Molecular Diagnostic Tools and Techniques in Disease Management

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Introduction

Accurate identity and early detection of pathogens is a crucial step in disease management and environmental monitoring. The failure to adequately identification and detect plant pathogens using traditional, culture based morphological strategies has led to the development of nucleic acid based molecular approaches. Molecular diagnostic began to develop a real momentum after the introduction of polymerase chain reaction (PCR) within the mid-1980s. To date, an increasing number of agricultural research and studies center is adapting molecular techniques for habitual detection of pathogens. With the advances in molecular biology and biosystematics, the strategies to be had have developed significantly inside the final decade, and besides conventional PCR other technologically superior methodologies along with the real time PCR and microarrays which allows limitless multiplexing functionality have the ability to carry pathogen detection to a brand new and improved stage of efficiency and reliability.

Detection of specificity and sensitivity

Sensitivity and specificity are numeric measures of effectiveness of a detection system. Diagnostic specificity is described as a measure of degree to which the technique is stricken by non-target components present in a sample, which may bring about false positive responses. Diagnostic sensitivity is described as a degree to hit upon the target pathogen within the sample, which may bring about false negative responses. Thus, a high degree of diagnostic accuracy is characterized by means of the ability to discover, real and precisely the target microorganism from a sample without interference from non-target components. The high degree of sensitivity of molecular methods made pre-symptomatic detection and quantification of pathogens possible. One of the most important advantages that molecular based detection has over conventional diagnostic detection strategies is the high specificity. That is the capacity to distinguish intently related organisms.

The specificity of PCR, be it conventional or real-time, relies upon the designing of proper PCR primers which are unique to the goal organism. Highly conserved gene areas are frequently the target for designing primers. Closely associated microbial species regularly range in a single-nucleotide polymorphism (SNPs) to few bases in such genes. A wide variety of inhibitors are reported. Although their mode of action isn't always clear, those inhibitors are believed to interfere with the polymerase activity for amplification of the target DNA.

Determination of viability

Nucleic acid-based detection techniques currently carried out in pathogen detection are based on nucleic acid hybridization or PCR. These strategies may be designed to hit upon both DNA and mRNA. Whereas DNA based detection approach is frequently more straightforward than that of mRNA, the steadiness of DNA ends in the opportunity that DNA based methods yield superb results from non-possible or dead pathogens. One of the main goals of pathogen detection system, besides figuring out the presence and lack of the pathogen, is the viability since in the occasion of fine result, it is crucial to recognize whether the pathogen detected poses chance to crop production, public health or food safety. In order to circumvent this problem many studies consider enrichment culturing (BIO PCR) instead of direct PCR. While the system allows the detection of best viable cells and facilitates in removal of feasible PCR inhibitors, it isn't appropriate approach for quantitative assay.

Pathogen quantification

Quantification of a pathogen upon its detection and identification is an important component as it could be used to estimate its ability risk regarding disorder development, establishment and unfold of inoculum and monetary loss. In addition, it provides statistics for well-informed disease control decisions. PCR is good for detection of small quantity of the target. Three PCR variants namely restriction dilution PCR, kinetic PCR and competitive PCR were used for quantitative evaluation of DNA. However, all are based on end point measurements of the amount of DNA produced which makes estimation of initial concentration of DNA and quantification rather problematic. A low abundance target with excessive genetic similarity to a microarray probe might produce a more potent hybridization signal in comparison with a higher abundance goal that has low similarity to the equal microarray probe. Due to the advancement of fluorogenic chemistry, a second generation PCR known as real time PCR has come to be a rising approach for the detection and quantification of microorganisms within the environment.

In PCR the target DNA sequence is amplified over a number of denaturation-annealing-extension cycles. In a conventional PCR, handiest the very last concentration of the amplicons can be monitored the use of a DNA binding fluorescent dye. However, in the quantitative real time PCR, the concentration of the amplicons is monitored at some point of the amplification cycles by the use of a collection of fluorescent reagents. The fluorescence intensity emitted during this procedure displays the amplicons concentration in real time.

Multiplexing

Crops may be infected with the aid of several pathogens and they'll be present in plant life in complexes. Therefore, it is important to develop appropriate technology that may detect multiple pathogens simultaneously. Multiplex PCR, a PCR variant that is designed to amplify multiple targets by means of the usage of multiple primer sets within the equal reaction, has been applied in lots of tests. Multiplex PCR assays can be tedious and time ingesting to establish and generally requiring lengthy processes. Among the drawbacks of such variation PCR assays are that the sensitivity is decreased notably and the number of different targets to be amplified in one assay is limited.

Moreover, the dynamic variety of the target in the sample to be tested isn't continually reflected in the final results of the test. The real-time PCR offers better multiplexing opportunities; however, multiplexing remains limited via the supply of dyes emitting fluorescence at extraordinary wavelengths. Thus, detection of extra than few pathogens is currently now not possible, by the use of these systems. In the beginning, DNA microarray was designed to look at gene expression and generate single nucleotide polymorphism (SNP) profiles and it is currently a brand new and emerging pathogen diagnostic technology which in theory, gives a platform for limitless multiplexing functionality. The principle of microarray is the hybridization of fluorescently labelled sequences or goals to their complementary sequences spotted on strong surface, including glass slides, serving as probes. The unlimited functionality for simultaneous detection of pathogens makes microarrays to be a method with a potential capability of detecting all applicable pathogens of a specific crop. In plant pathology, the technique was carried out for figuring out oomycete, nematode, bacterial and fungal DNA from pure cultures.

Conclusion and future outlook

Currently more and more research centre and laboratories are using molecular methods for detection and identification of pathogens. The development of more versatile robust and cost-effective systems, allowing for greater sensitivity and specificity, elevated throughput and detection of multiple microbes will continue over the coming years. Pathogen detection is only the first step; quantification and isolate characterization are crucial elements in diagnostics. Diagnostic technology is moving from qualitative to quantitative and there is no doubt that most tests will be quantitative in the future. Microarray-based technology is the most suitable technique for multiple pathogen detection in a single assay. Currently microarrays can be expensive for routine application. However, with reducing fabrication costs, the cost per sample will be significantly lower. The effort to add a quantitative aspect to microarrays must continue and more work is needed to address the challenges of working on environmental samples where contaminants (humic matter, organic substances, heavy metals etc.) may interfere with DNA hybridization and affect the performance of microarrays. Adding innovative molecular tools for differentiating viable from non-viable organisms should be given emphasis in developing diagnostic assays.

However, while the specificity and sensitivity of detection of pathogens are greatly progressed and pathogen detection is becoming simpler and faster, there are still important challenges, technical and economic nature, which want to be addressed to ensure the emergence of reliable detection machine for routine applications. Pathogen detection is simplest the first step; quantification and isolate characterization are crucial factors in diagnostics. Diagnostic generation is shifting from qualitative to quantitative and there may be absolute confidence that most tests might be quantitative within the future. Microarray-based era is the maximum suitable approach for a couple of pathogen detection in a single assay. Adding revolutionary molecular tools for differentiating viable from non-viable organisms should accept emphasis in developing diagnostic assays.

Reference

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