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Metagenomics and its Role in Plant Pathology

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SUMMARY

With the advancement in technology new tools are coming into existence for efficient utilisation in the field of science. Metagenomics is one of the most recent technologies which make identification of several microbes residing in an ecosystem easier. Since, the microbes have huge diversity; identity of millions of them is still unknown, and the field of metagenomics permits us to get an insight into it. The technique can be efficiently utilised in the field of plant pathology in detection of plant pathogens leading to an adequate management of plant disease.

INTRODUCTION

Metagenomics can be defined as the study of genetic material recovered directly from environmental samples. It is a broad field and can also be referred as environmental genomics, ecogenomics or community genomics. It is based on studies of ecological diversity of uncultured and cultural microorganisms using molecular biology. For the metagenomic analysis of microbial populations in phytopathology, the total content of nucleic acids involved with the disease is used. Because of its ability to reveal the previously hidden diversity of microscopic life, metagenomics offers a powerful lens for viewing the microbial world that has the potential to revolutionize understanding of the entire living world. As the price of DNA sequencing continues to fall, metagenomics now allows microbial ecology to be investigated at a much greater scale and detail than before. Recent studies use either "shotgun" or PCR directed sequencing to get largely unbiased samples of all genes from all the members of the sampled communities.

Steps and process involved in metagenomics:

- Sample collection
- DNA extraction
- Sample pre-preparation
- Sample analysis
- PCR
- DNA sequencing
- DNA microarray
- Bioinformatics studies
- Results and interpretation

In metagenomics, the construction of genomic libraries with direct isolation of DNA from soil microorganisms is required. DNA must be pure and intact, but is commonly contaminated with substances of soil in the sampling sites, and therefore the construction of genomic libraries needs the removal of these products. This is important when working in metagenomic isolation of soil microbes. For construction of genomic libraries, vectors commonly used are plasmids due to their high cloning efficiency, especially if *Escherichia coli* is used, where the optimal insertion size is 40 kb. Cloning a DNA fragment length leads discernment metagenomic populations of specific soil microorganisms, which includes genomes of many previously unknown organisms. For example, when the forest soils were analysed it indicate that certain area tested has an abundance of acid-bacteria and that the population of this bacterium contains large portions of this gene that induces the production of acids. This was determined after assessing the production of the clone. Genomic libraries can be constructed with 1µg of soil metagenomic DNA which may contain 30 000 to 50 000 clones with approximate size of 35 kb (Lee et al., 2004). The library represents several hundreds of thousands of microbial genomes.

Role of Metagenomics in Plant Pathology

Detection and diagnosis forms an inevitable part of plant pathology as accurate diagnosis of plant pathogen will result in effective management of a plant disease. Metagenomics play an eminent role in detection

of pathogens in complex environments. Many of the times a plant disease is caused by a complex of multiple microbes; the most conventional method of culturing microbes is not feasible for several microbes especially biotrophs; in many plant diseases the causal organism is not known; a number of soil microbes and the interactions among them affecting plant heath are concealed under soil, all these factors provides a room for the modern technology to come into action and provide us with appropriate results. This is where metagenomics can play a great role. The study of uncultured microbes may be carried out by metagenomics, which gives a broad sight for the investigation of microorganism origins and function in the environment. Metagenomic sequencing of a plant infected by viruses involves the sequence of all pathogens present, the extraction of RNA from infected plants and the production of complimentary DNA by random amplification method, and finally the sequence of possible plant pathogens. RNA viruses and viroids can be sequenced in any stage of the infection process. RNA is extracted and the viral cDNA is obtained; the sequencing can be done only with mRNA and rRNA from several phytoplasmas, bacteria and fungi present in the sample.

The detection of seedborne pathogens, which tend to become widely distributed across many nations due to current trends in specialized production areas. Untargeted detection techniques provide the ability to quickly test from the presence of multiple pathogens in a single analysis. Amplicon metagenomics enables identification of new sequences from still unknown microorganisms and provide preliminary information about their phylogenetic collocation. Shotgun sequencing can offer significant amounts of information on a new causal agent of plant diseases, as the near-complete or entire genome of an organism can be obtained by sequencing. This sequence data can greatly contribute to risk assessment by national regulatory agencies. The technique does, however, include a risk of prematurely identifying commensal or saprophytic organisms as pathogens. Rapid detection of outbreaks and determination of their origin using the genome sequence of a pathogen is gaining prominence in applications related to food security.

Advantages of metagenomics:

- One of the uppermost advantages of the present technique is its power to study many microorganisms in a single experiment that could not be possible with the conventional microbiology methods.
- It eliminates the need to grow organisms in the laboratory, thus eliminating the biases associated with traditional, cultivation-based methods like plate counts.
- Besides, the sequencing-based metagenomic technique is highly accurate and faster. We can also quantify the amount of viral load.
- Can retrieve unknown gene sequences leading to the "discovery" of novel microorganisms and functional genes.
- Prior knowledge of the microbial community composition or function is not needed for the analysis.

Disadvantages of metagenomics:

- The techniques are so costly.
- Sequencing may miss important microorganisms present in the sample at low concentrations
- Functional genes usually not analyzed
- So many microbes are still unknown to us therefore we don't have enough information to compare and study novel microbe sequences.

CONCLUSION

Metagenomics provide a new rationale and effective methodology for identifying the primary causative agents. It has emerged as a modern tool for the detection of several unknown microbes present in different environments. Like every other technology, it also has some merits and demerits but making the best utilisation of all the merits of a technology is need of the hour, for effective management of several plant diseases. If properly utilised the technique can be very helpful for prompt detection of pathogens in the planting material to be traded across boundaries and can prevent the spread of the pathogens. It also is an important tool for the detection of beneficial microbes present in the rhizosphere that can promote plant health.

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