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Advances in bacterial identification and characterization: methods and applications

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Abstract

Characterization and identification of bacteria and microorganisms are crucial in several fields such as medical, agricultural, and industrial microbiology. Conventional phenotype-based identification methods use low-precision scoring systems and therefore are affected by species phenotypic variations. These methods have low levels of reproducibility, which results in a decrease in the accuracy coefficient. Furthermore, conventional phenotype-based identification techniques include several methods, such as observation of growth and colony morphology, biochemical characterization, and conventional available biochemical methods, that are less accurate. Molecular-based methods have better strategies to identify and characterize microorganisms and bacteria. The development of molecular-based techniques has improved our ability to identify bacterial species in culture-dependent and culture-independent samples. Most of these techniques are only capable of identifying single bacterial strains or small groups of organisms at a time. However, some methods can be used to identify and characterize the bacterial communities in a range of hundreds to thousands of single strains. We also know that each of these methods has weaknesses and shortcomings that limit their application and usability. However, some of these methods have the strengths and potentials to improve conventional methods and to compensate for their shortcomings. In the present review, we highlighted recent progress in the field of bacterial characterization and identification using molecular-based techniques and discussed their abilities and limitations.

1. Introduction

Environments, such as soil, foods, etc., are natural resources for many microbial species. These species have been isolated, characterized, and identified for economic and industrial applications (Escobar-Niño et al. 2014; Mohseni, Ebrahimi, and Chaichi 2015; Emruzi et al. 2018; Assareh et al. 2012), medicine, and health approaches (Abd Alfadil et al. 2018; Zarei et al. 2011). These approaches have led to a dramatic increase in microbial studies, and in the same vein, in recent years, various techniques have been

developed to identify and characterize bacterial genomes. Many studies have reported that genomic sequencing relies on the growth of bacteria in the culture medium (Tashakor et al. 2017; Gholami et al. 2015). In contrast, in novel genomics, large amounts of DNA template achieved from cell cultures are required for genomic sequencing. However, most bacterial species remain uncultured and could not be sequenced (Lasken and McLean 2014). Methods for identifying bacteria need to be improved owing to the importance of these microorganisms, and focus on these techniques is necessary.

In this review, we will investigate the induction and recovery of bacteria from culture-dependent and culture-independent samples based on the recent developments in bacterial identification and characterization. The strengths and limitations of these techniques on molecular and genetic levels are also addressed (Table 1).

Table 1. Methods for identification and characterization of bacterial strains, as well as their strengths and limitations.

Methods	Strengths	Limitations	References
16S rDNA PCR	1) Study of the microbial diversity in systems 2) Extension of the biodiversity patterns	1) Restricted by the short read length obtained, sequencing errors 2) Difficulties in the measurement of OTUs	(Sogin et al. 2006; Quince et al. 2009; Youssef et al. 2009; Huse et al. 2010; Saxena et al. 2014; Mitani et al. 2005)
Multiplex PCR	1) Identification of more than two bacterial strains genes 2) Less cost and time to obtain products 3) Can be used in a Real-time PCR format	1) Analysis of the limited number of genes in one reaction 2) Need to pre-identification to species level	(Casey and Bosworth 2009; Rajtak et al. 2011; Cleven et al. 2006)
Microarray-based microbial identification	1) Monitoring an individual or small samples of organisms 2) No need to pre-culture bacteria 3) Less total assay time for microbial identification	1) Difficulty distinguishing between closely related bacterial species solely by 16S rDNA probes 2) There is no information about strain-specific characterizations 3) Has not been widely used in the identification of bacterial species	(Cleven et al. 2006; Gentry and Zhou 2006; Ye et al. 2001)
Multiple displacement amplification (MDA)	1) Does not require single cells culturing 2) The use of random hexamer primers 3) Longer Amplicons produced by the φ29 DNA polymerase than those achieved by PCR	1) Highly susceptible to contamination 2) The loss of some sequences due to the significant amplification bias of single cells 3) Need the constitution of the amplified DNA library	(Raghunathan et al. 2005; Salas and de Vega 2016; Detter et al. 2002)

Table 1 (continued)

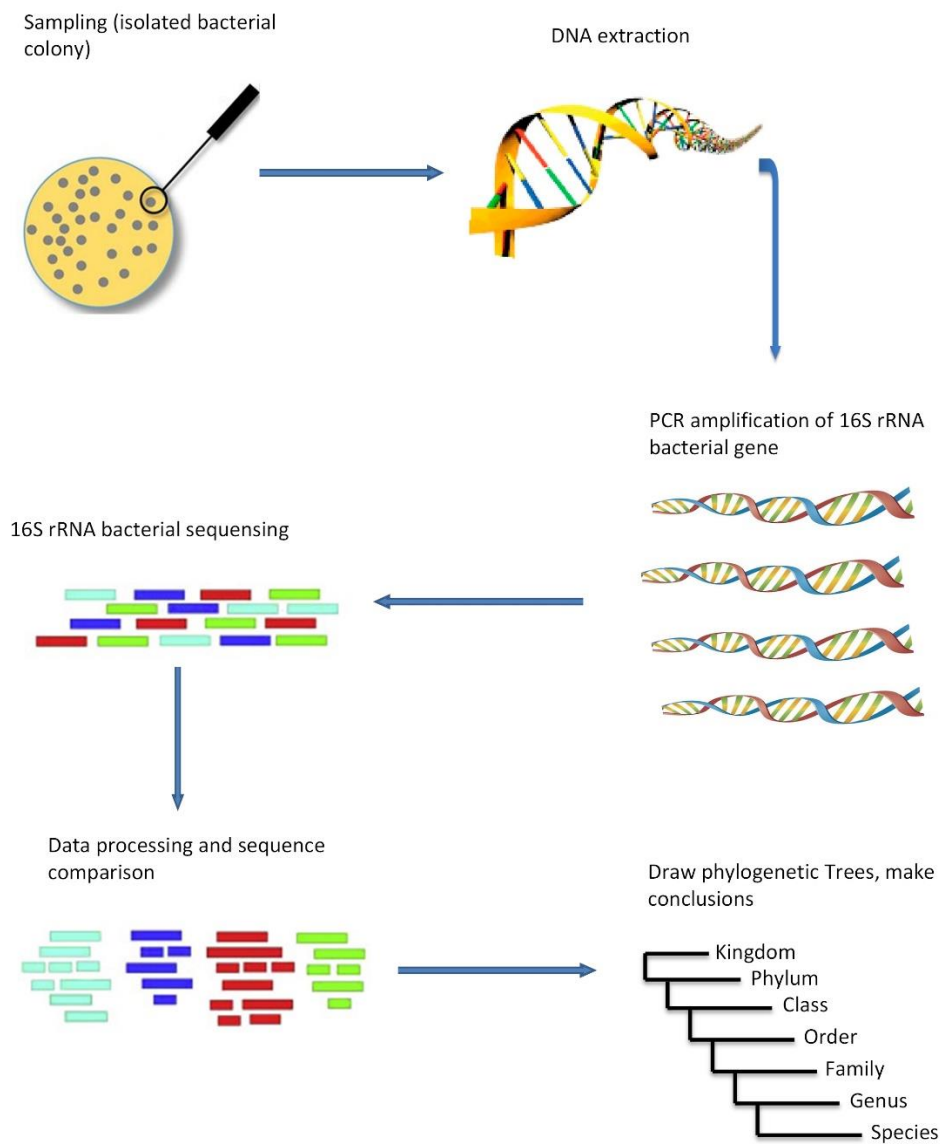| Methods	Strengths	Limitations	References
Fluorescence in situ hybridization (FISH)	1) Bacterial identification within complex mixtures of other bacteria 2) It could be useful as a routine technique, but probably leads to errors and contamination	1) Expensive test 2) Need to pre-identification procedures 3) Concerns about the sensitivity of the FISH as a microscopic method	(Bottari et al. 2006; Schmiedel et al. 2014)
Massive DNA sequencing	1) Amplification of the 16S rDNA sequence for all of the bacterial species with minimum bias 2) Identification of all mixed bacterial species 3) Determination of antimicrobial resistance genes of bacteria without isolation and purification in the medium	1) Bias in the relative amplification efficiency of 16S rDNA from the heterogeneous samples 2) Reliable 16S rDNA sequencing only when the samples contain a single bacterial species	(Cai, Caswell, and Prescott 2014; Suzuki and Giovannoni 1996)
Real-time PCR	1) Higher sensitivity, accuracy, and possibility of determination of DNA amplification 2) Can be quantitative or semi-quantitative	1) Analysis of a limited number of genes in one reaction. 2) Pre-identification to species level	(Cai et al. 2005; Cleven et al. 2006)
Random amplification of polymorphic DNA-PCR (RAPD-PCR)	1) Suitability for work on anonymous genomes 2) The applicability of this method is for limited quantities of DNA 3) High efficiency and low expense	1) Difficulties in distinguishing homozygotes and heterozygotes 2) Susceptibility to alterations in the quality of DNA, PCR conditions and components	(Kumari 2014; Arif et al. 2010)
Restriction fragment length polymorphism (RFLP)	1) Abilities in the molecular epidemiology of infectious outbreaks 2) High specificity and reproducibility 3) Ability to give the relative amounts of different bacteria in a sample	1) Low discriminatory power 2) Expensive to run	(Tabit 2016; Mitani et al. 2005)

Table 1 (continued)

Methods	Strengths	Limitations	References
Amplified fragment length polymorphism (AFLP)	1) High discriminatory power 2) The discriminatory potential of genomic DNA	1) Higher probability of producing inconclusive results because of DNA degradation 2) Requires expertise to run analysis	(Tabit 2016; Franco-Duarte et al. 2019)
Pulsed-field gel electrophoresis (PFGE)	1) High discriminatory power 2) Possible to cleave band from gel for amplification and sequencing	1) Labor-intensive with about four days to complete the protocols 2) High inconclusive result 3) The same separation of DNA sequences of different bacterial species	(Klaassen, van Haren, and Horrevorts 2002)

2. The 16S rDNA PCR analysis

The 16S ribosomal deoxyribonucleic acid (rDNA) sequencing analysis is a standard method for the direct identification of microbial populations in environmental samples, which were used for the first time by Carl Woese as a phylogenetic marker to discover three kingdom classification schemes (Woese and Fox 1977). In this method, DNA extracted from the environmental sample is used as a template for 16S rDNA amplified by the polymerase chain reaction (PCR) using universal primers. A mixture of 16S rDNA fragments from different cells in the environmental samples are then cloned and subsequently sequenced (Jo, Kennedy, and Kong 2016; Zarei et al. 2012; Khaleghinejad et al. 2015; Babashpour et al. 2011). The achieved sequences are analyzed, and taxonomic affiliations performed using bioinformatics alignments against sequence databases (Figure 1) (Rosselli et al. 2016). This method has been adopted for the study of the microbial diversity in systems from oceans (Whalan

and Webster 2014; Mohit et al. 2014; Biers, Sun, and Howard 2009) to soils (Rahman et al. 2014; Gholami D. et al. 2014b, 2014a; Luo et al. 2014), as well as Antarctic lakes (Møller et al. 2013; Murray et al. 2012), and Antarctic soils (Frank-Fahle et al. 2014; Niederberger et al. 2008). Moreover, many parallel sequencing methods for the identification and characterization of microbial communities rely on the amplification of the 16S rDNA, which has led to the extension of biodiversity patterns (Sogin et al. 2006). However, this technique is restricted by the short read length obtained, sequencing errors (Quince et al. 2009), differences originating from the different fragments selected (Youssef et al. 2009), and difficulties in measurement of operational taxonomic units (OTUs) (Huse et al. 2010). In addition, when using single-gene markers, the assessment of diversity is challenging because of the prevalence of horizontal gene transfer and the concern inherent in bacterial species (10-12). Furthermore, where there are closely related bacterial species, the resolution of the 16S rDNA is limited (Poretsky et al. 2014).

Sampling (isolated bacterial colony)

DNA extraction

PCR amplification of 16S rRNA bacterial gene

16S rRNA bacterial sequensing

Data processing and sequence comparison

Draw phylogenetic Trees, make conclusions

Kingdom
Phylum
Class
Order
Family
Genus
Species

Figure 1. Outline of the 16S rRNA sequencing. DNA extraction is followed by 16S rRNA gene amplification, then the PCR product is cloned and sequenced. Finally, the sequences are blasted, and bioinformatics tools are used to draw the phylogenetic tree.

3. Multiplex PCR assays

The multiplex PCR identifies more than two genes of bacterial strains. This has resulted in less cost and time to obtain products compared to regular single PCR assays (Casey and Bosworth 2009). Furthermore, this method can be used in a Real-time PCR format for multiple different targets by measuring the melting temperature of PCR amplicons with conventional double-strand DNA dyes such as SYBR Green (Rajtak et al. 2011; Li et al. 2017).

However, there are limitations to using the multiplex PCR, including the limited number of genes that can be analyzed in one reaction. Also, we need a pre-identification to species level when applying multiplex PCR (Cleven et al. 2006).

4. Microarray-based microbial identification

Multiplex PCR is not suitable to be used in multiple bacterial identifications due to challenging conditions in the setup process and validation. The DNA microarray is an advanced method for multiple microbial identifications. The development of this method dramatically helps microbiologists to monitor an individual or small samples of organisms (Gentry and Zhou 2006; Cleven et al. 2006; Cao et al. 2011). In microarray methods, the hybridization-based detection of multiple targets occurs (Loy and Bodrossy 2006; Bodrossy and Sessitsch 2004).

In DNA microarray technology, the desired DNA probes (ordered probes specific to target genomes) are spotted on a nonporous solid surface in a lattice pattern. The target DNA is then labeled with a reporter molecule, such as fluorescence dye, and then hybridized to the probe. Next, specific target probe duplexes are detected by assessing the fluorescent signals related to each spot on the DNA chip (microarray). There are two types of DNA microarrays: i) a PCR product-based DNA microarray and ii) an oligonucleotide-based DNA microarray. Both types of DNA microarrays are applied to identify bacterial species (Sato et al. 2010; Ye et al. 2001). In both methods, the hybridization between labeled DNA in the sample and probe DNA is performed. Microarrays can be fabricated either by spotting pre-existing DNA, such as cDNA microarrays or by direct synthesis of oligonucleotides on the solid surface. Finally, data are analyzed based on the differences between expression profiles (according to the color type of wells) to identify specific genes involved in bacterial species (Figure 2).

Conventional identification of bacterial strains often relies on the culture base, while in microarray base analysis, the bacteria do not require pre-culture. Therefore, in conventional identification, the cultivation yield can be identified in days or up to a week after sampling. Additionally, while the cultivation of bacteria under laboratory conditions is difficult, in the microarray technique, the total assay time for microbial identification is only three hours, and this time includes the DNA extraction, PCR, and steps of microarray in sequence (Järvinen et al. 2009; Marlowe et al. 2003; Bekal et al. 2003). However, microbiologists tend to identify bacteria at the genus and/or species level. This is a problem because distinguishing between closely related bacterial species solely by 16S rDNA probes is difficult. The preferred strategy to solve this problem is by sequencing the whole 16S rDNA (Janda and Abbott 2007).

The main drawback of assays using oligonucleotide probes that rely on rRNA (e.g., the rRNA microarray technique) is that it allows only species identification using the rRNA probes. It does not give us any information about strain-specific characterizations (Cleven et al. 2006).

Therefore, this method is not widely used in the identification of bacterial species because optimizing and validating the PCR as well as the steps of the microarray is difficult. Some studies have proposed resolving all of the mentioned problems by using whole DNA microarray technology, which allows

the simultaneous identification of a wide variety of genes (Cleven et al. 2006; Ye et al. 2001).
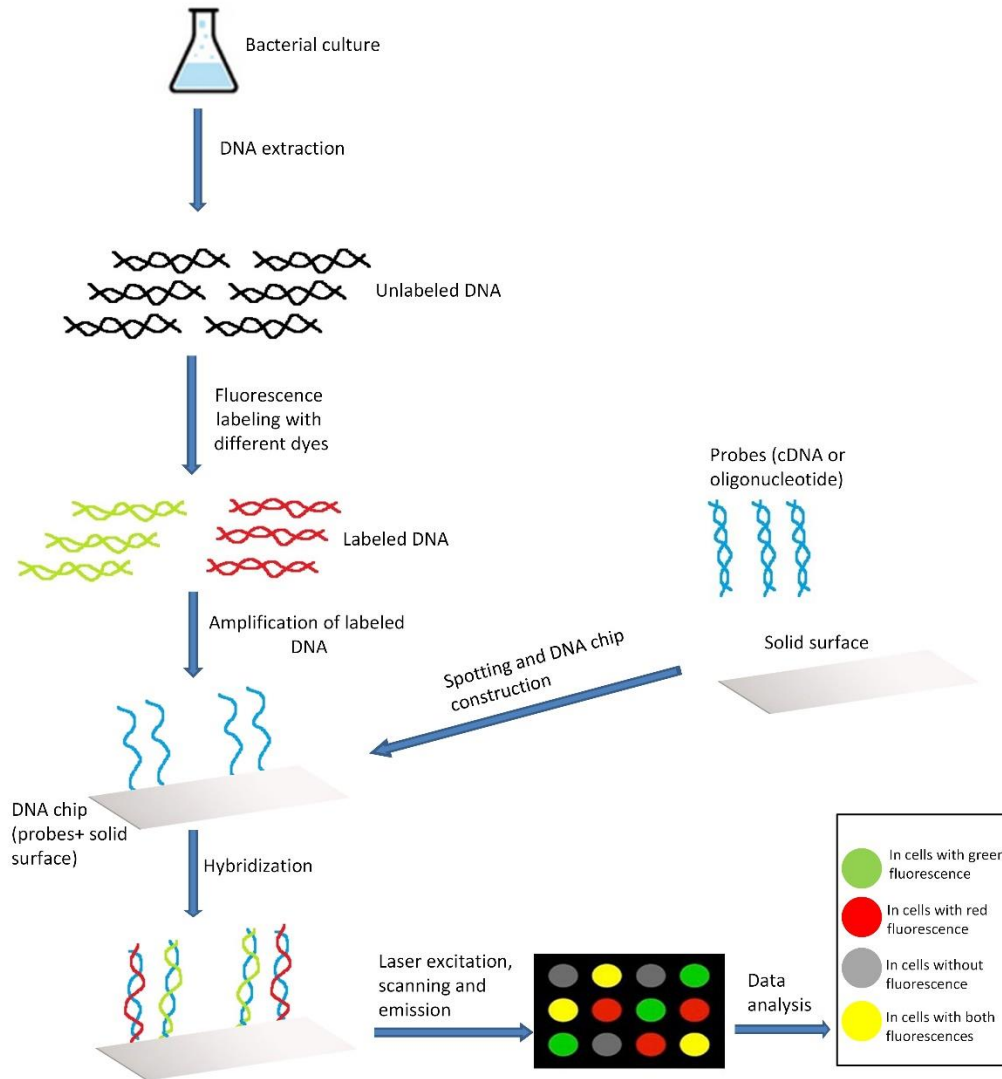
Figure 2. The workflow of bacterial identification using DNA microarray. DNA was extracted from bacterial samples, labeled with fluorescence dyes, and hybridized to the spotted pre-existing DNA probes or direct synthesis of oligonucleotides on the solid surface face. Finally, scanning was performed by a laser scanner, and data analysis processed by computer tools.

5. Multiple displacement amplification

The multiple displacement amplification (MDA) amplifies DNA templates using φ 29 DNA polymerase and random primers (Dean et al. 2001; Ellegaard, Klasson, and Andersson 2013). In this method, a single bacterial genome is amplified more than several- billion-fold. This method is used for the identification of new bacterial species, polymorphism analysis, and characterization

of pathogens (Raghunathan et al. 2005). In this process, the bacterial cells are sorted into a microtiter plate by Fluorescence-activated cell sorting (FACS). Next, the single cells are lysed and total-genome amplification is carried out (Figure 3).
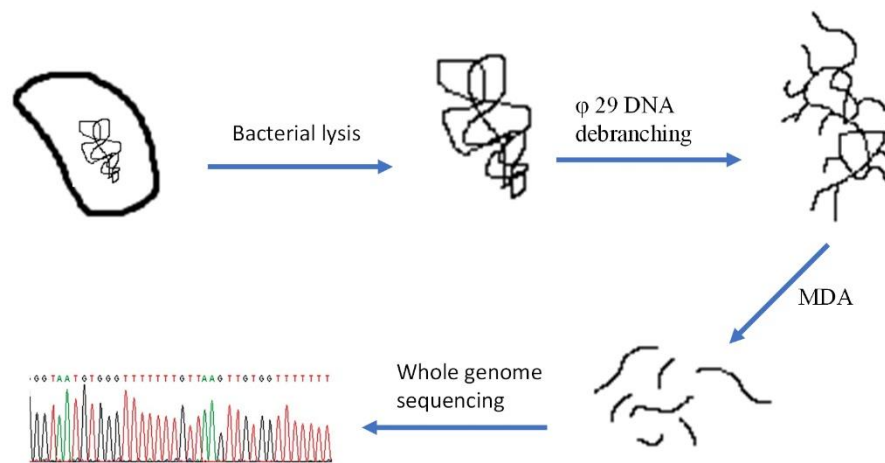

Figure 3. The MDA is a DNA amplification technique. This method sequences the single cell's DNA of uncultured bacteria.

After obtained whole genomes by MDA, it is highly recommended that the identities of the single or population amplified genome should be confirmed by 16S rDNA sequencing. Furthermore, amplified genomes can also be sequenced by a shotgun method on a range of high-throughput platforms.

6. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) (Jensen et al. 2015) is a well-established culture-independent method for bacterial identification that uses fluorescence oligonucleotide probes, which rely on 16S rDNA sequence for bacterial identification of the genus and/or species level (Cai, Caswell, and Prescott 2014). It is hard to identify bacteria within complex mixtures of other bacteria when using this specific research tool. Moreover, the FISH, despite its extraordinary abilities, is an expensive test, and pre-identification procedures are required (Bottari et al. 2006). The protocols for this method follows four steps: 1) Sample containing the target cells is fixed and permeabilized to allow penetration of the fluorescence probes into the cells, 2) The fixed cells are hybridized in a buffer containing the fluorescein-labeled probes at a specific temperature and under stringent conditions, 3) then the resultant is washed to remove unbound probes, and 4) finally, visualization and photographic analysis of the hybridized cells are performed by epifluorescence microscopy or flow cytometry (Figure 4).
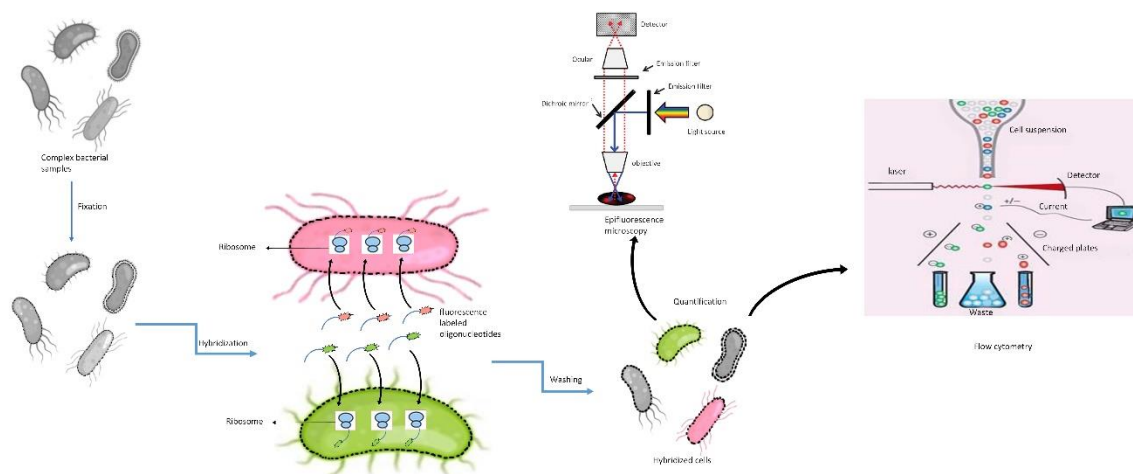
Figure 4. Fluorescence in situ hybridization is a rapid and accurate procedure for the identification of bacterial communities in different media. In this method, the sample is fixed to stabilize the cells and permeabilize the cell membranes. The FISH probes are added, and the 16S rRNA of the target bacteria are then hybridized with the FISH probes while the excess probes are washed. The resultant is subsequently analyzed by flow cytometry or epifluorescence microscopy.

The FISH method has the potential to approve data obtained with culture and PCR based methods. Where amplification-based techniques cannot be considered as routine, the FISH method is a helpful diagnostic tool for bacterial identification (Mallmann et al. 2010). Furthermore, this method can be used in the identification of the more abundant or invasive species in mixed infections (Kornreich et al. 2012; Schmiedel et al. 2014). The FISH method can also be useful, as routine techniques probably lead to errors and contaminations. However, there are concerns about the sensitivity of the FISH as a microscopic method. As the probe panel needs to be enlarged, the identification of the small microorganisms, such as intracellular microorganisms, with FISH remains challenging. In these cases, specific PCR assays will be able to identify the bacterial species (Schmiedel et al. 2014).

7. Other techniques for bacterial identification

7.1. Colorimetric sensor array

The colorimetric sensor array is a rapid identification method of the bacteria grown on nutrient media such as nutrient agar. This method is a simple research tool used for bacterial studies and optimization of bacterial production in the fermentation process (Carey et al. 2011).

7.2. Massive DNA sequencing

Specific PCR methods and DNA microarrays mentioned above can identify only earlier described bacterial species, and likely, novel or emerging bacteria will not be detected. This problem can be overcome by using universal primers to amplify and also sequencing in all bacterial species. The standard universal primer for the identification of bacterial is the 16S rDNA sequence. However, 16S rDNA sequencing is reliable only when the samples contain a single bacterial species. The bias in the relative amplification efficiency of 16S

rDNA from heterogeneous samples is yet another problem in this method (Suzuki and Giovannoni 1996).

Massive DNA sequencing is a method that amplifies the 16S rDNA sequence for all of the bacterial species with minimum bias, and therefore, identifies all mixed bacterial species (Hosokawa et al. 2017). The antimicrobial resistance genes of bacteria can be determined by this method without isolation and purification in the medium (Cai, Caswell, and Prescott 2014).

7.3. Real-time PCR

A molecular technique applied to monitor the amplification of the DNA or RNA sequence is known as real-time PCR. It is the cyclic reaction in which the gene of interest is amplified and quantified (Valones et al. 2009). Real-time PCR requires a thermocycler equipped with an optical system to receive fluorescence. This technique also needs computer software for analyzing the data of the reaction. The emitted fluorescence produces a signal in direct proportion with the amount of PCR products. The recorded fluorescence signals in each cycle exposed the amount of amplified product. The primer design is based on the alignment sequence of over one million bacteria 16S rDNA gene sequences. The design of the required primers was performed by software designers utilizing the RefSeq sequences of the GenBank databases. Real-time PCR was done for semi-quantification of the mRNA expression of genes containing the SYBR Green real-time PCR Master Mix primers and an adequate amount of cDNA (Valones et al. 2009; Dariush et al. 2019).

This method has many advantages over conventional PCR, including higher sensitivity, accuracy, and the possibility of determination of DNA amplification using fluorescence intensity. Real-time PCR has various applications within research laboratories, and this method can be quantitative or semi-quantitative (Franco-Duarte et al. 2019).

Real-time PCR is a more specific and inexpensive method compared to gel-based PCR assays. Also, it is possible to use one set of universal primers, such as 16S rRNA, and multiple specific probes to identified different genes of bacterial strains. Although the universal primers amplify the abundant bacteria in which false-negative results are probable, especially if a smaller population of bacteria is used (Cai et al. 2005).

7.4. Random amplification of polymorphic DNA-PCR

The random amplification of polymorphic DNA (RADP)-PCR engaged shorter primers with a length of 8-12 nucleotides with optional sequences that bind to the nonspecific sites on the template DNA of microorganisms. This helps in preparing a unique profile for bacterial identification because of the amplification of repetitive regions of template DNA (Jones and Kortenkamp 2000). This type of PCR can use isolated DNA or bacterial lysates then subject them to amplification in the presence of RAPD primers and magnesium to increase nonspecific annealing (Baker, Crumley, and Eckdahl 2002). Next, agarose gel electrophoresis is conducted on the amplified products to produce unique RAPD fingerprints. This method can be used to

identify a wide range of bacterial species that have not been identified because no prior knowledge of the target genomic sequence for RADP-PCR is required (Saxena et al. 2014). The main advantages of this method are its suitability for anonymous genomes (in which RADP-PCR sequencing enables researchers to obtain all the benefits of genomic sequencing without needing to share any particular information) and that it is very applicable where only limited quantities of DNA are available. Furthermore, this technology has high efficiency and low expense. The primers used in this technique are easy to purchase and do not require basic genetic and genomic information (Kumari 2014). However, homozygotes and heterozygotes cannot be distinguished by the current technology. This technique is very susceptible to changes in the quality of DNA, PCR conditions, and components leading to alterations of the amplified fragments (Arif et al. 2010; Kumari 2014).

7.5. Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) is a DNA variation that exploits polymorphisms in homologous DNA sequences to identify bacterial strains. The RFLP engages restriction enzymes, which can cut amplified DNA into DNA fragments. The DNA fragments are separated by agarose gel electrophoresis to produce unique patterns of bands for each bacterial strain. The bonding patterns are very similar in this method if the bacterial strains are closely related. Therefore, the RFLP is a suitable tool to survey the molecular epidemiology of infectious outbreaks to determine one or more pathogens involved in the outbreak (Mitani et al. 2005).

7.6. Amplified fragment length polymorphism

In amplified fragment length polymorphism (AFLP), restriction enzymes fragment genomic DNA, and ligated adaptors then amplify a subset of restriction fragments. In this method, primers are complementary to the adaptor sequences but have certain unique nucleotides; thus, only a small number of restriction fragments are selectively amplified. Gel electrophoresis is used to analyze the AFLP fingerprints and determines the yielding of distinct DNA fragments from a single strain genomic DNA. The application of AFLP in an intensive care unit (ICU) to investigate *Pseudomonas aeruginosa* in an outbreak has received a lot of attention (Bukholm et al. 2002). When there is no knowledge of the bacterial genomic sequence, the AFLP is a useful tool to determine the high specificity and discriminatory potential of genomic DNA (Franco-Duarte et al. 2019). However, there is more probability of producing inconclusive results because of DNA degradation. Also, this technology is very specialized and requires expertise to run the analysis (Tabit 2016).

7.7. Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) is a laboratory technique that separated large fragments of DNA and is a beneficial procedure for the characterization and identification of bacterial strains for epidemiological studies. In this technique, enzymes and detergents are applied to pure bacteria in agarose that release chromosomal DNA. The restriction enzymes cut agarose plugs at specific regions to produce a

restricted number of DNA fragments. Next, an electric current is applied to these plugs which are then subjected to alternate rotations in a magnetic field causing the DNA fragments to separate by fragment size, and finally, the banding patterns emerge (Parizad, Parizad, and Valizadeh 2016). This technique has challenges such as time-consuming protocols and high false results (Klaassen, van Haren, and Horrevorts 2002).

7.8. Metagenomics approaches

Metagenomics is a vital advancement method in which total DNA obtained from environmental samples is sequenced (Greninger 2018; Howe and Chain 2015; Datta et al. 2020). This method has revealed novel insights into a broad range of environmental samples. The process of metagenomic begins as an extension of local sequence alignments, and then each of the sequencing read is blasted with the other ones. Then, the highest-scoring pairs are selected, and overlapping sequences are identified for extension into the more extensive contiguous sequences. These sequences are developed for Sanger sequencing or next-generation sequencing (NGS) technologies (Howe and Chain 2015). Moreover, microbiome projects, such as the Human Microbiome Project (HMP) and Gut Microbiome Project (GMP), have been initiated to highlight the importance of metagenomics and un-cultural microorganisms (Datta et al. 2020).

Metagenomics has two specific sequencing strategies: amplicon sequencing in which the 16S rRNA gene is used as a phylogenetic marker, and shotgun sequencing that sequences whole given genomic DNA within

a sample (Rausch et al. 2019; Gholami et al. 2018). The use of the 16S rRNA gene has proven to be a cost-effective phylogenetic marker strategy for microbiome study. However, the protocols of the PCR-based phylogenetic marker are susceptible to biases by sample preparation and sequencing errors (Langille et al. 2013). Moreover, 16S rRNA gene amplicon sequencing is restricted to taxonomic classification at the genus level according to the available database used, so it gives us only limited functional information (Walsh et al. 2018). Therefore, these limitations of amplicon-based bacterial community studies raise concerns about the reproducibility of 16S rRNA gene amplicon sequencing and have resulted in an increased interest in developing techniques, such as shotgun metagenomics, with more accuracy and reproducibility for preparation and sequencing of amplicon libraries (Gohl et al. 2016; Faith et al. 2013).

Unlike 16S rRNA gene amplicon sequencing that only targets the 16S rRNA gene, shotgun metagenomics sequences the whole genomic DNA from bacteria (Brumfield et al. 2020). In this method, the library preparation includes random segmentation and adapter ligation. The typical process of using shotgun metagenomics data for the analysis of bacterial taxonomy is comprised of quality trimming and comparison to a reference database to produce a taxonomy profile. As the shotgun metagenomics sequencing gives all genetic information in the bacteria, the data can be used for other applications such as antibiotic resistance gene profiling and metabolic function profiling (Laudadio I 2019).

Shotgun metagenomics examines the functional relationships between hosts and bacteria by determining the functional content of samples directly (Faith et al. 2013; Walsh et al. 2018). Therefore, this method can detect unknown bacterial life that would otherwise remain unclassifiable (Rinke et al. 2013). However, the high cost of shotgun metagenomics and bioinformatics needs have further hampered its use for large-scale microbiome analysis (Walsh et al. 2018).

Obtaining sufficient sequences from the many organisms within the ecosystems is difficult. The abundance of different communities had led to some genomes being covered over many years, while others are covered by a handful of sequencing reads (Howe and Chain 2015). Also, the assembly of genes and single genomes is a challenge because of the high diversity of large microbial communities (Zhang et al. 2018; Alves et al. 2018).

8. Conclusion

The review has attempted to give a brief overview of past and current molecular techniques for the identification and classification of bacterial species. Sensitive and rapid detection of bacteria is now possible because of significant advances that have occurred in PCR and microarray assay. The development of the PCR and molecular genetic sequencing techniques has made it possible for culture-independent bacterial identifications. In recent years, a high-throughput 16S rDNA sequence in parallel to other high-throughput DNA sequencing techniques has proven more beneficial for the identification of novel bacterial species. Real-time PCR has been applied for the

sensitive identification of bacteria in different resources, including food, water, and animal/human tissues. Additionally, the microarray assay investigates the diversity of bacterial communities. At the same time, there are some technical difficulties, such as the extraction of materials from specific and complex environmental or biological samples and the availability of specific target sequences for the identification of specific bacteria, that need to be addressed. A significant challenge for all sequencing technologies is the validation of the technologies to establish the sensitivity and specificity of these methods for bacterial identification.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Ethical approval

This article does not contain any studies involving human participants or animals performed by any of the authors.

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