Crowdsourcing to create national repositories of microbial genetic resources: fungi as a model

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To address the challenging task of identifying, obtaining and cataloging the different microbes in a country with wide-ranging environments and habitats, we present a crowdsourcing model. With fungi as the prototype, we discuss approaches for rapid collection and identification of strains from environments and habitats that might lead to novel genes of industrial importance. Also, we expect the use of easy culture preservation methods to promote colleges as mini culture-collection centres and serve as the initial focal point in a national research initiative. Our model envisions the concerted involvement of undergraduate students, faculty, industries, national laboratories and culture-collection repositories to rapidly build a large assemblage of rare fungal strains and enhance the biodiversity resource of a country.

Keywords: Biodiversity, crowdsourcing model, fungi, microbial culture.

AN appreciation of species diversity is important for understanding the global ecosystem. The biodiversity of each state/country is a ticket to its prosperity because it enables the development of a framework for long-term conservation and facilitates targeted bioprospecting initiatives that can potentially catalyse industrial innovation. Local inventories even in a limited geographical context have tremendous value in disparate areas such as sustainability and biotechnology. Thus, the motivation to build national repositories comes from recognizing the broader implications of vast collections of microbial cultures as genetic resources for a sustainable future in addition to developing storehouses of taxonomic value in teaching and research[1,2]. The human gut microbiome project has revealed the ecology of diverse microbial communities, with clear implications for understanding normal and pathological conditions. Similarly, inroads into microbial consortia in various natural habitats will inform efforts to understand the evolutionary driving forces for dominance of select lineages in complex communities and the associated impact on the configuration and functioning of the respective ecosystem.

Identifying, obtaining and cataloging the different microbes in a country with wide-ranging environments and habitats is a challenging task. Therefore, we propose here a crowdsourcing blueprint that integrates resource accumulation, training of college students, and resource and benefit sharing by all the stakeholders. Crowdsourcing was introduced as an innovative, web-based business solution that combines the collective intellectual and technical expertise of a large network of individuals to address an unsolved problem. A major focus of the somewhat modified crowdsourcing model that we propose is to provide B Sc/M Sc students majoring in life sciences opportunities to participate in a national research initiative and experience first-hand the excitement of scientific discovery. We have chosen fungi as candidate organisms to illustrate our model.

Why build a fungal repository?

Fungi are heterotrophic organisms exhibiting saprotrophic (i.e. capable of deriving nutrition from decaying organic material) and mutualistic or antagonistic interactions with plants, animals and humans. It is hard to imagine terrestrial life without fungi – they are the major decomposers of organic matter and are integral to biological networks with other organisms, beneficial and otherwise.

Although fungi are hyperdiverse with an estimated 1.5 million species globally[3] (see ref. 4 for alternative estimates), only about 7% of them are currently known and no more than 1% from these species has been studied as part of fundamental and applied research[5,6]. This shortcoming in fungal research needs to be addressed if we are to materialize diverse payoffs, some of which we highlight here. For instance, an appreciation of the soil fungal diversity (including decomposers and mycorrhizal forms) has significant implications for agricultural productivity. Here, accumulation of data on fungal diversity in a small

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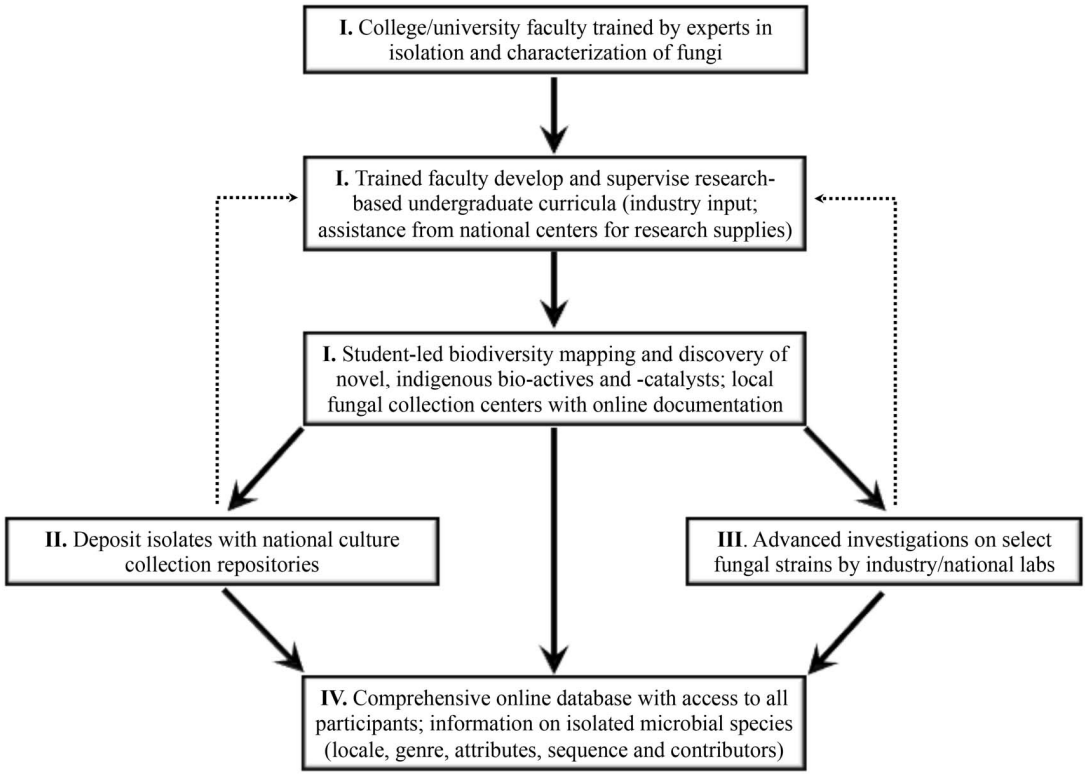

Figure 1. Roadmap for the crowdsourcing initiative to build national repositories. For simplicity, many details including administrative mechanisms have not been included.

scale from different locations would make the biodiversity data-mining exercise highly manageable while ensuring that the collective efforts culminate in a large repository for bioprospecting. From an industrial perspective, fungal enzymes currently used in different processes come from just five genera of fungi[7], clearly an under utilization of the vast fungal potential to produce bioactives and catalysts[8]. In this context, the likelihood of identifying novel fungal genes which encode either for entire pathways for biogenesis of secondary metabolites or for individual enzymes with unusual attributes, is higher in some parts of the world (e.g. the tropics) where there is high diversity of organisms on which the fungi depend for their survival[3]. Thus, the economic and ecological impact of fungi on humans and human-related activities lends support to building national fungal repositories.

Roadmap for crowdsourcing efforts to build a national repository

The highly successful Phage Hunters Integrating Research and Education (PHIRE) program in the US that is funded by the Howard Hughes Institute is inspiring and instructive both for its scope and success in establishing mechanisms that enable undergraduate and even high-school students to engage in independent scientific research. By providing simple procedures and protocols for mycobacteriophage isolation and characterization, motivated students with little or no prior exposure to advanced techniques or biological concepts are trained to become scientific explorers. We emulate some aspects of the PHIRE program in our four-phase crowdsourcing strategy (Figure 1). Although the initiative elaborated below is for the Indian context, the model should be equally applicable to other countries as well.

Phase I

The number of fungal isolates obtained from different habitats constitutes the base of the biodiversity mapping enterprise and, by extrapolation, the bioprospecting pyramid. This exercise could be a daunting task for India, a large country. One way to render the problem more tractable initially would be to restrict the collection of fungi to less-studied habitats and extreme/stressed environments. The expectation is that organisms with unusual traits (due to novel genes) are likely to be favoured in these challenging environments. The focus would include, for example, endophytic/litter fungal inhabitants of plants from different types of forests and deserts, colonizers of herbivore rumen and dung, symbionts from seaweeds and sponges, and opportunistic fungi from polluted, hypersaline and heavy-metal contaminated

Table 1. Some methods for isolating fungi from less-studied habitats or with unique traits

Characteristics of fungi	Key attribute of suggested method
Cellulolytic fungi	Incubation in cellulose-supplemented growth medium as the sole carbon source.
Halotolerant and halophilic fungi	Incubation in NaCl-amended growth medium.
Thermophilic fungi	Incubation in suitable medium at high temperatures.
Endophytes of roots, leaves and stems	Isolation from plant tissues on simple medium following surface sterilization.
Soil fungi	Isolation by dilution plating, baiting, soil steaming or Warcup method.
Litter and dung fungi	Incubation of litter or herbivore dung in moist chambers.
Aquatic fungi	Incubation of water sample with boiled seeds or cellophane strips.
Alkaliphilic fungi	Incubation of alkaline soil in growth medium with pH > 9.

soils[9]. Fungi from such little explored habitats have already afforded unexpected benefits. For example, fungi colonizing koala faeces produce hydrolases of industrial importance[10], while rumen fungi produce interesting glycosidases[11].

College/university students majoring in biology, biochemistry, biotechnology and microbiology will be the key players in the undertaking to isolate fungi from different habitats. For instance, students in a degree-granting institution located in an industrial belt might concentrate on isolating fungal strains from effluent-affected soils. After a few months, each participating college/university would have several isolates of fungi from a specific environment that it chose to study. At this stage, the focus of the project would shift from isolation to screening of the isolated fungi for production of novel enzymes or bioactivities using simple agar plate-based assays. For both stages, the experiments are rather straight forward requiring simple equipment: a pressure cooker for sterilizing growth media, a table-top wooden inoculation chamber fitted with a germicidal UV lamp, petri dishes, conical flasks, microcentrifuges and growth incubators.

Methods of media preparation and isolation of fungi from different habitats and environments are well established[12–14]. While most fungi could be isolated using simple media, the medium composition or incubation methods could also be altered to isolate fungi with specific characteristics[15] (Table 1). These fungal isolates could be identified based on microscopic attributes described in standard manuals[13,16–19], and grown in suitable media prior to tests of various bioactivities. To identify those fungi that produce anti-algal, -fungal or -bacterial metabolites, the autobiogram method which is both rapid and simple could be used[20]. Similarly, to visualize production of specific enzymes by the isolated fungi[21], agar plates infused with suitable substrates could be employed. The assay conditions can be adjusted to screen for activities under industrially relevant physicochemical conditions (temperature and high/low pH).

For the above plan to be successful, college faculty need to attend a short training programme by experts on isolation, culturing and testing of fungi. This training could be a part of the mandatory refresher courses conducted by the various universities and organized by the University Grants Commission for college teachers. Trained faculty can then undertake to supervise the short-term projects of the students and also train more mentors in subsequent refresher courses. Importantly, professional development of teachers will result from these research activities that they supervise.

To ensure that students have an enriching research-based curricula, it would be useful to have a centralized mechanism for distribution of protocols and reagents, similar to the NCERT kits now being distributed to schools in India. A national centre (e.g. Microbial Type Culture Collection (MTCC), Chandigarh) could play a role in this undertaking.

The approaches mentioned above are expected to provide a common platform that will ensure uniformity in training while maintaining rigour in the scientific pursuits. Such safeguards are needed if crowdsourcing is to be effective and reliable. In fact, while social networking has been used effectively for biodiversity mapping, a common refrain associated with such citizen science is the variability in data-gathering and documentation[22].

Phase II

Although industrially important fungi can be isolated from different habitats of the country, their potential can be fully realized only with coordinated academia–industry or academia–national research institute partnerships. We list some suggestions in this regard. First, once colleges/universities express willingness to participate in the national initiative, local chemical/biotechnology companies or national research laboratories could hold workshops where they present in broad strokes (without sharing proprietary information) longstanding problems that continue to challenge their respective industry and whose solutions could come from fungal research. Such exchanges will stimulate students to consider new directions while planning their screening activities. Second, national funding agencies [Department of Biotechnology (DBT) or Department of Science and Technology (DST)] that have identified thrust areas should establish new mechanisms for enabling these ties. For example, the research focus might be on a microbial disease that afflicts

a cash crop in a certain locale. Based on the premise that an endophyte from resistant plants in this locale might synthesize an antimicrobial compound that protects against this pathogen, colleges in this locale should be able to work together with the Principal Investigators and Ph D students of DBT/DST-funded research projects on this topic. Special incentives in the form of additional research budgets should be provided to encourage involvement of undergraduate students in such *de novo* research. Collectively, these different kinds of partnerships will sharpen the problem-solving skills of college students and possibly motivate a few of them to choose a scientific research career.

A college which identifies a strain/consortia with valuable properties might not have the wherewithal to fully reap the potential and would have to hand it off to another institute/industry with dedicated facilities (e.g. high-throughput spectrophotometric/fluorometric assay capabilities) and sophisticated expertise (e.g. metagenomic DNA sequencing to reveal valuable novel gene variants in non-culturable fungi). Therefore, it is important to develop at the outset intellectual property- and benefit-sharing formulas that ensure recognition and compensation of all stakeholders.

Phase III

As large-scale maintenance of cultures is costly and requires some troubleshooting expertise, colleges could serve as mini-collection centres and maintain a few cultures of fungi isolated from a special habitat. By subculturing the fungi on agar slants prepared in 1.5-ml Eppendorf tubes, the space required can be minimized[23]. Five such slants per isolate can easily be prepared and topped with either sterile water or mineral oil, placed in sterile polythene covers and refrigerated. Such cultures survive for several years, circumventing the need to subculture often. Following this approach, the Vivekananda Institute of Tropical Mycology (VINSTROM), Chennai has isolated and is preserving ~1000 endophyte isolates obtained from mangroves, forests trees and marine plants in southern India. The VINSTROM collection has isolates that produce novel enzymes or anti-algal, -fungal, -insect, -bacterial and -oxidant metabolites[24–26]; some of them exhibit unique traits such as heat-tolerant spores[27].

All colleges would also submit to national centres the potential strains that they have isolated and obtain accession numbers for them. They should also maintain a registry with information about the students and faculty involved in the isolation of the strains. The national centres would confirm the taxonomic identity and, if confirmed to be unique, also maintain the isolates submitted by the various colleges. Today, MTCC holds a total of only about 9000 microbial cultures, which includes bacteria and fungi (mtcc.imtech.res.in/aboutmtcc.php). Similarly, the National Fungal Culture Collection, Pune, has about 2800 different fungi (nfcci.dinpl.com/about_us.php). With crowdsourcing, these numbers will increase rapidly.

Phase IV

Akin to PhagesDB.org, we propose a portal for collation of all the fungi identified by the crowdsourcing initiative. This central warehouse of information will collate important data on all fungi, including their geographical/biological source. The portal should also serve as a forum that establishes networks between researchers and policy analysts. While individual colleges will be encouraged to independently showcase their findings, a shared website that has the entire compendium will be valuable. Moreover, given the plummeting costs of genome sequencing, it is likely that many of these fungi will be sequenced in the coming years and the sequences will be part of a comprehensive database, which needs to be maintained. One of the national culture collection centres could undertake these responsibilities provided additional resources and manpower are available to them. In this regard, those actively inolved with the CSIR-led open source drug discovery programme can provide valuable pointers and play advisory roles.

Summary

We hope that the above plan will motivate faculty and educational policy-makers to consider the desirability of and strategies for incorporating research-based curricula in the education of young scientists. Beta testing of the model in a small scale before wide implementation is critical. Moreover, we realize that it will be useful to integrate this initiative with ongoing DBT-led public–private partnership efforts to explore microbial diversity for producing industrially valuable products. With appropriate planning, it is our hope that college students can begin to view the formulation, testing and execution of a research project as a doable and enjoyable exercise. When such training activities are effectively integrated to build valuable genetic resources, the broader impact of the research undertaking gains added significance.

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