Establishing a national fungal genetic resource to build a major cog for the bioeconomy

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Global conservation activities of animals and plants to protect endangered species are laudable. Similarly, various national and international bodies have recognized the value of preserving different types of microbes, the 'hidden-constituents' that are present in all habitats. However, conservation of microbial biodiversity has generally not been a priority in the world. We present a roadmap for creating a national genetic resource for fungi, whose diversity reflects their remarkable fitness for the rich and varied habitats and environments in India. In addition to offering fine prospects for research-based higher education, this national asset will accelerate technology development and the bioeconomy.

Keywords: Bioeconomy, culture collections, fungi, national genetic resource.

IN India, sustained efforts by the Government, researchers and the general public have contributed to the success of conservation programmes such as Project Tiger (launched in 1973), mirroring comparable global initiatives aimed at protecting iconic animals (e.g. the giant panda) or plants (e.g. cycads). The conservation of microbes by maintaining culture collections has similarly been endorsed by various groups as equally valuable, due to the economic potential[1] and the critical role of microbes in the success of habitat-preservation programmes. Yet, conservation of microbes, especially fungi, has not been a priority either in India or elsewhere (with some exceptions, see below). Even the Member States of the Rio Convention on Biological Diversity did not emphasize conservation of fungi[2], leading to David Hawksworth's sobriquet for fungi as 'the orphans of Rio'!

Fungi cannot be classified as either plant or animal. In fact, their unique structural, growth and reproductive properties lend appeal to fundamental and applied studies of these diverse organisms. Because of the tremendous variations in their life histories and ecological strategies, Heilmann-Clausen *et al.*[3] have suggested customized, case-specific approaches to achieve success with respect to their conservation. We extend this idea by arguing that India, home to unique fungi in its rich habitats and ecosystems, could focus on conservation and development of fungi that hold potential for its growing bioeconomy.

Why have fungi not been given the importance they deserve?

Arguably, fungi constitute the most species-rich kingdom and are estimated to support 1.5 million species[4]. In fact, large-scale environmental metagenomics suggests revising this number to ~5 M. Fungi are ubiquitous and occur in soil, fresh water and sea water, either as symbionts or parasites of plants and animals. They live in extreme habitats having unusually high levels of salt, acidity, alkalinity, temperature, heavy metals or ionizing radiation. They perform important ecosystem functions, including nutrient recycling by acting on simple and recalcitrant substrates. Despite enormous species and functional diversity, fungi have typically not been considered for conservation efforts due to various reasons[5]. Foremost, their microscopic nature (certainly not as fetching as a tiger or an elephant for non-mycologists and policy makers) has led to an undervaluation of their roles in maintaining different habitats. Second, while it is easier to estimate the loss of habitats and extinction of larger species (the major drivers for conservation efforts), there are limited data indicating loss of fungal species due to elimination of habitats. Finally, the general negative perception is that fungi cause either disease or spoilage of food and materials. This is partly due to the fact that even the few papers in conservation-centric journals that deal with the role of fungi focus on their roles as pathogens rather than their vital contributions to new technologies or all four categories of ecosystem service as recognized

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by the Millennium Ecosystem Assessment[2,3,6] (e.g. aiding the roots of a plant to acquire soil N and P).

Why study fungi?

Microbial resources can be employed to address many of the current global challenges pertaining to health, food, energy and environment. Being one of the most ancient eukaryotes to colonize land[7], fungi have evolved to survive in diverse ecological niches with varying growth conditions. Such remarkable ecological fitness, the basis of their competitive edge, is partly due to phenotypic versatility, which in turn stems from genetic variability attributable to genetically different nuclei in their mycelia[8] and acquisition of novel genes through inter- and intra-kingdom gene transfers[9,10]. Such fungal diversity offers fine prospects for scientific exploration, manpower training and industrial exploitation. In fact, this contention is supported by research on an ecological group called the endophytes, conducted over the last two decades by the Vivekananda Institute of Tropical Mycology (VINSTROM), Chennai.

Endophytes infect plants but do not cause any disease in them. VINSTROM has about 1000 endophyte cultures, which were isolated from trees of Mudumalai forest in the Western Ghats, plants of Arunachal Pradesh, mangrove trees of Pichavaram in Tamil Nadu, and marine algae/seagrasses on the coast of Tamil Nadu. VINSTROM's global research collaborations have uncovered the biotechnological potential of some of these cultures. Endophytic fungi elaborate anticancer metabolites and plant-growth regulators[11], antimalarial metabolites[12], antialgal, antifungal, antibacterial and insecticidal metabolites, and novel enzymes for an array of industries[13–18]. If VINSTROM, a small laboratory with minimum resources and a singular focus on one ecological group of fungi (endophytes), could identify several fungal candidates with potential biotechnological traits, a more concerted nationwide effort could reliably be expected to identify numerous fungi with desirable characteristics.

Why another fungal culture collection centre in India?

India has many biodiversity hot spots and supports different ecosystems such as forests, grasslands, wetlands, deserts and mangrove ecosystems (coastal and marine). India's biodiversity is largely unknown, as exemplified by periodic discoveries of new plant and animal species[19,20]. Knowledge of the country's biodiversity offers a route to build national bioassets.

About 75% of the industrial fungal enzymes come from merely five genera of fungi[21], reflecting a phylogenetic bias in the investigations to date, which incidentally have uncovered at most 7% of all predicted fungi. Since the richness of fungal species increases towards the equator[22] and the tropics are expected to harbour most of the predicted but unknown fungal species, exploration of the different ecosystems of India offers profitable grounds for bioprospecting.

It is instructive then to examine the fungal preservation and taxonomic identification efforts in India and elsewhere[23]. Although ~27,500 (15,500 terrestrial litter, 327 coprophilous and 450 endophytic) fungi have been described in the scientific literature from India[24], most of these have not been preserved as live cultures for further use, thus limiting their potential for development. The scorecard in India for culture collection is as follows: the National Fungus Culture Collection of India (NFCCI), Pune, holds a collection of about 2800 strains of different groups of fungi (http://nfcci.aripune.org/about_us.php); the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, has over 9000 cultures, including actinomycetes, bacteria, fungi, yeasts and plasmids (http://mtcc.imtech.res.in/aboutmtcc.php) and the National Bureau of Agriculturally Important Microorganisms has 700 species belonging to 250 genera of fungi. In contrast, 30,000 fungal cultures are maintained by the Commonwealth Agricultural Bureau International, England[1] and 50,000 strains at the Centraalbureau voor Schimmelcultures, the Netherlands (http://www.cbs.knaw.nl/index.php/collection). Enhancing the fungal biodiversity inventory in India to these levels should be undertaken immediately, as it offers a long-term mechanism to catalyse innovations in education, research and industry. Towards this goal, we propose building the Fungal Genetic Resource of India (FUNGEN), an initiative whose scope and mission exceed that of a conventional repository. Unlike the conventional repositories which house taxonomy-based collections of cultures, FUNGEN would focus on a trait (phenotype)-centred collections[25]. Moreover, the development, planning and implementation of FUNGEN will exploit an unusual alliance of multiple stakeholders in our society.

What is the operational scope of FUNGEN?

Foremost, FUNGEN's mission will adhere to the guidelines set forth by the World Federation of Culture Collections, a global monitor of all collection activities. The overarching goal of FUNGEN will be to harness the technological potential associated with the fungal biodiversity in the less-studied habitats of India, using a crowdsourcing model that exploits a collaborative network of educational institutions, national research institutes and industries[26] (Figure 1). Skill development and curricular enhancement in different parts of the country should be a high priority of FUNGEN; manpower development in the various processes involved such as isolation, culturing and identification of fungi, molecular

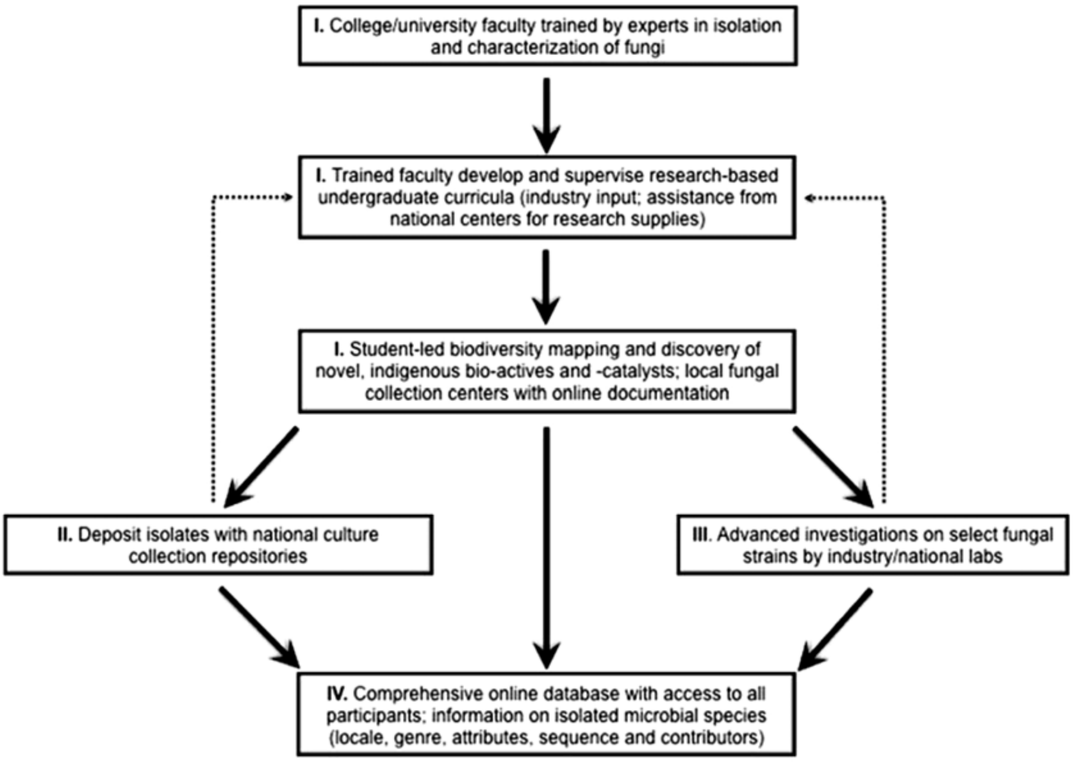

Figure 1. Crowdsourcing model to build a national microbial repository (reproduced with permission from Suryanarayanan and Gopalan[26]).

techniques and screening organisms for specific bioactivities will add a heuristic approach to higher education.

As to fungal biodiversity, our sampling space will be guided by Suryanarayanan and Hawksworth[27], who have identified many of the little explored and extreme habitats [e.g. corals, sponges, deep-sea sediments, resins, gums, soils (alkaline, acidic, hypersaline, contaminated with heavy metals), on and in insects, in the gut and dung of wild animals, plant roots and shoots]. These sources will be studied within the framework of research objectives to identify: (i) fungal metabolites that are effective against drug-resistant *Plasmodium falciparum* or *Mycobacterium tuberculosis*, nosocomial infection-causing yeasts, crop pests and pathogens, and (ii) enzymes for clinical therapy and for industry (e.g. plant biomass-deconstruction enzymes that are active at high temperature/salinity and low pH). This list will be widened based on evolving industrial or societal needs.

How will FUNGEN be operated?

FUNGEN should be physically located in one of the major cities with excellent infrastructure and skilled manpower. The management team should be led by a director with a long-standing record of leadership and scientific accomplishments in mycology, and assisted by two senior personnel with complementary expertise in educational outreach and industrial liaison. Several staff scientists (M S, Ph D level) will be needed to perform distinct functions: isolation of fungi from challenging sources, design of high-throughput screening methods for bioactives and enzymes, maintenance of fungal cultures submitted from different locations and associated informatics (a searchable database of fungi, including name, location, key attributes, registry of personnel involved; fungal genome sequences, etc.), development of teaching kits and training of college/university instructors. Appropriate administrative support will be essential to keep the centre running smoothly.

While FUNGEN will seek to identify strains with valuable properties and work actively with industry and national institutes, it will be greatly aided by a crowdsourcing effort to rapidly build this national fungal repository[26] (Figure 2). The plan envisions the recruitment of B Sc/M Sc students in colleges to participate in this national initiative while experiencing first-hand the execution of a research project and the excitement of scientific discovery. FUNGEN has two critical roles to play in this regard. First, it will train college faculty in isolation, testing and culturing of fungi from different habitats. For example, students in Andaman islands, West Bengal, Rajasthan and Kerala could be involved in isolating fungi from the Andaman Sea, Sunderban mangroves, soils of the Thar Desert, and endophytes or litter fungi from near the Silent Valley respectively. Second, although each of

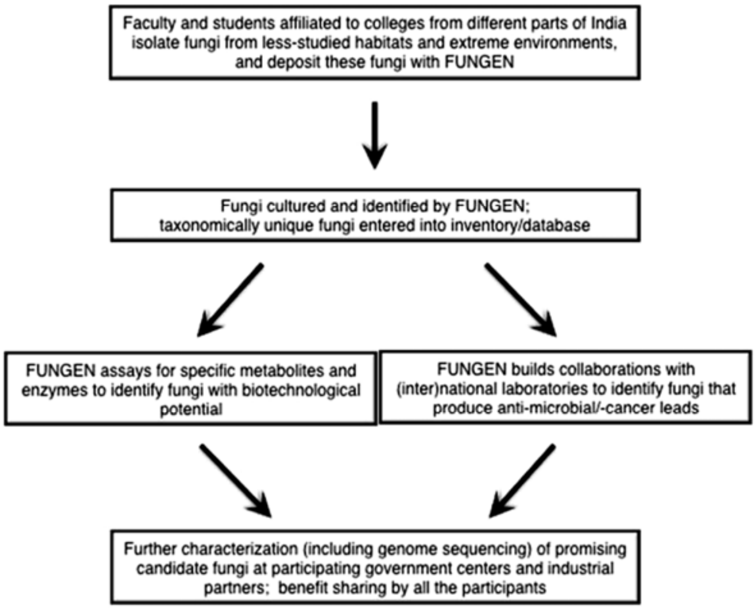

Figure 2. Framework for the overall functioning of FUNGEN.

the participating colleges can serve as a mini-collection centre, FUNGEN will obtain each isolate and provide an accession number. FUNGEN will initially parse these collections not by taxonomic identification but by the rapid biodiversity assessment method[28] that uses culture and morphological characteristics. Unique isolates will be maintained for subsequent taxonomic identification and inclusion in the central inventory.

Involvement of such multiple mini-centres of collection ensures rapid build-up of cultures and provides solutions to several logistical problems related to transfer of material in a timely fashion. More importantly, it also allows a certain degree of pride and ownership for the 'crowdsourcing' students in each region. Of course, there are challenges imposed by geography and the absolute need for uniformity in training while maintaining rigour in scientific pursuits. FUNGEN could share its training responsibilities with NFCCI and MTCC, with each serving as a regional training centre. If so, the future location of FUNGEN should be chosen so as to triangulate the country suitably. For the research-based curricula to be uniform and consistent, FUNGEN will need to develop a centralized framework for professional development of teachers and for protocols and reagents to be used for fungal isolation. Participant feedback will allow refinement of initial pilots for subsequent iterations.

How will FUNGEN be financed?

The cost for maintaining a culture collection nominally includes those for staff, acquisition and maintenance of strains, data management, utilities and consumables. However, there is no standard financial model suitable for all culture collection centres[1]. A cost-sharing plan involving State and Central Governments is needed. While teacher salaries, travel costs and infrastructure are logical fiscal responsibilities of the local colleges and their respective State Governments, the Central Government should bear the costs of overall operation of FUNGEN. The corporate social responsibility mandates should be expanded to encompass industrial contributions for the creation of such national repositories and for the training of the future workforce. After the first few years, FUNGEN will also generate revenue from sale of biological materials/intellectual property and research grants/contracts.

Summary

FUNGEN will provide an umbrella to integrate a decentralized network of experts, research scholars and existing facilities towards the shared objective of fungal collections and usage. A valuable precedent is provided by Liaud *et al.*[29], who screened fungal isolates housed in the International Centre of Microbial Resources, France, for production of organic acids. Clearly, public availability of biomaterials and coordinated efforts between FUNGEN and relevant partners will enhance the value of its collection several fold (see Furman and Stern[30] for other examples), while fully exploiting the biodiversity resources to accelerate India's research and bioeconomy.

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