CHAPTER 1

INTRODUCTION

1.1. Introduction

The kingdom fungi encompasses an array of organisms that are diverse and complex like those of plants and animals with an approximately estimated quantity of 5.1 million species (O'Brien et al., 2005; Taylor et al., 2010; Blackwell, 2011), of which only 10% have been described with proper names. It is one of the largest groups of organisms on the planet and they are considered to be the second largest group after insects. Nearly 10% of the known fungal species occurring in the world are now discovered and yet the vast majority of fungi are still unknown to us. It is estimated that about 14,000 species from the 1.5 million fungi existing on the planet develop fruiting bodies and are considerably enough in number to be classified as macro-fungi or mushrooms (Chang, 2006). The name 'Mushroom' is believed to originate from the French term "mousseron", which comprised both edible and venomous varieties of mushrooms (Ramsbottom, 1953). Initially, the term mushroom was used only to the edible representatives of macro-fungi. Even in the ancient literature, mushrooms have been mentioned in Chinese, Greek, Indian, Roman, and other kind of books (Acharya and Sarkar, 2020). They were considered as strength food by Greek warriors, Romans considered it as a food of the gods and elixir of life by the Chinese (Valverde et al., 2015). Among the Aztecs of South America, mushrooms were referred to as "teo-nonacte" i.e. flesh of God and it was worshipped (Thakur et al., 2012). It is believed that the "somrus" mentioned in Rigveda and other ancient Indian literature referred it as a decoction of mushroom, which is variously known as Ksumpa (in Sanskrit), Kukurmutta (in Kavaka), Chhatra (in Chhatra), Khumbi (in Khumbi) and Bhoomikavak (in Hindi) (Wasson, 1969). A review of the literature shows that nearly 700 species of higher Basidiomycetes are pharmacologically active (Mizuno, 1995; Wasser, 2002).

In terms of a broader perspective, one can describe the mushroom as a macro-fungus with a characteristic fruiting body that is either epigeous or hypogeous and is sufficiently large to be observed with naked eyes and selected by hand (Chang and Miles, 1992). Consequently, they do not have to be basidiomycetes, aerial, fruiting or edible. It is also considered that mushrooms may be ascomycetes, can be growing underground, non-fleshy in touch and not necessarily palatable. Although this definition is not that perfect, but it may be recognized as a reasonable terminology to assess the diversity and their number on earth (Ukwuru et al., 2018).

A mushroom is typically an umbrella-shaped fruiting body made up of a pileus (Cap) and stipe (i.e., stem-like structure). Pileus can be conical, flat, or even spherical; sporophore formed from several thin layers stacked together on the lower surface of pileus; pores are also found on some mushrooms; there are several types of mushrooms, based on their morphology, such as bracket fashioned, gilled, poroid, smooth or stiped, and attached on woods of tree trunks; they may be cup-shaped caps with or without stipes, coralloid, dentoid, or hood-like, etc. (Pacioni and Lincoff, 1981).

For millennia, wild mushrooms have been considered as nutritionally rich and delicious food items (Breene, 1990; Chang and Miles, 1992; Manzi et al., 1999). Besides their nutrient-dense value, wild edible mushrooms contain a variety of pharmaceutical compounds (Bobek et al., 1995; Bobek and Galbavý, 1999) and currently, several species of them have been established as having therapeutic properties (Wasser and Weis, 1999; Ozen et al., 2011; Vaz et al., 2011b, 2011a; Khatun et al., 2012). Moreover, mushroom extract has been used in several parts of the world as a potent remedy for the inhibition and cure for numerous diseases for

thousands of years (Kidd, 2000; Israilides and Philippoussis, 2003). Numerous varieties of mushrooms that are traditionally used are *Auricularia, Flammulina, Ganoderma, Grifola, Lentinus, Trametes* (*Coriolus*) and *Tremella* have been shown to possess significant medicinal qualities (Wasser, 2002; Ajith and Janardhanan, 2007).

1.2. Diversity of Fungi

1.2.1. Fungi worldwide

Hawksworth (1991) hypothesized that from the 1.5 million projected species and nearly 100,000 described taxa, only 7% of the world's fungal diversity has been described. In the last 10–15 years, some progress has been made in the understanding of diversity of fungi occurring in tropical forests but it is much less than expectations. Among the most important studies, Hong Kong is to be specifically mentioned due to fourfold increase in number of known species of fungi within a decade and documented more than 150 species for the first time (Hyde, 2003). These include under studied host plants, as well as distinct ecological niches including plant litter in tropical rivulets. It has been noticed that whensoever field visits are undertaken, by teams for short durations or by students studying in tropical regions, substantial numbers of previously unknown fungi are discovered continuously and described too. According to Schmitt and Mueller, (2004) at least 0.6 million species are estimated to be present on earth. Analysis of data on fungal diversity of 25 studies conducted in Asia, Europe and North America showed that the wealth of fungal species was significantly higher than the abundance of plants, which found to be consistent with estimates of species numbers given by Hawksworth, (1991). In addition, other researchers have estimated the number of species to be beyond 13 million, but the exact position is perhaps 25% while other species are yet to be known (Kaur et al., 2019).

1.2.2. Indian Scenario

The number of fungal species is compared at global and national levels and status is provided in **Table 1.1**. Available literature at the national level indicates that Ascomycotina and Deuteromycotina are relatively least represented. Nevertheless, this does not necessarily mean they are rare, but the current situation may be a result of a lack of exploration and data on these groups. As per Manoharachary et al., (2005), nearly 205 new taxa are described from India and in the last 70 years, there has been 10 doubling in the number of species being registered that colonize varied habitats.

Table 1.1: An approximate estimate of fungal diversity in India (Manoharachary et al., 2005)

Phyla	Global	India	Percentage
Myxomycotina	450	380	84.44
Mastigomycotina	308	205	66.55
Zygomycotina	55	50	90.91
Ascomycotina	2000	745	37.25
Basidiomycotina	357	232	64.98
Deuteromycotina	4100	468	11.41
Total	**7270**	**2080**	**28.61**

India has a very long history of using wild mushrooms as food and medicine. Several researchers from India have reported innumerable applications of mushrooms including food and medicine (Kaul and Kachroo, 1974; Purkayastra and Chandra, 1985; Sarkar et al., 1988; Bhatt and Lakhanpal, 1988, 1989; Kaul, 1993; Rai et al., 1993; Harsh et al., 1993, 1996; Sharma and Doshi, 1996; Sharda et al., 1997; Barua et al., 1998; Adhikary et al., 1999; Singh and Rawat, 2000; Boruah and Singh, 2001; Sagar et al., 2005; Sharma et al., 2009a; Karwa and Rai, 2010; Giri et al., 2012).

There are nearly 1,069 species that are used as food across the globe (Boa, 2004), from them almost 300 species of wild mushrooms are used as food in the African continent (Rammeloo and Walleyn, 1993).

1.3. Micro-fungi

Foreigners began studying fungi in India after they began collecting fungi in Britain and sending specimens to European laboratories for identification (Gugnani, 2021). Corda (1842) made the first record of Hyphomycetes in India and subsequently, Cunningham and Barclay, (2010) continued to study Indian fungi. Becklay (1886) contributed to our understanding of rust in the vicinity of Shimla. Mucorales, Ustilaginales, and Uredinales were some of the orders contributed by Cunningham, (1927).

The 'Father of Indian Mycology', Sir E. J. Butler initiated and facilitated large-scale collections and research related to mycology and phytopathology in India (Gautam et al., 2021). In 1905, he created the “Herbarium Cryptogamae Indiae Orientalis (HCIO)”, a national fungus herbarium. HCIO was reassigned in 1934 to the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi. The Fungi of India was compiled by Butler and Bisby, (1931) which was revised several times and updated by Sarbhoy et al., (1986); Sarbhoy and Agarwal, (1996) and Jamaluddin et al., (2004). *Helminthosporium* species on cereals were studied by Mitra, (1931, 1923). Several Indian species were named and described by Mitter and Tandon, (1937, 1930). Several interesting hyphomycetes have been reported since then by different workers from across the country (Mundkur, 1938; Ramakrishnan, 1949).

In addition to the richest biodiversity centre in Asia, India is also a centre of research on hyphomycetes diversity, with a high proportion of tropical species and genera were described first from this country (Bilgrami and Rizwi, 1991; Jamaluddin et al., 2004). There are now inclusive accounts of hyphomycetous fungi from India (Subramanian, 1971; Bhat, 2010; Mukerji and Manoharachary, 2010).

According to Léveillé, coelomycetes were first described in 1846 by using leaves of *Saussurea* sp. and discovered the first pycnidial fungus, *Ascosporasordidula*, which was renamed *Septoriasordidula* by Saccardo, (1884). The first Indian researchers to study this group were Subramanian and Ramakrishnan, (1953, 1952). A coelomycete fungus *Plagionema* was described with lateral and apical appendages, which was later reclassified as *Ciliochorella* Syd (Subramanian and Ramakrishnan, 1953). The same research group also contributed to the taxonomy of coelomycetes by describing some taxa (Subramanian and Ramakrishnan, 1955, 1954).

Mathur, (1979) provided a checklist of Indian coelomycetes collected from 1846 to 1977 in his book "The Coelomycetes of India". He listed 235 genera, 1527 species, and 33 varieties. Several fungi were described by Muthumary and coworkers (Muthumary, 1999, 1987a, 1987b, 1987c, 1986a, 1986b, 1986c; Muthumary and Masilamani, 1989; Muthumary and Sutton, 1986). Muthumary (1999) for the first time provided a monograph on Septoria species in India. Based on the host taxonomy and conidial measurements, she given a key to the 83 species in the monograph. Subsequently another important contribution was "Indian Coelomycetes" by Muthumary, (2019) contains descriptions and illustrations of 142 species of 78 genera.

1.4. Macro-fungi

Different researchers have studied macro-fungi diversity from different parts of India from time to time. Chahal, (1963) identified several edible fungi from Punjab. Tapwal et al., (2013) reported thirty species of macro-fungi in Assam's Jeypore Reserve Forest. These species belong to 26 genera and are distributed in 17 families. Further research work on macro-fungi in Gujarat by Ch et al., (2013) and Jammu and Kashmir was done by Anand et al., (2014). Similarly, Karwa and Rai, (2010), documented nearly 153 species of mushrooms from Central India (Maharashtra). Additionally, various research groups conducted an intensive survey of the mycofloral diversity in Central India (Sharma et al., 2009; Sharma et al., 2011a, 2011b; Sharma et al., 2009a). Swapna et al., (2008) reported 778 species of macro-fungi from 101 genera in 43 families in Karnataka while Thiribhuvanamala et al., (2013) recorded 23 species of macro-fungi from Tamil Nadu. Several hundred species of wood-degrading, non-gilled Agaricomycotina belonging to 27 families and more than 100 genera are described by Prasher, (2013) from Uttarakhand. Twenty-one species of mushrooms were recorded by Semwal et al., (2014), and two were found to be of Ascomycetes. Senthilarasu, (2014) studied macro-fungi from Mahabaleshwar and Mulshi in Maharashtra, representing 10 genera and 9 families. Pushpa and Purushothama, (2012) recorded 90 species from Karnataka belonging to 19 families, 28 of which were new to India. Prasher and Deepaliashok, (2012) documented 13 species of polyporoid fungi from 5 families and 10 genera from Himachal Pradesh while Dhingra et al., (2014) recorded 295 species belonging to 89 genera of non-poroid Agaricomycetous fungi from Himachal Pradesh.

In recent years, fungal taxonomy has got a boost and given rise to a natural classification based on the molecular tool (Hibbett et al., 2007; Shenoy et al., 2007;

Thambugala et al., 2015; Tian et al., 2015; Wang et al., 2015). As a result, an intergeneric shifting of species, as well as the introduction of new genera to the correct position of previously identified species, and the reassignment of these genera to various families, orders, and classes have occurred.

1.5. Study area – Gujarat state

The state of Gujarat is surrounded by the Arabian Sea at its southwest end and is geographically located on western part of India between 20° 6' N t° 24° 42' N and 68° 10' E t° 74° 28' E. With a landmass of 1,96,204 square kilometres (75,755 square miles), the state is unevenly distributed and has nearly 20 lakh hectares of forest cover. The major concentration of forest cover is in the Saurashtra region. Temperatures in the state range from 1°C to 46°C. Different types of forests have developed as a result of the variation in geophysical, bio-geographical and climatic conditions. Depending on the region of the state, it receives varying amounts of rainfall and the northern part is scrub forest and falls under arid-to semiarid regions of India (Rajput et al., 2015).

Gujarat state is rich with a prodigious diversity of natural ecosystems, which ranges from moist deciduous forests to pure desert environments (Tadvi, 2013). For Gujarat state, Champion and Seth (1968), recognised following four types of forests: 1) moist deciduous tropical forest; 2) dry deciduous tropical forest; 3) thorn forest in northern part of the state and; 4) littoral and swamp forests. The southern part of the state has tropical moist deciduous forests dominated by teak (*Tectona grandis*), which requires moderate rainfall. In tropical moist deciduous forests, teak is frequently associated with *Terminalia tomestosa* and *Anogeissus latifolia* etc. The state's central and Saurashtra regions are home to tropical dry deciduous forests. It contains *Anogeissus*

latifolia, *Boswellia serrata*, *Diospyros melanoxylon*, *Euphorbia nerrifolia*, *Tectona grandis* and *Wrightia tinctoria*, as dominant species. Savanna type grasslands are also found in the area. Northern thorn forests in northern Gujarat are typically characteristic to Kachchh and Saurashtra (including some part of Bhavnagar, Junagadh and Rajkot Districts). This forest type is characterized by the presence of different species of Acacia such as *A. arabica*, *A. nilotica*, *A. senegal*, *A. catechu*, *A. leucophloea*, *Capparis aphylla*, *Carrisa carandus* and *Zizyphus mauratiana*. Mangroves grow along the intertidal coastal areas of Kachchh, Porbandar, Rajkot, Dwarka and Jamnagar Districts. There are three foremost species viz. *Avicennia marina*, *Rhizophora mucronata* and *Ceriops tagal* are recorded from this forest type. Even though Gujarat has a diverse climate, geography, and vegetation; information on fungal diversity is scarce, and there is no comprehensive list of fungal species occurring in this area. The map of the study area has been shown in **Fig. 1.1**.

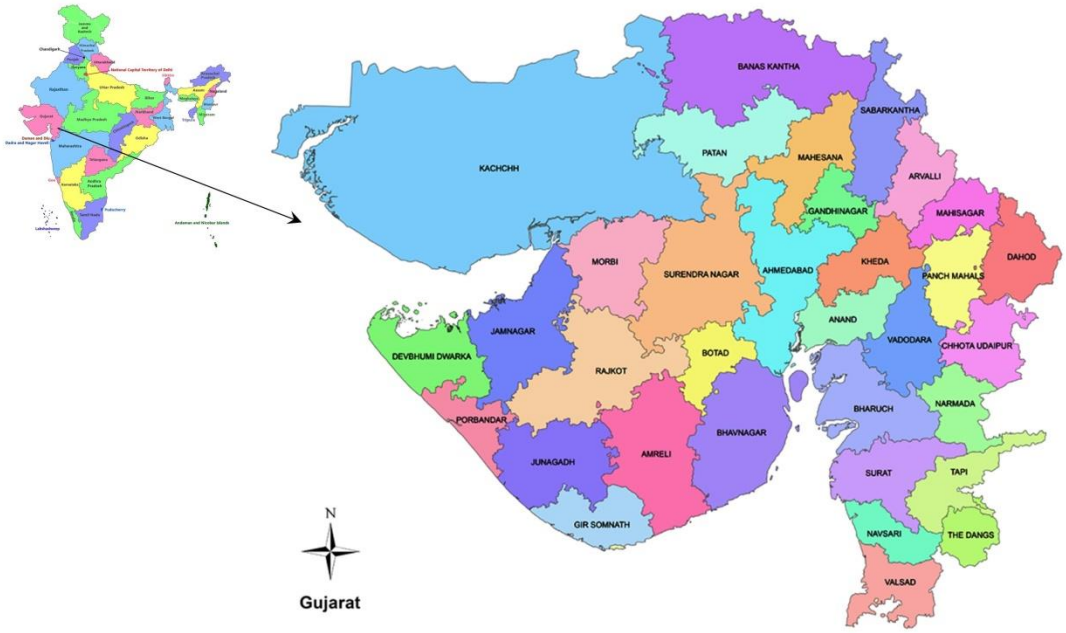

Fig. 1.1: Study area map

1.6. Molecular systematics of fungi

There is increasing applications of molecular tools to obtain unique features for the taxonomic study and phylogenetic relationships among fungi (Zambino and Szabo, 1993). It is almost imperative to use molecular tools to ensure that the inoculum is from the correct species. In contrast to morphological characters, molecular tools provide a more accurate method for identifying organisms. DNA techniques are rapid and more reliable to establish the identity of wild mushrooms and their authentication. Except for Restriction Fragment Length Polymorphism (RFLP), all DNA markers are based in some way on polymerase chain (PCR) reactions. Direct sequencing using PCR products became common with the advent of cycle sequencing methodologies (Murray, 1989) or repetitive nuclear DNA such as ribosomal DNAs (Savard et al., 1994). It is considered to be one of the most powerful techniques available for phylogenetic analyses (Nei, 1987).

Various molecular tools like Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), RFLP or DNA sequence analyses (ITS, nLSU, nSSU, mtSSU and RPB2) can overcome the limitations of morphological identification of fungal strains. It is possible to combine molecular methods with morphological methods to identify fungi reliably (Khush et al., 1992; Calonje et al., 1997). Through the application of molecular techniques, fungal systematics has been transformed tremendously. Initially, research on fungal molecular systematics focused largely on the rDNA and its encoding genes (Bruns et al., 1991; Hibbett, 1992) since the rDNA occurs in high copy number, which facilitates cloning and southern hybridization studies. Reverse transcriptase and other methods can be used to sequence rDNA directly (Anderson et al., 1987; Kohn et al., 1988; Guadet et al., 1989; Baldwin et al., 1995).

It is the gene cluster that codes for the rDNA that is the most commonly uses DNA region for molecular identification of filamentous fungi. Interspersed conserved sequences in the rDNA cluster allow for the amplification of intervening variable sequences. Molecular diagnostics can be developed by targeting the nuclear rDNA and mitochondrial genes because of their multiple occurrences together with their ubiquitous nature. Specific sequences are identified at different taxonomic levels due to the variation in sequence conservation within the gene cluster (Bainbridge, 1994; Blackwell, 2011).

1.6.1. Nuclear ribosomal DNA

Genes encoding ribosomal RNA (rRNA) of fungi are located in tandem arrays of transcribed and non-transcribed DNA segments on a single chromosome, which seems to be extremely conserved (Wipf et al., 1999). On a common precursor transcript, ITS (Internal Transcribed Spacer) contains a small segment of non-functional RNA between structural ribosomal RNAs. ITS sequence comparison is widely used in taxonomy and molecular phylogeny since it can be amplified from relatively small amounts of DNA and has a high degree of variation between closely related species. For sequencing the region of fungal DNA, the ITS region is the most commonly used. Molecular systematics is most useful at the species level and within the species (Rajaratnam and Thiagarajan, 2012).

Due to its high copy number and conserved and variable regions, the nuclear rDNA repeat unit is a curious region of the genome for examining polymorphisms. The genes responsible for encoding structural RNA reside in the ribosomes, which have a high copy number of tandemly repeated sequences. DNA regions that encode nuclear ribosomal RNA (rDNA) are popular targets for genetic studies. A fungal rDNA repeat

contains tandemly arranged genes, which are divided into three subunits namely, the small subunit (SSU), the 5.8S subunits, and the large subunit (LSU or 25S-28S). A region of internal transcribed spacer 1 (ITS 1) is located between the 5.8S and SSU genes, and a region of internal transcribed spacer 2 (ITS 2) is present between the 5.8S and LSU genes. In this rDNA gene repeat, transcription occurs as a single unit, then splicing is performed to remove the spacer regions. Intergenic spacers (IGS) are found between each transcript. It is possible to analyse the evolutionary histories of groups of taxa that differ in age since divergence by examining regions within a repeat that have different rates of evolutionary change. There are highly conserved regions encoding the rRNA genes (SSU, 5.8S, LSU), although moderately variable regions, called divergent domains, are dispersed throughout the gene regions (Bruns et al., 1991).

1.6.2. Internal transcribed spacer (ITS) regions

For examining the closely related species, ITS of nuclear ribosomal repeats may be useful. Since these two spacer genes do not encode a gene product, they evolve at a much faster rate than the genes for ribosomal subunits (Bruns et al., 1998) and therefore, probably, it is the most widely sequenced DNA region. Molecular systematics has been used for the discrimination within the species and even among the species level (e.g., to recognise geographical races). The ITS and IGS (Intergenic spacer) regions of the rRNA can sometimes show variation among individual repeats due to their higher degree of difference compared to other generic regions like SSU and LSU.

The ITS region is perhaps the most significant in this organism for molecular systematics. Primer targeting of ribosomal genes flanked by the ITS region is ideal

due to their high conservation. Owing to their high variability, ITS regions in fungi help to distinguish the closely related species (Hibbett et al., 1995). On the other hand, data on nucleotide sequence emerged from nuclear and mitochondrial rDNA coding areas do not provide sufficient variation to conclude phylogenetic relationships between closely related species (Hibbett and Vilgalys, 1993, 1991); therefore, are only valuable at the generic level authentication. Numerous reports are available on the analysis of ITS regions to establish the taxonomy of fungal species (Moncalvo et al., 1995a, 1995b; Gottlieb et al., 2000).

In ITS regions, certain segments have significantly more variability than others. Moncalvo et al., (1995a) reported the occurrence of nucleotide substitutions in ITS 1 and 2 were similar, but they found that differences were mostly located near the termini of ITS 1, whereas they were mostly located near the centre of ITS 2. Furthermore, ITS 2 is usually the region of nucleotide divergence between newly diverged taxa. According to Gottlieb et al., (2000), the ITS 1 data set did not provide a high level of resolution for internal phylogenetic branches as the ITS 2 data set. The ITS region has generally been considered aa a useful region for molecular identification of fungi at a species level (Lee et al., 2006; Yadav et al., 2007).

White et al., (1990) introduced designing a list of primers for PCR amplification and sequencing of fungal nuclear and mitochondrial rDNA, and since then these regions have been most often studied in fungal molecular systematics. Phylogeny derived from the sequence data of ITS rDNA has been used to detect and identify diverse lineages among closely related taxa or complexes of fungi. Therefore, ITS rDNA has become a prevalent tool to detect fungal phylogenetic species (Hibbett et al., 1995). Nevertheless, Taylor et al., (2000) claimed that a phylogenetic approach for

identifying fungal species should not be built on a single gene phylogeny, rather it should be based on multiple gene phylogenies. Moreover, degrees of variation in intron and exon may provide valuable information for fungal systematic and evolutionary studies at different taxonomic levels.

There is a possibility that the flexible sequences of the spacer regions (ITS and IGS) contain sequences that are mutual at the taxa level, and several species-specific sequences are identified in such regions (White et al., 1990; Mills et al., 1992; Lévesque et al., 1994; Bridge and Arora, 1998; Edel, 1998). In molecular systematics studies at the species level, the ITS region polymorphisms have been extensively applied to a range of fungi (Tsai et al., 1994; Vogler and DeSalle, 1994), plants (Wendel et al., 1995; Waalwijk et al., 1996a, 1996b) and animals (Baldwin et al., 1995). ITS regions are often so variable that they cannot be aligned accurately between genes. Species within a genus are usually described using them (Moncalvo et al., 1995a, 1995b; Yan et al., 1995). Many systematic questions have been addressed by rDNA analysis through PCR and RFLP (Bruns, 1995). Fungal taxa are commonly analysed using the more variable ITS region of rDNA (Anderson et al., 2003, 2001; Horton, 2002; Pryor and Gilbertson, 2000). The schematic diagram of the rDNA region of fungi and commonly used ITS primers has been shown in **Fig. 1.2**.

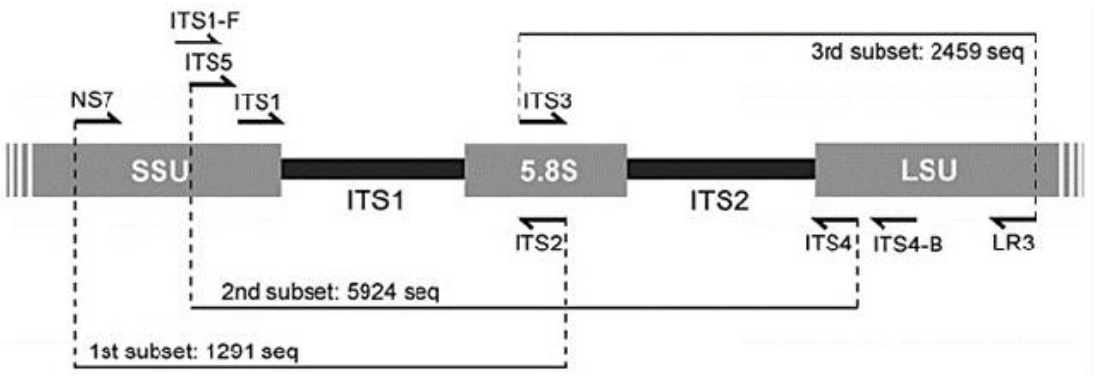

Fig. 1.2: Diagram of the rDNA region of fungi and ITS primers (Adapted from Bellemain et al., 2010)

1.6.3. DNA Barcoding

Currently, DNA sequencing is being used to study evolutionary and genetic relationships and is crucial for the quick identity of organisms. Therefore, scientists are using a short DNA sequence, called a DNA barcode, to identify species. Paul Hebert and colleagues proposed the use of DNA barcodes (Hebert et al., 2003). At first, DNA barcoding was carried out on insects and subsequently, it was extended to all organisms to develop a database (Begerow et al., 2010). As a global standard for biospecies identification, the international barcode of Life Consortium was formed in 2004 (Valentini et al., 2009). There are many advantages to DNA barcoding compared to traditional identification systems; i) access to molecular data, ii) recognition of damaged or fragmented organisms, iii) identification of immature specimens, iv) DNA barcoding plays a vital role in identifying and authentication of specimens if morphological characteristics cannot be determined and v) when specimens have polymorphic life cycles, they can be easily identified (Frézal and Leblois, 2008).

In DNA barcoding, species identification is achieved by isolating genomic DNA and amplifying it with primers specific to the target DNA barcode marker. The amplified and sequenced DNA is compared with a library of reference barcodes resulting from known species. If the sequence of an unknown species closely matches with one of the sequence in the barcode library, it is identified. Sequences that do not closely match will be innovative barcode sequences of unknown species (Hajibabaei et al., 2007). Hebert et al., (2003) established the cytochrome oxidase C (COI) as a genetic marker for phylogenetic studies of insects, birds, and fish; however, the use of COI as a marker for other eukaryotic organisms and found it an unreliable marker.

Accurate identification plays a crucial role in the biological conservation of species and fungal research. The most promising technique for identifying fungi at the species level is DNA barcoding. There are 1.5 million fungi in the world, but only 1, 08,574 of them have been barcoded. The identification of universal DNA barcode markers for fungi is a major obstacle to studying fungal biodiversity. NCBI is accumulating the generated data and centres for a growing collection of fungal sequences. As a result, it provides an easy way to identify fungal species. There are several common DNA barcode markers, such as COI (Seifert et al., 2007), ITS sequences (Bellemain et al., 2010), LSU rRNA gene (Stockinger et al., 2010), SSU rRNA gene (Mouhamadou et al., 2008), and ribosomal polymerase B1 and B2 (Seifert, 2009). The ITS region is a highly evolving region compared to the 18S rRNA gene, resulting in larger sequence variations between closely related species in the microbiota. The ITS region has therefore been used as a DNA barcode for fungi identification. Since the ITS region contains many copies, it is possible to amplify DNA from samples that contain a very low amount of DNA (Begerow et al., 2010; Chase and Fay, 2009). A fresh sample must therefore be used to isolate the DNA.

As compared to COI, ITS has a powerful resolving power for *Chrysomyxa rust* fungi (Feau et al., 2011). According to Dentinger et al. (2011), the COI gene is not a suitable DNA barcode for the identification and authentication of mushrooms and associated species and sequences obtained using the ITS region were compared with COI sequences and found that COI has a less diverging sequence. Introns frequently found in COI are responsible for less powerful amplification. Thus, ITS is the important marker for the DNA barcode for fungi (Dentinger et al., 2011). The ITS region of edible mushrooms was used as a marker for genetic classifying and phylogenetic analysis by Avin et al., (2012).

A web platform helps in gathering and use of DNA barcode data, Barcode of Life Data Systems (BOLD, launched in 2005), which offers an integrated environment for assembling and using DNA barcode data. The specimen, distributional and molecular data will be collected and managed online. It will also include the analytical tools to support their validation. Recently, BOLD system improved the version in October 2013 that comprises iterative improvements that support data collection and analysis as well as new modules that enhance data distribution, citation and annotation. The flow diagram of the DNA barcoding pipeline has been shown in **Fig. 1.3**.

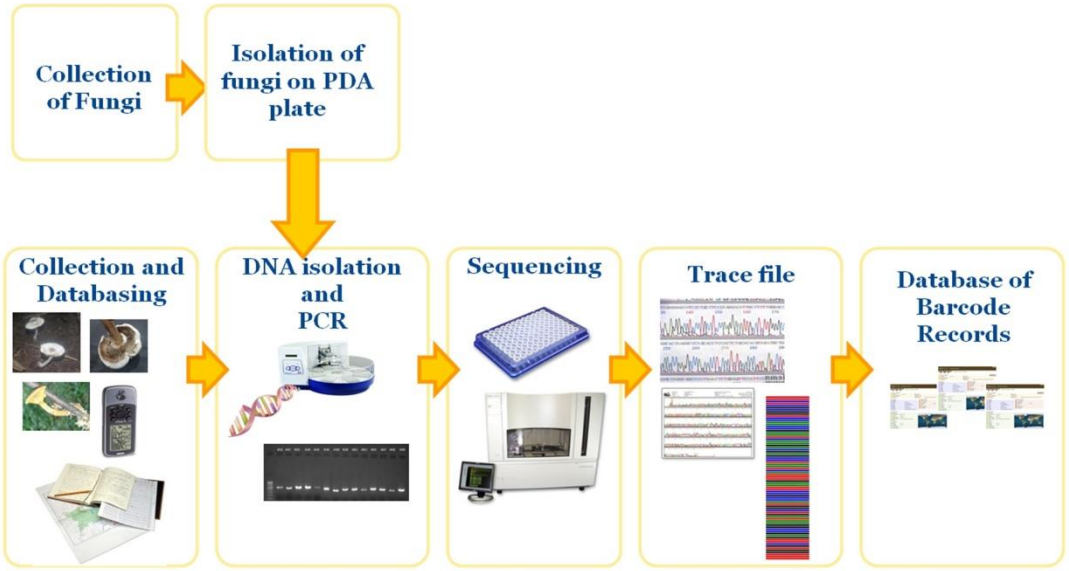

Fig 1.3: Pipeline of DNA barcoding

1.7. Potential of fungi

A wide range of living species found in nature has developed some extremely specific "chemical adaptations" that cover a wide range of biological roles. With rapid technological advances in the past 50 years, scientists now have access to an arsenal of techniques and laboratory tests that enable them to identify potentially useful secondary metabolites with astonishing accuracy. For the separation of these compounds, therapeutic plants, protists, different fungi and animals are used. The earth's uncountable plant and fungal species hold massive potential for discovering novel chemicals.

The fungi are a vital source of bioactive compounds. A multifaceted structure of these compounds allows them to inhibit their molecular targets selectively. The use of fungi in biotechnological processes has been invaluable in both primeval and current times (Demain, 1999), contributing to the development of mycotechnology. In the industrial context, different strains of fungi are used to manufacture enzymes, pigments, polysaccharides, polyhydric alcohols, lipids, glycolipids and vitamins. The fungi that

produce secondary metabolites are tremendously beneficial in terms of healthiness and nutrition (Grabley and Thiericke, 1998). Additionally, manipulating the genome and optimizing the environment friendly circumstances during the process of fermentation that has aided the procedure of creating new products.

Among animals and humans alike, fungi play a crucial role in disease management. Several commercial products used in medicine were processed by fungi at the start of the twenty-first century. Among the topmost 10 record breaking drugs are two anticholesterol statins, semisynthetic penicillin (amoxicillin) and the immunosuppressant cyclosporin A. Their combined annual revenue exceeds $1 billion. Over the period 2004-2008, amoxicillin was estimated to cost 1.7 billion dollars and cyclosporine to cost 1.4 billion dollars (Smith and Ryan, 2009). Micafungin, a new antifungal; mycophenolate, an immunosuppressive; rosuvastatin, a cholesterol-decreasing drug; and cefditoren compound are among the newly approved drugs (Sharma and Sharma, 2016).

Nearly 2,50,000 species of fungi make the Kingdom fungi, of which 75% are Ascomycetes (90% are filamentous fungi and the rest are yeasts) and 25% are Basidiomycetes. Historically, fungi have positively affected the world economy, i.e., by producing food, pharmaceuticals, enzymes, and chemicals of commercial significance. Besides several diseases in animals, they also cause economic loss by spoilage of millions of foods crops every year.

The versatility of fungi allows them to thrive at pH levels between 2.5 and 11, at temperatures between 5 and 60°C and at salt concentrations greater than 4 M (Sharma and Sharma, 2016). As a result of their large metabolic diversity, they make use of several carbon and nitrogen sources from hemicelluloses to form simple glucose. Using established metabolic pathways, fungi make unique complex molecules. These

compounds produced by the fungal cultures are often released into the environment, the recovery is less cautious. Looking to the enormous application and tremendous potential of bioactive compounds from fungal species, the present study is undertaken to investigate "Therapeutic evaluation of fungi from Gujarat"

The research work was undertaken with the following objectives:

Objectives

1. Collection, isolation, purification and identification of different species of fungi using classical and molecular methods
2. Establishment of DNA barcodes for the identified fungi and utilization of bioinformatics strategies for their taxonomic studies
3. Screening of various fungi to examine their therapeutic applications
4. Characterization of biomolecule of interest for their therapeutic applications.

1.8. References

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