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Review

Novel Molecular Techniques for Identifying Agricultural Microorganisms

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Abstract: Agriculture involves activities aimed at improving soil quality for food production. In this environment, microorganisms play a vital role, positively and/or negatively affecting plant growth. Given this impact, knowing the microbiota associated with agricultural systems and phytopathogens is crucial. The microbial culture method has proven ineffective in identifying microorganisms in agricultural systems, and more effective methods with greater scope for their identification currently exist. This review compiles updated information on new methods for studying microorganisms in the agricultural system, such as metagenomics, and new proposals for microorganism identification methods, such as Raman spectrometry, nanotechnology, and phytopathogen biosensors. In addition, it discusses the strengths and limitations of the new methods for microorganism identification.

Keywords: agricultural system; microorganisms; microbial identification methods

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1. Introduction

Microbiology focuses on isolating and identifying microorganisms (bacteria, fungi, and viruses), both known and unknown. This work requires a long and careful procedure using different strategies to identify microorganisms at the genus and species level [1]. Microbiological culture media have been a key tool for successfully isolating microorganisms, while biochemical reactions and phenotypic characteristics are key elements for their identification [2]. Currently, diverse culture media are used, depending on the sample and its associated microorganisms. However, depending on the type of microorganism and its growth on the culture medium, it will take time for a visible colony to form, which is the main criterion for isolating microorganisms. The time to obtain a colony often varies, but it is estimated to take 24–48 h for fast-growing microorganisms. Subsequently, the colonies are selected based on their phenotypic characteristics (e.g., size, color, edge, and surface) and Gram staining for bacteria and reseeded to obtain biomass (24 h). Later, in the case of bacteria, they are subjected to a panel of biochemical assays that take 24–48 h to obtain the results and subsequent identification. This procedure is successful, and, in some laboratories, it is routine and is considered the gold standard method [2].

Despite its virtues, the gold standard method has disadvantages, one of which is that most microorganisms associated with a biological or inorganic sample, such as soil, cannot be grown in standard laboratory culture media. Indeed, it is estimated that 90% of the microorganisms associated with a biological sample are unculturable [3], so only a small microbial fraction is recovered [4]. Another disadvantage is that its procedures require substantial time and, in some circumstances, standardized techniques for the correct interpretation.

Other alternative methods have been developed in recent decades to improve the identification of microorganisms, especially unculturable ones. Nucleic acid-based techniques, such as the amplification of specific DNA sequences by polymerase chain reaction

(PCR) [5], and immunological techniques, such as the enzyme-linked immunosorbent assay (ELISA) [1], are suitable for reducing the time required to detect and identify microorganisms and are highly sensitive. They also do not require the isolation of the microorganism for identification. Other new techniques for identifying microorganisms have been developed, such as fluorescence in situ hybridization (FISH), matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), and nanoparticle-based sensors. However, they require knowledge about the target microorganism, such as the sequences of specific genes or common/specific antigens, to develop adequate protocols for microbial detection. Moreover, they require high-tech equipment and qualified technicians, which limits their use [6]. In this review, we will address different novel strategies for microbial identification.

2. Nucleic Acids

Using nucleic acids from microorganisms as target molecules for identification has been crucial. One technique that uses these molecules is PCR, which has significantly reduced the identification time. This versatile technique has also been coupled with numerous other techniques, including isothermal amplification, high-resolution melting analysis (HRMA), next-generation (NGS) and third-generation (TGS) sequencing, and additional amplification strategies that are currently emerging. We will highlight some of them below.

2.1. Isothermal Amplification

Isothermal amplification methods are conducted at a specific temperature, so they do not require a thermocycler. We briefly discuss some of these methods. Rolling cycle amplification begins with a single strand of circular nucleic acid hybridizing with a specific primer. Then, a DNA polymerase (e.g., the high-fidelity Phi29) performs complementary strand synthesis on the primer several times [7].

Strand-displacement amplification involves aligning random hexamer primers to a double-stranded template DNA, followed by single-stranded DNA synthesis by a high-fidelity DNA polymerase. This process results in the displacement of the template DNA strand during synthesis [8].

Helicase-dependent amplification involves a double-stranded DNA template being opened by a DNA helicase, leaving regions of single-stranded DNA where specific primers hybridize. Then, these primers are extended by a DNA polymerase, generating new DNA [9].

Recombinase polymerase amplification involves a primer bound to a recombinase proteinase complex, which recognizes its specific sequence and recombines by opening the strands of the template DNA. Single-stranded DNA-binding proteins stabilize the strand unbound by the primer. Then, the recombinase protein separates, leaving the primer free to be extended by a DNA polymerase such as Bsu, forming the complementary strand [10].

Loop-mediated amplification (LAMP) is conducted in two steps. In the first step, four primers bind to the target DNA, two at each end. Next, a DNA polymerase with DNA strand displacement activity synthesizes the complementary strand, which forms a loop structure at one end. A loop structure also forms at the other end of the template DNA strand, generating a bell-shaped DNA structure. In the second cyclic amplification step, the primers hybridize to the loop region of the DNA stem-loop, initiating the synthesis and amplification of the DNA strands [11].

These methodologies offer the advantage of not requiring a thermocycler, which reduces cost. In addition, they are more specific and exhibit higher performance in some instances, as exemplified by LAMP. A variant of LAMP was developed, the reverse transcription loop-mediated isothermal amplification (RT-LAMP) method [12,13], which has enabled the rapid detection of pathogenic viruses of agricultural interest, such as the potato leafroll virus [14] and the chrysanthemum stem necrosis virus from chrysanthemum and

tomato [15]. Another variant of LAMP is the immunocapture RT-LAMP method, which detects strains associated with *Citrus tristeza* virus symptoms [16].

2.2. High-Resolution Melting Analysis (HRMA)

HRMA is complementary to PCR and is performed after DNA amplification (post-PCR). It is based on the generation of melting curves that depend wholly on the amplicon sequence [17]. The melting curves generate a fingerprint that can be used to identify microorganisms [18]. Furthermore, when HRMA was coupled with a machine that reads curve classification algorithms, it was 90% accurate in microbial identification from positive culture samples. An HRMA-PCR assay has been developed to identify black *Aspergillus* species in grape simplex [19]. Moreover, HRMA-PCR identified and differentiated 28 fungi belonging to the genera *Alternaria*, *Penicillium*, *Epicoccum*, *Fusarium*, and *Trichoderma* from 28 wheat endogenous fungal isolates [20].

2.3. Next-Generation Sequencing (NGS)

Current state-of-the-art DNA sequencing techniques comprise three generations. First-generation DNA sequencing is based on the chain termination method and offers a nucleotide read length of 500–1000 base pairs (bp). Second-generation DNA sequencing, also known as NGS, includes pyrosequencing, sequencing by synthesis, and sequencing by ligation. These methods provide nucleotide read lengths of 50–500 bp. TGS employs single-molecule sequencing, with read lengths of approximately 10 kilobases (kb). First-generation sequencing is exemplified by the Sanger method; second-generation sequencing is represented by commercial platforms by Roche (Indianapolis, IN, USA), Illumina (San Diego, CA, USA), and Applied Biosystems (Norwalk, CT, USA); and TGS is represented by platforms from Pacific Biosystems (PacBio; Menlo Park, CA, USA) and Oxford Nanopore Technologies (ONT; Oxford, UK).

It is evident that NGS methods have revolutionized the DNA sequencing field since they have significantly accelerated the throughput and reduced the time required to obtain results. However, these methods have a significant drawback in that they produce short nucleotide reads. Advances in sequencing chemistry have enabled the generation of real-time nucleotide reads, which correspond to TGS. TGS can generate sequencing reads longer than 10 kb, which is crucial for enhancing and facilitating the quality of genome assembly and the analysis of genomic structure [21]. The first TGS technology was the HeliScope platform, which uses fluorescently labeled nucleotides followed by library preparation [22]. However, it is expensive and slow, and the sequencing has high error rates and produces very short reads. While Illumina introduced a library preparation kit for synthetic long reads, the methodology uses classical short-read sequencing [21].

TGS technologies have been developed to address the limitations of NGS. In recent years, TGS technologies have grown and improved read lengths. Currently, two different TGS platforms have been developed: PacBio and ONT [23–25].

The typical sequencing workflow for NGS and TGS includes library preparation, sequencing, data analysis, and bioinformatics [26]. The description of this workflow is complex and outside the scope of this review; for more details about these platforms, we recommend reading the review [27]. However, we will briefly mention the principles of these two technologies. The PacBio technology is called Single Molecule, Real-Time (SMRT) and is based on silicon chip wells. These wells, or zero-mode waveguides (ZMWs), are microscopic chambers where high-fidelity DNA sequencing reactions occur in real-time [24]. The DNA to be sequenced is fragmented, circularized, and ligated with primers that generate loops at the ends of the fragments. These processed fragments are loaded into and immobilized at the bottom of the ZMWs. In the ZMWs, labeled nucleotides are added by the enzyme DNA polymerase, which is bound to the DNA fragments, to begin DNA replication. When a nucleotide is incorporated into the newly synthesized strand, fluorescent light is recorded and associated with a specific base [25]. The main benefits of PacBio technologies are average read lengths of 15,000–20,000 bp, allowing easy genome

assembly; high sequencing accuracy, up to 99.9%; the ability to sequence DNA with high GC content; and direct methylation detection.

The ONT technology is similar to PacBio. However, it uses a nanopore system in which the DNA or RNA fragment passes through a microscopic hole in a nanopore embedded within an electroresponsive membrane, during which changes in electrical current due to the varying properties of the nucleotide bases are detected in real-time [28,29]. This technology has read lengths of 10 kb to 4 megabases.

The two technologies have potential applications in genomics, transcriptomics, epigenomics, and metagenomics. Metagenomics focuses on studying microbial communities living in natural ecosystems in symbiosis with plants or animals, which is achieved by analyzing the total microbial genetic material from the collected sample [30]. Sequencing hypervariable regions 1–6 of the 16S ribosomal RNA (rRNA) gene has been the most widely used method to characterize and classify microbial communities [31]. Two approaches are used in metagenomic analysis: 16S rRNA and shotgun sequencing. The 16S rRNA sequencing aims to identify microorganisms at the genus or even species level for taxonomic classification. Shotgun sequencing aims to completely sequence the genomes of microorganisms to provide greater genomic coverage and data.

While NGS has been widely used for metagenomics, it is limited because it does not provide good phylogenetic resolution, with results reaching the genus level but rarely the species level because NGS cannot sequence the entire 16S rRNA gene [32]. TGS technologies can sequence longer DNA fragments, making them suitable for metagenomic analysis [33]. Given this advantage, TGS technologies can be applied in agriculture. PacBio sequencing successfully identified the microbiome profile of soils contaminated with heavy metals [34]. Similarly, ONT sequencing was used to explore the microbial communities associated with extreme soils from different locations, such as the cryoconite holes on Svalbard glaciers, the Greenland Ice Sheet, and the Austrian Alps [35].

Toxin contamination is another problem in agriculture that occurs in soil and water and affects plants. Shiga-toxin-producing *Escherichia coli* (STEC) is the most common cause of diarrheal diseases and can be present in agricultural waters. Nanopore sequencing has been used to rapidly detect and classify STECs in contaminated irrigation water via genome characterization [36].

Metagenomics studies have greatly increased knowledge of the composition, functionality, and dynamics of the soil microbiome, helping to understand that plants can withstand climatic changes through intimate interactions with microbial communities in their environment. Soil metagenomics data have helped to provide potential tools for designing and evaluating ecosystem restoration strategies for sustainable agriculture [37]. In addition, cereal metagenomics has revolutionized food security by harnessing the beneficial interactions between cereals and their microbiota [38].

Legume-based crop rotation is commonly recognized for its efficiency in mitigating greenhouse gas (GHG) emissions. A soybean-radish rotation field study was designed to determine how the microbial community contributes to GHG emissions. Metagenomics showed a decrease in the relative abundance of Proteobacteria and an increase in the relative abundance of *Acidobacteria*, *Gemmatimonadetes*, and *Chloroflexi* in these crops. In addition, the metagenomic data clarified that bacterial carbohydrate metabolism substantially increased during the rotation process while formaldehyde assimilation, methanogenesis, nitrification, and dissimilatory nitrate reduction decreased. These findings demonstrate that the response of the soil bacterial microbiome is linked to GHG-associated metabolism during soybean-radish rotation [39].

Plant-associated diazotrophs are related to plant nitrogen supplementation. One study examined the diazotrophic community in multiple compartments (soil, epiphytic, and endophytic niches of the root, leaf, and grain) of three cereal crops (maize, wheat, and barley). Its metagenomics data showed that their diazotrophic communities were dominated by a few taxa, represented by *Methylobacterium*, *Azospirillum*, *Bradyrhizobium*,

and *Rhizobium*. In addition, 37 new species were identified that have genes related to multiple nitrogen metabolic processes [40].

Metagenomics studies have revealed the function of plant microbiota, such as a microbiota-driven regulatory pathway controlling root branching plasticity that could contribute to plant adaptation to different ecosystems [41]. Another study demonstrated the contribution of the microbiota to novel cadmium tolerance mechanisms in wheat and the changes in soil fungal pathogens that increase plant damage [42]. *Metagenomics is a powerful tool for understanding plant–microorganism interactions, and several ongoing studies are using this approach to advance knowledge of the agricultural microbiome to help improve, restore, and adapt to the climate adaptation of agricultural crops*.

2.4. Fluorescence In Situ Hybridization (FISH)

FISH has the peculiarity of visually detecting microorganisms in a sample, allowing the interaction of microorganisms with their environment to be explored. Therefore, FISH does not require the isolation and cultivation of microorganisms. FISH involves designing a fluorescently labeled oligonucleotide (probe) that specifically recognizes a DNA sequence in the genome of the target microorganism [43]. This fluorescently labeled oligonucleotide is introduced into the cells, where it hybridizes with the genomic DNA under specific temperature, humidity, and dark conditions. The successful and specific binding of the oligonucleotide with its target DNA is visualized under a fluorescence microscope. FISH can be applied to different samples, such as tissue biopsies and soil samples, to examine microorganisms of interest [44]. It can be quantitative by changing the microbial concentration and allowing the localization of the microorganisms in the sample.

The unicellular *alga Chlamydomonas reinhardtii* is a model organism for photosynthesis, chloroplast genesis, and flagellar motility [45]. Using fluorescent compounds to detect algae is complicated by the autofluorescence of chlorophyll. Therefore, *Chlamydomonas reinhardtii* is used as a model to standardize fluorescence labeling. FISH has been used to determine the in situ distributions of specific messenger RNAs and proteins in *Chlamydomonas reinhardtii* [46]. The major order in the Basidiomycota phylum corresponds to the order Sebacinales, mycorrhizal fungi, distributed across all continents in subtropical climates and associated with orchids, liverwort thalli, and Ericaceae as ectomycorrhizal and endomycorrhizal fungi [47]. They are also symbiotic with various monocotyledonous and dicotyledonous plants [48], including crop plants such as barley, maize, and tomato. For the first time, the intimate association between *Serendipita indica* and *Rhizobium radiobacter* was reported using FISH, showing the *Rhizobium* bacterium to be a plant growth enhancer [49]. In addition, adding nitrate to the rice root increased the number of nitrate-reducing bacteria detected by FISH, including members of the genera *Bacillus*, *Dechloromonas*, and *Aquaspirillum* [50]. Reducing nitrate in the root inhibits methanogenesis via competition for electron donors and the accumulation of toxic intermediates [51]. While the *Nitrospirillum amazonense* strain CBAmC is a nitrogen-fixing bacterium used for sugarcane inoculation, little is known about its establishment and colonization in sugarcane cultures. FISH showed that the CBAmC strain establishes itself on different sugarcane tissues, especially in the root [52].

3. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS detects the molecular mass/charge of a microorganism's proteins, producing a pattern of mass spectral fingerprints unique to a microorganism that can be helpful for microbial identification. MALDI-TOF MS is a fast and sensitive method that can detect microorganisms from a microbial colony at a genus and species level [53].

The MALDI-TOF MS is a soft ionization method, allowing the microorganism's proteins to be ionized and evaporated [53]. The different ionized proteins are separated by mass via their TOF, the starting point for detecting intact microorganisms. The detector generates spectral signals for each protein in a mass-to-charge (m/z) detection range from

1000 to 20,000, within which most ribosomal proteins are found [54]. Finally, the mass spectrum obtained is compared with those stored in a database to identify the microorganism (Figure 1). In addition, the biological sample is prepared by mixing it with a matrix, which is then crystallized. Different compounds can be used as matrices, and their selection will depend on the biomolecules to be analyzed and the type of laser used. The matrix comprises small acidic molecules with strong absorption at the laser wavelength used. The most common compounds that function as a matrix are 2,5-dihydroxybenzoic acid, alpha-cyano-4-hydroxyciannamic acid, sinapinic acid, ferulic acid, and 2,4-hydroxy-phenyl benzoic acid.

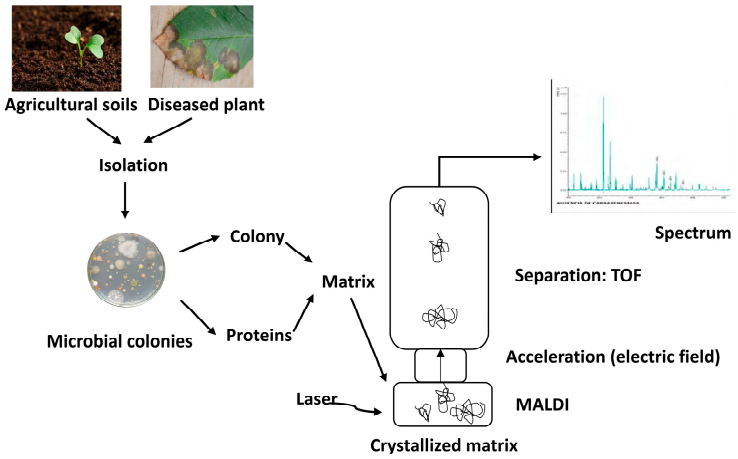


Figure 1. Matrix-assisted laser desorption ionization (MALFI) time-of-flight (TOF) mass spectrometry. Microorganisms are isolated from agricultural soil or diseased plant samples and grown in culture media. Next, a microbial colony or proteins extracted from the microorganism are mixed with the matrix, placed on the MALDI plate, and allowed to crystallize. Then, a laser beam desorbs the proteins, and the ionized proteins travel through the flight tube and are separated by mass (m) and charge (z), allowing their identification via the m/z spectrum.

Considering the MALDI-TOF MS conditions is crucial since they can alter reproducibility. Indeed, large changes in conditions can cause significant alterations in the m/z spectrum of the same microbial species. These conditions are the type of MALDI-TOF MS instrument, the matrix, the age of the microorganism, the sample, the matrix ratio, the culture medium, and growth conditions.

The advantage of MALDI-TOF MS is that microbial identification is fast since it is executed from a colony grown in a culture medium, saving time in identifying microorganisms. While acquiring the equipment can be expensive, running costs are generally low [55].

Endophytic bacterial diversity in the culturable fraction of the roots, stems, and leaves of high selenium-accumulating plants *Stanleya pinnata* and *Astragalus bisulcatus* was assessed using MALDI-TOF MS, identifying the bacteria *Variovorax*, *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Paenibacillus*, *Advenella*, *Pantoea*, and *Arthrobacter* [56].

Regarding phytopathogens, *Pantoea stewartii* subsp. *Stewartii*, which causes Stewart's wilt in sweetcorn, was isolated and identified by MALDI-TOF MS from different parts of infected plants [57]. In addition, while the pathogens *Acidovorax oryzae*, which causes bacterial stripe in rice, and *Acidovorax citrulli*, which causes bacterial fruit blotch in cucurbit plants, are very difficult to differentiate, MALDI-TOF MS combined with Fourier transform infrared spectroscopy successfully differentiated them [58]. Moreover, MALDI-TOF MS differentiated 43 strains of the genera *Dickeya* and *Pectobacterium* at the species level [59]. Furthermore, MALDI-TOF MS analysis of 54 *Trichoderma* strains isolated from soil samples collected from garlic and onion crops revealed the genetic variability of *Trichoderma* species, identifying *T. asperellum*, *T. asperelloides*, *T. afroharzianum*, *T. erinaceum*, *T. hamatum*,

T. lentiforme, *T. koningiopsis*, *T. longibrachiatum*, and two new species, *T. azevedoi* sp. nov. and *T. peberdyi* sp. nov [60].

In addition to identifying microorganisms of agricultural interest, MALDI-TOF MS has been used to rapidly identify microorganisms in biofertilizers with high precision, safety, and efficacy to improve their quality. Other examples of MALDI-TOF MS applications are identifying an endophytic bacterium in a biofertilizer for canola crops [61] and evaluating raw cheese as a source of microorganisms for a biofertilizer [62].

4. Sensors

Despite the effectiveness of the above methods, new technologies are needed that are easier, faster, more specific, more sensitive, more profitable, and more appropriate for real-time monitoring at low cost and without requiring highly qualified personnel. One alternative with all these characteristics is biological sensors, which are also specific to the microorganism of interest. Compared to the standard methods, these sensors generate results in less time and are easy to use; they are practically ideal for field applications, which is vital for agriculture.

The development of sensors to detect microorganisms has increased in recent years. A sensor is an analytical device that can specifically recognize a microorganism through the interaction of a molecule on its surface with a molecule or phenotypic characteristic of the microorganism. This interaction produces a physicochemical translation signal that is converted into an electrical signal [63]. Sensors commonly comprise two units: a receptor molecule that specifically recognizes a component of the microorganism of interest (named a recognition element) attached to the sensor platform, and a translator that converts the recognition event into a measurable and interpretable signal. The recognition element can take various forms, such as tissue, cells, enzymes, antibodies (Abs), nucleic acids, microorganisms, and organelles. The signal translator also varies; the most common is an electrochemical signal, but it can also be a magnetic, micromechanical, optical, piezoelectric, thermometric signal, or a combination of one or more of them [64].

While one application of these sensors is identifying microorganisms of agricultural interest, this does not indicate that it is their main application since they have been used in three agrifood areas: food safety, food quality, and process control. Regarding food safety, sensors are used to detect xenobiotic compounds such as additives, pesticides, fertilizers, heavy metals, bacterial toxins, and mycotoxins [65]. Regarding food quality, the sensors are used to assess food composition (sugars, amino acids, alcohols, organic acids, and cholesterol) and shelf life (compounds involved in rancidity, maturity, and freshness index) [66]. Regarding process control, the sensors are used to monitor fermentation, pasteurization, and cheese making [67].

4.1. Immune-Based Sensors

Immunosensors are a type of sensor that detects a target antigen (Ag) present on the microorganism of interest via a specific Ab, forming a stable Ag–Ab complex that serves as a capture agent. This type of sensor mainly uses nanoparticles modified with Abs to allow specific recognition of the target Ag present on the microorganism of interest. Some nanoparticles are designed to generate a colorimetric response, which occurs because the Ag–Ab complex causes their aggregation, inducing a color change [68] (Figure 2). This immunosensor is widely used for bacterial detection, mainly with Ab-conjugated gold nanoparticles that aggregate after their specific interaction with the target bacteria [69]. In other immunosensor systems, Ag–Ab binding can generate a non-color-based, measurable signal for the sensor translator system. Immunosensors can be classified into three main types based on the signal translator mode: electrochemical (amperometric, conductometric, impedimetric, and potentiometric), optical, and piezoelectric [70].

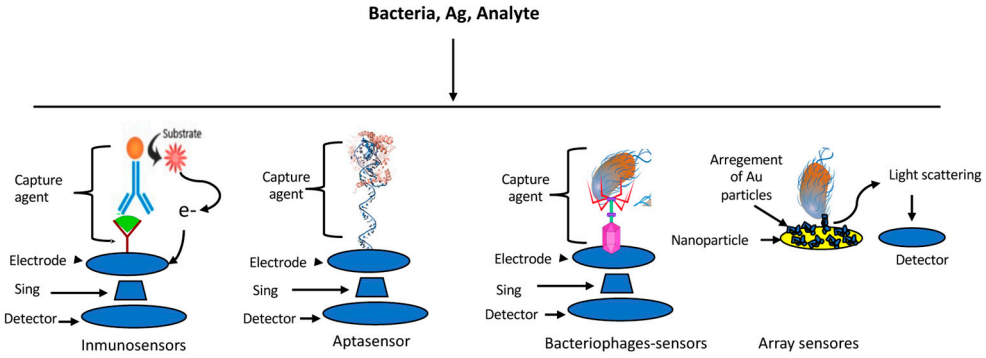


Figure 2. Bacteria, antigens (Ag), or target analytes are exposed to different sensors. Their recognition elements specifically capture the target compound, generating a chemical reaction or change in the environment that is captured by an electrode. Then, the electrode sends an electrical, optical (e.g., color or fluorescence), or vibrational signal, which is detected and interpreted by a device. In immunosensors, the sandwich system consists of an antibody coupled to an enzyme. In aptasensors, the recognition elements (e.g., a nucleic acid or peptide) vary, with a specific bacteriophage functioning as a recognition element in bacteriophage-sensors. In sensor arrays, the recognition element is a nanoparticle with its own design characteristics (i.e., size, arrangement, and surface), and its binding to the microorganism generates light scattering.

4.1.1. Electrochemical Immunosensors

Immunosensors often use an electrochemical translator. The principle of this method is that the surface of the immunosensor acts as an electrode, which is attached to a specific enzyme coupled to an Ab or fluorescent molecule. Ag recognition by the Ab activates the enzymatic or fluorescence signal, which the surface electrode captures as an electrochemical signal. Sandwich-type electrochemical immunosensors use two Abs: the capture Ab and the detection Ab (often labeled with an enzyme or fluorescent molecule). The capture Ab is linked to the immunosensor and recognizes the target microorganism. Later, the detection Ab is added, which also recognizes the target microorganism and produces a signal via enzymatic activity. The product of the catalytic reaction generates an electrical charge that is detected by the sensor's electrode [71], which converts this signal into an electrical signal, such as current, resistance, or voltage, that can be measured and displayed on the detector [71].

An electrochemical immunosensor was developed to detect and monitor an enzyme from *Agrobacterium* strain CP4 in transgenic plants. Since the enzyme 5-enolpyruvylshikimate-3-phosphate synthase isolated from *Agrobacterium* strain CP4 (CP4-EPSPS) is only present in transgenic crops, it can be used as a biomarker. A portable electrochemical immunosensor was designed to detect CP4-EPSPS and was successfully applied to transgenic crops. It detected CP4-EPSPS quickly and with high sensitivity (0.050 ng mL^{-1} of CP4-EPSPS), making it a cost-effective sensor for monitoring transgenic plant crops [72].

Detecting mycotoxins in food is crucial for food safety since their consumption can harm human health. An electrochemical immunosensor incorporated into a microfluidic cell was developed to detect the citrinin (CIT) mycotoxin produced by *Aspergillus*, *Monascus*, and *Penicillium* species. This immunosensor could efficiently detect CIT in cereal samples, specifically rice samples [73]. Another electrochemical immunosensor was designed to detect the microtoxin ochratoxin A (OTA) in wine grapes (Cabernet Sauvignon, Malbec, and Syrah) post-harvest tissues [74]. These studies highlight the importance of using immunosensors to detect unwanted microbial compounds.

4.1.2. Optical Immunosensors

Optical immunosensors detect optical changes caused by the Ag–Ab interaction, which generates an evanescence field when reflected and incident rays interfere with each other. An example is the fiber-optic immunosensor, in which the Ag–Ab reaction is

immobilized on the surface of the fiber and a fluorescent light is produced when a laser beam is incident, which is detected and measured by the sensor. This type of sensor has been used to detect *Clostridium botulinum* toxins in agricultural foods, with the botulinum toxin detected at very low concentrations of 5 ng/mL within one minute with high specificity [75]. Detecting botulinum toxin in industrialized foods is vital to their quality control because its consumption causes severe neurological damage in humans.

4.1.3. Piezoelectric Immunosensors

Piezoelectric immunosensors are made of quartz crystal materials and have an Ab or Ag immobilized on their surface. An external alternating electric field or a pH change can be applied to this material to cause an oscillation frequency proportional to changes in the quartz crystal mass. The interaction of the Ab with the Ag changes the oscillation frequencies when subjected to an external alternating-electric field. Notably, this system can be affected by factors such as conductivity, electrode morphology, density, dielectric constant, liquid temperature, and viscosity. This system has been used to detect *Bacillus anthracis* spores in agricultural soils [76]. Detecting *Bacillus* spores is crucial since they cause zoonotic diseases such as anthrax, which can be fatal in humans.

4.2. Aptasensors

Monoclonal Abs are widely used in immunosensors and have many advantages for identifying microorganisms. However, they are difficult to obtain, with their production and purification being a long and expensive procedure. In addition, their solubility and stability are important. Therefore, an alternative for sensors is aptamers, which are small molecules with a unique arrangement that allows the recognition of specific molecules. These aptamers can be peptides, short single-strand DNA or RNA sequences (20–60 nucleotides), which form a structural fold, allowing the recognition or binding of the bacterium, proteins, or enzymes with high affinity and specificity [77] (Figure 2). There is great interest in aptamers due to advantages such as low production cost, easy production, low immunogenicity, high chemical stability, high binding affinity, and high reproducibility.

Nucleic acid aptamer sensors are the most popular and use various signal translation strategies, including colorimetry, chemiluminometry, electrochemistry, fluorometry, and fluorescence. Aptamer-based sensors are designed to recognize different targets. For example, they can recognize toxins, proteins, or nucleic acids from a pathogen or the whole cell [78].

Aptasensors have been used in agriculture. For example, an aptasensor was used to detect OTA in red wine samples with 100% efficiency, indicating its applicability for OTA detection with the advantage of being economical, specific, sensitive, fast, and easy [79]. T-2 toxin is the most potent and toxic mycotoxin produced by various *Fusarium* species. It can affect human health and is widely distributed in field crops and stored grain. An electrochemical aptasensor with a nonenzymatic signal amplification strategy was developed to detect the T-2 toxin. It was used to detect the T-2 toxin in beer samples and could have potential applications in analyzing other foodstuffs [80].

4.3. Bacteriophage-Based Sensors

Aptamers have some limitations, mainly in their structural stability, with their half-life being short when exposed to the environment or changes in temperature or pH, making them ineffective in detecting microorganisms. A different approach is to use microorganisms as sensor recognition elements, such as bacteriophages, which express proteins that specifically recognize components of their target bacterium, helping them to act as a selector for it. The disadvantage of using bacteriophages is that they only recognize a specific bacterium. However, they have the advantages of being cheap, easy to produce, and having greater stability than aptamers and Abs, enabling them to tolerate large environmental changes (Figure 2). This type of sensor is an alternative for detecting pathogens in plants of agricultural interest. These bacteriophage-based sensors have detected bacteria

such as *E. coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *Xanthomonas campestris* [81]. *X. campestris* is important because it is a phytopathogen of *Philodendron scadens* subsp. A new bacteriophage-based nano-biosensor was developed, and the electrochemical impedimetric method was fully optimized and applied to quantitatively detect *E. coli O157:H7* in food. The sensor detected this foodborne pathogen with high specificity and an excellent recovery rate of 90.0–108% [82]. This *E. coli* serotype is common in food and is the primary cause of foodborne diarrheal outbreaks in the population.

4.4. Array-Based Sensors

As previously mentioned, a sensor typically contains the recognition element as part of its composition. However, some sensors, such as array-based sensors, do not use a recognition element to detect and discriminate against microorganisms [83]. Array-based sensors are mainly nanoparticles with different sizes, chemical compositions, and physicochemical characteristics. Consequently, the nanoparticle arrangement will confer different affinities toward different bacterial species. Therefore, the binding of a bacterial species to a plasmonic nanoparticle causes different signals in the form of light scattering, fluorescence emission, or UV absorbance levels (Figure 2).

Based on the characteristics of their nanoparticle array, gold-silver alloy nanoclusters were developed to detect and discriminate sulfur-producing bacteria [84]. This type of sensor has also been used to detect different types of agricultural compounds. For example, the colorimetric sensor array real-time monitoring system with multivariate analysis was developed to efficiently discriminate potato varieties with different types and degrees of corruption [85]. Similarly, a smartphone-based colorimetric sensor array system and gas chromatography technique effectively differentiated rice varieties and had the advantages of being simple, rapid, and low cost [86].

4.5. Optoelectronic Nose

This system is very similar to the previous one. However, its arrangement consists of different compounds that act as substrates or indicators for the products released by a bacterium, generating a unique response pattern, mainly colorimetric, that acts as a specific optical fingerprint for each bacterium. This system can act as an olfactory system because bacteria produce volatile organic compounds (VOCs) that are collected and respond to different substrates or indicators (recognizing aromas). The generated fingerprint profile can be compared with data from a predefined library for bacterial identification [87]. An optoelectronic nose was developed with 36 indicators to detect different VOCs. When this system was applied to a bacterial mixture (*P. aeruginosa*, *Enterococcus faecalis*, and *E. coli*), the chemical or color responses of the indicators produced different fingerprints for each bacterium, allowing their classification with high sensitivity [88]. Detecting these bacterial species associated with fecal contamination in food crops is important as an indicator of agricultural irrigation water quality.

5. Optical Detection Methods

Another alternative for detecting microorganisms is using light as the main component. This type of method falls within the category of optical methods. Due to their simplicity and speed in detecting microorganisms, these methods have gained significant attention and have been applied in different areas, including agriculture. Their basic principle is the interaction between light and microorganisms; that is, when light of different wavelengths falls on a microorganism, it will change (absorption, scattering, polarization, interference, or fluorescence), which is then detected. This light change will depend on the intrinsic characteristics of the microorganism, reducing the problems of misinterpretation or variability. These methods require isolated or pure microorganisms for detection. One of the most efficient optical methods for detecting microorganisms is vibrational spectroscopy.

5.1. Vibrational Spectroscopy

Vibrational spectroscopy determines the vibrational changes in molecules (mainly chemical bonds) when energy is applied to them. Each molecule has different vibrational behaviors, generating characteristic spectra. Therefore, the molecules present on the surface of a microorganism can be detected, enabling its identification [89]. Vibrational spectroscopy requires less microbial biomass than standard molecular identification methods, with identification from a microbial colony taking six to eight hours [90]. These vibrational techniques have the advantage of being faster than other techniques. The vibrational spectrum can also be obtained directly from the habitat of the microorganism without extra processing, making this technique simpler and faster. Another advantage of spectroscopic techniques is that they can potentially identify microorganisms without isolation and culturing.

5.1.1. Raman Spectrometry

Infrared and Raman (RS) spectroscopy are the most widely used spectroscopies and are based on optical vibration. Both spectroscopies are complementary, and RS is the most commonly used for identifying microorganisms. In RS, the interaction between a photon and the chemical bonds of a molecule results in a transfer of energy between them, producing an inelastic scattering event due to the energetic change of the incident photon, which is measurable via the wavelength. The wavelength change is based on the specific vibrational modes the molecules maintain within a sample, producing a unique RS pattern [91] (Figure 3).

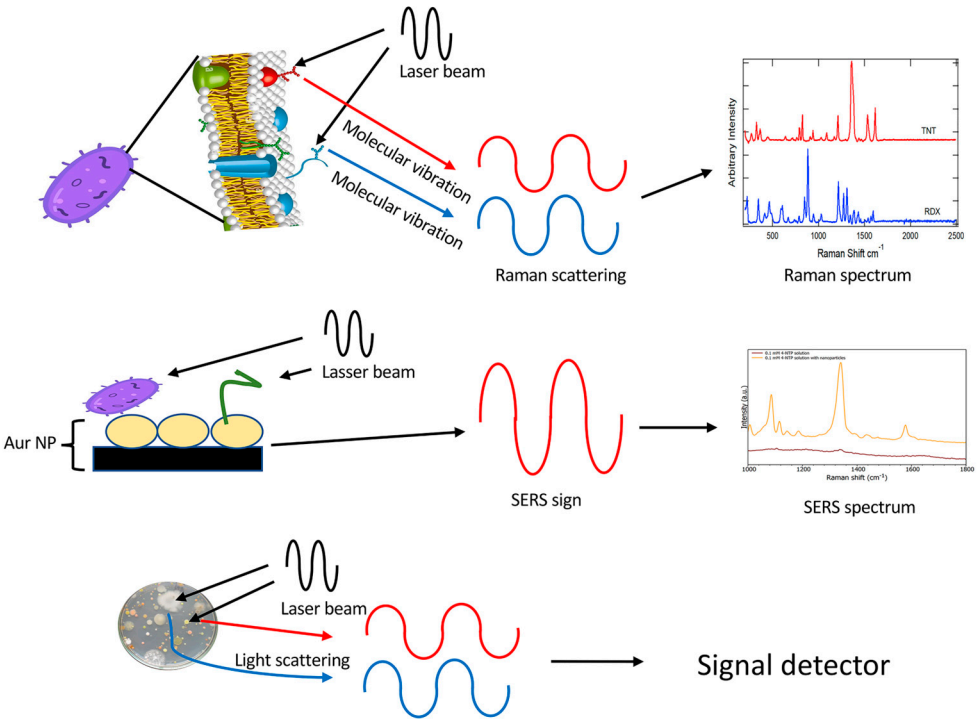


Figure 3. Optical system. Raman spectrometry (RS) consists of shining a longer-wavelength laser beam on the surface of a cell, exciting the molecules, and generating vibrations, producing a shorter wavelength recorded in the Raman spectrum. Surface-enhanced RS (SERS) is like RS but uses a nanoparticle (NP) to enhance the Raman signal. In polarization, when a laser beam hits a colony, it disperses at different wavelengths, which is detected.

RS is applied to biological samples because it has the advantage that their water content does not interfere with the RS spectrum, allowing the samples to be used directly or in humid conditions, reducing sample preparation protocols, and providing results closer to reality. Another advantage of RS for microbial detection is that Raman scattering can be performed at any wavelength, which is favorable because it can be coupled with

other systems. For example, using visible wavelengths for excitation allows RS to be integrated into an optical microscope. Using Raman micro spectroscopy is of great interest in identifying bacteria in liquid suspension without culturing. The amplitude of the incident beam provides flexibility in the different components of RS, such as the excitation wavelengths, detectors, and optical components required to acquire Raman spectra.

However, RS has one important limitation: the produced signal has a weak range, decreasing sensitivity. In addition, RS can interfere with other optical phenomena, such as the autofluorescence or absorption of a sample, causing problems in providing appropriate results. Therefore, the vibrational spectrum collected through the spontaneous production of Raman photons requires highly sensitive detection hardware, long exposure times, and high excitation power compared to other optical techniques. However, an alternative to overcome this Raman limitation is surface-enhanced RS (SERS).

RS has been used to detect bacterial cancer disease in tomatoes (*Lycopersicon esculentum* L.) caused by the bacterium *Clavibacter michiganensis* subsp. *michiganensis*. This bacterium was detected in tomato crops as a preliminary diagnostic with a reduced detection time compared to the gold standard method [92]. In addition, RS was used to characterize diseased plants based on biochemical markers and spectral anomalies in asymptomatic infected plants to allow their timely identification [93].

5.1.2. SERS

SERS is a recently developed RS modality that amplifies the intrinsic Raman signal. SERS uses metallic nanoparticles with rough surfaces and a colloidal aspect. This type of nanoparticle dramatically enhances the Raman signal of an absorbed molecule by 10^6–10^8-fold [94] (Figure 3). This enhancement is achieved via two main mechanisms: the transfer of electromagnetic charges and chemical charges. Regarding electromagnetic enhancement, the signal is amplified by the surface plasmons inside a metallic structure when it is excited. Regarding chemical enhancement, the signal is amplified up to 10^2-fold via the transfer of electrons between the nanoparticles and the absorbed and chemically bound molecules. SERS has enabled the detection of single molecules and very low concentrations of analytes, such as microbial samples, without sample preparation.

Both label-free and immunoassay-based SERS systems have been used to detect and identify bacteria. Label-free SERS is an attractive SERS modality because it amplifies the intrinsic Raman signals without modifying the metallic structures. Metal nanostructures can be incorporated externally or internally within bacterial cells. In contrast, SERS-immunoassays have been used to directly detect biomarkers, such as DNA released by cells or Abs expressed on their surface. While SERS immunoassays are like ELISA, the specific immunological matching with SERS can detect multiple species simultaneously with a single-wavelength laser. Therefore, SERS offers many advantages over conventional RS due to its strong signal, which allows microbial species and strains to be distinguished without performing a complex and multivariate analysis [95].

SERS has been used to detect fungi such as *Aspergillus niger*, *Saccharomyces cerevisiae*, *Fusarium moniliforme*, and *Trichoderma viride* in grain crops using colloidal Au nanoparticles. These fungi showed different Raman phenotypes, allowing their easy characterization by SERS. Combining SERS with multivariate statistical analysis, we identified various fungi quickly and accurately. Therefore, SERS can be applied to rapidly detect fungi in the food and biomedical industries [96].

In addition, identifying pesticides is relevant to environmental contamination in agriculture. SERS was used to develop a portable, fast, and specific device to detect low concentrations of the pesticide fenthion, which managed to detect it in plants at 10^{-8} mol/L [97]. Moreover, SERS was used to destructively and non-destructively detect the organophosphorus pesticides fenthion and triazophos in vegetable samples (cowpeas and peppers), detecting fenthion at 1.21×10^{-5} mg/kg and triazophos at 2.96×10^{-3} mg/kg under destructive conditions and fenthion at 0.13 ng/cm^2 and triazophos at 1.39 ng/cm^2 under non-destructive conditions [98].

Another application of SERS is the analysis of crop and soil nutrients. It has been used to quantify nutrients such as nitrogen, phosphorus, potassium, and micronutrients in plant tissue and soil samples. This information is important to optimize fertilizer application and evaluate agricultural product quality and soil health [99].

5.2. Polarization

Colony morphology is considered during microorganism identification since visual changes in colony morphology are characteristic of some bacterial genera. However, the disadvantage is that colony morphology is not constant and depends on several factors, such as growth conditions and cultural media. Shining a light on a microbial colony can generate some characteristic changes, which can be used for bacterial identification. Bacteria exhibit specific basic shapes, such as cocci, spirals, and rods. During their growth, they divide and organize differently depending on the genus, displaying different distinctive arrangements [100]. The polarization of light is sensitive to the microstructures of biological systems. Microstructural changes can be detected in bacterial colonies, which is one approach for microbial identification (Figure 3).

In polarimetry, the Mueller matrix is a tool for characterizing the interaction of various polarized light states with a sample. A polarization-based imaging modality was developed based on Mueller matrix polarimetry (MMP) to identify bacterial cultures from structural information about bacterial colonies. This technique could discriminate between *Lactobacillus rhamnosus*, *E. coli*, and *Rhodococcus erythropolis* [101]. In addition, MMP was used to determine structural changes in diseased tissue samples compared to healthy tissues [102]. Moreover, polarimetry has been used to automate plant phenotyping using high-dimensional image sensors, with the image data potentially helping to improve seasonal yield by monitoring crop health [103].

5.3. Laser Scattering

Similar to polarization-based identification, laser scattering is sensitive to colony morphology. The microstructure of a bacterial colony depends on cell shape and arrangement. Therefore, the light scattered by a bacterial colony generates a unique diffraction pattern, often called a scatterogram, which is captured using a camera and algorithmically analyzed to identify the colony. This technique uses elastic light scattering to collect information about the sample's morphology. This technique has been used to develop an automated detection system, named bacterial rapid detection using optical scattering technology [104], which was used to identify *Bacillus* species in rice cereal samples, including *B. thuringiensis*, *B. anthracis*, *B. subtilis*, *B. cereus*, and *B. megaterium* [105].

6. Conclusions

The use of different technologies for bacterial identification depends on the biological sample and its physicochemical and microbiological complexity. While the different technologies are promising, developing updated platforms or databases of the different microbial species is important since many of these technologies require comparative patterns or databases to identify microorganisms. Among these technologies, those that do not require microbial culturing have a great advantage, while those that require expensive equipment, making them inaccessible to some laboratories, have a great disadvantage. While some research laboratories have this equipment, it is evident that this represents a limitation for expanding new technologies and generating results in different areas of microbiology. Applying these new technologies in agriculture is necessary to deepen knowledge in agricultural microbiology, which will allow crops to be improved by applying biofertilizers with new microorganisms.

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References

1. Ahmad, F.; Babalola, O.O.; Tak, H.I. Potential of MALDI-TOF mass spectrometry as a rapid detection technique in plant pathology: Identification of plant-associated microorganisms. *Anal. Bioanal. Chem.* **2012**, *404*, 1247–1255. [CrossRef]
2. Larone, D.H. *Medically Important Fungi: A Guide to Identification*, 3rd ed.; ASM Press: Washington, DC, USA, 1995; pp. 190–192.
3. Huang, W.E.; Ferguson, A.; Singer, A.C.; Lawson, K.; Thompson, I.P.; Kalin, R.M.; Larkin, M.J.; Bailey, M.J.; Whiteley, A.S. Resolving genetic functions within microbial populations: In situ analyses using rRNA and mRNA stable isotope probing coupled with single-cell Raman-fluorescence in situ hybridization. *Appl. Environ. Microbiol.* **2009**, *75*, 234–241. [CrossRef]
4. Babalola, O.O.; Kirby, B.M.; Le Roes-Hill, M.; Cook, A.E.; Cary, S.C.; Burton, S.G.; Cowan, D.A. Phylogenetic analysis of actinobacterial populations associated with Antarctic dry valley mineral soils. *Environ. Microbiol.* **2009**, *11*, 566–576. [CrossRef]
5. Babalola, O.O. Molecular techniques: An overview of methods for the detection of bacteria. *Afr. J. Biotechnol.* **2003**, *2*, 710–713. [CrossRef]
6. Huang, W.E.; Li, M.; Jarvis, R.M.; Goodacre, R.; Banwart, S.A. Shining light on the microbial world: The application of Raman microspectroscopy. *Adv. Appl. Microbiol.* **2010**, *70*, 153–186.
7. Ali, M.M.; Li, F.; Zhiqing Zhang, F.L.; Zhang, K.; Kang, D.K.; Ankrum, J.A.; Le, X.C.; Zhao, W. Rolling circle amplification: A versatile tool for chemical biology, materials science and medicine. *Chem. Soc. Rev.* **2014**, *43*, 3324–3341. [CrossRef]
8. Paez, J.G.; Lin, M.; Beroukhim, R.; Lee, J.C.; Zhao, X.; Richter, D.J.; Gabriel, S.; Herman, P.; Sasaki, H.; Altshuler, D.; et al. Genome coverage and sequence fidelity of phi29 polymerase-based multiple strand displacement whole genome amplification. *Nucleic Acids Res.* **2004**, *32*, e71. [CrossRef]
9. Vincent, M.; Xu, Y.; Kong, H. Helicase-dependent isothermal DNA amplification. *EMBO Rep.* **2004**, *5*, 795–800. [CrossRef]
10. Piepenburg, O.; Williams, C.H.; Stemple, D.L.; Armes, N.A. DNA detection using recombination proteins. *PLoS Biol.* **2006**, *4*, e204. [CrossRef]
11. Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N.; Hase, T. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **2000**, *28*, e63. [CrossRef]
12. Misawa, Y.; Yoshida, A.; Saito, R.; Yoshida, H.; Okuzumi, K.; Ito, N.; Okada, M.; Moriya, K.; Koike, K. Application of loop-mediated isothermal amplification technique to rapid and direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in blood cultures. *J. Infect. Chemother.* **2007**, *13*, 134–140. [CrossRef]
13. Zhao, X.; Li, Y.; Wang, L.; You, L.; Xu, Z.; Li, L.; He, X.; Liu, Y.; Wang, J.; Yang, L. Development and application of a loop-mediated isothermal amplification method on rapid detection *Escherichia coli* O157 strains from food samples. *Mol. Biol. Rep.* **2010**, *37*, 2183–2188. [CrossRef]
14. Ho-Jong, J. Simple and rapid detection of potato leafroll virus by reverse transcription loop-mediated isothermal amplification. *Plant Pathol. J.* **2011**, *27*, 385–389. [CrossRef]
15. Suzuki, R.; Fukuta, S.; Matsumoto, Y.; Hasegawa, T.; Kojima, H.; Hotta, M.; Miyake, N. Development of reverse transcription loop-mediated isothermal amplification assay as a simple detection method of *Chrysanthemum stem necrosis virus* in chrysanthemum and tomato. *J. Virol. Methods* **2016**, *236*, 29–34. [CrossRef]
16. Selvaraj, V.; Maheshwari, Y.; Hajeri, S.; Yokomi, R. A rapid detection tool for VT isolates of *Citrus* tristeza virus by immunocapture-reverse transcriptase loop-mediated isothermal amplification assay. *PLoS ONE* **2019**, *14*, e0222170. [CrossRef]
17. Montgomery, J.L.; Sanford, L.N.; Wittwer, C.T. High-resolution DNA melting analysis in clinical research and diagnostics. *Expert. Rev. Mol. Diagn.* **2010**, *10*, 219–240. [CrossRef]
18. Yang, S.; Ramachandran, P.; Rothman, R.; Hsieh, Y.H.; Hardick, A.; Won, H.; Kecojevic, A.; Jackman, J.; Gaydos, C. Rapid identification of biothreat and other clinically relevant bacterial species by use of universal PCR coupled with high-resolution melting analysis. *J. Clin. Microbiol.* **2009**, *47*, 2252–2255. [CrossRef]
19. Xanthopoulou, A.; Ganopoulos, I.; Tryfinopoulou, P.; Panagou, E.Z.; Osanthanunkul, M.; Madesis, P.; Kizis, D. Rapid and accurate identification of black aspergilli from grapes using high-resolution melting (HRM) analysis. *J. Sci. Food Agric.* **2019**, *99*, 309–314. [CrossRef]
20. Cłapa, T.; Mikołajczak, K.; Błaszczyk, L.; Narożna, D. Development of high-resolution melting PCR (HRM-PCR) assay to identify native fungal species associated with the wheat endosphere. *J. Appl. Genet.* **2020**, *61*, 629–635. [CrossRef]
21. van Dijk, E.L.; Jaszczyszyn, Y.; Naquin, D.; Thermes, C. The third revolution in sequencing technology. *Trends Genet.* **2018**, *34*, 666–681. [CrossRef]
22. Thompson, J.F.; Steinmann, K.E. Single molecule sequencing with a HeliScope genetic analysis system. *Curr. Protoc. Mol. Biol.* **2010**, *92*, 7–10. [CrossRef]

23. Eid, J.; Fehr, A.; Gray, J.; Luong, K.; Lyle, J.; Otto, G.; Peluso, P.; Rank, D.; Baybayan, P.; Bettman, B.; et al. Real-time DNA sequencing from single polymerase molecules. *Science* **2009**, *323*, 133–138. [CrossRef]
24. Athanasopoulou, K.; Boti, M.A.; Adamopoulos, P.G.; Skourou, P.C.; Scorilas, A. Third-generation sequencing: The spearhead towards the radical transformation of modern genomics. *Life* **2021**, *12*, 30. [CrossRef]
25. Ardui, S.; Ameur, A.; Vermeesch, J.R.; Hestand, M.S. Single molecule real-time (SMRT) sequencing comes of age: Applications and utilities for medical diagnostics. *Nucleic Acids Res.* **2018**, *46*, 2159–2168. [CrossRef]
26. Ambardar, S.; Gupta, R.; Trakroo, D.; Lal, R.; Vakhlu, J. High Throughput sequencing: An overview of sequencing chemistry. *Indian. J. Microbiol.* **2016**, *56*, 394–404. [CrossRef]
27. Scarano, C.; Veneruso, I.; De Simone, R.R.; Di Bonito, G.; Secondino, A.; D'Argenio, V. The third-generation sequencing challenge: Novel insights for the omic sciences. *Biomolecules* **2024**, *14*, 568. [CrossRef]
28. Lin, B.; Hui, J.; Mao, H. Nanopore technology and its applications in gene sequencing. *Biosensors* **2021**, *11*, 214. [CrossRef]
29. Feng, Y.; Zhang, Y.; Ying, C.; Wang, D.; Du, C. Nanopore-based fourth-generation DNA sequencing technology. *Genom. Proteom. Bioinform.* **2015**, *13*, 4–16. [CrossRef]
30. Handelsman, J.; Rondon, M.R.; Brady, S.F.; Clardy, J.; Goodman, R.M. Molecular biological access to the chemistry of unknown soil microbes: A new frontier for natural products. *Chem. Biol.* **1998**, *5*, 245–249. [CrossRef]
31. Yang, B.; Wang, Y.; Qian, P.Y. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinform.* **2016**, *17*, 135. [CrossRef]
32. Winand, R.; Bogaerts, B.; Hoffman, S.; Lefevre, L.; Delvoye, M.; Braekel, J.V.; Fu, Q.; Roosens, N.H.; Keersmaecker, S.C.; Vanneste, K. Targeting the 16s rRNA gene for bacterial identification in complex mixed samples: Comparative evaluation of second (Illumina) and third (Oxford Nanopore Technologies) generation sequencing technologies. *Int. J. Mol. Sci.* **2019**, *21*, 298. [CrossRef]
33. Kim, C.; Pongpanich, M.; Porntaveetus, T. Unraveling metagenomics through long-read sequencing: A comprehensive review. *J. Transl. Med.* **2024**, *22*, 111. [CrossRef]
34. Hur, M.; Park, S.J. Identification of microbial profiles in heavy-metal-contaminated soil from full-length 16S rRNA reads sequenced by a PacBio System. *Microorganisms* **2019**, *7*, 357. [CrossRef]
35. Edwards, A.; Debbonaire, A.R.; Sattler, B.; Mur, L.A.J.; Hodson, A.J. Extreme metagenomics using nanopore DNA sequencing: A field report from Svalbard, 78° N. *BioRxiv* **2016**, *10*, 073965. [CrossRef]
36. Maguire, M.; Kase, J.A.; Roberson, D.; Muruvanda, T.; Brown, E.W.; Allard, M.; Musser, S.M.; Gonzalez-Escalona, N.G. Precision long-read metagenomics sequencing for food safety by detection and assembly of Shiga toxin-producing *Escherichia coli* in irrigation water. *PLoS ONE* **2021**, *16*, e0245172. [CrossRef]
37. Jagadesh, M.; Dash, M.; Kumari, A.; Singh, S.K.; Verma, K.K.; Kumar, P.; Bhatt, R.; Sharma, S.K. Revealing the hidden world of soil microbes: Metagenomic insights into plant, bacteria, and fungi interactions for sustainable agriculture and ecosystem restoration. *Microbiol. Res.* **2024**, *285*, 127764. [CrossRef]
38. Masenya, K.; Manganyi, M.C.; Dikobe, T.B. Exploring cereal metagenomics: Unravelling microbial communities for improved food security. *Microorganisms* **2024**, *12*, 510. [CrossRef] [PubMed]
39. You, X.; Wang, S.; Du, L.; Chen, Y.; Wang, T.; Bo, X. Metagenomics reveals the variations in functional metabolism associated with greenhouse gas emissions during legume-vegetable rotation process. *Ecotoxicol. Environ. Saf.* **2024**, *275*, 116268. [CrossRef]
40. Xiong, C.; Brajesh, K.; Singh, B.; Zhu, Y.G.; Hu, H.W.; Li, P.P.; Han, Y.L.; Han, L.L.; Zhang, Q.B.; Wang, J.T.; et al. Microbial species pool-mediated diazotrophic community assembly in crop microbiomes during plant development. *mSystems* **2024**, *9*, e0105523. [CrossRef]
41. Gonin, M.; Salas-González, I.; Gopaulchan, D.; Frene, J.P.; Roden, S.; Van de Poel, B.; Salt, D.E.; Castrillo, G. Plant microbiota controls an alternative root branching regulatory mechanism in plants. *Proc. Nat. Acad. Sci. USA* **2023**, *120*, e2301054120. [CrossRef]
42. Zheng, S.; Qi, J.; Fu, T.; Chen, Y.; Qiu, X. Novel mechanisms of cadmium tolerance and Cd-induced fungal stress in wheat: Transcriptomic and metagenomic insights. *Ecotoxicol. Environ. Saf.* **2023**, *256*, 114842. [CrossRef]
43. Moter, A.; Göbel, U.B. Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. *J. Microbiol. Methods* **2000**, *41*, 85–112. [CrossRef] [PubMed]
44. Moussata, D.; Goetz, M.; Gloeckner, A.; Kerner, M.; Campbell, B.; Hoffman, A.; Biesterfeld, S.; Flourie, B.; Saurin, J.C.; Galle, P.R. Confocal laser endomicroscopy is a new imaging modality for recognition of intramucosal bacteria in inflammatory bowel disease in vivo. *Gut* **2011**, *60*, 26–33. [CrossRef]
45. Harris, E.H. *Chlamydomonas* as a model organism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2021**, *52*, 363–406. [CrossRef]
46. Uniacke, J.; Colón-Ramos, D.; Zerges, W. FISH and immunofluorescence staining in *Chlamydomonas*. *Methods Mol. Biol.* **2011**, *714*, 15–29. [CrossRef] [PubMed]
47. Selosse, M.A.; Setaro, S.; Glatard, F.; Richard, F.; Urcelay, C.; Weiß, M. Sebacinales are common mycorrhizal associates of Ericaceae. *New Phytol.* **2007**, *174*, 864–878. [CrossRef] [PubMed]
48. Deshmukh, S.; Hückelhoven, R.; Schäfer, P.; Imani, J.; Sharma, M.; Weiss, M.; Waller, F.; Kogel, K.H. The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 18450–18457. [CrossRef]

49. Sharma, M.; Schmid, M.; Rothballer, M.; Hause, G.; Zuccaro, A.; Imani, J.; Kämpfer, P.; Domann, E.; Schäfer, P.; Hartmann, A.; et al. Detection and identification of bacteria intimately associated with fungi of the order *Sebacinales*. *Cell Microbiol.* **2008**, *10*, 2235–2246. [CrossRef]
50. Scheid, D.; Stubner, S.; Conrad, R. Identification of rice root associated nitrate, sulfate and ferric iron reducing bacteria during root decomposition. *FEMS Microbiol. Ecol.* **2004**, *50*, 101–110. [CrossRef]
51. Roy, R.; Conrad, R. Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane production by nitrate in anoxic rice field soil. *FEMS Microbiol. Ecol.* **1999**, *28*, 49–61. [CrossRef]
52. Schwab, S.; Hirata, E.S.; Amaral, J.C.A.; da Silva, C.G.N.; Ferreira, J.P.; da Silva, L.V.; Rouws, J.R.C.; Rouws, L.F.M.; Baldani, J.I.; Reis, V.M. Quantifying and visualizing *Nitrospirillum amazonense* strain CBAmC in sugarcane after using different inoculation methods. *Plant Soil* **2023**, *48*, 197–216. [CrossRef]
53. Giebel, R.; Worden, C.; Rust, S.M.; Kleinheinz, G.T.; Robbins, M.; Sandrin, T.R. Microbial fingerprinting using matrixassisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) applications and challenges. *Adv. Appl. Microbiol.* **2010**, *71*, 149–184. [PubMed]
54. Ryzhov, V.; Fenselau, C. Characterization of the protein subset desorbed by MALDI from whole bacterial cells. *Anal. Chem.* **2001**, *73*, 746–750. [CrossRef] [PubMed]
55. Bonnelly, R.; Calderon, V.V.; Ortiz, I.; Ovando, A.; Pinales, C.; Lara, W.; Mateo-Perez, S.E.; Cardenas-Alegría, O.; Ramos, R.T.; Rodriguez-Rodriguez, Y.; et al. Comparison of two bacterial characterization techniques for the genomic analysis of river microbiomes. *Appl. Microbiol.* **2023**, *3*, 1037–1045. [CrossRef]
56. Sura-de Jong, M.; Reynolds, R.J.B.; Richterova, K.; Musilova, L.; Staicu, L.C.; Chocholata, I.; Cappa, J.J.; Taghavi, S.; van der Lelie, D.; Frantik, T.; et al. Selenium hyperaccumulators harbor a diverse endophytic bacterial community characterized by high selenium resistance and plant growth promoting properties. *Front. Plant Sci.* **2015**, *6*, 113. [CrossRef] [PubMed]
57. Wensing, A.; Zimmermann, S.; Geider, K. Identification of the corn pathogen *Pantoea stewartii* by mass spectrometry of whole-cell extracts and its detection with novel PCR primers. *Appl. Environ. Microbiol.* **2010**, *76*, 6248–6256. [CrossRef] [PubMed]
58. Wang, Y.; Zhou, Q.; Li, B.; Liu, B.; Wu, G.; Ibrahim, M.; Xie, G.; Li, H.; Sun, G. Differentiation in MALDI-TOF MS and FTIR spectra between two closely related species *Acidovorax oryzae* and *Acidovorax citrulli*. *BMC Microbiol.* **2012**, *12*, 182. [CrossRef] [PubMed]
59. Šalplachta, J.; Kubesová, A.; Horký, J.; Matoušková, H.; Tesařová, M.; Horká, M. Characterization of *Dickeya* and *Pectobacterium* species by capillary electrophoretic techniques and MALDI-TOF MS. *Anal. Bioanal. Chem.* **2015**, *407*, 7625–7635. [CrossRef]
60. Inglis, P.W.; Mello, S.C.M.; Martins, I.; Silva, J.B.T.; Macêdo, K.; Sifuentes, D.N.; Valadares-Inglis, M.C. *Trichoderma* from brazilian garlic and onion crop soils and description of two new species: *Trichoderma azevedoi* and *Trichoderma peberdyi*. *PLoS ONE* **2020**, *15*, e0228485. [CrossRef]
61. Martínez-Hidalgo, P.; Flores-Félix, J.D.; Sánchez-Juanes, F.; Rivas, R.; Mateos, P.F.; Regina, I.S.; Martínez-Molina, E.; Igual, J.M.; Velázquez, E. Identification of canola roots endophytic bacteria and analysis of their potential as biofertilizers for canola crops with special emphasis on sporulating bacteria. *Agronomy* **2021**, *11*, 1796. [CrossRef]
62. Nunes, A.R.; Sánchez-Juanes, F.; Gonçalves, A.C.; Alves, G.; Silva, L.R.; Flores-Félix, J.D. Evaluation of raw cheese as a novel source of biofertilizer with a high level of biosecurity for blueberry. *Agronomy* **2022**, *12*, 1150. [CrossRef]
63. Singh, R.; Mukherjee, M.D.; Sumana, G.; Gupta, R.K.; Sood, S.; Malhotra, B.D. Biosensors for pathogen detection: A smart approach towards clinical diagnosis. *Sens. Actuators B Chem.* **2014**, *197*, 385–404. [CrossRef]
64. Chen, Y.; Qian, C.; Liu, C.; Shen, H.; Wang, Z.; Ping, J.; Wu, J.; Chen, H. Nucleic acid amplification free biosensors for pathogen detection. *Biosens. Bioelectron.* **2020**, *153*, 112049. [CrossRef] [PubMed]
65. Patel, P.D. (Bio)sensors for measurement of analytes implicated in food safety: A review. *Trac-Trends Anal. Chem.* **2002**, *21*, 96–115. [CrossRef]
66. Mello, L.D.; Kubota, L.T. Review of the use of biosensors as analytical tools in the food and drink industries. *Food Chem.* **2002**, *77*, 237–256. [CrossRef]
67. Ferreira, L.S.; De Souza, M.B.; Trierweiler, J.O.; Broxtermann, O.; Folly, R.O.M.; Hitzmann, B. Aspects concerning the use of biosensors for process control: Experimental and simulation investigations. *Comput. Chem. Eng.* **2003**, *27*, 1165–1173. [CrossRef]
68. Wilson, R. The use of gold nanoparticles in diagnostics and detection. *Chem. Soc. Rev.* **2008**, *37*, 2028–2045. [CrossRef]
69. You, Y.; Lim, S.; Gunasekaran, S. Streptavidin-coated Au nanoparticles coupled with biotinylated antibody-based bifunctional linkers as plasmon-enhanced immunobiosensors. *ACS Appl. Nano Mater.* **2020**, *3*, 1900–1909. [CrossRef]
70. Felix, F.S.; Angnes, L. Electrochemical immunosensors-a powerful tool for analytical applications. *Biosens. Bioelectron.* **2018**, *102*, 470–478. [CrossRef]
71. Cho, I.H.; Lee, J.; Kim, J.; Kang, M.S.; Paik, J.K.; Ku, S.; Cho, H.M.; Irudayaraj, J.; Kim, D.H. Current technologies of electrochemical immunosensors: Perspective on signal amplification. *Sensors* **2018**, *18*, 207. [CrossRef] [PubMed]
72. Gao, H.; Wen, L.; Tian, J.; Wu, Y.; Liu, F.; Lin, Y.; Hua, W.; Wu, G.A. Portable electrochemical immunosensor for highly sensitive point-of-care testing of genetically modified crops. *Biosens. Bioelectron.* **2019**, *142*, 111504. [CrossRef] [PubMed]
73. Arévalo, F.J.; Granero, A.M.; Fernández, H.; Raba, J.; Zón, M.A. Citrinin (CIT) determination in rice samples using a micro fluidic electrochemical immunosensor. *Talanta* **2011**, *83*, 966–973. [CrossRef] [PubMed]
74. Fernández-Baldo, M.A.; Bertolino, F.A.; Messina, G.A.; Sanz, M.I.; Raba, J. Modified magnetic nanoparticles in an electrochemical method for the ochratoxin A determination in *Vitis vinifera* red grapes tissues. *Talanta* **2010**, *83*, 651–657. [CrossRef] [PubMed]

75. Ogert, R.A.; Brown, J.E.; Singh, B.R.; Shriver-Lake, L.C.; Ligler, F.S. Detection of *Clostridium botulinum* toxin a using a fiber optic-based biosensor. *Anal. Biochem.* **1992**, *205*, 306–312. [CrossRef] [PubMed]
76. Oztuna, A.; Nazir, H.; Baysallar, M. Simultaneous *Bacillus anthracis* spores detection via aminated-poly(vinyl chloride) coated piezoelectric crystal immunosensor. *J. Coat.* **2014**, *2014*, 256168. [CrossRef]
77. Zhou, J.; Rossi, J. Aptamers as targeted therapeutics: Current potential and challenges. *Nat. Rev. Drug Discov.* **2017**, *16*, 181–202. [CrossRef]
78. Prieto-Simón, B.; Samitier, J. Signal off aptasensor based on enzyme inhibition induced by conformational switch. *Anal. Chem.* **2014**, *86*, 1437–1444. [CrossRef] [PubMed]
79. Alhamoud, Y.; Li, Y.; Zhou, H.; Al-Wazer, R.; Gong, Y.; Zhi, S.; Yang, D. Label-free and highly-sensitive detection of ochratoxin A using one-pot synthesized reduced graphene oxide/gold nanoparticles-based impedimetric aptasensor. *Biosensors* **2021**, *11*, 87. [CrossRef]
80. Lu, X.; Wang, L.; He, B.; Zhao, R.; Bai, C.; Zhang, Y.; Ren, W.; Jiang, L.; Suo, Z.; Xu, Y. AgPdNFs and AuNOs@GO nanocomposites for T-2 toxin detection by catalytic hairpinassembly. *Mikrochim. Acta* **2023**, *190*, 120. [CrossRef]
81. Peng, H.; Chen, I.A. Rapid colorimetric detection of bacterial species through the capture of gold nanoparticles by chimeric phages. *ACS Nano* **2018**, *13*, 1244–1252. [CrossRef]
82. Abdelhamied, N.; Abdelrahman, F.; El-Shibiny, A.; Hassan, R.Y.A. Bacteriophage-based nano-biosensors for the fast impedimetric determination of pathogens in food samples. *Sci. Rep.* **2023**, *13*, 3498. [CrossRef]
83. Li, B.; Li, X.; Dong, Y.; Wang, B.; Li, D.; Shi, Y.; Wu, Y. Colorimetric sensor array based on gold nanoparticles with diverse surface charges for microorganisms identification. *Anal. Chem.* **2017**, *9*, 10639–10643. [CrossRef] [PubMed]
84. Yang, J.Y.; Yang, T.; Wang, X.Y.; Wang, Y.T.; Liu, M.X.; Chen, M.L.; Yu, Y.L.; Wang, J.H. A novel three-dimensional nanosensing array for the discrimination of sulfur-containing species and sulfur bacteria. *Anal. Chem.* **2019**, *91*, 6012–6018. [CrossRef] [PubMed]
85. Wu, Y.; Zhang, J.; Hu, X.; Huang, X.; Zhang, X.; Zou, X.; Shi, J. A visible colorimetric sensor array based on chemo-responsive dyes and chemometric algorithms for real-time potato quality monitoring systems. *Food Chem.* **2023**, *405*, 134717. [CrossRef] [PubMed]
86. Arslan, M.; Zareef, M.; Tahir, H.E.; Guo, Z.; Rakha, A.; Xuetao, H.; Shi, J.; Zhihua, L.; Xiaobo, Z.; Khan, M.R. Discrimination of rice varieties using smartphone-based colorimetric sensor arrays and gas chromatography techniques. *Food Chem.* **2022**, *368*, 130783. [CrossRef] [PubMed]
87. Li, Z.; Askim, J.R.; Suslick, K.S. The optoelectronic nose: Colorimetric and fluorometric sensor arrays. *Chem. Rev.* **2019**, *119*, 231–292. [CrossRef] [PubMed]
88. Carey, J.R.; Suslick, K.S.; Hulkower, K.I.; Imlay, J.A.; Imlay, K.R.; Ingison, C.K.; Ponder, J.B.; Sen, A.; Wittrig, A.E. Rapid identification of bacteria with a disposable colorimetric sensing array. *J. Am. Chem. Soc.* **2011**, *133*, 7571–7576. [CrossRef]
89. Maquelin, K.; Choo-Smith, L.P.; Kirschner, C.; Ngo-Thi, N.; Naumann, D.; Puppels, G. Vibrational spectroscopic studies of microorganisms. In *Handbook of Vibrational Spectroscopy*; Chalmers, J.M., Griffit, P.R., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2006. [CrossRef]
90. Maquelin, K.; Kirschner, C.; Choo-Smith, L.P.; Ngo-Thi, N.; Van Vreeswijk, T.; Stämmler, M.; Endtz, H.; Bruining, H.; Naumann, D.; Puppels, G. Prospective study of the performance of vibrational spectroscopies for rapid identification of bacterial and fungal pathogens recovered from blood cultures. *J. Clin. Microbiol.* **2003**, *41*, 324–329. [CrossRef]
91. Harz, M.; Rösch, P.; Popp, J. Vibrational spectroscopy-a powerful tool for the rapid identification of microbial cells at the single-cell level. *Cytometry A* **2009**, *75*, 104–113. [CrossRef]
92. Vallejo-Pérez, M.R.; Navarro-Contreras, H.R.; Sosa-Herrera, J.A.; Lara-Ávila, J.P.; Ramírez-Tobías, H.M.; Díaz-Barriga Martínez, F.D.; Flores-Ramírez, R.; Rodríguez-Vázquez, Á.G. Detection of *Clavibacter michiganensis* subsp. michiganensis assisted by micro-Raman spectroscopy under laboratory conditions. *Plant Pathol. J.* **2018**, *34*, 381–392. [CrossRef]
93. Vallejo-Pérez, M.R.; Sosa-Herrera, J.A.; Navarro-Contreras, H.R.; Álvarez-Preciado, L.G.; Rodríguez-Vázquez, Á.G.; Lara-Ávila, J.P. Raman spectroscopy and machine-learning for early detection of bacterial cancer of tomato: The asympthomatic disease condition. *Plants* **2021**, *10*, 1542. [CrossRef]
94. Vo-Dinh, T.; Liu, Y.; Fales, A.M.; Ngo, H.; Wang, H.N.; Register, J.K.; Yuan, H.; Norton, S.J.; Griffin, G.D. SERS nanosensors and nanoreporters: Golden opportunities in biomedical applications. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2015**, *7*, 17–33. [CrossRef]
95. Premasiri, W.R.; Moir, D.T.; Klempner, M.S.; Krieger, N.; Jones, G.; Ziegler, L.D. Characterization of the surface enhanced Raman scattering (SERS) of bacteria. *J. Phys. Chem. B* **2005**, *109*, 312–320. [CrossRef] [PubMed]
96. Wang, H.; Liu, M.; Zhao, H.; Ren, X.; Lin, T.; Zhang, P.; Zheng, D. Rapid detection and identification of fungi in grain crops using colloidal Au nanoparticles based on surface-enhanced Raman scattering and multivariate statistical analysis. *World J. Microbiol. Biotechnol.* **2022**, *39*, 26. [CrossRef]
97. Zhao, H.; Cui, X.; Zhang, P.; Zhou, M.; Liu, C.; Shi, X.; Ma, J. Surface-enhanced Raman spectroscopy detection for fenthion pesticides based on gold molecularly imprinted polymer solid-state substrates. *Appl. Spectrosc.* **2024**, 37028241253860. [CrossRef]
98. Lv, M.; Pu, H.; Sun, D.W. A tailored dual core-shell magnetic SERS substrate with precise shell-thickness control for trace organophosphorus pesticides residues detection. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2024**, *316*, 124336. [CrossRef]

99. Xu, S.; Guo, Y.; Liang, X.; Lu, H. Intelligent rapid detection techniques for low-content components in fruits and vegetables: A comprehensive review. *Foods* **2024**, *13*, 1116. [CrossRef] [PubMed]
100. Shapiro, J.A. The significances of bacterial colony patterns. *Bioessays* **1995**, *17*, 597–607. [CrossRef]
101. Badieyan, S.; Dilmaghani-Marand, A.; Hajipour, M.J.; Ameri, A.; Razzaghi, M.R.; Rafii-Tabar, H.; Mahmoudi, M.; Sasanpour, P. Detection and discriminate on of bacterial colonies with Mueller matrix imaging. *Sci. Rep.* **2018**, *8*, 10815. [CrossRef]
102. Chen, Y.; Chu, J.; Xin, B.; Qi, J. Mechanical stability of polarization signatures in biological tissue characterization. *Biomed. Opt. Express* **2024**, *15*, 2652–2665. [CrossRef]
103. Krafft, D.; Scarboro, C.G.; Hsieh, W.; Doherty, C.; Balint-Kurti, P.; Kudenov, M. Mitigating illumination-, leaf-, and view-angle dependencies in hyperspectral imaging using polarimetry. *Plant Phenomics* **2024**, *6*, 0157. [CrossRef]
104. Kim, K.P.; Singh, A.K.; Bai, X.; Leprun, L.; Bhunia, A.K. Novel PCR assays complement laser biosensor-based method and facilitate listeria species detection from food. *Sensors* **2015**, *15*, 22672–22691. [CrossRef]
105. Singh, A.K.; Sun, X.; Bai, X.; Kim, H.; Abdalhaseib, M.U.; Bae, E.; Bhunia, A.K. Label-free, non-invasive light scattering sensor for rapid screening of *Bacillus* colonies. *J. Microbiol. Methods* **2015**, *109*, 56–66. [CrossRef]

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