Molecular Diagnosis of Fungal Pathogens in Cotton

Bruce R. Lyon and L. Augusto Becerra-LopezLavalle
Australian Cotton Cooperative Research Centre, School of Biological Sciences A12,
The University of Sydney, Sydney, NSW 2006

Summary

Micro-organisms can be identified by the size or sequence of a portion of their DNA, and this information can suggest if they are likely to cause disease. Molecular plant pathology could revolutionise disease diagnosis in cotton crops by enabling fast and accurate identification of harmful fungi before severe disease symptoms develop.

Fungal pathogens of cotton

At least thirty different fungal pathogens are capable of infecting cotton, and the diseases that they cause can be of considerable economic importance. Diseases such as Verticillium wilt, Fusarium wilt and Black root rot have the potential to inflict serious losses on cotton crops. The organisms responsible for Alternaria leaf spot and Phytophthora boll rot can also cause significant damage during some seasons. Moreover, seedling establishment can be greatly influenced by a complex of fungal pathogens that affect young cotton plants.

Plant pathologists have traditionally studied plant diseases by recognising the characteristic symptoms of a disease or by physically isolating the disease-causing organism from infected plant tissue. A crucial step in diagnosing whether a disease is widespread in a crop is therefore to survey plants for disease symptoms or the presence of the suspected pathogen. Subsequent surveys can then determine if disease control methods have been effective.

Classical identification of plant pathogens

Classical microbiological techniques employed in plant pathology have relied on sampling plant tissue, soil or other relevant material and incubating it on synthetic growth media in the laboratory. Micro-organisms that grow from these samples can be observed for tell-tale visual characteristics such as colony appearance and colour. Alternatively they can be viewed under the microscope for distinctive cell shapes or reproductive structures, or subjected to other growth tests that differentiate particular strains.

Although classical microbiological techniques can be time consuming and laborious, they have been used to successfully identify the causal agents of numerous diseases of cotton and other crops. However, some researchers have recently estimated that less than 1% of microbial species will be detected under such circumstances, due to the selectivity of growth media and conditions. Hence, the vast majority of microbes from the environment that are not stimulated to grow under laboratory conditions will be overlooked.

Molecular identification of plant pathogens

Molecular methods offer an entirely new approach to the diagnosis of disease in crop plants. A DNA amplification technique known as the polymerase chain reaction (PCR) can be used to multiply specific regions of the DNA of any micro-organism living in or around a plant.

The internal transcribed spacer (ITS) region of the ribosomal DNA of fungi is one region that can be targeted in this procedure (Figure 1). Using the PCR technique, it is not necessary to firstly purify the organism and grow it to produce a visible colony of cells, and so the selective nature of laboratory culture can be avoided.

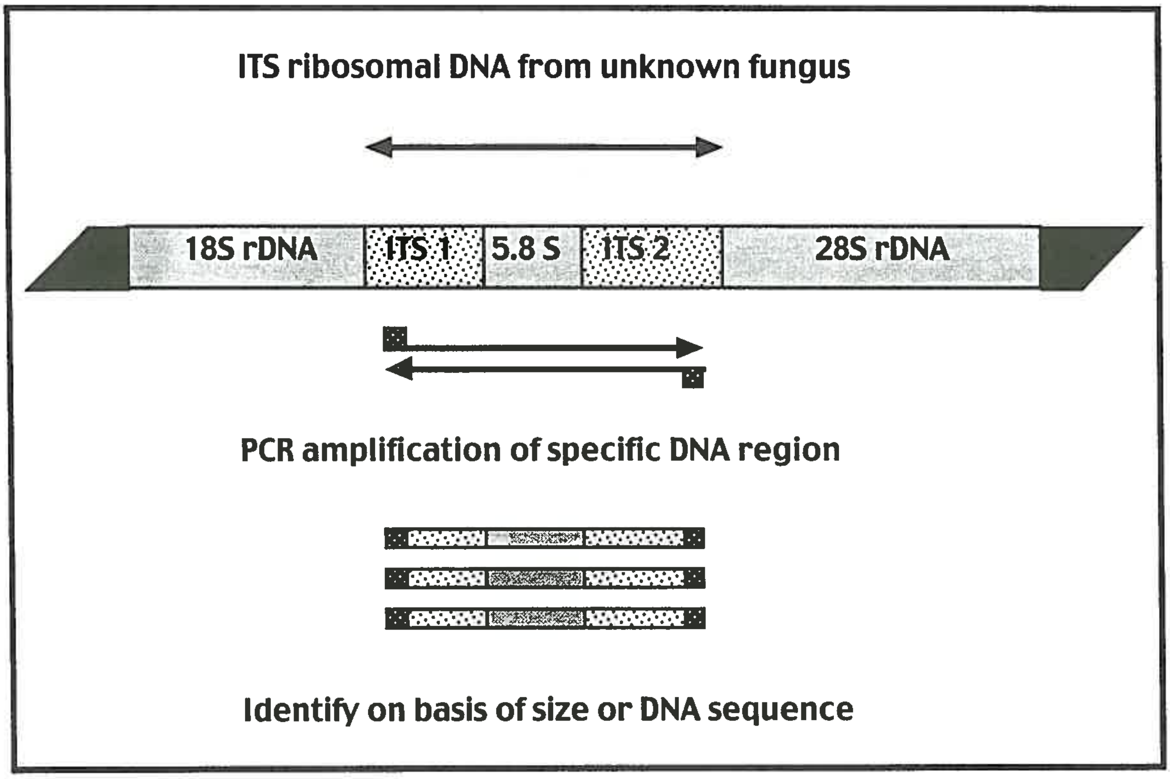

Figure 1. Diagram of the ribosomal DNA (rDNA) region of a fungal chromosome showing the location of the internal transcribed spacer (ITS) region. The ITS DNA region can be multiplied using the polymerase chain reaction (PCR) for the identification of potential plant pathogens.

Once a region of DNA is multiplied using the PCR, the sequential order of the four constituent molecules or 'bases' (designated by the letters A, C, G or T) in the DNA can be determined. Known as the DNA 'sequence', the order of bases in a given piece of DNA is usually unique to an organism, and can therefore act like a signature to confirm its identity. An unknown microbe can thus be identified by the size or sequence of a portion of its DNA, and this knowledge can in turn suggest whether that organism might cause disease.

Plant disease diagnosis using the World Wide Web

Researchers can now use the World Wide Web to access large international databases of DNA sequence information gathered from a host of organisms. Scientific research using such databases is known as Bioinformatics. New data is being added at an exponential rate, but already around 4,000 sequences for the well-studied ITS DNA region exist for fungi. A survey of this database revealed that, of 31 recognised fungal pathogens of cotton, 80% could be identified to the level of genus using currently available sequence data (Table).

ITS DNA sequences have been employed to help identify some 60 fungal isolates detected in the vascular tissues of cotton plants collected from a field near Narrabri, NSW in 1997. DNA sequences were determined for 15 representative fungi, and these were matched to the DNA database. Using this data, we were able to identify amongst others, four different species of *Fusarium*, two species of *Phomopsis*, a species of *Cladosporium* and a strain of *Alternaria alternata*. All of these fungi are known or potential pathogens of cotton.

Table. Fungal diseases of cotton, the causal organisms, and the number of complete DNA sequences for the internal transcribed spacer (ITS) region recognised at genus or species level.

Disease in Cotton	**Causal fungus**	**Genus**	**Species**
Anthracnose	*Glomerella gossypii*	1	0
Seedling Disease Complex	*Glomerella cingulata*	1	0
Ascochyta Blight	*Ascochyta gossypii*	0	0
Seedling Disease Complex			
Phytophthora Boll Rot	*Phytopthora capsici*	50	0
Diplodia Boll Rot	*Diplodia gossypina*	1	0
Alternaria Leaf Spot	*Alternaria macrospora*	24	0
Cercospora Leaf Spot	*Mycosphaerella gossypina*	2	0
Phomopsis Leaf Spot	*Phomopsis* spp.	115	8
Rhizoctonia Leaf Spot	*Rhizoctonia solani*	1	0
Seedling Disease Complex			
Areolate Mildew	*Mycosphaerella areola*	2	0
Powdery Mildew	*Salmonia malachrae*	0	0
	Leveillula taurica	0	0
Black Root Rot	*Thielaviopsis basicola*	1	1
Seedling Disease Complex			
Phymatotrichum Root Rot	*Phymatotrichum omnivorum*	0	0
Sclerotium Stem & Root Rot	*Sclerotium rolfsii*	1	0
Charcoal Rot	*Macrophomina phaseolina*	2	2
Southwestern Cotton Rust	*Puccinia cacabata*	56	0
Tropical Rust	*Phakopsora gossypii*	0	0
Seedling Disease Complex	*Fusarium moniliforme*	125	0
	Fusarium roseum	125	0
	Pythium aphanidermatum	3	1
	Pythium debaryanum	3	0
	Pythium heterothallicum	3	0
	Pythium irregulare	3	0
	Pythium polytylum	3	0
	Pythium splendens	3	0
	Pythium sylvaticum	3	0
	Pythium ultimum	3	1
Fusarium Wilt	*Fusarium oxysporum*	125	14
Seedling Disease Complex			
Verticillium Wilt	*Verticillium dahliae*	21	2
Witches' Broom (Escobilla)	*Colletotrichum gossypii*	0	0

Molecular diagnosis of cotton diseases

In complementary work, a comparison was made of the DNA sequences for the ITS regions of two important vascular wilt pathogens of cotton, namely *Verticillium dahliae*, the cause of Verticillium wilt, and *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov*), which is causing outbreaks of Fusarium wilt in cotton. The DNA sequence data has been used to design very specific molecular diagnostic assays that can be used to distinguish single or dual infections of cotton plants by these important pathogens (Figure 2).

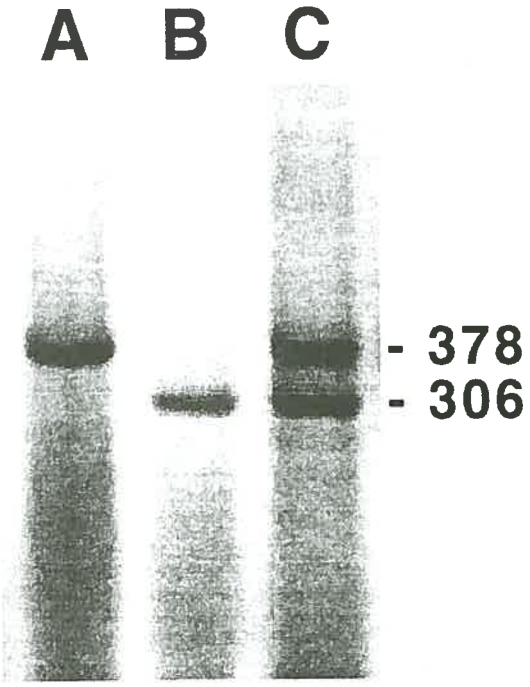

Figure 2. Analysis of DNA isolated from the vascular tissues of cotton plants inoculated with (A) *Fov*, (B) *V. dahliae* or (C) both *Fov* and *V. dahliae*. DNA regions of 378 and 306 bases in size are diagnostic for *Fov* and *V. dahliae*, respectively. The presence of the organisms deep within vascular tissue implies that, unlike other closely related strains, these isolates of *F. oxysporum* and *V. dahliae* are able to successfully infect cotton plants.

Molecular methods that utilise DNA sequence information can thus be employed to broadly survey the range of organisms infecting cotton plants or living in the soil around them. Furthermore, such methods can be fine-tuned for assays that can diagnose the presence, and even estimate the abundance, of specific plant pathogens. Such diagnostic assays would provide valuable information for the development and implementation of appropriate control strategies for diseases in cotton crops.

Acknowledgments

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Reference

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