



THE POLYMERASE CHAIN REACTION (PCR) AND PLANT DISEASE DIAGNOSIS : A REVIEW

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Abstract

Polymerase chain reaction (PCR) is a powerful scientific technique in molecular biology that amplifies genes or any fragment of DNA, making thousands to millions of copies of particular DNA sequence or from one molecule. With this system, minute amounts of DNA are often replicated very rapidly and thereby amplified to such an extent that the DNA becomes easy to detect, study and use for any given purpose. Three essential steps to PCR include (a) melting of the target (b) annealing of two oligonucleotide primers to the denatured DNA strands, and (c) primer extension by a thermostable DNA polymerase. Newly synthesized DNA strands serve as targets for subsequent DNA synthesis as the three steps are repeated up to 35-50 times. The specificity of the tactic derives from the synthetic oligonucleotide primers, which base-pair to and define each end of the target sequence to be amplified. The use of PCR grew rapidly in plant pathology, as in other disciplines, with the introduction in 1988 of *Thermus aquaticus* (Taq) DNA polymerase. This enzyme exhibits relative stability at DNA-melting temperatures, which eliminates the need for enzyme replenishment after each cycle of synthesis, reduces PCR costs and allows automated thermal cycling.

Key words : Polymerase chain reaction, Plant Disease Diagnosis, *Thermus aquaticus*

Introduction

In Scientific American, Karry Mullis summarized the procedure: "Beginning with one molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in a day. The reaction is easy to execute. It requires no quite a tube, a couple of simple reagents, and a source of warmth." He was awarded the Nobel prize in Chemistry in 1993 for his invention, seven years after he and his colleagues at Cetus first put his proposal to practice. However, some controversies have remained about the intellectual and practical contributions of other scientists to Mullis' work, and whether he had been the only inventor of the PCR principle.

Advantages

The technique offers several advantages compared to more traditional methods of diagnosis :

1. Organisms need not be cultured prior to their detection by PCR.
2. The technique possesses exquisite sensitivity, with

the theoretical potential to detect a single target molecule in a complex mixture without using radioactive probes.

3. It is rapid and versatile.
4. Unlike serology, the development of reagents with narrow or broad specificities is accomplished almost at will with lower cost.

Application of PCR

Selective DNA isolation

PCR allows isolation of DNA fragments from genomic DNA by selective amplification of a selected region of DNA. This use of PCR augments many methods, like generating hybridization probes for Southern or northern hybridization and DNA cloning, which require larger amounts of DNA, representing a selected DNA region. PCR supplies these techniques with high amounts of pure DNA, enabling analysis of DNA samples even from very small amounts of starting material.

Amplification and quantification of DNA

Because PCR amplifies the regions of DNA that it

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targets, PCR are often used to analyze extremely small amounts of sample. This is often critical for forensic analysis, when only a trace amount of DNA is out there as evidence. PCR can also be utilized in the analysis of ancient DNA that's tens of thousands of years old. These PCR-based techniques are successfully used on animals, like a forty-thousand-year-old mammoth, and also on human DNA, in applications starting from the analysis of Egyptian mummies to the identification of a Russian tsar.

Quantitative PCR methods allow the estimation of the quantity of a given sequence present during a sample—a technique often applied to quantitatively determine levels of organic phenomenon. Real-time PCR is a longtime tool for DNA quantification that measures the buildup of DNA product after each round of PCR amplification.

PCR in diagnosis of diseases

PCR permits early diagnosis of malignant diseases like leukemia and lymphomas, which is currently the highest-developed in cancer research and is already getting used routinely. PCR assays are often performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity that's a minimum of 10,000-fold above that of other methods. PCR also permits identification of non-cultivable or obligate or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses from tissue culture assays and animal models. The basis for PCR diagnostic applications in microbiology is that the detection of infectious agents and therefore the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes.

Viral DNA can likewise be detected by PCR. The primers used got to be specific to the targeted sequences within the DNA of an epidemic, and therefore the PCR are often used for diagnostic analyses or DNA sequencing of the viral genome. The high sensitivity of PCR permits virus detection soon after infection and even before the onset of disease. Such early detection may give physicians a big lead in treatment. The amount of virus ("viral load") during a patient also can be quantified by PCR-based DNA quantitation techniques.

More sensitive monitoring of microbial infection or colonization of individual plants can be accomplished with PCR, especially since it is now possible to amplify target sequences in situ in tissue or individual cells (Chiu *et al.*, 1992; Nuovo *et al.*, 1992). Natural microbial populations or genetically engineered organisms or their nucleic acids are often sensitively monitored in soil, insect vectors, water, or air by PCR. Observing the movement of genetic

elements through plants or microbial populations will also be facilitated by PCR. For example, PCR could be used to follow fungal dsRNAs or linear dsDNA plasmid migration during hyphal anastomosis. Some fungal dsRNA elements are possible determinants of virulence (Azzam & Gonsalves, 1991), while others confer hypovirulence to their host fungi. For instance, one viral dsRNA confers hypovirulence to its host, the chestnut blight fungus, *Cryphonectria parasitica*, and rapid and sensitive monitoring of this genetic element will facilitate its use as a biological control agent of chestnut blight (Choi & Nuss, 1992).

Other applications

PCR is often employed in phylogenetic studies to amplify genetic material, usually ribosomal or transfer RNAs or the spacers between them. Amplified sequences are compared and used to discern evolutionary relationships of organisms, including plant pathogens or soil microorganisms (Barry *et al.*, 1991; Kocher, 1992; Mazzarella *et al.*, 1992; O'Neill *et al.*, 1992). For example, Lee & Taylor (1992) use the internal transcribed spacers of the rDNA region to infer a phylogenetic tree of several *Phytophthora* species. Recently, analysis of sequences of ribosomal-protein genes demonstrated that aster yellows-type mycoplasma like organisms (MLOs), which are pathogenic for members of the genus *Oenothera*, are more closely related to *Acholeplasma laidlawii* (a nonsterol-requiring acholeplasma) than they are to sterol-requiring animal mycoplasmas (Lim & Sears, 1992). Phylogenetic studies facilitated by PCR are also used to measure biodiversity in particular ecosystems. In addition, denaturing gradient gel electrophoresis of amplified 16S ribosomal RNAs from complex ecosystems has recently been employed to profile microbial communities (Muyzer *et al.*, 1993).

Classification of pathogen isolates may also be simplified using a combination of morphological and molecular characteristics. For instance, obtaining the sexual stage of *G. graminis* var. *graminis* takes weeks in the laboratory, and it would be faster to identify this fungus based on its morphologically characteristic adhesive cells (hyphopodia) and a positive PCR test (Elliott *et al.*, 1993). PCR is already being used to advance studies of host-pathogen interactions. Cloning or gene-synthesis strategies for pathogen or pathogen-induced host genes or cDNAs often include PCR procedures (Atreya *et al.*, 1992; Choi & Nuss, 1992; Hayes & Buck, 1990; Jaeck *et al.*, 1992). PCR could also be used to construct pathogen genomic or cDNA libraries (Zhang *et al.*, 1992), or could be used to construct libraries of host or pathogen genes that are differentially expressed during the infection process

(Duiguid & Dinauer, 1992; Hara *et al.*, 1991). In addition, genetic mapping is facilitated by PCR-generated markers used in linkage studies (Mazzarella *et al.*, 1992; Tragoonrung *et al.*, 1992; Versalovic *et al.*, 1991) that will likely assist in mapping and cloning disease resistance loci of host plants.

Other applications of PCR include DNA sequencing to work out unknown PCR-amplified sequences during which one among the amplification primers could also be utilized in Sanger sequencing, isolation of a DNA sequence to expedite recombinant deoxyribonucleic acid technologies involving the insertion of a DNA sequence into a plasmid or the genetic material of another organism. Bacterial colonies (*E. coli*) can be rapidly screened by PCR for correct DNA vector constructs (Borja and Ponz, 1992). PCR can also be used for genetic fingerprinting; a forensic technique wont to identify an individual or organism by comparing experimental DNAs through different PCR-based methods.

Some PCR 'fingerprints' methods have high discriminative power and may be wont to identify genetic relationships between individuals, like parent-child or between siblings, and are utilized in paternity testing. This technique can also be wont to determine evolutionary relationships among organisms.

Conclusions

Because of its sensitivity, PCR will continue to be used diagnostically to detect genomes or antigens of microorganisms that are scarce, difficult to culture, or difficult to identify once cultured. Pathogen screening of seeds, stored grain, micropropagated tissue culture, or vegetatively propagated plants will be assisted by PCR or related techniques. PCR or immuno-PCR may prove to be more sensitive and reliable methods for detection of toxins, pesticides, or other undesirable chemicals, microorganisms, or ingredients in our food. For example, PCR was used to detect wheat contamination (wheat-specific DNA) in dietary non-wheat products (Allmann *et al.*, 1992), and it was used detect the human pathogen, *Listeria monocytogenes*, in naturally contaminated food samples. As PCR methods for detection of pathogens become available, more research will focus on using these as tools to study pathogen populations, biology, ecology, variability, and host-pathogen interactions.

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