

Microbial Genetic Resources: Some Aspects and Prospects

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Nature is bountiful of microbes and fungi which form backbone for mankind. Only 5 to 70% of microbes and fungi are available in the form of cultures/germplasm. There is a need to unearth the hidden microbial and fungal wealth using innovative methods. Aspects and prospects of microbial genetic resources are discussed in this manuscript.

Key Words: Biodiversity, Cultures, Fungi, Microbes, WFCC

Introduction

Microbiome is a unique term applied to denote the prokaryotes and some fungi like yeasts which colonize and survive in different ecological niches under varied ecological conditions including extremophilic habitats. Microbes have a greater role in waste recycling, recycling of elements, plant productivity, management of biotic and abiotic stress, in industry, agriculture, pharmaceuticals and others. It is known that several agricultural practices including non-judicious use of pesticides, fungicides, weedicides, biocides, and over-fertilization besides human interference have caused damage to the ecological functioning of an ecosystem which in turn resulted in the loss of helpful microbes and fungi that are essential for plant and soil health. Thus, the complications that arose have impacted the influence of microbes and their role in agro-ecosystems.

The microbial world and microbial germplasms are the unseen national and international resources that deserve greater attention. Microbes include viruses, bacteria, mycoplasma, actinomycetes, fungi, algae and others (Wald, 2001).

Few microbial species were described till 1995, but later more than 2000 microbial species have been added. Maybe as of today, 400 bacterial species must have been encountered from India. However, the actinomycetes in particular with reference to India need to be attempted with utmost care and attention due to the fact that only around 30 to 40 actinomycetes are reported from India. The stipulated rules of CBD and National biodiversity Authority do not permit the exchange of microbial or

fungal cultures, though they permit the scientists to discuss with their respective partners in person. As per the international nomenclatural rules, one has to deposit the new taxon of microbial and fungal germplasm, in two culture collections in two countries.

Microbial and Fungal Diversity

The diversity of microbes existing on earth is still not known fully to mankind and it is mainly because out of millions of organisms existing and colonising diversified ecological niches have not been discovered fully. Only 5 to 10% of microbes and fungi have been discovered and 95% remain unexplored in nature. The exploration of such wealth may take thousands of years. At present what we know about microbial diversity is only a drop of water present in Atlantic Sea. It is known that microbes have been playing a very important role with reference to mankind besides being the foundation of the coming bioeconomy. The Convention of Biological Diversity (CBD) makes *ex situ* of conservation of microbes and fungi as mandatory. These organisms have become increasingly important and coordinated with shared best practices including data systems and engaged with many genome programs envisaged by the World Federation for Culture Collections (WFCC). Article 9 of CBD mandates that every country needs to have *ex situ* microbial germplasm repository to manage the microbial and fungal diversity unique to the country. Microbial and fungal genetic resources represent a complete genome, genes and some parts of gene that are relevant to human welfare. Genetic resource can be referred as the diversity of microbial and fungal and other

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living entities or species that make up an ecosystem. Mutation, genetic drift, gene flow and selection act as evolutionary factors due to the fact that every population may contain a distinctive gene pool different from other population. Thus, genetic resources are complementary to type culture collections and biodiversity collections. Microbial resource centres may maintain diversified groups of microbes and fungi whereas genetic resources may maintain thousands of isolates including hundreds of mutants at the same genetic locus. Genetic resources are not configured as intellectual property due to the fact that they are the creations of nature but not that of the human mind. It is important to control biodiversity loss, as humans are dependent on plants, animals, microbes, fungi and others for their survival. In this context, it is imperative to mention that 1/3rd of global microbial and fungal diversity exists in India and there is a need to explore diversified habitats for the microbes and fungi for human sustenance.

Fungi are heterotrophs, flourish in various environments as saprophytes and also as pathogens due to the absence of chlorophyll and are able to proliferate on materials originating from animals, plants, and others. Their diversified existence in a complex ecosystem enriches the life of man and other living beings. Fungal diversity is immeasurable and its loss will near irretrievable damage to the intricate network of life. Fungi form prospective candidates of the biological diversity omnipresent in the environment and attracted greater attention from many due to their vital role in human welfare. There is a great threat to the fungal world due to the deterioration of the environmental conditions. Many fungi have become already endangered and it's high time that everyone should work for the protection of nature in terms of biodiversity. Recently it has been estimated that 13 million fungi may exist on earth of which only 150000 species are so far described and India is bestowed with 29000 fungal species (Karon *et al.*, 2004, Manoharachary *et al.*, 2005, Smith, 2012).

Enumeration, Isolation, Growth and Identification of Microbes and Fungi

Various habitats form microcosms and are inhabited by a wide range of microbes and fungi whose predominance is influenced by various ecological factors and biological factors. The population and functional dynamics of microbes and fungi differ from habitat to habitat. The complex activities happening in each ecological niche due to microbes and fungi put tremendous influence in

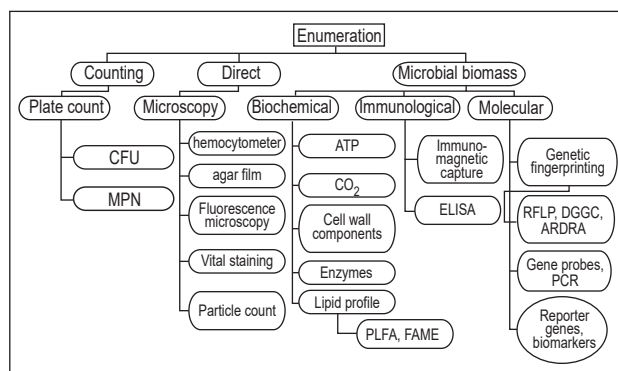


Fig. 1. Enumeration, Isolation, Growth and Identification of Microbes

the form of beneficial activities for human sustenance. Therefore, methods of isolation, enumeration, culturing of microbes and other activities form important aspects of understanding microbial and fungal diversity. The study of a microbial and fungal population in a given habitat is a challenging task due to the fact that some organisms may be represented by large numbers or some by small numbers. Therefore, various approaches have been evolved to enumerate microbes. After enumeration and isolation of microbes and fungi, different culture media have to be selected for their growth and multiplication.

Synthetic, semi-synthetic and non-synthetic or natural media are used for culturing the microorganisms. Enriched media contains substances that encourage the growth and multiplication of required organisms. This is followed by isolation, growth and characterization of microorganisms. Identification of microorganisms and fungi is based on morphological characters, molecular characters and serological techniques including DNA

Conservation of Microbes and Fungi

Biopreservation is nothing but the preservation of the integrity and functionality of the organism. The main aim is to maintain the organism alive, and uncontaminated without affecting the original status and also without causing any variation and mutation. Unlike for plants and animals, less attention is paid for the conservation of microbes and fungi. Microbial germplasm deposition form backbone and is the gold mine for all the ecosystems services including production of novel therapeutics. Community and ecosystem preservation is very difficult to understand as per current technology. Hence, gene pool in the form of extracted DNA has to be preserved. Researchers have to focus on preservation of intact

communities including co cultures, enrichment cultures and natural communities.

It is of immense importance to culture non-culturable microbes and fungi. Preservations include agar slant culture, agar slant cultured with paraffin oil, saline suspension, cryopreservation, preservation in dry vacuum, freeze drying (lyophilization) and refrigeration. Fungi form an important component for human welfare and therefore deserve to be conserved *in situ* and *ex situ* methods. Some of the fungi like rusts, smuts, powdery mildews and others cannot be cultured artificially. Therefore, they have to be maintained on living hosts which is considered in-situ conservation. It is also possible to preserve them in the form of herbarium specimens wherein the fungal spores can survive for years together. In some fungal species, *ex situ* conservation include preservations in agar slants, glycerol oil, soil culture, water culture, cryopreservation, preservation on dry vacuum, lyophilization and refrigeration (Om Prakash *et al.*, 2013). Further, the identification of microbes and fungi is an important aspect due to the fact that many microbiologists and fungal taxonomists have expired, some of them are not in a position to render help in identification due to bad health and there are only few endangered taxonomists who can be helpful in the identification of bacteria and fungi. Unfortunately, scientific organisations, governmental organisations, research institutions, policymakers, and universities have not emphasized the importance of taxonomy and taxonomists though taxonomy is the mother of all sciences. Taxonomy is important as it is useful to identify the species that are useful to mankind.

Microbial Genetic Resources

Mother earth possesses greater biomass and rich diversity of microbes and fungi. Microbial and fungal culture collections have a long history of making significant contributions to various branches of science. The stakeholders of microbes, fungi and others have found it difficult to convince the funding agencies and others with a common and unified voice. The world's largest microbial and fungal collection centre, American Type Culture Collection (ATCC), was established in 1925. ATCC distributes microbial cultures which are certified and recognized under Budapest treaty. Further, the whole genome sequence that is generated for microbes

is not publicly available. Regulatory issues like Nagoya protocol on access and benefit-sharing as well as biosecurity issues impose a new compliance burden on collection staff. Thus there are diversified issues and problems that need perfect answers. Diverse living collections are guardians of characterized germplasm for agriculture and health which form the foundation for biotechnology (Kovin, 2017; WFCC 2010). Some collection centres are more than a hundred years old. A total of 476 culture depositories are registered with World Federation of Culture Collection, but only a few are funded. However, the nonfunded organisations are getting closed, thus paving the way for losing millions of pounds. All the national and international societies and also organisations have to be united to stress up on funding microbial and fungal resource centres around the world. Table 1 shows the microbial germplasm centres located around the world and India.

Table 1. Microbial spectrum barcoding

Microbes	India	World
Viruses/bacteria etc.	1200	15000
Algae	8000	40000
Fungi	29000	1,50,000

Asia

Countries and Regions	Culture Collections	Cultures
Bangladesh	1	25
China	48	343,831
Chinese Taipei	2	75,746
Hong Kong	2	3,201
India	33	221,241
Indonesia	22	15,774
Iran	23	112,486
Israel	4	776
Japan	26	265,863
Korea (Rep. of)	26	175,183
Malaysia	13	16,828
Mongolia	3	7,131
Nepal	1	14
Pakistan	9	4,168
Philippines	6	4,197
Qatar	1	2,300
Saudi Arabia	1	9
Singapore	3	6,289
Sri Lanka	9	997
Thailand	66	124,597
Viet Nam	4	10,306
Total	303	1,390,962

Africa

Countries and Regions	Culture Collections	Cultures
Egypt	6	2,973
Morocco	2	1,742
Nigeria	3	385
Senegal	1	210
South Africa	4	13,660
Uganda	1	550
Zimbabwe	2	702
Total	19	20,222

America

Countries and Regions	Culture Collections	Cultures
Argentina	15	10,280
Brazil	89	137,292
Canada	20	88,741
Chile	5	2,491
Colombia	4	8,035
Cuba	13	6,440
Ecuador	3	4,481
Mexico	18	9,757
USA	37	343,835
Uruguay	1	20
Venezuela	3	3,476
Total	208	614,848

Europe

Countries and Regions	Culture Collections	Cultures
Armenia	1	11,520
Austria	2	6,070
Belarus	1	1,645
Belgium	7	295,229
Bulgaria	4	12,807
Czech	14	14,995
Denmark	3	112,066
Estonia	5	30,412
Finland	2	10,857

Conclusion

There is a need to strengthen biodiversity, taxonomy, conservation, and utilization of microbes and fungi.

Table. Holding in important culture collection centres of India

S. No	Name	Bacteria	Fungi	Yeasts	BGA
1	NCIM, Pune	1400	950	600	20
2	NFMC, Bharatidasan University, Tiruchirappalli	---	----	----	290
3	ITCC, IARI, New Delhi	20	3800	--	--
4	MCC, Pune	149314	15338	--	--
5	MTCC, Chandigarh	1124	1245	575	---
6	NAIMCC, NBAIM, MAU, UP	3049	4179	63	356
7	NCCPF, Chandigarh	4341	---	---	----
8	NCDC, Karnal	400	15	20	---
9	NFCCI, Pune	3050	-	-	-

Countries and Regions	Culture Collections	Cultures
France	38	98,685
Germany	15	107,024
Greece(Hellenic Rep.)	8	7,962
Hungary	8	15,451
Ireland	1	380
Italy	20	49,972
Kazakhstan	2	398
Latvia	1	1,452
Macedonia	1	12
Netherlands	6	106,275
Norway	2	3,028
Poland	10	9,206
Portugal	9	14,940
Romania	2	760
Russian Federation	29	68,427
Slovak	4	4,825
Slovenia	3	17,142
Spain	5	12,821
Sweden	4	52,940
Switzerland	4	3,965
Turkey	12	7,769
U.K.	19	87,415
Ukraine	11	18,695
Uzbekistan	4	3,436
Yugoslavia	2	897
Total	259	1,189,478

Oceania

Countries and Regions	Culture Collections	Cultures
Australia	34	97,120
Fiji	1	49
New Zealand	6	28,148
Papua New Guinea	1	270
Total	42	125,587

Source: World Data Centre for Microorganisms and World Federation for Culture Collection.

Human resource development is essential. Hence, skilled scientists/technicians human resources need to be developed in the whole world. Microbial genetic resources contain several metabolites that are useful

to mankind. Therefore, germplasm centres have to be funded adequately to encourage diversity, taxonomy and conservation studies and research.

Acknowledgements

Thanks to NASI, Prayagraj for encouragement and to Dr Anil Kumar Saxena, Ex-Director NBAIM for encouragement and support.

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