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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

REVIEW ARTICLE BIOSCIENCE RESEARCH, 2019 16(2): 1763-1775. **OPEN ACCESS**

Application of Using DNA Barcoding Genes in Identification of Fungi Species, a Review

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Kingdome fungi are characterized by having a great biological diversity on land and plays important role as organic materials degraders in ecosystems. Although there are more than 1.5 million species of fungi colonizing our land, the numbers of fungi species identified are less than 5 % of fungi species expected to exist, This is because of using traditional methods that are based on morphological characteristics for identification of fungi species. Currently, DNA sequences based identification (DNA barcoding) has became as an effective method for identification and taxonomic studies of known and unknown fungal species. Moreover, DNA barcoding has been shown to be more reliable, accurate and quick identification method for genus to species level. In this paper, The history of DNA barcoding in fungi was introduced and application of using most useful DNA barcoding genes in identification of fungi species were shortly reviewed at topics including internal transcribed spacer (ITS) of rDNA, the 28S rRNA (the nuclear large subunit, LSU), and the 18S rRNA gene (the nuclear small subunit, SSU), β-tubulin (*BT*), Translation elongation factor 1α (*TEF-1α*), Second largest subunit of RNA polymerase II (*RPB2*), minichromosome maintenance protein (*MCM7*), and Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). Universal primers of DNA barcodes genes for fungi identification were included as well.

Keywords: Fungi, DNA barcoding, rDNA, ITS, *TEF-1α*

INTRODUCTION

Fungi is considered the second largest group on the planet as they show high species diversity, where the number of fungi species is estimated between 1.5 -5.1 million (Hawksworth, 1991; O'Brien et al., 2005; Blackwell, 2011). Many fungi have a great value and usefulness in human's life and health because of its economic and medical value. Moreover, fungi are considered as a natural decomposer in the ecosystem. Alongside many species also are considered harmful and have the potential to cause diseases for humans, plants and animals (Borman et al., 2008).

In spite of the great importance of fungi with its diverse species of up to a millions. The identification of fungi species remains late, where estimated species identified and described are less than 5 % of their expected number on the planet. (Hawksworth, 1991). Part of this problem is because of the conventional methods used in fungi identification. These methods usually depend on the identification and description of fungi through the cultural characteristics and microscopic description within the laboratory. As a result, these methods requires great effort, knowledge and experience in the science of fungi, which usually make the process of classification of fungi difficult and advances slowly (Nilsson et al., 2011). Moreover, in some fungi species, a study of morphological characteristics which may be missing, misleading, and morphologically similar

have made the identification of fungal species a major challenge for mycologists using the conventional methods (Seifert, 2011).

DNA sequences have become the main reference in the identification of fungal species (Hebert et al., 2005). The use of DNA sequencing in the identification and classification of fungi species has enabled fungal scientists to overcome the challenges to using conventional methods (Atkins & Clark, 2004 ; Sun & Guo, 2012). Molecular techniques such as a DNA barcoding have recently been the main tool in identification of fungi (Purty &Chatterjee, 2016). The DNA barcode is considered as a short gene sequence found in organisms' genome which is unique completely to differentiate organisms' identity to species level. The employ of DNA barcodes for the identification of new species is arising as a effective tool to resolve species boundaries and to measure species variation . In many cases, these DNA barcodes can be used as a basis for the detection of new taxa (Costion et al., 2011).

Identification of a fungal species by DNA barcoding tool is done by amplifying specific region of a gene by Polymerase Chain Reaction (PCR) (Purty &Chatterjee, 2016). Generally, DNA barcoding utilizes short DNA sequences (500- to 800-bp) in species discrimination among most living organisms using PCR primers that are usable for the wide taxonomic group (Conrad et al., 2012). There are several criteria that are taken into account in any ideal DNA barcode. Of these are short piece of DNA and universally usable. Moreover, it shows large variation among species than within species and is conservative within species and among fungi species. In addition, the ideal DNA barcode is preferred in case it has high rate of PCR amplification (Hollingsworth et al., 2009).

DNA barcoding sequence amplified by PCR is then compared against a sequence database, such as GenBank sequence database (GenBank) the European Nucleotide Sequence Archive of the Molecular Biology Laboratory, (EMBL); and the DNA Data Bank of Japan, (DDBJ). The sequences comparing in those sequence databases usually are carried out by an individual Basic Local Alignment Search Tool (BLAST) to identifies species based on sequence similarity (Altschul et al., 1990; Taylor et al. 2000; Huzefa at al., 2017).

Many Mycologists have been contributing to attempts finding typical conserved DNA barcodes sequences which would be useful for identification of most fungi groups . Several DNA barcodes (genetic markers) have been investigated to resolve challenges related to fungi identification and taxonomy. Short region sequences belong to the nuclear ribosomal DNA (rDNA) and protein-coding genes have been used extensively as proposed DNA barcodes.

The region of internal transcribed spacer (ITS) of rDNA has been used widely and got lots of attention and consideration among mycologists as DNA barcode marker for fungi (O'Brien et al. 2005; Buée et al. 2009). However, ITS has been found not to be having enough genetic variations in some fungi groups such as blue fungi and yeast species. The nuclear large subunit ribosomal RNA gene (LSU) has been proven to be having more sufficient sequence variations than ITS regions in yeasts species identification (Kurtzman and Robnett, 1998 ; Fell et al. 2000; Roe et al., 2010).

Protein-coding genes are generally used more in building phylogenetic trees to infer relatedness between fungi groups and genera (Schoch et al., 2009). Actually, Protein-coding genes have shown strong ability for determining relationships at different taxonomic levels (Schoch et al., 2009). The β-tubulin sequence gene has been used successfully as a reliable DNA barcode in species separations for *Penicillium, Fusarium,* and *Aspergillus* (O'Donnell and Cigelnik, 1997; Frisvad and Samson, 2004) and Translation elongation factor 1-α has proven as reliable DNA barcode for *Fusarium*, Trichoderma and *Hypocrea* in species separation (Druzhinina et al. 2005; O'Donnell et al., 2010). In addition, the largest subunit of RNA polymerase II (RPB1) has shown a reliable capability as a DNA barcode for fungi groups including Basidiomycota, zygomycota (Matheny et al., 2002; Tanabe et al., 2004).

However, fungi universal barcodes have not been definitively finalized formally but many studies have proposed potential DNA barcodes . Therefore, our information on DNA barcoding for fungi identification needs to be improved and reviewed as most fungi identification today is carried out using emerging molecular methods that are based on DNA sequence in identification of fungi species . In this article we review most DNA barcoding genes used todays in fungi species identification.

History: DNA barcoding for fungi

Since long time, morphological characteristics including conidia or spore shape and structures containing spores have been conventionally used as only way for grouping and identification fungal cultures (Hyde &Abd-Elsalam, 2010) However, Identifying of fungal cultures mainly on morphological characteristics is considered hard work and time consuming, particularly when well-skilled mycologists are not available to examine the fungal culture characteristics. In addition many cultures do not produce spores, therefore, absence of morphological characteristics may be resulting in misidentification.

DNA sequence-based tools have appeared as a strong tool for studying fungi identification and taxonomy (Hibbett et al., 2011). The employ of DNA sequences to obtain important formation about identification of biological species of living organisms is not newly introduced , in fact, DNA barcoding was first proposed before more than a decade ago (Pecnikar & Buzan, 2014). DNA barcoding actually started 2003 when a Canadian scientists Paul Hebert and co-workers from University of Guelph in Canada wrote a paper titled "Biological identifications through DNA barcodes " stated that we can use a short DNA sequences from certain regions in genome to identify species, he called these sequences barcodes (Hebert at al. 2003).

The employ of DNA sequences in understanding variation among organisms has started early in the 70's. But real use of DNA sequences has not started until PCR and sequencing were developed. Certain DNA region of cytochrome c oxidase I gene (COI) of mitochondria has been proved as potential DNA barcode for animals' identification. CO1 was proved not to be a good genetic marker for fungi as it has very low rate PCR amplification (Schoch at al., 2012).

The first report published was 1982 using 5S rRNA gene sequences to explore the relatedness among different fungi groups (Dutta and Ojha, 1972; Walker and Doolittle 1982). After several years, another important paper published by White et al., (1990) that was the base of identification of fungi species based on DNA sequences. This paper revealed many of the PCR primers to be used to amplify universal fungal ITS regions of rDNA. Those developed primers are still in use in most today's fungal laboratory work. The ITS sequence was recommended as the genetic marker region to be used by many mycologists at an international conference held by the Consortium for the Barcode of Life (CBOL) in 2007

(Schoch & Seifert, 2011). Since then, Looking at other genes that can be used as candidates for barcode regions has started with a search focusing on protein coding regions. Nowadays, ITS is still a promising DNA barcoding candidate for fungi kingdom (Schoch & Seifert, 2011).

After some DNA barcoding genes were determined, Mycologists were asked also for establishment of usable databases to deposit obtained sequences of known species and origins, So that, sequences deposited in databases can be used in sequences comparing for identification by mycologists worldwide (Schoch & Seifert, 2011). Researchers have then employ DNA sequences for fungi species identification and taxonomy. The obtained sequences were compared with databases of reference sequences for identification and then deposited in databases such as mainly GenBank , EMBL or DDBJ. For example, the Barcode of Life Data Systems (BOLD) contains millions DNA sequences belonging to selected DNA species barcodes, that are close up to more than 70,000 organism species including fungi. Meanwhile, GenBank has more than 150,000 sequences of ITS region, Those ITS sequences deposited represent nearly 14,000 species (Schoch & Seifert, 2011). However, the challenges of obtaining the appropriate DNA barcode have not been absolutely solved so far and the search for the appropriate DNA barcode for fungi keeps on.

Appropriate fungal DNA barcoding genes

Internal transcribed spacer (ITS) of rDNA

The ITS region of rDNA is the most commonly DNA sequence used in molecular identification, and has been proposed as the DNA barcode for fungi identification (Schoch et al., 2012). Researchers have found it a reliable tool for identification fungal cultures at genus level to species level. The ITS regions are found in fungal genome as tandem repeats (Table 1) (Michot et al., 1983).

Table 1: Universal DNA barcoding Primers for PCR amplification of fungi

Primer name	Sequence (5′->3′)	Reference
ITS1	TCCGTAGGTGAACCTGCGG	White et al. 1990
ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990
BMB-'B'	CCGTCAATTCVTTTPAGTTT	Lane et al. 1985
BMB-'C'	ACGGGCGGTGTGTPC	Lane et al. 1985
BMB-BR	CTTAAAGGAATTGACGGAA	Lane et al. 1985
BMB-CR	GTACACACCGCCCGTCG	Lane et al. 1985
5.8SR	TCGATGAAGAACGCAGCG	(Hopple & Vilgalys, 1995) (Hibbett&Vilgalys,1993) (Hibbett&Vilgalys,1991)
LR7	TACTACCACCAAGATCT	(Hopple & Vilgalys, 1995) (Hibbett&Vilgalys,1993) (Hibbett&Vilgalys,1991)
LR5	TCCTGAGGGAAACTTCG	(Hopple & Vilgalys, 1995) (Hibbett&Vilgalys,1993) (Hibbett&Vilgalys,1991)
LR0R	ACCCGCTGAACTTAAGC	(Hopple & Vilgalys, 1995) (Hibbett&Vilgalys,1993) (Hibbett&Vilgalys,1991)
LR16	TTCCACCCAAACACTCG	(Hopple & Vilgalys, 1995) (Hibbett&Vilgalys,1993) (Hibbett&Vilgalys,1991)
LR3R	GTCTTGAAACACGGACC	(Hopple & Vilgalys, 1995) (Hibbett&Vilgalys,1993) (Hibbett&Vilgalys,1991)
EF1-1018F	GAYTTCATCAAGAACATGAT	Stielow et al. 2015
EF1-1620R	GACGTTGAADCCRACRTTGT	Stielow et al. 2015
Btub2Fd	GTBCACCTYCARACCGGYCARTG	Stielow et al. 2015
Btub4Rd	CCRGAYTGRCCRAARACRAAGTTG TC	Stielow et al. 2015
fRPB2-7cF	ATG GGY AAR CAA GCY ATG GG	Stielow et al. 2015
fRPB2-7cR	CCC ATR GCT TGY TTR CCC AT	Stielow et al. 2015
gpd1	CAACGGCTTCGGTCGCATTG	Berbee et al., 1999
Gpd2	GCCAAGCAGTTGGTTGTGC	Berbee et al., 1999

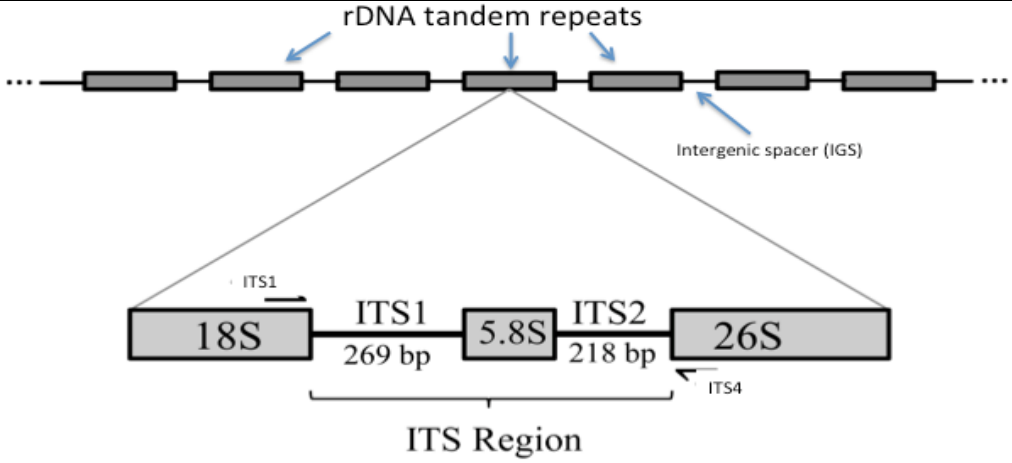

Figure1: The ribosomal RNA genes (rDNA) of fungi found as tandem repeats (Zhang et al., 2015)

ITS regions including internal transcribed spacer1 (ITS1) and internal transcribed spacer 2 (ITS2) are variable DNA sequence areas of rDNA cluster genes which also contain the 18S rRNA gene (the nuclear small subunit, SSU), the 5.8S rRNA gene, the 28S rRNA gene (the nuclear large subunit, LSU) (Table 1). These rDNA cluster genes are flanked by 5' external transcribed sequence (5' ETS) and 3' ETS (Michot et al .,1983).

The rDNA tandem repeats are found in thousands copies in fungal genomic DNA and are detached by non-transcribed region called intergenic spacer (IGS) (Michot et al. 1983). Many features have made the ITS region as a target for identification and taxonomy based studies ; ITS region contains conserved flanked sequences among all fungi species, Therefore, a universal primers were easily developed and used successfully among almost all fungi species(Baldwin et al., 1995; Song et al., 2012). Moreover, ITS has small size of DNA sequence (600bp) including (ITS1+ITS2) and is easily amplified and detected by PCR from small tiny of fungal DNA as rDNA cluster genes are located in thousands copes in fungal genome. In addition, it has been confirmed that ITS regions has a high genetic sequence variations among closely related fungi genera and even related species (White et al. 1990; Baldwin et al. 1995; Song et al., 2012). In additions, mutations within the ITS areas of the rDNA cluster genes happen with greater rate than other rDNA genes, this leads to more genetic sequence variations within this region. Therefore , ITS regions is preferred more than other rDNA genes for the identification and molecular systematics of fungi species (Iwen et al., 2002).

Previous studies on DNA barcoding using rDNA cluster genes as a sequence target focusing mainly on 18S rDNA gene [Geiser et al.2007; O'Donnel et al.,2008; Ertz et al.,2004]. Even though the genetic sequence variations within rDNA cluster genes has been successful used for molecular systematics of fungi at genus level. However, it was revealed that those genes have less mutations frequently, therefore, they have a limited sequence differences compared with ITS regions , making mycologists prefer ITS than other rDNA cluster genes in sequence comparing for identification (Einsele et al., 1979; Kitada et al., 1991; Makimura et al., 1994; Haase et al., 1995; Kappe et al., 1996; de Hoog et al., 1998;Sugita & Nakase 1998; Iwen et al., 2002).

The ITS DNA regions are the most repeatedly sequenced by mycologists to be used in taxonomy and identification at the genus to species level. It was estimated that more than 100 000 ITS sequences were dropped in the GeneBank, and around of 61 % of ITS sequences deposited were annotated and classified to species level (Feliner & Rossello, 2007; Benson et al., 2008; Nilsson et al., 2008; Nilsson et al., 2009). The ITS sequences deposited to date has reached up to 1000 000 sequencesaGenBank(https://www.ncbi.nlm.nih.gov/genbank/). This big number of ITS sequences deposited showed that fungi species identification system has been developed based on ITS1 and ITS2. Mostly mycologists has been using this system today as known in fungi identification at genus to species level (Nilsson et al., 2009).

Meyer and Paulay (2005) performed a study using rDNA cluster genes as a sequence target including SSU, LSU, ITS regions to analyze 700 fungal cultures representing more than 200 taxa of different phyla (). This study strongly supported ITS SSU and LSU as it has high PCR amplifications and sequence variations. Interestingly, ITS regions were proven to be a potential DNA barcode for species discrimination of Dikarya (Ascomycota and Basidiomycota) and had a better identification power than SSU and LSU. Surprisingly , ITS regions had lowest identification power for fungi taxa that were outside of Dikarya such as Mucoromycotina, Chytridiomycota, and Blastocladiomycotina (Meyer and Paulay, 2005). This study obviously revealed that ITS is ineffective for species discrimination of such taxa. But, The ITS region was also effective to be useful for species discrimination of fungi members in Zygomycetes (Schwarz et al., 2006).

The SSU sequence variations were used to classify a fungus culture above genus level and it did well taxonomic job at high levels (White et al., 1990). In addition, It was reported also using LSU sequence polymorphism in identification of fungal cultures at genus level and higher taxonomic levels (Vilgalys et al., 1990; Rehner et al. 1995; Raja et al., 2017). However, ITS regions including ITS1 and ITS2 have been proven as the best at species identification level. Some mycologists combined sequences of both LSU and ITS regions, their studies revealed that the combined sequences did well job in identification of fungi cultures to species level. In fact, using ITS alone have shown satisfied identification results to species level (Schoch et al., 2011; Raja et al., 2017).

Many authors reported that analyzing ITS sequences of fungi genera such as *Alternaria*, *Aspergillus*, and *Penicillium* confirmed that ITS has a unique sequence variations to differentiate between fungi genera. Moreover, it was shown that ITS1 region has more variations than ITS2 in comparing sequences among fungi genera. Similar work was performed on genera from fungi phyla Basidiomycota and Ascomycota showed that ITS1 has more variability than ITS2 (Ryberg et al., 2008; Mullineux & Hausner, 2009; Blaalid et al., 2013).

Mycologists in workshops agreed on the idea to accept the ITS region as a potential candidate of genetic barcoding marker for fungi identification and molecular taxonomy (Bruns, 2001). So that, it is essential to reflect the roles of ITS DNA region variations in describing and determination borders among species. Nevertheless, one of the setbacks of using the ITS region alone is that ITS regions usually show low variability generally within different species of some genera such as *Cladosporium, Fusarium*, and *Trichoderma*. Therefore, ITS region can not be considered as a typical DNA barocoding for all fungi groups. Similar findings showed also low Intra-species ITS polymorphisms in fungi species such as *Fusarium* species (IWEN et al. 2002; Raja et al. 2017) and reported that intra-species ITS sequence variations within *Aspergillus* species were insignificant.

Although many articles have indicated that big sequence polymorphism has been found among ITS regions of fungi groups. In spite of this, in some cases, the sequence variability within the ITS region is less than 1% among species. Therefore, at this point, the ITS sequence would not be enough to be used alone in fungi species discrimination and even it would be a way much harder to separate strains of a single species as ITS sequence variability between strains are very low. Using ITS sequences alone that may have vey low variability between strains and even between some species results in misrepresenting fungi identification (Gaskell et al., 1979; Okeke et al., 1992; O'Donnell et al., 1998; IWEN et al., 2002). In these cases, morphological and phenotypic characters are involved to avoid misidentification when there are high nucleotides sequence similarity within ITS regions. Whether ITS was the best correct DNA barcode used for fungi species identification is still a debate question, even though ITS sequences have been broadly employed as a DNA sequence based tool for fungi species identification for decades.

With more and more ITS sequences deposited in the GenBank, the ITS regions is still the best DNA barcoding as ITS sequences deposited in GenBank covering about 13 350 species (Nilsson et al. 2009). But, this number is so small and it is 0.9% of the estimated fungal species on the land. The field of the DNA sequences based identification may be considered the fungi field where development goes so slowly and mainly painstaking (Nilsson et al., 2009;). Hopefully, recent studies have shown that GenBank contains around 172 000 of the entire fungal sequence of ITS covering nearly 2500 genera, 15 500 species and more than 57% of those sequences binomially named (Schoch et al.,2012; Raja at al., 2017).

Protein coding genes as a candidate for DNA barcoding

Proteins –coding genes marker have resulted in a better consistent identification and have been used for systematic studies in fungi (Geiser et al. 2004). It was proven that phylogenetic analysis of protein coding genes usually do better in discriminating related species more than ITS regions usually do (Bruns, 2001), Furthermore, DNA barcoding protein coding genes usually do a better job in separation of unsolved fungi species taxonomy. Among protein coding genes that have been used are β-tubulin (*BT*)" (Geiser et al., 2007), the gene *BT* is responsible for coding a protein, once the protein is polymerized, it forms microtubules that are the main element of the cytoskeleton. Microtubles have vital roles in DNA segregation and structural support (Findeisen et al., 2014). Translation elongation factor 1α (*TEF-1α*) (O'Donnell et al.,2008), is considered as an elongation factor belonging to a group of proteins which are involved in protein formation in the ribosome mainly through translational elongation (Alberts et al., 2002). Second largest subunit of RNA polymerase II (*RPB2*) (Ertz et al., 2002), is the gene that codes and forms large subunit of nucleus RNA polymerase enzymes. This enzyme is known by transcription of certain DNA regions to form mRNAs (Young et al.,2003). Minichromosome maintenance protein (*MCM7*) (Raja et al., 2011), is the gene that is responsible for producing a mini-chromosome maintenance proteins (MCM) and has an important role in beginning of eukaryotic replication (Nakatsuru et al., 1995). Glyceraldehyde 3-phosphate

dehydrogenase (*GAPDH* or *gpd*) is a gene coding for enzyme playing a primary role in glucose break down through glycolysis process (Tarze et al., 2007).

These genes usually are candidates as potential genetic markers in addition to ITS regions. Moreover, protein-coding genes have many introns, which usually accumulate mutations , thereby , evolving rapidly, This make mycologists prefer them as the potential candidates to be used at higher taxonomic levels (Schoch et al., 2009). Researchers have used protein coding genes combined with ITS regions to re-evaluate the taxonomic placement of many fungi based on phylogentic trees constructed by combining DNA sequence data of both ITS regions and protein coding genes such as *TEF-1α*, BT, or *RPB2*. Building tress based on combined sequences of these genes was efficient in re-evaluating of *Diaporthe* species (Udayanga et al., 2012a). Many studies preferred *MCM7* and *RPB2* genes more than other protein coding genes based on their efficacy in constructing phylogenetic trees and high rate of PCR amplifications of ascomycetes (Raja et al., 2011). Additionally, combining of *MCM7* gene sequence with LSU sequence was effective to infer phylogentic relationships of fungi species from ascomycetes (Aguileta et al., 2008).

The *BT* gene has been reported as better DNA barcode than ITS regions of the rDNA genes in separating *Aspergillus* species (Geiser et al., 2007). In phylogenetic study covered the nectriaceous fungi, it was stated that BT was proven as a potential candidate to be a DNA barcode for the nectriaceous fungi due to BT gene sequences had high inter-species and low intra-species variations combined with high PCR amplification (Zhao et al., 2011).

The EF-1α gene sequences of nectriaceous fungi have revealed sequence variations that are significant and are of importance when used in studying of systematics of *Trichoderma*, *Fusarium* species. But, EF-1α gene had low PCR amplifications compared with BT gene (Zhao et al. 2011). Translation elongation factor 1α (*TEF-1α*) gene were assessed as a DNA barcode marker of dermatophytes which showed a high identification capacity, high inter-speciesvariability, and low intra-species variations that are mostly useful to separate167 strains of 30 species of dermatophytes species (Mirhendi et al., 2015).

The partial sequences of *TEF-1α*, *BT* and *RPB2* genes were combined with ITS sequences regions to build phylogenetic trees that were used in identification of *Neofusicoccum kwambonambiense* and *N. parvum* which are fungal pathogens on strawberry fruits in Brazil (Lopes et al., 2014). These genes were also useful in taxonomy of unresolved fungi species of low ITS sequences variability, *Trichoderma* is the genus of fungi belonging to Ascomycota which is known by important medical and industrial uses. However, the phylogenetic and systematics of this genus have stayed hard to resolve by ITS regions as ITS did not show signification variations among *Trichoderma* species (Mukherjee et al., 2013). However, The *TEF-1α* and *RPB2* genes were proven to have enough sequence variability to resolve identification and taxonomy of *Trichoderma* species (Lu et al., 1999; Lu et al., 2004).

The protein coding genes have been successfully used in reclassification some misidentified fungi species when combined with ITS region sequences. The fungus *Rhizoctonia leguminicola*, which is resposable for producing mycotoxins such as slaframine and swainsonine, which lead to neurological problems to grazing animals. The original classification of this fungus was misplaced as it was classified on morphological characters. Based on sequences of ITS regions and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene, it was clear that placement of this fungus in the genus *Rhizoctonia* was incorrect , and therefore the new genus *Slafractonia* within the phylum Ascomycota was established (Alhawatema et al., 2015).

The *gpd* and *BT* genes are housekeeping and conserved proteins in fungi, the *gpd* and *BT* genes have power for resolving relationships among species of lichenized fungi and even at strains of a single species of lichenized fungi (Myllys et al.,2002). Such study , revealed the ability of the gpd gene for identification of mycorrhizal fungi species. 1.2-kb fragments of the *gpd* gene from mycorrhizal fungi such as *Boletus*, and *Amanita* were amplified and sequenced. Those sequences showed enough variations for significant identification (Kreuzinger et al., 1996).

The phylogeny between *Alternaria radicina* and *A. carotiincultae* was re-investigated by studying a nucleotide sequence variability within *TEF-1α*, *BT*, and *gpd* genes. *A. radicina* and *A. carotiincultae* were separated mainly by *TEF-1α*, and BT genes, Yet, *gpd* gene sequences variations failed to discriminate between *A. radicina* and *A. carotiincultae* (Park et

al., 2008). The phylogenetic relationship of species of the *Pyrenophora* genus were evaluated based on DNA regions of both rDNA ITS and *gpd* genes. Analysis of sequences data showed ITS and *gpd* genes are useful DNA barcode for identification of *Pyrenophora* species (Zhang et al.,2001).

The phylogenetic relationship of *Stemphylium phaseolina* and *S. variabilis* were evaluated. Maximum parsimony (MP) and maximum likelihood (ML) of phylogenetic tress were constructed based on the combined DNA sequences of both ITS and *gpd* genes. The findings in this study indicated that combined sequences of gpd and ITS could be used to separate between closely related species (Kiela et al., 2017). Additionally, The *gpd* gene sequence has been shown of a great importance for inferring and trace the evolutionary relationship among fungal human pathogens such as *Penicillium marneffei* that is an intracellular dimorphic fungus and is evolutionary related to human athogenic fungus *Ajellomyces capsulatus* based on *gpd* sequences analysis (Thirach et al. 2008).

Universal primers for potential fungal DNA barcodes

In most labs today, Universal primers of fungal DNA barcodes have been almost developed (Table 1). The ITS regions are amplified by using universal ITS1+ITS4 primers (White et al. 1990). Mycologists are usually interested to amplify the first of 900 bp of the LSU as it has been proven to have a significant sequence variation to employ in phylogenetic studies. This regions can be amplified successfully using primers 5.8SR + LR7, and sequenced using primers LR5, LR16, LR0R, and LR3R (Hibbett&Vilgalys, 1991; Hibbett&Vilgalys,1993 ;Hopple & Vilgalys, 1995). The labs amplify the SSU region using the conserved primers BMB-B, BMB-BR, BMB-C, BMB-CR (Lane et al. 1985). The universal primers for DNA barcoding protein genes were developed as well. The partial protein coding genes sequence of *TEF-1α* is amplified using Primers EF1-1018F and EF1-1620R (Stielow et al. 2015). The two conserved sequence primers Btub2Fd and Btub4Rd were used to amplify the partial β-tubulin (BT) gene (Stielow et al. 2015). Among the potential DNA barcoding coding protein is second largest subunit of RNA polymerase II (*RPB2*), which is targeted using primers fRPB2-7cF and RPB2-7cR (Stielow et al. 2015). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH* or *gpd*) that has been a target for amplification using primers gpd1 and gpd2 (Berbee et al., 1999).

CONCLUSION

Mycologists working in fungi taxonomy and identification will benefit from progress in the DNA barcoding, specifically to overcome challenges arisen when studying morphological characters that are so complicated to analyze when these morphological characters between fungal species are similar or absent which finally results in misidentification. Today, the ITS region of rDNA is considered as a first place in molecular identification and the ideal DNA barcoding for most fungi groups such as fungi phyla Ascomycota and basidiomycota . However, ITS has shown limited variability in some fungi taxa such as *Cladosporium*, *Fusarium*, *Trichoderma*.
In cases (Cladosporium, *Fusarium* ,Trichoderma and other fungal groups out of ascomycetes and basidiomycetes) where ITS showed low sequence variations to separate fungi species or strains of a single species, The Proteins –coding genes such as β-tubulin (BT), Translation elongation factor 1α (*TEF-1α*), and second largest subunit of RNA polymerase II (*RPB2*) are combined with ITS data in these cases to increase resolution among related species in phylogentic analysis. Although LSU and SSU are recommended at higher levels of taxonomy, combining the entire ITS with LSU sequence (first 900bp) resulted in a better species-level identification. When fungal culture is misidentified and has unknown taxonomy, morphological characteristics have to be studied side by side with phylogenetic trees that are constructed based on ITS and protein coding genes sequences to have a better species-level identification as ITS alone would not enough in these cases, even though ITS sequence of unknown cultures can be utilized in comparisons with reference sequences from databases GenBank, EMBL and DDB.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors express their deep appreciations for all participants and their guidance.

AUTHOR CONTRIBUTIONS

All authors shared in this review.

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REFERENCES

Aguileta G, Marthey S, Chiapello H.; Lebrun MH, Rodolphe F, Fournier E, Gendrault-Jacquemard A,Giraud T, 2008. Assessing the performance of single-copy genes for recovering robust phylogenies. Syst. Biol. 57: 613−627.

Alberts B et al., 2002. Molecular Biology of the Cell, 4th ed. New York: Garland Science.

Alhawatema MS, Sanogo S, Baucom DL, Creamer R, 2015. A search for the phylogenetic relationship of the ascomycete *Rhizoctonia leguminicola* using genetic analysis. Mycopathologia 179:381–390

Altschul SF, Gish W, Miller W, Myers EW, Lipman, DJ,1990. Basic Local Alignment Search Tool. J Mol Biol. 215: 403−410.

Atkins SD, Clark IM , 2004. Fungal molecular diagnostics: a mini review. Journal of Applied Genetics 45: 3–15.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ 1995. The ITS Region of Nuclear Ribosomal DNA: A Valuable Source of Evidence on Angiosperm Phylogeny. Annals of the Missouri Botanical Garden. 82 (2): 247–277.

Berbee ML, Pirseyedi M, Hubbard S, 1999. Cochliobolus phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91: 964-977

Blaalid R, Kumar S, Nilsson RH, Abarenkov K, Kirk P, Kauserud H, 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. Mol. Ecol. Resour. 13: 218–224

Blackwell M, The Fungi: 1, 2, 3 … 5.1 Million Species?. Am. J. Bot. 98: 426−438

Borman AM, Linton CJ, Miles SJ, Johnson EM, 2008. Molecular identification of pathogenic fungi. J Antimicrob Chemother 61: 7–12.

Bruns TD, 2001. ITS Reality. Inoculum 52: 2–3.

Bruns TD, Arnold AE , Hughes KW, 2008. Fungal networks made of humans: Unite, FESIN, and frontiers in fungal ecology. New Phytol. 177: 586–588.

Buée M, Reich M, Murat C, Morin E, Nilsson RH, et al., 2009. Pyrosequencing analyses of forest soils reveal an unexpectedly high fungaldiversity. New Phytol. 184: 449-456.

Conrad LS, Keith AS, Sabine HC, Vincent R, John LS, André CL, Wen C and Fungal , 2012.Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Studies in Mycology 81: 1-26.

Costion C. et al., 2011. Plant DNA barcodes can accurately estimate species richness in poorly known floras. PloS ONE 6:11 de Hoog GS, Bowman B, Gra¨ ser Y, et al., 1998. Molecular phylogeny and taxonomy of medically important fungi. Med Mycol. 36: 52–56.

Druzhinina IS, Kopchinskiy AG, Komoń M, Bissett J, Szakacs G, Kubicek CP, 2005. An oligonucleotide barcode for species identification in Trichoderma and Hypocrea. FungGenet Biol. 42: 813–828

Dutta SK, Ojha M, 1972. Relatedness between major taxonomic groups of fungi based on the measurement of DNA nucleotide sequence homology. Mol Gen Genet 114: 232– 240.

Einsele H, Hebart H, Roller G, et al., 1997. Detection and identi.cation of fungal pathogens in blood by using molecular probes. J Clin Microbiol. 35: 1353–1360.

Ertz D, Miadlikowska J, Lutzoni F, Dessein S, Raspé O, Vigneron N, Hofstetter V, Diederich P, 2008. Towards a new classification of the Arthoniales (Ascomycota) based on a three-gene phylogeny focussing on the genus Opegrapha. Mycol Res experimentally infected tissues. Journal of Clinical Microbiology 44: 340–349.

Feliner GN, Rossello JA , 2007. Better the devil you know? ´ Guidelines for insightful utilization of nrDNA ITS in specieslevel evolutionary studies in plants. Mol Phylogenet Evol. 44: 911–919.

Fell JW, Boekhout T, Fonseca A, Scorzetti G, Statzell-Tallman A, 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2

domain sequence analysis. Int J Syst Evol Microbiol. 50:1351–1371.

Findeisen P, Mühlhausen S, Dempewolf S, Hertzog J, Zietlow A, Carlomagno T, Kollmar M, 2014. Six subgroups and extensive recent duplications characterize the evolution of the eukaryotic tubulin protein family. Genome Biol Evol. 6(9): 2274-88

Frisvad JC, Samson RA , 2004. Polyphasic taxonomy of Penicillium subgenus Penicillium—a guide to identification of food and air-borne terverticillate penicillia and their mycotoxins. Stud Mycol. 49: 1-173.

Gaskell GJ, Carter DA, Britton WJ, Tovey ER, Benyon FHL, Lovborg Y, 1997. Analysis of the internal transcribed spacer regions of ribosomal DNA in common airborne allergenic fungi. Electrophoresis 18: 1567–1569.

Geiser DM, Jimenez-Gasco MD, Kang SC, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O"Donnell K , 2004. A DNA sequence database for identifying Fusarium. European Journal of Plant Pathology 110: 473–479.

Geiser DM, Klich MA, Frisvad JC, Peterson SW, Varga J, Samson RA, 2007. The current status of species recognition and identification in Aspergillus. Stud Mycol. 59:1–10.

Geiser DM., del Mar Jiménez-Gasco, M., Kang S, Makalowska I ,Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'Donnell K, 2004. Fusarium- DNA sequence database for identifying Fusarium. Eur. J. Plant Pathol. 110:473-479.

Haase G, Sonntag L, van de Peer Y, Uijthog JMJ, Polbielski A, Melzer-Krick B, 1995. Phylogenetic analysis of ten black yeast species using nuclear small subunit rRNA gene sequences. Antonie van Leeuwenhoek Int J Genet. 68: 19–33.

Hawksworth DL, 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycol Res. 95(6): 641–655.

Hebert PDN, Cywinska A, Ball SL, de Waard JR, 2003. Biological identifications through DNA barcodes. Proc. R. Soc. London B. 270: 313-321.

Hebert PDN, Gregory TR, 2005. The promise of DNA barcoding for taxonomy. Syst. Biol. 54:852-859.

Hibbett, D. S., A. Ohman, D. Glotzer, M. Nuhn, P. M. Kirk, and R. H. Nilsson. 2011. Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. Fungal Biology Reviews 25: 38-47

Hibbett DS, Vilgalys R, 1991. Evolutionary relationships of Lentinus to the Polyporaceae: evidence from restriction analysis of enzymatically amplified ribosomal DNA. Mycologia 83: 425-439.

Hibbett DS, Vilgalys R, 1993. Phylogenetic relationships of the Basidiomycete genus Lentinus inferred from molecular and morphological characters.

Hollingsworth PM, Forrest LL, Spouge JL et al, 2009. A DNA barcode for land plants. Proc Natl Acad Sci USA. 106:12794–12797

Hopple JS, Vilgalys R, 1999. Phylogenetic relationships in the mushroom genus Coprinusand dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. Molecular Phylogenetics and Evolution 13:1–19.

Hopple, JS, Vilgalys R, 1994. Phylogenetic relationship among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. Mycologia 86: 96-107.

Hyde KD, Abd-Elsalam K, Cai L, 2010. Mycotaxon 114, 439−451.

Iwen PC, Hinrichs SH, Ruppy ME, 2002. Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. Med Mycol, 40: 87–109.

Kappe R, Fauser C, Okeke CN, Maiwald M, 1996. Universal fungus species primer systems and group-species hybridization oligonucleotides for 18S rDNA. Mycoses . 39: 25–30.

Kiela BCR , Vijai B, Sabine B, 2017. Aetiology of stemphylium blight on lentil in Canada. Canadian Journal of Plant Pathology 39:(4) 422-432.

Kitada K, Oka S, Kimura S, Shimada K, Nakamura Y,1991. Diagnosis of Pneumocystis carinii pneumonia by 5S ribosomal DNA amplication. J Protozool. 38: 90S–91S.

Kreuzinger N, Podeu R, Gruber F, Göbl F, Kubicek CP, 1996. Identification of some ectomycorrhizal basidiomycetes by PCR amplification of their *gpd* (glyceraldehyde-3-phosphate dehydrogenase) genes. Appl Environ Microbiol. 62 (9) 3432-3438

Kurtzman CP, Robnett CJ, 1998. Identification

and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26 S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek. 73:331–371

Lane DJ, Pace B, Olsen GC, Stahl DA, Sogin ML, Pace NR, 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci USA. 82: 6955-6959.

Liu YJ, Whelen S, Hall BD, 1999. Phylogenetic relationships among Ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol. 16:1799–1808

Lopes UP, Zambolim L, Pinho1 DP, Barros AV, Costa H, Pereira OL, 2014. Postharvest rot and mummification of strawberry fruits caused by Neofusicoccum parvum and N. kwambonambiense in Brazil. Tropical Plant Pathology 39(2):178-183

Lu B, Druzhinina IS, Fallah P, Chaverri P, Gradinger C, Kubicek CP, Samuels GJ, 2004. Hypocrea/Trichoderma species with pachybasium-like conidiophores: teleomorphs for T. minutisporum and T. polysporum and their newly discovered relatives. Mycologia 96: 310−342.

Makimura K, Murayama SY, Yamaguchi H, 1994. Detection of a wide range of medically important fungi by the polymerase chain reaction. J Med Microbiol . 40: 358–364.

Matheny PB, Liu YJJ, Ammirati JF, Hall BD , 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (Inocybe, Agaricales). Am J Bot 89:688–698.

Meyer CP, Paulay G, 2005. DNA barcoding: Error rates based on comprehensive sampling. PLoS Biol. 3(12): 422.

Michot B, Bachellerie JP, Raynal F, 1983. Structure of mouse rRNA precursors. Complete sequence and potential folding of the spacer regions between 18S and 28S rRNA. Nucleic Acids Research, 11 (10): 3375–3391.

Mirhendi H, Makimura K, de Hoog GS, Rezaei-Matehkolaei A, Najafzadeh MJ, Umeda Y, Ahmadi B, 2015. Translation elongation factor 1-α gene as a potential taxonomic and identification marker in dermatophytes. Med Mycol. 53(3): 215-24.

Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M. Trichoderma: Biology and Applications; CABI, 2013. Oxford, 1-10.

Mullineux T, Hausner G, 2009. Evolution of rDNA ITS1 and ITS2 sequences and RNA secondary structures within members of the fungal genera Grosmannia and Leptographium. Fungal Genetics and Biology 46: 855–867.

Myllys L, Stenroos S, Thell A, 2002. New genes for phylogenetic studies of lichenizedfungi: glyceraldehyde-3-phosphate dehydrogenase and beta-tubulin genes. The Lichenologist: 34(3) 237-246.

Nakatsuru S, Sudo K, Nakamura Y (March 1995). Isolation and mapping of a human gene (MCM2) encoding a product homologous to yeast proteins involved in DNA replication. Cytogenet Cell Genet. 68 (3-4): 226–30.

Nilsson RH, Abarenkov K, Larsson K-H, Kõljalg U, 2011. Molecular identification of fungi: rationale, philosophical concerns, and the UNITE database. Open Appl Inf J. 5:81–86.

Nilsson RH, Ryberg M , Abarenkov K , Sjokvist E, Kristiansson E, 2009. The ITS region as a target for characterization offungal communities using emerging sequencing technologies. EMS Microbiol Lett. 296: 97–101.

Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson KH & Ko˜ljalg U , 2006.Taxonomic reliability of DNA sequences in public sequences databases: a fungal perspective. PLoS ONE 1: 59.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R , 2005. Fungal community analysis by large-scale sequencing of environmental samples. Appl Environ Microbiol. 71: 5544-5550.

O'Donnell K, Sutton DA, Fothergill A, McCarthy D, Rinaldi MG, Brandt ME, Zhang N, Geiser DM, 2008. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the Fusarium solani species complex. J Clin Microbiol. 46(8): 2477-90.

O'Donnell K, Sutton DA, Rinaldi MG, Sarver BA, Balajee SA, et al., 2010. Internet-accessible DNA sequence database for identifying fusarium from human and animal infections. J Clin Microbiol. 48: 3708-3718.

O'Donnell K, 1992. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete Fusarium sambucinum (Gibberella pulicaris). Curr Genet. 22: 213– 220.

O'Donnell K, Cigelnik E, 1997. Two divergent intragenomic rDNA ITS2 types within a

monophyletic lineage of the fungus Fusarium are nonorthologous. Mol Phylogenet Evol. 7:103–116.

Okeke CN, Kappe R, Zakikhani S, Nolte O, Sonntag H-G. Ribosomal genes of Histoplasma capsulatum var. duboisii and var. farciminosum. Mycoses 41: 355–362.

Park MS, Romanoski CE and Pryor BM ,2008. A re-examination of the phylogenetic relationship between the causal agents of carrot black rot, Alternaria radicina and A. carotiincultae. Mycologia 100:(3) 511-527.

Pecnikar ZF, Buzan EV, 2014. 20 years since the introduction of DNA barcoding: from theory to application. J Appl Genet. 55: 4–52.

Purty RS, Chatterjee S, 2016. DNA Barcoding: An Effective Technique in Molecular Taxonomy. Austin J Biotechnol Bioeng 3(1), 1059.

Raja HA, Miller AN, Pearce CJ, Oberlies NH, 2017. Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community. J Nat Prod. 80: 756−770.

Raja HA, Schoch CL, Hustad VP, Shearer CA, Miller AN ,2011. Testing the phylogenetic utility of *MCM7* in the Ascomycota. MycoKeys 1:63–94.

Rehner SA, Samuels GJ, 1995. Can. J. Bot. 73: S816−S823.

Ryberg M, Kristiansson E, Sj€okvist E, Nilsson RH ,2008. An outlook on the fungal internal transcribed spacer sequences in GenBank and the introduction of a web-based tool for the exploration of fungal diversity. New Phytologist 181: 471–477.

Schoch CL, Seifert KA, 2011. DNA barcoding in fungi. In Access Science. McGraw-Hill Education.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, et al., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci USA. 109: 6241-6246.

Schoch CL, Sung GH, López-Giráldez F, Townsend JP, Miadlikowska J, et al., 2009. The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst Biol. 58: 224-239.

Schoch CL, Sung, GH, López-Giráldez F, Townsend JP, Miadlikowska J, Hofstetter V, Robbertse B, Matheny PB, Kauff F, Wang Z, 2009. Syst. Biol. 58: 224.

Schwarz P, Bretagne S, Gantier JC, Garcia-Hermoso D, Lortholary O, Dromer F, Dannaoui E ,2006. Molecular identification of Zygomycetes from culture and

Seifert KA, 2009. Progress towards DNA barcoding of fungi. Mol Ecol Resour. 9(1):83–89.

Seifert KA, 2009. Progress towards DNA barcoding of fungi. Molecular Ecology Resources 9: 83–89.

Song J, Shi L, Li D, Sun Y, Niu Y, Chen Z, Luo H, Pang X, Sun Z, 2012. Extensive Pyrosequencing Reveals Frequent Intra-Genomic Variations of Internal Transcribed Spacer Regions of Nuclear Ribosomal DNA. PLoS One 7(8).

Stielow JB, Lévesque CA, Seifert KA, Meyer W, Irinyi L, Smits D et al., 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Mol Phyl Evol Fungi. 35: 242– 263,

Sugita T, Nakase T, 1998. Molecular phylogenetic study of the basidiomycetous anamorphic yeast genus Trichosporon and related taxa based on small subunit ribosomal DNA sequences. Mycoscience. 39: 7–13.

Sun X, Guo L-D ,2012. Endophytic fungal diversity: review of traditional and molecular techniques . Mycology 3: 65–76.Syst Bot 18: 409-433.

Tanabe Y, Saikawa M, Watanabe MM, Sugiyama J, 2004. Molecular phylogeny of

Tarze A, Deniaud A, Le Bras M, Maillier E, Molle D, Larochette N, Zamzami N, Jan G, Kroemer G, Brenner C (April 2007). "GAPDH, a novel regulator of the pro-apoptotic mitochondrial membrane permeabilization". Oncogene. 26 (18): 2606–20.

Tanabe Y, Saikawa M, Watanabe MM, Sugiyama J,2004. Zygomycota based on EF-1alpha and RPB1 sequences: Limitations and utility of alternative markers to rDNA. Mol Phylogenet Evol. 30: 438–449.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher, MC, 2000 Fungal Genet. Biol. 31: 21−32.

Thirach S, Cooper CR, Jr and Vanittanakom N, 2008. Molecular analysis of the Penicillium marneffei glyceraldehyde-3-phosphate dehydrogenaseencoding gene (gpdA) and differential expression of gpdA and the isocitrate lyase-encoding gene (acuD) upon internalization by murine macrophages.

Journal of Medical Microbiology 57, 1322–1328.

Udayanga D, Liu XZ, Crous PW, McKenzie EHC et al., 2012a. A multi-locus phylogenetic evaluation of Diaporthe (Phomopsis). Fungal Diversity 56: 157–171.

Vilgalys R, Hester M,1990. J. Bacteriol. 172: 4238−4246.

Walker WF, Doolittle WF, 1982. Redividing the basidiomycetes on the basis of 5S rRNA sequences. Nature, 299: 723–724.

White TJ, Bruns T, Lee S, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Proto-cols: A Guide to Methods and Applications. Academic Press, Inc. New York. 315–322.

Young RA, 2003. RNA Polymerase II". Annual Review of Biochemistry. 60 (1): 689–715.

Zhang G, Berbee M, 2001. Pyrenophora Phylogenetics Inferred from ITS and Glyceradehyde-3-Phosphate Dehydrogenase Gene Sequences. Mycologia 93(6), 1048-1063.

Zhang W, Yuan Y, Yang S, Huang J, Huang L ,2015. ITS2 Secondary Structure Improves Discrimination between Medicinal “Mu Tong” Species when Using DNA Barcoding. PLoS ONE : 10(7): e0131185.

Zhao P, Luo J, Zhuang WY, 2011. Practice towards DNA barcoding of the nectriaceous fungi. Fungal Diversity 46:183−191.