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Anamorphic Fungi: An Overview

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Abstract: Anamorphic fungi or Deuteromycetous fungi, which reproduce by mitotic division, are ubiquitous and cosmopolitan in distribution. The natural classification of these fungi has always been questioned which persists even today. Molecular taxonomy is an emerging field is being sought to solve accurate identification, systematic and phylogenic problems. DNA barcode approaches is primarily concern for accurate identification. Conservation of these fungi should be work out with appropriate standards. Anamorphic fungi which have also being utilized biotechnologically to produce varieties of economically important products. Bioinformatics discipline is of much helpful in this field for data transformation and industrial applications. An appreciable attention is required to explore anamorphic fungi and their conservation with establishment of new culture collection centers. A better cooperation and coordination is also required among Mycologists at International level to have improved understanding for to harvest potentials of these fungi.

Key words: Anamorphic fungi · Taxonomy · Morphology · Molecular approaches

INTRODUCTION

Hawksworth [1] estimated that there are 1.5 million species of fungi, of which, only 75,000 species have been described so far. Several mycologists have tried to answer the question "where are the missing fungi?" Identification, taxonomic position and mapping of fungi are challenging task for mycologist around the world. The world of fungi provided a fascinating and almost endless source of biological diversity, with rich biosource.

The true fungi belong to kingdom Eukaryota that has 4 phyla, 103 orders, 484 families, 4979 genera and 75,000 species [2]. Among all the phyla anamorphic fungi or Deuteromycotina is discussed briefly to highlight the extent of taxonomic position or systematic diversity, conservation, biotechnological approaches, bioinformatics and some other important issues with special reference to Asian scenario.

Anamorphic fungi are ubiquitous and cosmopolitan in distribution covering tropic to pole and mountaintops to the deep ocean. They are known to colonies, multiply and survive in diversified habitats viz,. water, soil, air, litter, dung foam, etc. This group comprises 20,000 species belonging to 1700 genera, which can be placed in Hyphomycetes, Coelomycetes and Blastomycetes. So far 8000 species of these fungi are reported from India [3-7].

Anamorphic or conidial fungi, which sporulate asexually through mitotic divison, are also called `Fungi Imperfecti'. The need for independent classification of anamorphic fungi remained unfulfilled. Despite this, anamorphic features have contributed richly to the elucidation of fungal taxonomy. Only 10% of described anamorphic species are known to have teleomorphs, which belonging to either Ascomycotina or Basidiomycotina. The asexual stages are called anamorph and perfect stage is called teleomorph.

Numerous fungi are apparently or permanently anamorphic (anamorphic holomorphs) as they have lost the capacity to form a teleomorph. These fungi are traditionally considered as imperfect fungi because they lack perfect stage or fail to sexually reproduce. The absence of teleomorph prevented their assignment to a natural taxon, which necessitated selection of artificial non-sexual characteristics to describe and classify them. This genetic inability of many imperfects to reproduce sexually is a primitive or advanced condition and in contemporary mycology presents a taxonomic quandary. Alexopolous [8] provided excellent scientific rationale for

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excluding imperfect fungi from contemporary fungal systematic and expressed urgent necessity to develop logical and valid taxonomic system to determine their phylogeny.

At present, anamorphic fungi are classified on microscopic morphological features such as the size, shape, surface ornamentation of spore, their mode of formation (conidium ontogeny), etc. and phylogenic affinities by using anamorph-teleomorph relationship.

Molecular taxonomy, which is an emerging field, will be more useful in resolving specific taxonomic problems of these interesting fungi in order to stabilize a more practical classification of these fungi. A large number of characters derived through advanced techniques in resolving fungal systematic problem is the need of the hour.

Molecular techniques based on DNA analysis seem to offer a wide range of advantage. Molecular marker technologies based on polymorphisms found in DNA have facilitated research in a variety of disciplines such as Taxonomy, Phylogeny and Identification. These techniques include study of Isoenzymes, Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphic DNA (AFLP), Quantitative trait loci (QTLs), Variable Number Tendom Repeat (Minisatellite), Simple Sequence Repeat (microsatellite) and Internal Transcribed Spacer (ITS) region of the nuclear ribosomal DNA genes (rDNA). Ribosal gene (18S-5.8S-18S) sequence analysis has revealed genetic variation in within and between species. The gene of this region is available in high copy number and possesses highly conserved as well as variable sectors, which facilitates differentiation of taxa at different level [9]. They are useful for phylogenetic analysis among related species and/or strains with in a species.

The phylogenetic trees constructed based on variation in the non-transcribed spaces by using different software cluster analysis. The use of molecular data in fungal phylogenetics has allowed the placement of asexual fungi (anamorph fungi) near the organisms to which they are most closely related, something that was impossible with the morphological characters of sexual reproduction traditionally used in fungal classification. Attempts have already been made to distinguish between strains of species [10] characterization of species [11], re -identification of species using molecular marker [12]. Sequencing of fungal ribosomal RNA gene for phylogenetics [13], molecular evolution [14] and fungal molecular systematics [15]. DNA sequence data are being successfully used to linked anamorphs with a holomorph and provide phylogenetic placement for anamorphs with unknown teleomorph [16].

The latest concept of DNA barcode have been proposed and initiated for facilitate biodiversity studies, identify, associated sex and enhance forensic analysis. DNA barcoding involves sequencing a standard region of DNA (gene) as molecular tools. Various gene have been employed such as nuclear large ribosomal subunit [17], internal transcribe spacer [18], partial β-tubuline gene sequences [19], partial elongation factor 1-α (EF-1 α) [20] and mitochondrial gene cytochrome oxidase I (COI) also known as COX I [21]. It is rightly stated that proper identification reveals the correct biology of fungi. Consequently incorrect identification can potentially cause some problem especially in quarantine of plant pathogenic fungi. Therefore further studies on the efficacy of various DNA barcoding markers as DNA barcodes are needed.

In recent year mycologists have witnessed a real revolution in the biological sciences with the advent of powerful technologies and application. These new technologies have ushered biology into the “genomics era” where studies involving whole genome are possible. In fact, the beauty of this technology is that it transcends the answering of old questions and challenges us to formulate and address even more complex biological question. Genomics is the high throughput discovery and study of many genes simultaneously on a genome wide scale.

Genomic approaches encompasses four inter related areas: (i) structural genomics, which is primarily concerned with the determination of genome structure at the sequence level (ii) comparative genomics, which involves the molecular differences between organisms at different taxonomic level and (iii) functional genomics, which focuses on assigning function to the annotated ORFs.(IV) Computational genomics is the branch of computational biology that deals with the analysis of entire genome sequences [22]. The first step after acquiring sequence data is often the submittal of these data to a database. The availability and the accessibility of such databases by the public of researchers have proved to be instrumental in many respects.

Most importantly, these databases necessitated the production of standardized protocols and formats for data deposition, storage and retrieval. Some of the major database include the National Center for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/and the European Molecular Biology Laboratory (EMBL) http://www.embl-heidelberg.de/, two excellent resources for gene annotation. Mannhaupt *et al.* [23] have reviewed what is the genome of a filamentous fungus by using analysis of the *Neurospora* (ascomycetes) genome sequence.

Since the completion of sequencing of *S. cerevisiae* (ascomycetes) genome, the genome sequencing of number of anamorphic fungi have been initiated among many of them such as *Aspergillus nidulans* [24] and *Aspergillus fumigatous* [25] and *Aspergillus oryzae* [26] have recently been published and some of them are near to completion such as *Penicillium chrysoporium* (http://www.jgi.doe.gov/programs/whiterot.htm) and *Microsporum grisea* (http://www. fungalgenomics. ncsu. edu/index.htm). The availability of genome sequence of these fungi will provide valuable insight into genome plasticity and the evaluation of anamorphic fungi. The fungal genome sequences will also serve as a platform to identify gene function and the regulatory network that exists among these genes.

DNA microarray technology [27, 28] is gaining increasing popularity among researchers interested in gene expression profiling as is evidenced by published data generated by this technique. Technical improvements and innovations that rendered microarray analysis more reliable and cost effective are making this technique widely used by an ever-increasing number of researchers from different disciplines [29]. The first fifty microarray studies in filamentous fungi have been done by Breaksher and Mamany [30]. Anamorphic fungi are also being used to identify specific genes for its pathogencity and to derive novel drug.

An approach has also been to develop protein arrays to perform studies ranging from analysis of protein interactions to studying enzymatic activities. Uetz *et al.* [31] used protein arrays to perform large scale yeast two hybrid screens in an effort to identify protein- protein interactions in *S.cerevisiae*. Zhu *et al.* [32] used proteome chips to analyze protein activities in yeast. They made microarrays slides with proteins produced by the over expression of 5800 ORF from yeast. The arrays were subsequently used to detect protein-protein and protein l ipid interaction, which led to the identification of several new calmodulin-and phospholipid bindind proteins. Anamorphic fungi are being used to identify specific genes for wonder protein by protein array technology.

Proteomics involves the high throughput simultaneous study of protein in a cell [33]. Proteomic studies will ultimately go beyond the simple characterization of protein profile to more intricate protein analysis, investigating areas such as post translational modifications and protein-protein interactions [34]. A myriad of technologies are being developed to perform such studies. http://pfam.wustl.edu/index.html. is protein database from Washington University [35]. Analysis and annotation of protein from whole genomes has been reviewed by Mewes *et al.* [36].

Metabolomics aims at the quantification and identification of metabolites present in the sample of interest as well the analysis of intracellular metabolic fluxes resulting from the activity of different enzymes [37]. The need for metabolomic studies is becoming exceedingly urgent to complement other functional studies where, despite all the achieved advances, more is yet to be learned. http://www.genome.ad.jp/kegg / which is mainly a database of metabolic pathway.

Anamorphic Fungi and Biotechnology: Anamorphic fungi are considered as chemical factories. They are easily grown in relatively simple nutrient medium in industrial size fermenter and produce extra-cellular secretion like desired proteins, plant growth regulators, growth factors, hormones, vaccines, antigen, vitamins, drugs, antibiotics and variety of enzymes such as protease (cheese industry), alkaline lipase (detergents industries), cellulase,(paper industry), invertase (beverage industry) and produce taxol-drug which has anticancerous properties (Table 1). The diverse metabolic activities of fungi have also been exploited in fields such as environmental remediation [38].

In recent years, novel techniques have been developed to produce rare and valuable molecules to change hereditary traits of fungi so as to diagnose disease and cure them biotechnologically through proteins and polypeptides forming a new class of potential drug or through immunodiagnostically designed vaccine. Therefore, anamorphic fungi are the most promising in economic growth in the near future and deserve maximum attention. Now the role of fungi in Biotechnology is term as Mycotechnology [39]. The following website can be used for Importance of fungi http://www.cbs.knaw.nl/search_fdb.html. There are several methods are being used to genetics improvement of the fungal strain on which the industry depends has been key to improvements in yield and the reliability and quality of desirable products, decrease in the formation of unwanted products, reduction in the costs of nutrients, equipment, fuel, labor, transport and improvement in downstream processing.

Table 1: Biotechnological products from Anamorphic fungi

Categories	Products	Anamorphic fungi
Antibiotics	Cephalosporine	*Cephalosporium acremonium*
	Griseofulvin	*Penicillium griseofulvum*
	Penicilline	*Penicillium notatum*
	Baccatin A	*Gibbrella baccata*
Organic Acid	Citric acid	*Aspergillus niger*
	Kojic acid	*Aspergillus oryzae*
	Gluconic acid	*Penicillium purpuroqenum*
Enzymes	Lactase	*Aspergillus niger*
	8- amylase	*Aspergillus oryzae*
	Cellulase	*Tricoderma reese*
	Amylase	*Aspergillus niger*
	Lipase	*Geotrichum sp.*
Plant Growth Hormone	Gibberellins	*Fusarium moniliform*
Bioherbicides	Collego	*Colletotrichum gloesporiodis*
Biofungicides	Bioderma and Ecofit	*Trichoderma viridi*
	Binab T	*Trichoderma harzianum*
	Biofox C	*Fusarium oxysporum*
	Coniothyrin and Koni	*Coniothyrium minitans*
	Kalisena	*Aspergillus niger*
	Soilgard	*Gliocladium virens*
Bioinsecticides	Biotrol and Boverin	*Beauvaria bassiana*
	Metaquino	*Matarhizium anisopliae*
	Vertalac	*Verticillium lecanii*
Bio-Nematicides	Paecil	*Paecilomycetes lilacinus*
Dairy Products	Cheese	*Penicillium rocqueforti*
Steroids	Cortisone	*Aspergillus niger*
	Cortixalole	*Curvalaria lunata*
	Progestrone	*Aspergillus ochraceous*
Anti cancer	Taxol	*Pestalotiopsis microspora*
	Pyrrolosporin	*Micromonspora sp.*
	Myrocin C	*Myrothecium Verrucaria*
	Saintopin	*Paecilomyces sp.*
Vitamins	Vitamine B_{12}	*Eremothemium ashbyii*
	Vitamin A	*Rhodotorula gracilis*
Anti tumer	Pyrrolosporin	*Micromonspora sp.*
	Myrocin	*Myrothecium verrucaria*
	saintopin	*Paecilomyces sp.*

Anamorphic Fungi and Bioinformatics: Bioinformatics is the science of the data management systems in genomics and proteomics of life forms. It is comparatively young discipline in information technology and has progressed very fast in the last few years. Biotechnologists can access various database for research and exchange information for comparison, confirmation, storage and analysis practices by bioinformatics worldwide. As on date, there are a number of databases on specific genes and proteins pertaining to human, animals, plants, fungi, bacteria and other life forms. These are also being enriched and update through research in modern biology with the practice of bioinformatics.

These databases helped in new inventions in enabling life sciences to invent the useful products for human welfare. Such inventions attain importance in the present scenario of patents and WTO regime for the future development of Bioinformatics and they will have to play a vital role with the involvement of Internet tools and the World Wide Web (www). Largely the bases available in generic or specific forms gain the future r-DNA research. Thus, bioinformatics and biotechnology have to move hand in hand for their progress. However, bioinformatics can now be branded a bonofide discipline within information technology.

The first eukaryotic genome sequenced database was that of yeast (*S. cerevieceae*). After that, genome sequences of few fungi are known and several other species are under progress. There are many genome-sequencing projects have been launched, e.g.

(*http://www.aspergillus.man.ac.uk*). In concern to culture collection centers, 350 registered culture collection centers have data base with the list of conservation and supply of culture [40].

Anamorphic Fungi and Bioremediation: Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. Bioremediation is limited in the number of toxic materials it can handle but where applicable, it is cost effective [41]. Biodegradation, mineralization, bioremediation, biodeterioration, biotransformation, bioaccumulation and biosorption are some term with minor subtle differences but often overlapping. Biodegradation is the general term used for all biologically mediated breakdowns of chemical compounds and complete biodegradation leads to mineralization.

The roles of fungi in degradation of complex carbon compounds to inorganic elements are review by Bennett and Fasion, [42]. Whenever bioremediation figures as the topic of discussion, bacteria agents come into focus and fungi are much less studies. One should realize, however, the greater potential of fungi by virtue of their aggressive growth, greater biomass production extensive hyphal reach in soil. More research will be focused in future on using the diverse fungal flora for bioremediation. Future work will be more focused on the biotechnological aspects. It may be possible to clone the highly efficient degradative enzyme producing genes into bacteria and conversely, bacterial genes can be transferred to fungi which are suitable. There is no doubt, therefore, regarding fungi being harnessed more and more in environmental bioremediation in future.

Conservation of Anamorphic Fungi: Threat to fungi through out the globe is of serious concern since they are not only beautiful but also powerful and play a significant role in human welfare. Moore *et al.* [43] have suggested the following steps for fungal conservation: (i) conservation of habitats (ii) *In situ* conservation of mycological reserves/ecological niches and (iii) *Ex-situ* conservation especially for saprophytic species growing in culture by use of synthetic medium. Storage methods involving sterilization, mineral oil, soil, silica gel, freeze drying and cryopreservation at ultra low temperature are most successful methods for retention of both viability and conservations of characteristics of anamorphic fungi up to 10 to 20 years.

Fungi are very seldom legally protected, enabling managers to prevent damage to their habitat. In the absence of legal protection, some effort needs to be made to have code of practice or suggestive documents stressing the importance of fungal conservation. One of the tools that would help in conservation is inventorization. In most countries checklist of fungi are not available. However, such projects are operative under the umbrella of IUCN. The World Federation for Culture Collections (WFCC) has formulated guidelines for conservation. These guidelines should be followed by Culture collections centers to maintain appropriate standards [44].

Asian Scenario: Asia is considered one of the mega biodiversity centers of the world containing diverse ecosystems with many novel organisms having new genes and secondary compounds. There is a striking imbalance in prioritizing research between the macro and microorganisms in Asia. The macroflora and fauna get maximum attention and the microbial diversity remained neglected. Whatever, a little amount spent for the systematic research in microorganisms. The documentation of anamorphic fungal groups still remains unexplored or under explored. Our country is handicapped in terms of novel pharmaceutical products from potential anamorphic fungi; even though there is a global upsurge to tap these bioresources to a greater extent using potential biotechnological applications based on accurate species identification. Hence, greater attention is required on targeting research programmes that includes the exploration of anamorphic fungi, which is so far neglected in Asia. The potentialities of these organisms need to be inventorying in the immediate future for the benefit of human kind. This should be our highest priority. Since the pressure on our biodiversity is scaling in term of Intellectual Property Right (IPR) both by the developed countries as well as our neighbours (who share a large number of species with us). There is also a continued natural and biotic (including anthropogenic) pressure on our existing biodiversity.

Hence, in this crucial moment the concerned people must reprioritize their focus more on inventories and sustainable use of the country biotic wealth. It is rightly pointed out that the taxonomic research in Asia is greatly neglected. However, in the recent years efforts are being made by various agencies to fill up the gap through all Asia R and D project. Extensive research using molecular markers in progress in many institutions all over Asia.

DNA fingerprinting of some anamorphic fungi is being carried out and lot of efforts have been put in. This information has potential in strategic planning of future towards use of Anamorphic fungi in Asia.

To improve systematic research in Asia, a substantial increase in the number of mycologist, publication of manuals, increase in funding, conducting short-term training programs, establishing repositories and improving identification services are one of urgent need. The enthusiastic participation and intense involvement and rich contributions by young scientists are very much required in the field of Mycology both at the National and International level. Even though we have congenial atmospheres and vast resources of anamorphic fungi, it is unfortunate that a very few patents have been granted by the Asian countries.

The invention of novel antibiotic compounds, drugs, proteins, enzymes and other industrial products needs to be taken up and proper identification of anamorphic fungi is of also utmost importance. Therefore, the establishment of culture collection centers is a prerequisite. In India, there are only two culture collection centers that cover different group of anamorphic fungi, i.e. IMTECH, Chandigarh and IARI, New Delhi. Thus, a number of culture collection centers in different region of India are urgently needed which will serve as nodal agencies for supply of cultures. The impact of bioinformatics scenario on Indian biosciences especially in fungal molecular biotechnology can be seen both in tangible and non-tangible research and in development activities. Now a days, these fields grow in quantity as well as quality that can be seen through research papers published by Indian molecular mycologist. A better cooperation and coordination is required among mycologist at regional, national and international level for exchange of vital information in term of bioinformatics.

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