Review Article

The Need To Explore The Fungal Facet Of Biodiversity Of Arunachal Pradesh

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Abstract: Fungi, due their unique method of vegetative growth, absorptive nutrition and the presence chitinous cell wall differ from the other eukaryotic organisms considerably and hence are placed in a kingdom of their own. Fungi perform many ecosystem functions including degradation of macromolecules in nature culminating in nutrient recycling and aid in the growth and performance of plants by forming mycorrhizal associations. Fungi are a source of many novel secondary metabolites exhibiting antibiotic, anticancer, and cholesterol reducing properties. Many fungal metabolites have been successfully marketed as pharmaceutical products. Fungi also produce many enzymes which are used by industries in various processes. Despite such desirable technological properties, fungi are not given due importance in biodiversity enumeration and conservation programmes. It is estimated that there are about 1.5 million species of fungi on this planet; of this, only 7% is currently known to science. It is essential to look for the undescribed fungi since many of these could represent new species harbouring novel genes which could find use in pharmaceutical, agricultural and other industries. Places with rich biodiversity should be explored for novel fungal species and varieties. Here we stress the importance of exploring the fungal wealth of biodiversity-rich Arunachal Pradesh by identifying some of the environments and habitats which need to be studied. A collection of fungal cultures isolated from the less-studied and extreme habitats of Arunachal Pradesh could be created with the help of students and faculty; these could later be screened for the production of industrially important bioactive metabolites and enzymes.

Key words: Biodiversity, Fungi, Mycorrhiza, Lichen, Endophyte

Introduction

Fungi with their unique mode of growth and nutrition belong to a separate kingdom of their own. They perform very important ecosystem functions such as supporting and aiding plant growth, increasing resistance of plants including crops to different abiotic and biotic stressors and by recycling nutrients in the biosphere. Although the lowest estimate of the number of species of fungi on earth is 1.5 million, hardly 7% of this huge number has been identified. It is believed that most of yet to be discovered 93% of the estimated 1.5 M species could be found in the tropics as the tropics support a high diversity of plants and animals on which the fungi depend for their survival. In this context, it is imperative that the fungal diversity of Arunachal Pradesh is explored as it falls within the Eastern Himalayan region, a biodiversity hotspot of India. The exercise would enhance the bioeconomy of India.

Fungi represent one of the earliest eukaryotic groups to have colonized land and as a consequence, have evolved multiple strategies to exploit various habitats and ecological niches for their heterotrophic mode of survival (Heckman *et al.,* 2001). They exist as parasites, pathogens and symbionts of plants and animals and also as saprotrophs. They have evolved adaptations to exploit a variety of nutrients sources and occupy different ecological niches and habitats such as fresh water, seawater, soil, dead organic matter and plants and animals. Fungi have evolved tolerance to survive in extreme environments such as cold, heat, low or high pH, heavy metals, radioactivity, high pressure, and abnormal concentrations of sugars or salt. Fungi perform various ecosystem functions including nutrient cycling. It has been estimated that the fungal kingdom has the largest number of species which varies from 1.5 million to 5 million (Blackwell, 2011; Hawksworth, 2012). However, of these, only around 7% are currently known (Hawksworth, 2012) and the major share of the remaining 93% of the undiscovered fungi is expected to be in the tropics where the diversity of other life forms is rich (Hillebrand, 2004; Arnold and Lutzoni, 2007). Therefore earnest efforts to discover the unknown fungi are needed as new species potentially represent novel genes which could be exploited for improving food, pharmaceutical and agricultural industries (Suryanarayanan and Hawksworth, 2005). The total number of fungal species reported from India is about 27,500 which includes 15,500 from plant litter, 327 on herbivore dung, 450 endophytic fungi (Bhat, 2010). Earnest efforts to discover the unknown fungi are important since apart from increasing the number of known species, new species potentially represent novel genes which could be exploited for improving food, pharmaceutical, and agricultural industries (Suryanarayanan and Hawksworth, 2005) (Fig. 1). World over, almost all the efforts to document and conserve biodiversity are focused on only charismatic animals and plants while microbes, especially the fungi, are least considered. Although Arunachal Pradesh falls within the biodiversity hotspot region of South Asia, its biodiversity has been poorly recorded (Borges, 2005). This is more so for fungi since most fungi are microscopic and rarely attract the attention of conservationists, naturalists and policy makers. Arunachal Pradesh which supports different unique habitats and ecosystems as well as a high diversity of endemic plants and animals is certain to have many new species of fungi representing novel genetic makeup. Hence, this state has to be explored assiduously for new fungal species. Here, we highlight some of the habitats and niches which could be studied for fungi of Arunachal Pradesh. Such studies are bound to enhance the biodiversity wealth and bioeconomy of India (Suryanarayanan *et al.,* 2015).

Soil

Soils are complex ecosystems rich in microbes including fungi. They are populated by saprotrophic fungi, resting structures (spores and sclerotia) of plant pathogenic fungi and fungi which cause diseases in soil-borne organisms like nematodes and insects. The interaction among soil fungal species and between soil fungi and other resident organisms leads to competition among these organisms. One method of overcoming the inetrsepcific competition in the soil is by antibiosis which involves antibiotic production by fungi for eliminating susceptible species from the environment. Thus, the study of soil for fungi will not only identify new fungal species but also novel antibiotics- a critical need in the present times owing to the rapid and widespread evolution of drug resistance among the pathogenic microbes. Furthermore, soils harbour fungal species which are antagonistic to plant parasitic nematodes and insects. For instance, soil fungi such as *Arthrobotrys* which live in soil and also near the roots of crop plants (rhizosphere) produce hyphal traps to capture and kill plant-parasitic nematodes. Furthermore, such nematophagous fungi produce anti-nematode metabolites like nematotoxin (Niu and Zhang, 2011). Species of *Trichoderma*, a common soil fungus is used universally as biocontrol agent to control fungal pathogens of crops such as *Fusarium* and *Pythium* species. Thus, screening soils for fungi could reveal those species that could be used as biological control agents of pests.. It would be worthwhile screening soils of unique environments of Arunachal Pradesh such as those of Dibang Valley (located about 1700 meters above mean sea level). Soils samples collected from different forests and mountains of Arunachal

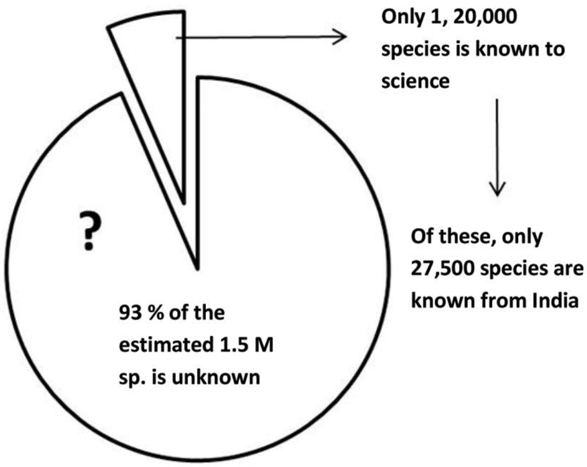

Fig. 1. Of the 1.5 Million species of fungi that are estimated to exist today, only 7% has been described; of these known species, 27,500 species have been reported from India.

Pradesh could be baited with specific substrates (e.g. cellulose, animal hair etc.) to isolate specific fungi capable of utilizing these substrates. Fungi present in habitats which are fire-prone have evolved certain adaptations. Suryanarayanan *et al.,* (2011) reported that some mesophilic fungi in the fire-prone forests of the Western Ghats in southern India produce thermotolerant spores which can withstand exposure high temperatures; some these fungi also utilize furaldehydes carbon source (Govindarajulu *et al.,* 2014). Furaldehydes are toxic volatile organic compounds produced in large amounts when plant biomass is burnt. Furaldehyde utilizing fungi could find use biofuel production. In biofuel production from plant biomass, the mandatory pre-treatment step evolves a lot of furaldehydes which need to be removed before fungi which convert cellulose to glucose could be used. It is envisaged that if organisms utilizing furladehydes as carbons source are introduce after the pre-treatment step, they can remove the toxic chemicals and make the substrate suitable for the conversion of cellulose to glucose. Such a method would cut down considerably the expense involved in biofuel production. The Jhum kheti or slash-and-burn method of cultivation presents a similar environment as that of the fire prone forests mentioned above. The people of Tirap District of Arunachal Pradesh continue to practice Jhuming cultivation due to the undulating hilly terrains existing here (Wangpan and Tangjang, 2012). It is suggested that the soils from agricultural fields subjected to Jhuming cultivation be studied for the presence of fungi capable of utilizing toxic furaldehydes.

Fresh water

Ascomycetous fungi (and their asexual states) of the orders Leotiales, Pezizales, Rhytismatales, Eurotiales, Amphisphaeriales, Diaporthales, Hypocreales, Halosphaeriales, Sordariales, Xylariales, and Pleosporales have been reported

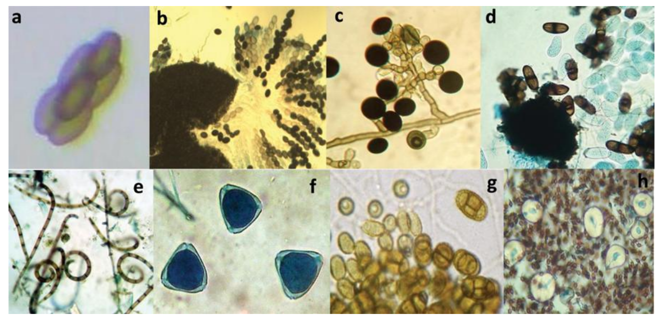

Figure 2. Photomicrographs of fungi isolated by VINSTROM from different substrates. a. Ascospore of *Saccobolus* sp. from elephant dung; b. *Sordaria* sp.; c. *Nigrsopra oryzae* d. *Lasiodiplodia theobromae* (note 2-celled dark conidia) isolated as endophytes from leaves of forest trees of the Western Ghats; e. Spores of *Troposporella* sp.; f. *Catenularia* sp.; g. *Pithomyces* sp.; h. *Emericella nidulans* isolated as soil fungi from forests of the Western Ghats.

to occur in fresh water lakes, flowing streams and rivers. They degrade plant materials in the water bodies and generally produce radiate asexual spores or ascospores (sexual spores) which are sticky. These fungi could be isolated by collecting the foam found in flowing waters. Many new species have been identified among the aquatic fungi throughout the world. Shearer and Raja (2010) provide an excellent website depicting the, world record and ecology of fresh water fungi; it also serves as an excellent pictorial identification manual. Recently, Sati *et al.,* (2014) described the occurrence five species of aquatic fungi from Kumaun Himalayas. Various rivers and their tributaries in Arunachal Pradesh would be excellent habitats for studying these fungi since the waters here are unpolluted. Another interesting aspect would be to study the diversity of fungi that might exist in natural hot springs of Arunachal Pradesh. Bora *et al.,* (2006) reported the presence of two such hot springs in Arunachal Pradesh; one in Dirang area of West Kameng district and the other in Kitpi area of the Twang district. The temperature of these waters is around 40°C. Apart from documenting the diversity of fungi present in such extreme environments, the fungi should be screened for the production of thermostable enzymes. Thermostable enzymes are highly sought after by industries and bioprospecting organisms from extreme environments such as hot springs is likely to identify such enzymes. Similarly, fungi existing in the snow clad regions of mountain tops have to examined both for their diversity and cold-tolerant enzymes since such enzymes are also in demand by industries.

Herbivore dung

The coprophilous fungi pass through the gastro intestinal tracts of herbivores without being digested germinate and grow on the dung. The dung is a special habitat as it is an isolated island of temporary source of nutrients. Hence, only fungi which are adapted to exploit this special habitat survive in it (Fig. 2a). A fresh dung sample incubated in moist chamber could be observed periodically for the occurrence of fungi. Initially, phycomycetes appear and deplete the simple sugars present in the dung, followed by the ascomycetes and basidiomycetes. A study of the coprophilous fungi colonizing the dung of Koala bear in Australia showed that some of them produce novel plant biomass destructuring enzymes which could be of use in biofuel production (Peterson *et al.*, 2011). A study of the dung fungi of herbivores of Arunachal Pradesh could be undertaken with a view to identify fungi producing novel enzymes of biomass degradation. The dung of animals such as the mitun, deer (Ami), Musk deer (Ala) and Red panda (Ayi minjini) found in Anini of Dibang Valley of Arunachal Pradesh (Biodiversity News Letter, CPEB, 2015) could function as a novel source of coprophilous fungi.

Insects

Some fungi of the genera *Beauveria, Metarhizium, Nomuraea, Paecilomyces, Cordyceps* and *Entomophthora* are entomopathogenic. They are parasitic on certain insects. Spores of an entomopathogenic fungus infect the insect, germinate and grow in the haemocoel. It may produce toxins to kill the insect and when the environmental conditions (humidity, temperature etc.) are conducive, the fungus emerges out of the body of the dead insect and produces spores to infect another individual. As entomopathogenic fungi do not attack all types of insects and have a restricted host range, they are being tried for their efficacy to function as biological control agents to control agricultural insect pests. This idea has gained prominence due to the harmful effects of chemical pesticides on the environment and the rapid development of resistance to such chemicals by insect pests (Charnley and Collins, 2007). The insect fungi are a source of chitinolytic enzymes as they produce these enzymes to penetrate the exoskeleton of the insect to infect and to emerge out after the death of the insect. Since chitin modifying enzymes (groups of chitinase, chitosanase and chitin deacetylase) have enormous economic potential in pharmaceutical and other industries, it would be profitable to prospect for insect fungi in Arunachal Pradesh. There is an excellent scope for finding

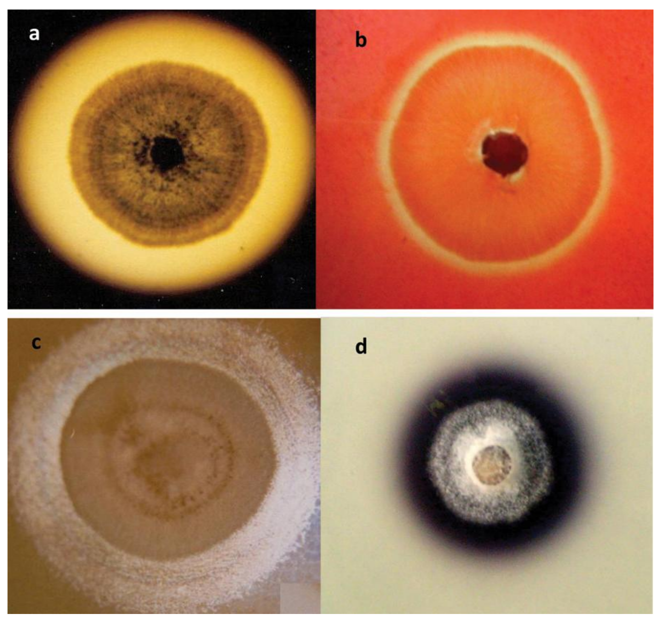

Fig. 3. Qualitative agar plate assays to determine the secretion of certain enzymes by fungi. (a). Fungus grown in starch agar medium and flooded with iodine solution shows a clear zone around the growth indicating that it produces amylase. (b). Fungus grown in cellulose amended-agar medium and flooded with Congo –red showing a clear yellow zone around the growth indicating production of cellulase. (c). Formation of precipitate around the growth when the fungus is grown in agar medium amended with Tween 20 confirms lipase activity. (d). Formation of a blue zone around the growth when the fungus is grown in the presence of á naphthol shows the formation of laccase.

suitable biocontrol agents and novel chitin modifying enzyme producing fungi here since the insect diversity is high.

Plants

Fungi are associated intimately with plants either as pathogens, symbiotic endophytes or passive residents on tissue surfaces. A brief description and importance of the endosymbiotic fungi (endophytes and mycorrhiza) are given below.

(i) Endophytes

These are mainly ascomycetous fungi which infect living plant tissues and reside in them for short or long periods without causing any apparent ill effect to the plants (Fig. 2 b-d). Many recent studies indicate that the diversity of endophyte harboured by tropical plants is extraordinarily high (Arnold *et al.*, 2000, 2007; Arnold and Lutzoni, 2007). Fungal endophytes increase the ecological fitness of the host plants by increasing their tolerance to high temperature and drought (Rodriguez *et al.*, 2009). The presence of endophytes in plants also protects them from insect pest attack. These fungi also produce many metabolites such as anticancer, antiinsect, antibacterial, antifungal and antialgal compounds which have potential to be used in industries (Suryanarayanan *et al.*, 2009, 2010). In a few cases, the endophytic fungi have been reported to produce in culture the economically important metabolite which is produced by their host plant. An endophyte isolated from the tissues of the Taxus tree produces taxol, the anticancer drug which is originally obtained from the tree host. Endophytes also produce many industrially important enzymes such as lipase, cellulase, xylanase, amylase, pectinase and protease (Govindarajulu *et al.*, 2011; Suryanarayanan *et al.*, 2012; Thirunavukkarasu *et al.*, 2015). Apart from recording the diversity of endophytes, these fungi as well as fungi isolated from other habitats described here could be screened for extracellular enzyme production by simple agar plate assay (Fig. 3) (Govindarajulu *et al.*, 2013). Although this assay is qualitative and not quantitative, it is simple and could be used to rapidly identify candidate fungi among numerous isolates for further quantitative studies. Here again, it remains to be seen if the endophyte diversity in Arunachal Pradesh is as high as its plant diversity. A concerted effort to study the endophytes of in this region could very well identify new fungi.

(ii) Mycorrhiza

Mycorrhizal fungi form mutualistic association with roots of plants. There are broadly two types of these fungi, (i) arbuscular mycorrhizal (AM)fungi which show a wide distribution, and (ii) ectomycorrhizal fungi which are more specific in their distribution and many of them form macro fruit bodies on the soil/root surface. These fungi derive nutrients from the photosynthates of the leaves. The host plants gain from the mycorrhizal association in many ways. The mycorrhizal fungi protect the host plants from root disease causing organisms and toxic stresses. They extract the insoluble minerals from the soil and provide them to the host plants and also improve the absorption of soil water by plants. It is now accepted that mycorrhizal associations determine to some extent the structure of plant communities. The diversity of mycorrhizal fungi is not full known. Although one estimate says that there are around 7750 species of ectomycorrhizal fungi, it is expected that there are at least 20000 species of this group of fungi (Rinaldi *et al.*, 2008). The importance of mycorrhizal association is highlighted by orchids. Orchids produce very small seeds with limited reserve food to support the young plant immediately after germination. The mycorrhizal fungus with which an orchid has an obligate association provides the necessary nutrients to the developing plant until it produces leaves and becomes autotrophic. There are many orchid species found in Arunachal Pradesh and hence an investigation of the mycorrhizal association of the different orchid species here would be interesting.

In general, the following can be stated. About 80% of the state of Arunachal Pradesh has forest cover (Hajra and Mudgal, 1997) and there are many reports on rare and endemic plants from this state (Ambrish and Amadudin, 2006). A study by Roy and Behera (2005) indicates that certain areas of the state including West and East Kameng, Lower Subansiri, Lohit, Changlang and Tirap districts support rich diversity of plant life. Similarly, parts of many plants (shrubs, trees and climbers) are being used by the local people of the state for various medicinal purposes (Namsa *et al.*, 2011). Plants with such unique status such as endemism, ethnobotanically acclaimed e.g. Amachi, Apichi, Ebichi, Ratachi and Mishimi teeta used by the Mishmi tribe of Dibang valley and from species rich forests e.g. Talle valley, have to be investigated for their endophyte assemblages and mycorrhizal associations.

Plant litter

Plant litter (leaf, bark, twig and fruits) present on the floor of the forest forms a natural substratum for the growth of saprotrophic fungi. These fungi colonize the litter and degrade it thereby aiding immensely in the recycling of nutrients. Generally, the litter is initially colonized by the so called sugar fungi which survive on the simple sugars present in the litter. After the depletion of simple sugars, these fungi are replaced by the asomycetous fungi which derive their nutrients from the litter by digesting the more complex sugars such as cellulose and hemicellulose by elaborating cellulase and hemicellulase enzymes. In the final stage of this succession on litter, the basidiomycetous appear and deconstruct the remaining lignin by producing lignin degrading enzymes. One could observe some overlap in the fungal succession stages as co-occurrence of different fungi may occur sometimes. As the diversity of litter fungi is substantial, it is necessary to study this ecological group occurring in various forest types found in Arunachal Pradesh.

Lichens

Lichen is not a single organism but is formed by the association between a fungus and a photosynthetic organism such as a cyanobacteruim or an alga; some lichens have both these as partners. The biology of a lichen is different from that of its either partner. Lichens are the first to colonize many substrates which are usually unfit for the growth of other organisms and their growth on such substrates slowly make them suitable for different colonizers. Many lichens are very sensitive to pollutant and hence they are used as pollution indicators. Lichens are also known to produce an array of biochemical and pigments. Some are edible. Studies like the one by Gupta *et al.,* (2014) on the diversity of lichens in Western Himalaya are needed in the Eastern Himalaya, especially in Arunachal Pradesh.

Mushrooms

Macrofungi, especially the mushrooms of Arunachal Pradesh need special attention with reference to the traditional knowledge accrued by the local people on their medicinal and food value. It is pertinent to mention here the study of Das *et al.,* (2014) which identifies two new species of macrofungi from North Sikkim. Similarly, investigations are needed on the diversity of wood rot fungi which abound in the forests of Arunachal Pradesh. Wood rot fungi are basidiomycete fungi that grow on both standing and fallen tree trunks. They produce cellullase and lignin degrading enzymes to cause the rot of wood and derive nutrition.

Fungi as sources of biocatalysts for industries

Considering the enormous biodiversity and variety of habitats that a state like Arunachal Pradesh supports, it is pertinent to elaborate here the need to explore fungi for their enzymes which find use in industries. One desirable property here is that the fungi secrete their enzymes to digest the large molecules to derive their nutrition. Thus it is relatively easy to harvest these extracellular enzymes for industrial applications. Non-food based industries such as textile, leather and biofuel production and food industries including baking, dairy, fruit juices, brewing, distilling and protein hydrolysis use enzymes. Of the approximately 260 different types of enzymes currently used by these industries, nearly 60% are of fungal origin; however, most of these enzymes come from merely five genera of fungi *viz.* *Aspergillus*, *Penicillium*, *Trichoderma*, *Rhizopus* and *Humicola* (Østergaard and Olsen, 2010). This underscores the importance of screening other species of fungi for their enzymes as this exercise is bound to reveal many enzymes which are more suitable for various industrial processes than the ones currently being used. Examples include salt-tolerant and thermostable and cold tolerant enzymes or enzymes with better catalytic abilities with reference to their stability and substrate conversion. The lignin degrading enzymes such as lignin peroxidase, manganese peroxidase, and laccase produced by the wood rot fungi play an important role in degrading lignin in the wood which one of the most recalcitrant biomolecules. Apart from being studied for improving the process of biomass degradation these enzymes are also used for bioremediation since they degrade complex organic compounds like azodyes and DDT. Another group of fungal enzymes with high potential for industrial use is the chitin modifying enzymes (CME). The CME include chitinase, chitosanase and chitin deacetylase enzymes. They find use in would healing, biological control of insect pests, drug delivery and bioremediation. Arunachal Pradesh with its unique and high bio and habitat diversities offers an excellent opportunity for bioprospecting for such fungal enzymes.

Fungi as a source of bioactive molecules

It well known that fungi are a good source of various secondary metabolites exhibiting bioactivities; these include plant growth regulation, microbial inhibition and molecules showing novel antibiotic, antioxidant, lipid modulating, and antifungal action. There is a dire need for more efficient metabolites showing these activities than the ones currently available. Again, exploration of fungi for such metabolites from the less studied habitats such as the ones existing in different parts of Arunachal Pradesh would be profitable.

Having established that the diversity of various groups of fungi of Arunachal Pradesh have to be explored, we suggest here that the crowdsourcing model by proposed by Suryanarayanan and Venkat Gopalan (2014) and Suryanarayanan *et al.,* (2015) be followed to create rapidly a genetic resource of fungal cultures unique to Arunachal Pradesh. These models envisage, apart from involving students and faculty for creating such a genetic resource, the enhancement of bioeconomy of the country.

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