. Using the developed DNA barcoding protocols, sequence data of EU regulated plant pests and phylogenetically related species was generated and will be made publicly available using the Q-Bank database.

The list of phytoplasma diseases that are of EU quarantine are summarized in table 1. It is important to underline that the quarantine list a number of diseases but it is well known that different phytoplasmas are associated to the same disease in diverse parts of the world. Therefore, phytoplasmas listed in table 1 were selected as main representatives of those associated with the same disease worldwide. Based on this knowledge and in order to provide the most useful and robust tool possible a number of phytoplasma strains belonging to the majority of described taxons were barcoded and will be deposited in the QBank.

Materials and methods

Fresh or frozen phytoplasma infected plant material from periwinkle or from natural plant hosts was used after a chloroform/phenol extraction of total nucleic acid (Prince *et al*., 1993). This DNA was then employed for sequencing the marker regions selected for reliable identification of quarantine phytoplasmas; for all markers a 400 - 600 bp fragment is suggested for the use of the barcode system.

A total of 154 phytoplasma strains from 15 ribosomal groups were employed for barcode sequencing. Besides strains in periwinkle also 36 strains in natural infected plants such as napier grass, grapevine, plum, jujube, apple, pear, spartium, pine tree, hibiscus and erigeron were employed. Strains belongs to the official collection of micropropagated phytoplasmas (Bertaccini, 2010), or were collected or provided by colleagues listed below. Specific protocol and primers are in validation phase in order to provide a quick and reliable tool for identification of quarantine phytoplasmas. The selected barcode regions are within the 16Sr DNA, tuf and SecA genes. The produced barcode sequences will be uploaded in the publicly available database that is in preparation (Qbank) where protocols for nucleic acid extraction and primers for amplification will also be available after the end of the validation process.

Table 1. List of EU quarantine phytoplasma-associated diseases.

Name of the disease	'*Candidatus* Phytoplasma'	16Sr DNA grouping of phytoplasmas Qbol target*	Other 16Sr DNA phytoplasmas associated with the disease*
Elm phloem necrosis	'*Ca.* P. ulmi'	16SrV-A	None
Peach rosette		16SrIII	16SrI
Peach X disease		16SrIII-A	None
Peach yellows		16SrIII	16SrI-B/16SrXII
Strawberry witches' broom		16SrI-C	'*Ca*. P. fragariae', 16SrI-B, 16SrXII
Apple proliferation	'*Ca.* P. mali'	16SrX-A	None
Apricot chlorotic leafroll	'*Ca.* P. prunorum'	16SrX-B	None
Pear decline	'*Ca.* P. pyri'	16SrX-C	None
Palm lethal yellowing		16SrIV	16SrI/16SrXXII
Witches' broom on *Citrus*	'*Ca.* P. aurantifolia'	16SrII-B	16SrVI, 16SrIX
Grapevine flavescence doreé		16SrV-C/-D	None
Potato stolbur		16SrXII-A	16SrI-A, 16SrI-C, 16SrII
potato purple top wilt	'*Ca*. P. americanum'	16SrXVIII-A	16SrVI

* Groups and subgroups are according with Lee *et al*., 1998 and as in Bertaccini and Duduk, 2009.

Results

For a total of 26 phytoplasma strains all the three barcode sequences were produced, while for 54 strains 16SrDNA sequences were obtained, and for 118 and 89 strains, respectively, tuf and secA barcode sequences were obtained.

For the naturally infected plants, 4 strains were sequenced in the 16S rDNA, while 74 sequences were obtained for tuf gene and 54 sequences for the secA gene. The project is still in progress and more sequences are under production.

Discussion

The three barcode sequences employed in the project will allow to unambiguously detect and identify quarantine phytoplasmas in a short time and with no need of specific phytoplasma expertise in the laboratory. From the research carried out, the tuf gene fragment was shown to be able to differentiate the majority of phytoplasmas enclosing those in the EU quarantine list (table 1) and together with the 16S rDNA appears to be very helpful in barcoding of phytoplasmas. Further work is in progress toward increasing number of sequences to be deposited in the Qbank.

Acknowledgements

The work was carried out as part of the project "QBOL: Development of a new diagnostic tool using DNA barcoding to identify quarantine organisms in support of plant health" EU/FP7.

We would like to especially thank Matt Dickinson, Geofrey Kawube, Michel Dollet, Bojan Duduk, Karen Gibb, Helena Guglielmi-Montano, Jean Hanson, Ing Ming Lee, and Bern Schneider for providing some of the strains.

A particular thank to Michael Kube and Jelena Mitrovic for producing and providing some of the 16SrDNA sequences together with the relative trace files and to Jennifer Hodgetts for allowing the use SecA primers prior to their publication.

References

BERTACCINI A., 2010.- [online] URL: http://www.ipwgnet.org/index.php?option=com_content&view=article&id=29&Itemid=5 [accessed 25 April 2011].

BERTACCINI A., DUDUK B., 2009.- Phytoplasma and phytoplasma diseases: a review of recent research.- *Phytopathologia mediterranea*, 48: 355-378.

HANNER R., 2009.- Data Standards for BARCODE Records in INSDC (BRIs).- *Database Working Group, Consortium for the Barcode of Life, proposed 6 November; 2005; revised 26 March 2009.*

HEBERT P. D. N., CYWINSKA A., BALL S. L., DEWAARD J. R., 2003.- Biological identifications through DNA barcodes.- *Proceedings of the Royal Society of London. Series B, Biological Sciences*, 270: 313-321.

LEE I-M., GUNDERSEN-RINDAL D., DAVIS R. E., BARTOSZYK I., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.

PRINCE J. P., DAVIS R. E., WOLF T. K., LEE I-M., MOGEN B. D., DALLY E. L., BERTACCINI A., CREDI R., BARBA M., 1993.- Molecular detection of diverse mycoplasmalike organisms(MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs.- *Phytopathology*, 83: 1130-1137.

Corresponding author: Assunta BERTACCINI (e-mail: bertaccini_a@biblio.cib.unibo.it), Dipartimento di Scienze e Tecnologie Agroambientali, Patologia vegetale, *Alma Mater Studiorum*-University of Bologna, viale Fanin 42, 40127, Bologna, Italy.