Metagenomics and Meta Transcriptomics

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Shotgun metagenomic research has been of significant importance in the characterization of the taxonomic and functional profiles of microbial communities during the past two decades. Next-generation sequencing (NGS)-based metagenomics technologies were initially employed in the clinical domain for the purpose of pathogen detection (Gu *et al*., 2019). Subsequently, these technologies have gained increasing prominence in the field of plant disease diagnostics (Piombo *et al*., 2021). Shotgun metagenomics is a technique that enables the comprehensive sequencing of the complete genomes of microorganisms found in a given sample. This sample might include many sources, such as symptomatic or asymptomatic host plants, as well as soil and other environmental matrices. This approach is considered reliable and effective for the detection and identification of pathogens. Consequently, a meticulous diagnosis has been established (Mechan *et al*., 2020). This technology offers a significant benefit by eliminating the need for prior isolation of pathogens in culture. This is particularly advantageous for obligate pathogens, which cannot be cultured. Additionally, this technology does not rely on specific probes or primers for individual pathogens, thereby avoiding the biases commonly associated with PCR and metabarcoding amplification.

The complexity of the analysis involved in sequencing data necessitates a thorough examination of potential flaws. Following the quality control of the reads and the assembly of metagenomic contigs, the data undergoes a process known as "binning" to generate entire genomes. By enabling the construction of comprehensive genomes through the use of specialized software tools like BUSCO (Simao *et al*., 2015) and CheckM (Parks *et al*., 2015), the process of binning makes it easier to identify novel pathogens. But this method has problems when used on samples with fragmented genomes. This is likely because of things like not enough coverage or the presence of very different communities with closely related species (Knight *et al*., 2018). Shotgun metagenomics, when implemented with a high sequencing depth, enables accurate taxonomic identification at the species level. This method is particularly effective for identifying the most prevalent species, especially those with whole genomes stored in databases. In recent literature, notable instances of achieving species-level resolution through the utilization of high-throughput sequencing (HTS) technologies have been documented.

For instance, Yang *et al*. (2022) employed the Oxford Nanopore Technologies MinION sequencing platform to differentiate between the boxwood blight fungal pathogens *Calonectria pseudonaviculata* and *Calonectria henricotiae*. A comparative analysis of the capabilities of two third-generation sequencing devices, namely MinION by Oxford Nanopore Technologies and Sequel by Pacific Biosciences. The objective of their investigation was to evaluate the efficacy of these instruments in the identification and diagnosis of fungal and oomycete infections found in Pinaceae and Solanum tissues. This assessment was carried out using a metagenomic method.

The integration of metagenomics and meta-transcriptomics is of significant importance in order to comprehensively understand the genetic capabilities and metabolically active species within the entire microbiome. High-throughput sequencing (HTS) plays a crucial role in the field of meta-transcriptomics since it enables the sequencing of the entire transcriptome. This comprehensive approach facilitates the identification of isoforms, unique transcripts, alternative splice variants, and subsequently, genomic variants. In their study, Garalde *et al*., (2018) employed Oxford Nanopore Technologies to perform direct sequencing of natural RNA. This approach circumvented the need for reverse transcription and amplification, enabling the acquisition of whole RNA sequences. The annotation of expressed genes is facilitated by the absence of

introns, which allows for rapid identification. However, in the context of metagenomics, the accurate taxonomic classification of fungal transcripts at the species level heavily relies on the presence of full genomes. In a recent study, Chialva *et al.,* (2019) employed an RNA-seq dataset that had been previously created for tomato plants in order to identify and analyze the taxonomic and functional diversity of the root microbiota.

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