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DNA BARCODING STRATEGIES IN VARIOUS ORGANISMS, APPLICATIONS IN FOOD SAFETY, DISEASE CONTROL, AND FUTURE DIRECTIONS

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Abstract

DNA barcoding is a taxonomic technique used to detect the specie and establish phylogenetic relationship with other species. There is a great utility of using the DNA fragment for function as barcode. There is a specific DNA for each specie and by scanning such barcode, we easily identify the specific characteristic of any organism. DNA barcode is universal and different for each species but there is a limitation that still no single gene sequence has been discovered which can be used for each species including animals, plants, bacteria and viruses. Hence, there is the need of using different barcoded for all these different taxonomic groups. Identification of different species (endangered, new, or alien), food safety analysis, disease vector identification, monitoring of water quality and to sustain natural resources are some of important DNA barcoding's application. DNA barcoding has a huge potential in ecological analysis, biomonitoring, environment protection and biodiversity assessment. DNA barcoding enables to have genetic identification of different food products including crops, processed food, poultry food and fisheries. As DNA through every cell can be detected and identified and it cannot be changed, DNA barcoding make use of these features and make the diagnosis of food products easy. Many molecular techniques used for the detection of barcodes including PCR as well as loop mediated isothermal amplification have made possible the diagnosis and control of various diseases. DNA barcodes are also helpful and efficient in the bio surveillance as well as disease detection in order to monitor and target the damaging pathogens. If the new and efficient sequencing techniques are developed, it will bring revolution in DNA barcoding process as inexpensive and faster analysis will be performed.

1.1 Introduction

DNA barcoding is a technique which is one of the molecular methods to recognize the species and established phylogenetic relationship with other species. In this strategy, there is a great utility of using the DNA fragment for function as barcode. There is a specific DNA for each specie and by scanning such barcode we easily identify the specific characteristic of any organism. It is a swift and correct species identification system. It creates an attainable ecological system by using tiny DNA stretch instead of whole genome. This method is used for both prokaryotes and eukaryotes. The tiny DNA stretch is produced from pennant region of genome, which is act like a flag. This specific flag is different for different species such as cytochrome oxidase 1 (COI) for animal, matK and rbcL for plants, internal transcribed spaces (ITS) for fungus. It has many applications in different field such as agriculture, encouraging natural resources, preserve imperil species, protecting natural resources and identification of medicinal plants (V. Savolainen et al., 2005). For example, if we have a different collection of fishes and all have closely related to each other but during the speciation event, they might have undergone nucleotide changing specifically in that part of DNA fragment which experience moderate amount of changing during the course of evolution. So, such changing is used for DNA barcoding and although different species of fishes identify on the bases of these DNA barcode. The story was started in 1982, when Bemjamin Victor locate an unfamiliar fish in a shoal of Panama. By the finding of only single case, he was not able to demonstrate that it was a new specimen. So, the fish go on unsigned on his bureau for 25 years. Later, he was got off another larva in last year. By the use of barcoding, which is a new kind of DNA recognition technology. He demonstrated that it was a new kind of his riddle chunk, that both species were in fact a new specimen. DNA barcoding was devising in 2003 by Paul Herbert in the Guelph University, Canada. His aim was giving rise to a distinctive recognition marker for each specie which was based on short piece of DNA. Sunder organism would be a simple piece of work for sequencing this short piece of DNA. Dr. Herbert put forward the piece of gene called cytochrome c oxidase (CO1) as a satisfactory to this purpose. All the animals have cytochrome c oxidase I (COI) piece of DNA to fulfill that purpose. It should be well authentic, but not too much to act as definitive marker. Cytochrome c oxidase piece of DNA was simply taking out because it is one of the irritants to find out the gene outside the cell nucleus in mitochondria. (Hurbert et al., 2015). At beginning, the scientist used several morphological features, anatomical characteristic, histological characteristic, biochemical feature, cytogenetic, embryological, ecological characteristic. In case of animals, behavioral characteristic also used to identify a specie. So, due to taxonomic purpose different type of characteristics is utilized but presently molecular techniques have also been used to characterize the species. Particularly, DNA barcoding, is one of the techniques beside DNA hybridization. These are few authentic ways to identify the species.

1.2 Properties of DNA Barcoding

1.2.1 Discrimination

Barcoding region must be different for each species. Because in DNA barcoding, we are looking for single DNA locus which different in each specie.

1.2.2 Universtality

Barcoding typically amplified a region of DNA by PCR. So, there is need of primer that will be amplified consistently.

1.2.3 Robustness

Barcoding protocols amplify a region of DNA by PCR. There is also needed to select a locus that amplifies reliability and sequence well (Paul et al., 2010).

1.3 Database for DNA Barcoding

DNA barcodes are specific sequences in the genome of an organism, which are placed in Barcode of Life Data System (BOLD) database. It is an online ledge that composed of reference library of DNA barcodes, which can be used to allocate recognition of sequence of unidentified species. BOLD is a searchable storehouse for barcode records. It stores specimen data and imagine sequence as well as trace files. It gives us spotting software based on the contemporary barcode library. It also monitors the number of barcode sequences which records species protection (Morehead, 2020).

1.4 DNA Barcoding Methodology in Cancer Nanotechnology

DNA barcoding-based protein prescription method transformed the protein gesticulation into barcode oligonucleotide probes and amplified the signal by PCR amplification in order to achieve the easily offended detection. This methodology also useful for identification of low molecular weight surface marker of cancer cell. Weissleder group also developed photo-cleaved DNA barcoded antibodies which are used for recognizing multiplexed marker. DNA can be break by light at 350nm absorbance and added into solution where it amplified and analyzed by using gel electrophoresis. Anyhow, another amplification free method also accounts 90+ proteins in single cells by using DNA barcoded antibodies with fluorescent detection to look over inter and intratumor heterogeneity. This method also developed pathways to investigate drug response in clinical sample (Linmei Li & Yao Lu., 2018).

1.5 Meta-Barcoding

It is explained as the barcoding the DNA that grant a simultaneously unity of any texa. This unity lies mainly in same environmental sample. There are differences between the meta barcoding and DNA simple barcoding. In meta barcoding, the barcoding doesn't lie only on 1 special organism. On the other hand, DNA barcoding crisps on 1 specific organism. The other aim of metabarcoding is to identify or detect analyze configuration of species inside of a sample (Vu & Le, 2019).

1.6 Basic Steps of DNA Barcoding

The first step involves selection of appropriate DNA barcode marker. DNA barcode was built by means of reference database. DNA extraction and amplification should be carried out by using the PCR with specific primer. The amplified DNA fragment was sequence with the help of sequencer such as sanger sequencer and next generation sequencer. At last, bioinformatics analysis should be carried out (Kress et al, 2012).

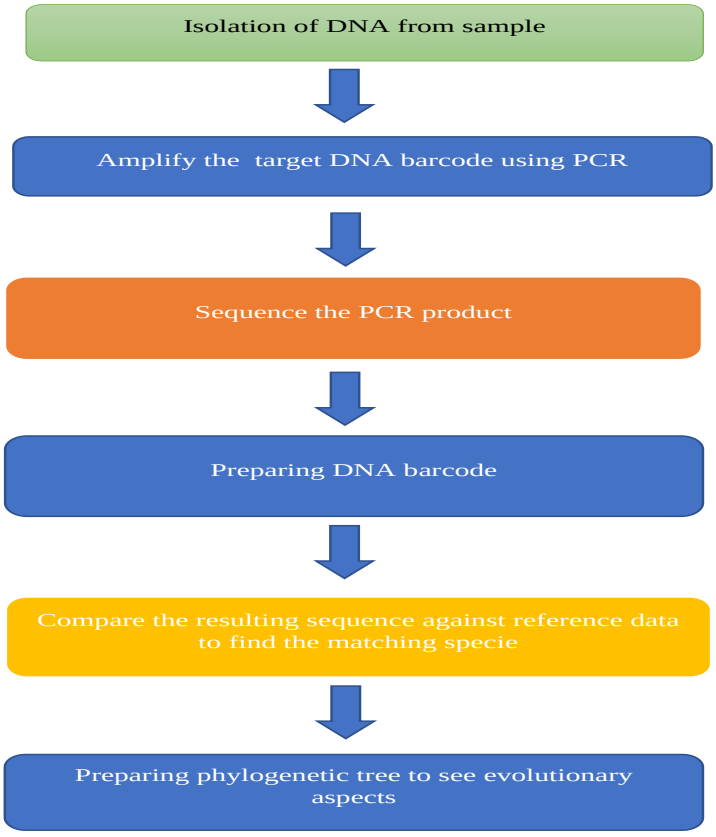

Figure 1. Flow Sheet Diagram of the Mechanism of DNA Barcoding

2.0 Barcoding of Different Species

In ideal cases, there is only 1 gene sequence will be used for all kind of species and groups including animals, plants, bacteria and viruses. But there is a limitation that still no 1 gene sequence has been discovered. Hence, there is the need of using different barcoded for all these different taxonomic groups or it will also depend on the type of study case.

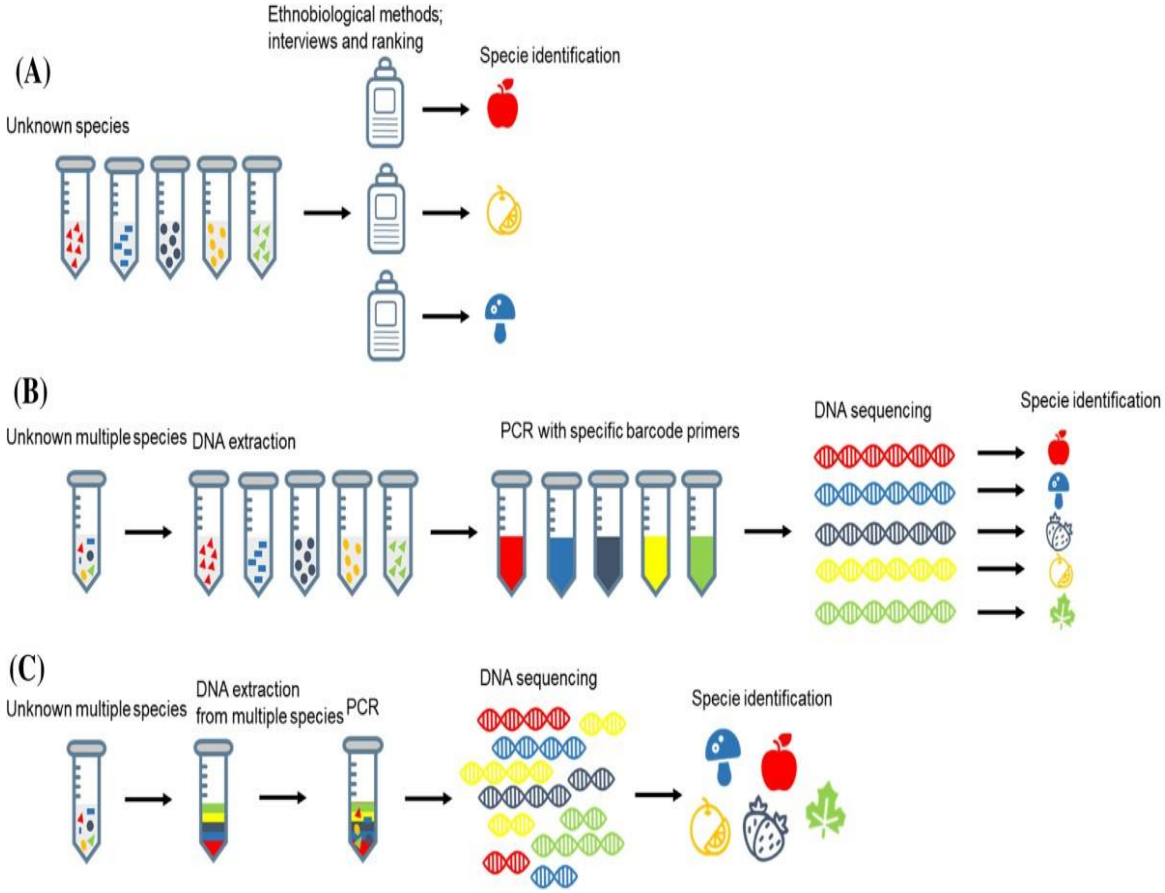

Figure 2. Method of DNA Barcoding for Multiple Species. (Dawan, J., & Ahn, J. 2022)

2.1 Barcoding Animals

There are various barcodes used for animals, but COI locus is the most frequently used barcode that is mitochondrial cytochrome C oxidase 1. Many other genes of the mitochondria are also used including 18S, Cytb and 12S. There are certain reasons that mitochondrial genes given preferences than the nuclear genes. The reasons include the absence of introns, the less recombination of mitochondria and the inheritance mode which is haploid mode. Some other reasons include the number of mitochondria present in the cell that is several thousand. All these mitochondria have many circular molecules of DNA. Although there is very limited tissue sample, mitochondria still are able to provide the abundant amount of DNA (Hebert et al., 2003).

2.2 Barcoding Plants

Mitochondria genes are not preferred in plants for DNA barcoding. There is the reason that very less mutation rates are shown by plant mitochondrial genes. For plants, many genes are present in the genome of chloroplast. Out of these, matK (K Gene) is the most dominant one that is promising itself or sometimes after being attached with some other genes. (Fazekas et al., 2008). In order to identify species, various markers having multi-locus have been used. These include ribosomal ITS DNA together with trnH gene, matK gene and rbcL genes. ITS is called as internal transcribed spacers. If we have to achieve the best identification and differentiation between plant species, 2 or more than 2 chloroplast genomic barcodes have to be used (Kress et al., 2007).

2.3 Barcoding Bacteria

A highly conserved gene is used for bacteria. The 16S gene which is small subunit of rRNA can be utilized for bacteria in order to perform DNA barcoding. (Janda et al., 2007) Various studies have indicated that in order to barcode bacterial DNA, COI, cpn60 or rpoB can be used as well. Cpn60 is type 2 chaperonin and rpoB is the beta subunit of polymerase enzyme of RNA that will serve as barcodes of DNA for bacteria (Smith et al., 2012).

2.4 Barcoding Fungi

The most difficult task is to barcode fungi as it is challenging. There are more than 1 primer combinations may be required. We may say that for some of the fungi groups, COI markers can be used but this is not true for all fungi groups. (Seifert et al, 2007) Hence, various other DNA markers are used for barcoding fungi. These other markers include ITS of rDNA and LSU of rRNA. LSU is the large ribosomal subunit of nuclear ribosomal RNA which can serve as marker for barcoding fungi (Dentinger et al., 2011).

2.5 Barcoding Viruses

For viruses to barcode, there are not any universal genes as with bacteria and fungi etc. These cannot be used to barcode viruses for differentiation or delimitation. Occasionally, on different basis like nucleic acid hybridization as well as host range and various other factors including electron microscopy and serological reactions, the viruses infecting plants are identified. Their specific group genes are identified by detecting through RT-PCR (Roossinck et al., 2011). As viruses lack universal genes, specific genes for viruses or sometimes for a group of viruses are identified and targeted for barcoding detection. For a virus of tomato brown rugose fruit, small replicase subunit region specific markers were developed for the detection (Alkowni et al., 2019).

There is another technology that is widely used now in order to diagnose and barcode viruses DNA named as RCA (Rolling Circle Amplification) (Jeske, 2018). This technology is an enzymatic process which is isothermal in which a short DNA/RNA primer are taken and then amplified to form a long ss RNA or DNA with the help of a DNA template of circular DNA and a polymerase enzyme e.g: Phi29 DNA polymerase. There are many advantages of using RCA technology over PCR. One of them is the ability of this technology to also identify circular DNA content without having any previous information or knowledge. There is the possibility of detecting viruses by using all these molecular technologies that can target the genomic regions. But still it is not possible and easy to detect the new viruses till that time when the whole genome of viruses has been decoded. (Jeske et al., 2018).

2.6 Barcoding Nematodes

Nematode barcoding is very important for the correct identification of them as well as many remedies for these phytopathogens as they cause various plant diseases. A combination of gene markers including mitochondrial COI and ITS were used in order to barcode a cyst nematode of potato that was distributed in Indonesia (Handayani et al., 2020). Four plant parasites species named as Aphelenchoides were detected using the mtCOI targeted regions (Sanchez-Monge et al., 2017). Various nematodes will be barcoded by QBOL. It will target 6 genome regions. These genomic regions include D1-D2 as well as D2-D3 of the LSU gene, IGS2, SSU, an RNA polymerase 3 fragment gene, subunit 1 and subunit 2 of COI genes of mitochondria. IGS2 is the 2nd intragenic spacer region and SSU is the small subunit of rRNA gene.

3.0 Applications of DNA Barcoding

Identification of different species (endangered, new, or alien), food safety analysis, disease vector identification, monitoring of water quality and to sustain natural resources are some of important DNA barcoding's application. DNA barcoding has a huge potential in ecological analysis, biomonitoring, environment protection, biodiversity assessment and removal of alien species from environment. DNA barcoding plays vital role in fields of ecology, biology, evolution, conservation etc.

3.1 General Applications

3.1.1 Identification of Species

Some short DNA sequences in genome of organism provides DNA barcode for their identification which are stored in (BOLD) in a database system. DNA barcoding has even ability to identify the species at larval stage where some of the characteristics of species are appeared for their identification. CITES has used DNA barcoding methods to monitor the illicit trade of threatened species. Invasive species can also be detected by DNA barcoding as it can easily differentiate between native species and invasive ones. On border control of species, when it is difficult to distinguish between morphological characteristics of different species, DNA barcoding helps (Buzan et al., 2013).

3.1.2 Identification of Disease Vector

With the help of DNA barcoding, scientists are able to identify those vectors that are responsible for disease in different animals and humans. A worldly mosquitoes DNA barcoding has been made as reference library of barcode that enables the less usage of different insecticides and scientists to point out the vector causing disease. DNA barcoding successfully detect the vector underlying the disease Leishmaniasis spread by sand flies. It involves detection of vector after analysis of DNA of 20 different species (Gang Wang et al., 2012).

3.1.3 Checking of Agricultural Pests

DNA barcoding also have a tremendous role in controlling agricultural pest by their identification even at larval stages. There is global tephritid barcoding that identify and controls fruit fly's drosophila melanogaster. Recently a new strategy under with the name of pollen DNA barcoding identify the origin of pollen species with high taxonomic resolution (Paul D. and N. Hebert 2005).

3.1.4 Water Quality Monitoring

One of the important sources of life is drinking water. Determination and management of different living species in water is necessary. By DNA barcoding a library of these species is created to identify them. In 2009 scientists were able to identify 184 different species in an aquatic sample with the help of DNA barcoding. Now different environmental agencies are using barcoding to check the water quality and to make better strategies for them (Akhtar et al., 2015).

3.1.5 Evaluation of Biodiversity

Biodiversity assessment can be done with less difficulty by the aid of DNA barcoding. The areas which are so enrich with different species are difficult to analyze. Even in many cases species become extinct before they are properly recorded in taxonomy. According to a study, more than ninety percent species are still waiting to be recorded. This is more frequent in developing countries where resources are not enough to do all this assessment. Here's the DNA barcode make this easier and time consuming to evaluate and keep record of the biodiversity in an area. Moreover, DNA barcoding is also helpful in conservation of rare species about which there is a danger of extinction soon in future (Buzan et al., 2013).

3.1.6 DNA Barcoding and Biomonitoring

DNA barcoding has a huge potential in detection of endangered species as well as the indication of different ecological conditions such as low level of oxygen at a place etc. It helps a lot in environmental conservation and ecological assessments. Some invasive alien species possess a threat to humans and environment. Due to tourism and trade these exotic species tends to increase in number and cause harm to environment. Their detection through barcoding by using BOLD is very important to eliminate them before they cause huge disaster (Buzan et al., 2013).

3.2 Application of DNA Barcoding in Food Safety

During Food storage and transportation there is a huge risk of food piracy and mislabeling as there were no standard reference for identification of food labeling. Now a days when there is revolution in agriculture and industrial production of food by using different advance technologies, so customers are more conscious about their health and want to know more about authenticity of food products. Advancements in bioinformatics and gene studies enable us to keep surety about food piracy. Here DNA barcoding comes into play, international platform of BOLD has a reference library having living species' barcodes of DNA. DNA barcoding enables to have genetic identification of different food products including crops, processed food, poultry food and fisheries. As DNA through every cell can be detected and identified and it cannot be changed so DNA barcoding make use of these features make the diagnosis of food products easy (Gianni Barcaccia, 2015).

3.2.1 Identification and Traceability of Food Products

The identification of food products and even their raw material is very necessary to prior to their consumption. DNA barcoding is very effective in proving origin of food and all type of its raw material. Moreover, if there is any kind of adulteration in food products then DNA barcoding immediately detect it. When it comes to seafood and poultry food then the identification of organism is also very necessary as the effectiveness of DNA barcoding is strongly rely upon on molecular variability of organism. If the organism is closely related to some other species than the resolution of DNA barcoding can be affected. So, the organism should have low intra species polymorphism in order to achieve high resolution (Andrea Galimberti, 2012).

3.2.2 DNA Barcoding and Agriculture Products

Before barcoding of DNA, it was a major concern for biologists to identify inter and intra specific species identification when it comes to agriculture and crop products. DNA barcoding sequence short chloroplast genome fragment of test plant which based on the nucleotide variability and compare them with available reference sequence in database such as BOLD. Agriculture products have to undergo processes before ending up into final consumable products. Sometimes maybe these procedures change the morphological structure of plants, but DNA can withstand these manufacturing strategies even present in small amount so is an authentic source of identification (Massimo Labra, 2014).

3.2.3 Use of Barcoding DNA for Dairy Products

These products are food products that made from mammalian milk. There is an increased chance of adulteration and allergies in milk-based products. So, authentication of these dairy products is one of the major concerns to minimize the risk of adulteration. DNA barcoding is that molecular tool which enables the trace and characterization of DNA from sample products like milk by having access to these mammalian species DNA. There is a universal marker named plastidial rbcl has been shown to have potential to detect traces of raw cow milk. This not only describe the composition of milk-based products but also the composition of microbial used in processed dairy products (Andrea Galimberti F. D., 2012).

3.2.4 Detection of Food Adulteration

Now a days there are increased chance of food fraud by mislabeling and erroneous description of food products as the food is processed and have no ability to distinguish components. The detection of food components through DNA barcoding by using barcode of life data system facilitates to have an insight into species taxonomy. So, whenever there is a problem regarding food safety arises, it will immediately be stopped from being reached to customers (Gianni Barcaccia, 2015).

3.2.5 Role in Food Manufacturing Processes

DNA barcoding not only involve in identification of plant and organism's species but also have a role food processing method. As the quality of food not only depends on its composition but also on manufacturing process of food. As the most common foods like bread, cheese, wine dairy products etc. are processed through biological methods such as fermentation and here the microorganisms have role. DNA barcoding controls the microorganisms throughout the food manufacturing by identify the microorganisms and their origins. This is also helpful in prevention of food from spoilage from microbes by identification of the species and effect of environment on them (Andrea Galimberti M. C., 2019).

3.3 Applications of DNA Barcoding in Disease Control

DNA barcoding is a technique that enables scientists from various fields to apply them in different areas. These fields involve forensic sciences, taxonomies, biotechnology and food industry. Apart from these fields, DNA barcoding can be applied in disease diagnostics and disease control. For metagenomics, DNA barcodes have been utilized. In this field, barcodes have helped in knowing both non culturable and culturable populations of microbes. Many molecular techniques used for the detection of barcodes including PCR as well as loop mediated isothermal amplification have made possible the diagnosis of various diseases and these barcoding genes brought a revolution in the capabilities of curing these diseases after diagnosis (Konwarh and Sharma, 2020).

3.3.1 DNA Barcoding in Disease Control & Diagnostics

There are many advantages of using the molecular techniques and molecular morkers (barcodes) for detecting and diagnosing diseases over all other methods. The advantages include the accuracy of these signatures, ability and reproducibility for quick detection of disease. DNA barcodes are also helpful and efficient in the bio surveillance as well as disease detection in order to monitor and target the damaging pathogens. There are various other benefits of using DNA barcodes for diagnostics as they are very important to analyze the pathogens and then we can limit the entry of these pathogens in new regions, and we can also then prepare or devise a new and efficient control strategy for that particular pathogen and disease. To date, various fungal, viral and bacterial pathogens have been identified, diagnosed and monitored successfully using this DNA barcoding technique.

3.3.2 Barcoding & Diagnosis of Plant Pathogens

In order to control the spreading of disease, the plant pathogens have to be detected early as well as the health of the plant. This strategy enables us to prepare and devise an effective managing practice. Disease results not only be defined as caused by the only main pathogen but also some other diverse groups and microbial communities are also the reason for disease cause, and these are called as the microbiomes. The plant pathogens include viruses, bacteria, fungi, nematodes, phytoplasmas and oomycetes. Detecting their genes and markers and barcoding them will help to diagnose as well as control the disease specifically caused by these agents. Molecular barcoding has gained a huge importance from many years but there are various challenges faced in order to develop and apply the molecular techniques for diagnosis to help the plant pathogens to be detected (Li et al. 2019).

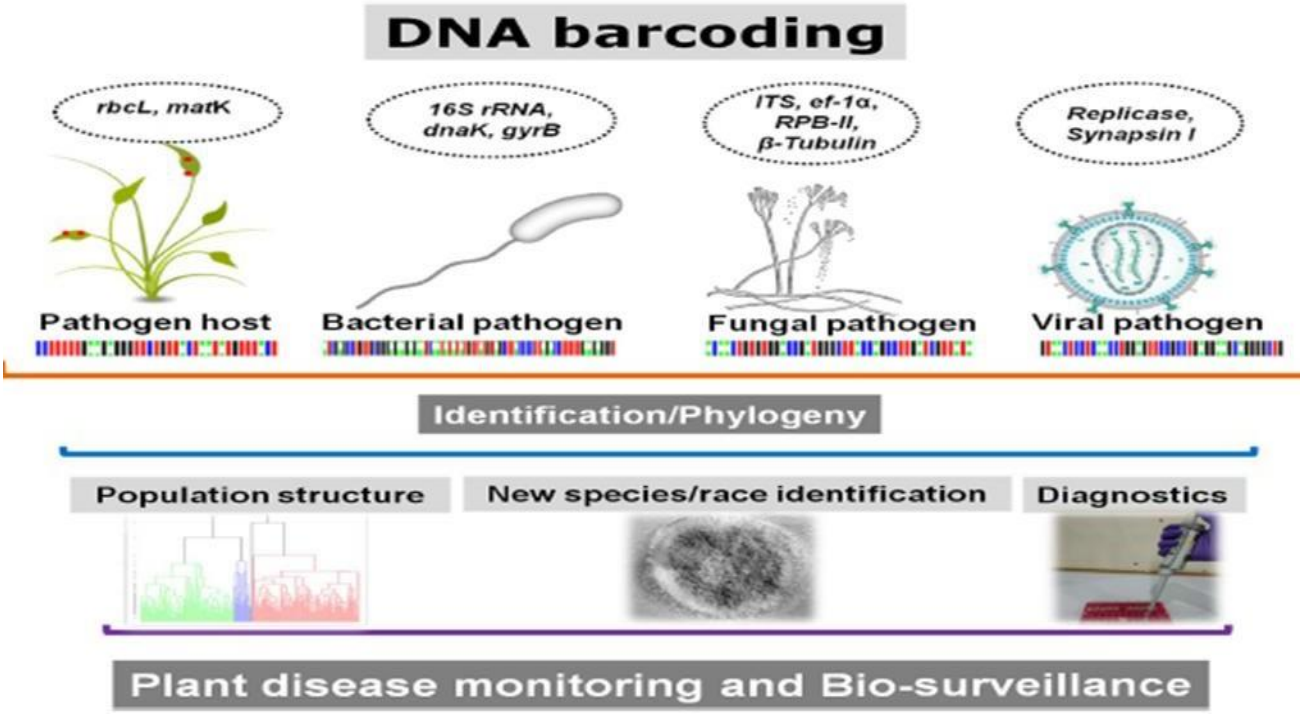

Figure 3. (Schematic Picture Representing the Applications of this Barcoding DNA Technology in Diagnosis and Disease Control) (Choudhry et al, 2021)

3.3.3 Meta Barcoding & Detection of Disease

Technique or method used to amplify barcodes the gene parts from different environments including soil or tissues of the plants. In order to get inside of the microbial communities.

Meta barcoding is an effective method that used the universal primers as it is a high throughput screening method. Using these microbial communities to detect the changes in environment is an old method therefore, the scientists need to identify and devise high throughput techniques that are more reliable and efficient to complete these targets. Metabarcoding has various applications including biomonitoring the changes in environment, studying biodiversity and exploring the microbial ecology (Laura et al., 2020). Beside these all advantages and applications of meta barcoding, this methodology is specifically used for different aspects that involves detecting the complexes of various plant diseases, naming and cataloguing them and finally discovering and identifying plant microbiomes.

3.3.4 Plant Disease Detection & Meta Barcoding

Currently, the idea of meta-barcoding increased comprehensively because of its use in diseases in the detection process of plants, a scientist implemented meta barcoding to account grapevine body with thes multiplex infectious agent, the corresponding visualization in plant picking the ITS (ITS 1, 5.8 S, and ITS 2), larger sub unit and SSU domains (Morales-Cruz et al. 2018). A fascinating technique were employed by Tremblay et al. (2019) to show interactivity of plant of a beehive describing samples of pollen pellet by meta barcoding. This reported many plant pathogens for importance of agricultural ways like these, Colletotrichum sp, Fusarium sp., Alternaria species and Pythium sp. attacking ITS1 intergenic domains for fungi and also for oomycetes, ATP9-NAD9 for the Phytopthora sp. and for ITS 2 for plant species (Tremblay et al. 2019). Metabarcoding is very helpful for the disease significance. Abdelfattah et al. (2016) showed that Myco sphaerella citri named to affect oily spot disease in citrus was not showing through process of metabarcoding describing interaction of other species affecting the disease in area of southern Italy (Abdelfattah et al., 2016). For understanding of microbial interactivity, information obtained through this process is useful. That can show significant role in management of the diseases.

3.3.5 Combination Process of DNA Barcodes for Identification of Medicinal Plants

Scientists employed the combination of DNA markers when plants cannot be detected by a single locus. Most common one marker that include trnH-psbA, matK, rbcL and ITS are used. Mishra et al. (2017) describes effectiveness of barcoding DNA method for establishing species surroundings in Terminalia, Combretaceae by employing the regions of ITS and matK ITS. But the useful one includes ITS and trnH-psbA as showed by Kress et al. (2005) to detect flowering plants. Significantly, Chen et al. (2014) detected traditional materials that were in listed for the traditional pharmacopeias of India, Korea, China, Europe and of America by employing the of ITS 2 + psbAtrnH barcode loci. with this data, the TCM barcode (TCMD) was emerged. For treatment (neurological diseases) like hemiplegia, sciatica, emaciation, cervical spondylosis, and neuralgia, the roots of the country mallow Indian are employed, "Bala" and applied for powering of the CNS process.

The study implemented by Wang et al. (2020) demonstrates the best one used combination is that ITS + psbA-trnH for the detection of Stephania species and of Menispermaceae. For the treatment of disorders such as dysentery, fever, tuberculosis and cancer, Stephania species are employed vastly.

4.0 Limitations & Short Comings of DNA Barcoding

Though it emerged as much significance, but it has many shortcomings that are following:

4.1 DNA Barcode Gap

Main thing that proved a disadvantage like in the strain type and of unknown strain (differences) matrices gone higher by use of taxon sampling study (Meier et al. 2008) by using the mean ones in place of the smallest, used interspecific distances for identifying the gap barcoding. It showed relating to DNA shows that none of single gene is ideal barcode like be same within the species though different among species. By disease forecasting and by surveillance., many things are happening to understand the pathosystem. The use of the Biosecurity protocols globally to show the spreadness of non-native infectious hosts via trade internationally, more commonly ornamentals (Luchi et al., 2020).

4.2 Physical Parameters

In physical parameters, it's not a simple way to combine DNA barcodes to ecological ways of barcoded taxon in query, because it is needed when it used for the purpose of biomonitoring. In aquatic systems for the detection of target DNA dependent on the DNA molecules concentration, caused by many factors involved. DNA molecules presence is also dependent on dispersion like, strength or the direction of currents. DNA movements in streams and lakes are not known significantly, that made the sampling tough. Some other factors can be helpful in affecting the target species, like some fishes have changes of movements on seasonal basis. Some fishes like these (crayfish or musssels) can release DNA in higher amounts (moulting, spawning). For distribution and quality, the DNA in soil is less known.

4.3 Barcode Reference Libraries

The main constraint of using the barcoding technique depends on barcode libraries of references for the purpose of taxonomic detection of used sequences in this process. That is true only if the reasonable reference is used. But, for the organisms like fungi and phytoplankton many of the databases are not complete. In addition, spelling mistakes are present in the currently available databases that have many misdetections.

4.4 Technological Bias

From sampling towards the bioinformatics analysis of data, the DNA barcoding includes methodological bias. PCR inhibitors can contaminate the DNA samples, apart that the most common or bigger source of error in DNA barcoding is primer bias. Efforts had been done to identify primers for use in taxonomic groups that are different, the isolation of DNA marker, and to

check the primers' design is a burdensome process. Apart that, PCR replication goes towards exponential increase in contamination. Use of samples of mitochondria-enriched or PCR-free methods to reduce biases, many approaches had been made but today, but DNA metabarcoding method is based on sequencing of amplicons in this process. (Pawlowski et al., 2018).

4.5 Lack of Standardization

But the boundaries of bioinformatics have been the domain of much discussion among users of DNA- barcoding. Technologies of sequencing are also emerging speedily, combined with the tools for the study of the DNA data obtained, and uniformity of these used techniques is needed quickly to show alliance and data distribution and sharing at larger time-scale. (Zhou et al., 2013)

4.6 Mismatches among Barcode-Dependent & Conventional Identification.

The direct comparison of taxa lists obtained by barcode-based recognition due to several causes, is significant to know that these listed obtained by the (morphological) detections never will occur. Preventing accurate taxonomic assignment sequences of eDNA, the most significant cause is the incompleteness and second one is the shortage of authenticity of the molecular references databases obtained. But Sequences that are linked to a missing one names will goes noy correctly recognition, taxa that is not occurring in databases references not to occur by eDNA (Pawlowski et al., 2018).

4.7 Estimates of Richness/Diversity

Mainly the method used of DNA Barcoding can be resulted in underestimate of species diversity. Several studies on this shows that artifacts are a main root cause of affecting the of inflated biodiversity. For low numbers of sequencing reads, the most burdensome issue is happening. Since many studies shows the reads that are of low frequency could be artifacts, these reads are eliminated by the data filtering methods. Thus, for allowing the differentiation in informative reads and in artifacts, there is a powerful need for more strong bioinformatics algorithms used. For improving testing process of bioinformatics algorithms employed; by allowing a suitable filtering process of artifacts, complete libraries of references would give a and so far, it would be achievable to obtain an authentic and true one species assignment (Zhan et al., 2014).

5.0 Future Perspectives & Directions

Effective and quick protocols are required for plant pathologists. DNA barcoding method or technique can achieve this problem in future. Methods and Dual DNA barcoding is achieving attention significantly for fungal pathogens (Hoang et al., 2019). There are numerous applications and advantages of using this technique in the disease diagnostics. For timely execution of management or control techniques, it can be handy in facilitating swift identification of phytopathogens. For the detection of the phytopathogens process on level of field can assist to limit the use of agrichemical compounds and can made the crop enhancement processes more greener one.

Super-barcodes" that has greatly enhanced the authenticity and accuracy of species detection and recognition. There is more need to or a requirement to synchronize in the taxonomists and on DNA-barcoding working, in the NGS. Right from the collection of samples to the point of selection of genomic domains for evaluation, the Meta- barcoding that are required to be handled and arranged accurately. Failing to overcome with the challenges occurring recently could exist for some reasons. For the commonly employed fungal infectious agents, and also in protists and of the animals (Taylor and Harris 2012), the search for barcode locus continues for universal barcode. There is need to collaborate with the thought that it has no individual importance, is needed with the taxonomy of 250 years, detection by morphologically, NGS and also by techniques used of genome skimming and isothermal amplification techniques and many other techniques. By using these we can fulfil the requirements of identifying newly species, detection of materials of unknown plants and disease or disorders diagnostics. For investigation of the Phyto medicinal and herbal product and accuracy by DNA barcoding with the aim of covering and by defending consumers from associated potential risks with product substitution and with the contamination in

this process. These techniques and methods could enhance the ethno-botanical and scientific philosophy of phytomedicinal and safe use of these medicines. For understanding the extension and improvement in DNA barcoding with special reference barcode for detection and of interaction of species, there is more need to do. Then recognition and identification of organism could make it reliable by employing the barcode with use of significant sequences of nucleotides involved. These are the major features, aims and goals of DNA barcoding that can be obtained by environmental scientists, molecular biologists and ecologists.

Conclusion

With advancements and improvements in PCR as well as sequencing techniques, DNA barcoding for both plants and animals will keep on improving. The sequencing technology is improving day by day and with the help of NGS, DNA barcoding process will be more applicable in different species with more efficiency. These sequencing techniques are very efficient for barcoding DNA extracted from collected samples from soil or animal intestines etc. It is only possible to look for overlooked species as well as the identification of potential cryptic species can be possible when a threshold in the target group has been achieved or established. Hence, DNA barcoding process has a key component in the form of establishment of robust thresholds for the delimitation of species. At the moment, there is not a single technique that may be applicable universally to classify and differentiate species as well identify them. However, it is proposed by various researchers that when single region of DNA is not enough for DNA barcoding, then we can apply 2 or more DNA regions in combination for barcoding. In the end, scientists predict that if the new and efficient sequencing techniques are developed, it will bring revolution in DNA barcoding
process as inexpensive and faster analysis will be performed. Consequently, DNA barcoding will not only diagnose and control different diseases but also revolutionize other fields of science like pharmacy, medicine, forensics and biotechnology.

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