Roadmap for culture collections in India to enrich themselves with indigenous fungal biodiversity

Rahul Sharma

Exploration of selected habitats

India is a tropical country with a wide variety of habitat types that harbour a great diversity of fungi[1]. These fungi include both beneficial forms like edible mushrooms, mould-producing industrial enzymes or other biotechnologically important products along with the ones like yeasts, indispensable for bread and wine industry and those that cause diseases in humans, animals and plants apart from a few that produce toxins. A large part of India's fungal diversity is still unknown, as evidenced by regular reports of new genera or species being published[2–8]. During a recent study of Maharashtra soils covering 31 districts, we have so far recovered 2 new genera and 9 new species of keratinophilic fungi[9]. These findings suggest that even well-studied locations in India like Maharashtra harbour many novel taxa that might possess biotechnologically important properties. Therefore, there is need for a fresh, systematic survey of India's soils (or other niches) to recover unknown fungi.

Bridging the gap between cultures in Indian collections and fungi

The number of fungal culture collections in India has risen considerably after the establishment of Indian Type Culture Collection (ITCC), New Delhi in 1936. However, the number of holdings of different culture collections has not reached international standards. Even if all fungal strains available in Indian culture collections are put together, they account for very little of the total fungal diversity known from the country. More than 27,000 species of fungi have been reported from India so far[1], but less than 5000+ species (not strains) are available in culture collections across the country, several of them being redundant species. Hence there is an urgent need to isolate different species of fungi from Indian soils or other niches so that we have the entire diverse genetic pool harbouring Indian confines for possible exploitation for biotechnology, medicine and industry. A nation-wide joint programme can be initiated by various culture collections in collaboration with selected local mycologists, covering all possible states encompassing different habitats. In the absence of a collaborating mycologist in a state, researchers/teachers at the local colleges or universities can be identified and trained at the culture collections for sampling and isolation of various groups of fungi. A model project was carried out by the present author in Maharashtra recently on keratinophilic fungi, which resulted in a collection of 729 soil samples from across 31 districts covering about >20,000 km by road in 2.5 years. The project has some interesting results, including several novel taxa that are keratinolytic and some with antifungal properties. Thus given the results obtained in the project, a similarly scaled-up project could be formulated to include other habitat niches to target isolation of diverse forms of fungi across geographic confines of India.

Screening of fungi, yeasts, wild mushrooms and other basidiomycetes

Once we have a large collection of fungi from the above-mentioned projects, which include not only multiple strains of few species but a large number of species of different genera belonging to various families (more than 536 families of fungi are known[10]), a large-scale screening programme could be initiated in-house at selected culture collections (NCMR, MTCC, NFCCI, NCIM, NCCPF, NCAIM and ITCC), or in collaboration with industry or other organizations like CDRI which have expertise in high-throughput screening for bioactive compounds. An important aspect to consider is their ability to produce secondary metabolites which have been extensively used in medicine because some of the most noted medically relevant compounds are of fungal origin, like cyclosporine (an immunosuppressant drug) used globally for organ transplants and penicillin that saved so many lives during World War II. All isolated fungi should then be screened for multiple activities like anti-cancer, antibiotic, anti-inflammatory, and for the production of enzymes (lipase, cellulase, protease, amylase, etc.) and secondary metabolites useful for agriculture, medicine, pharmaceuticals, food industry, etc. Apart from these beneficial fungi, we certainly need reference cultures of fungi that are agents of human, animal or plant disease for testing antifungals and fungicides. There is immense potential in the indigenous fungal diversity to provide a potential genetic resource for agriculture, medicine, as well as pharmaceutical and food industry. Instead of investing in strain improvement, industries would be willing to fund for the search of new, potential wild strains that are genetically stable and having specific bioactive properties.

Yeasts are an important group of non-filamentous Eumycotan fungi that are extremely important not only for the bread and wine industry, but also in human disease. Special project(s) could be formulated for yeasts after preliminary training of regional collaborators in various states across India. The primary habitats that could be focused are the flowers; fruits (fresh and rotten) collected in forest and non-forest locations, apart from human habitats like flour mills and jaggery units, etc. Native species of plants endemic to different parts of India are most suitable for yeast diversity. Beneficial yeasts are indispensable in bread and wine industry, which have been extensively exploited by the Western world (e.g. Germany and France have 200 kinds of bread and wine respectively), but indigenous yeasts are not fully exploited in India. Several yeasts are also responsible for human infections, and a collection of indigenous strains of clinical forms is essential for testing newly developed antifungal drugs. Several research groups are reporting novel mushroom and related wood-rotting species from India, but their cultures are not always available in the collections. Given the potentiality of wild mushroom species from Indian forests, explorative

projects could be initiated across the country to recover indigenous germplasm of different species on a large scale. To streamline the whole procedure from the collection of mushrooms to raising pure cultures and finally testing for bioactive properties, specified grants could be sought.

Services at the culture collections

Rapid identification of fungal pathogens of crops is important in agriculture. Special programmes with specific funding from the Government (Ministry of Agriculture) could be initiated for the identification of fungal pathogens of crops to directly support farmers. Fungal identification report supported by advice on the use of remedial measures in consultation with agricultural experts in a time-bound manner will help save/reduce crop losses and save human lives. There is also a need to identify and revise the list of serious plant pathogens that are dealt with by quarantine offices at the port of entry in the country. Although India already has alternative agencies for this, culture collections can help in training the technical staff at such agencies and upgrade them with the changing taxonomy of various plant pathogens. This will help prevent the entry of serious plant pathogens due to misidentification or changed taxonomy and nomenclature. Globally, the number of human fungal infections is increasing at an alarming rate even when numerous cases are not reported due to lack of a medical mycologist at most hospitals. Several of these infections are acquired during surgery carried out in rooms that have fungal spores in the air or due to people living in sick buildings, both of which could be avoided if proper mycological examination and hygiene of operation theaters or housing is maintained by a hospital or municipal staff. Regular training and expert advice are needed from culture collections to health workers across India with a specific technical team that may be hired in a separate programme jointly initiated with the Health Ministry, Government of India and NCCPF, Chandigarh. Culture collections can provide mycological services to construction firms/institutions for the examination, prevention and cure of sick buildings.

Strengthening fungal taxonomy at the culture collections

Over the years, fungal taxonomy in India has seen a slowdown mainly due to regular retirement of experienced taxonomists without replacement by competent fresh recruits. The situation has worsened due to the induction of molecular phylogeny methods in species recognition and description. Only a few research groups from India are now publishing good taxonomy papers based on molecular phylogeny in premier mycology journals, due to lack of experienced molecular fungal taxonomists. Like conventional taxonomy where one requires several years of experience to gain expertise, getting insight into molecular taxonomy (not a technique) of different groups of fungi also requires several years of work on a particular taxonomic group. This is because fungi are very diverse in their genetic make-up and require different genetic loci for characterization. Unlike bacteria, where the 16S rRNA gene is generally considered gold standard apart from DNA–DNA hybridization, it is now becoming clear that the ITS region of the rRNA gene is not suitable to characterize all groups of fungi, more so because of the highly incomplete NCBI database[11]. For example, β-tubulin (βTUB) gene for *Aspergillus* spp.[12] and translation elongation factor 1α (*TEF1*α) for *Fusarium* spp.[13]. Knowledge of interspecific and intraspecific variations in different genetic loci used in different genera is essential in making a correct taxonomic decision. The Indian fungal taxonomic scene could be improved in a time-bound manner through individual, in-depth training programmes and collaborative joint projects on specific groups of fungi, viz. ascomycetes, basidiomycetes (mushrooms), zygomycetes, etc. with various mycologists of the country. Novel taxa are not only important in terms of their taxonomic value/status, but also because they represent biological entities with different/novel sets of workable genes, and hence with potentially newer properties. From a recent project on keratinophilic fungi in Maharashtra, we can conclude that larger the area one scans, the more diverse forms one can recover, including novel taxa. Therefore, to obtain a few of the potential strains of filamentous fungi and yeasts for application, the collection of samples from a larger area is key instead of sporadic sampling. A few mycologists alone cannot do justice to the vast fungal diversity that India possesses. Development of molecular taxonomic base in India through in-depth workshops and short-term projects with researchers from across the country could help improve the situation by training research teams from various colleges and universities to explore the vast landscape of India and expand the fungal genetic resource at culture collections in a time-bound manner.

1. Manoharachary, C. *et al.*, *Curr. Sci.*, 2005, **89**, 58–71.
2. Sharma, R. *et al.*, *IMA Fungus*, 2013, **4**(1), 89–102.
3. Sharma, R. and Singh, S. K., *IMA Fungus*, 2013, **4**(2), 177–186.
4. Sharma, R. *et al.*, *IMA Fungus*, 2015, **6**(2), 337–343.
5. Das, K. *et al.*, *Mycotaxon*, 2015, **130**(1), 105–130.
6. Das, K. and Verbeken, A., *Crptogamie Mycol.*, 2011, **32**(4), 365–381.
7. Saluja, P. *et al.*, *Antonie van Leeuwenhoek*, 2012, **101**(4), 733–742.
8. Saluja, P. and Prasad, G. S., *FEMS Yeast Res.*, 2007, **7**(3), 482–488.
9. Sharma, R. and Shouche, Y. S., *Mycopathologia*, 2019; http:/doi.org/10.1007/s11046-019-00346-7.
10. Cannon, P. and Kirk, P., *Fungal Families of the World*, CABI, UK, 2007, p. 456.
11. Nilsson, R. H. *et al.*, *PLoS ONE*, 2006, **1**(1), e59, 1–4.
12. Samson, R. A. *et al.*, *Stud. Mycol.*, 2014, **78**, 141–173.
13. Short, D. P. *et al.*, *Fungal Genet. Biol.*, 2013, **53**, 59–70.

Rahul Sharma is in the Centre for Biodiversity Exploration and Conservation, 15 Kundan Residency, 4th Mile-Mandla Road, Tilhari, Jabalpur 482 021, India; *Present address: Department of Botany, Mata Gujri Mahila Mahavidyalaya, Civic Centre, Marhatal, Jabalpur 482 001, India.*
e-mail: rahulpremasharma@gmail.com