Final Draft

GUIDELINES

for

Management of Microbial Germplasm

submitted to

National Advisory Board for Management of Genetic Resources

National Bureau of Agriculturally Important Microorganisms
Kushmaur, Mau, Uttar Pradesh 275103, India

2014

ACKNOWLEDGEMENTS

A wide number of experts in the field of conservation and management of microbial germplasm were consulted in the preparation of these Guidelines. In particular, the following scientists of the National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau made the most significant contributions to the contents herein:

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Abbreviations and Acronyms

AFLP	Amplified fragment length polymorphisms
ARDRA	Amplified Ribosomal DNA Restriction Analysis
ATCC	American Type Culture Collection
CBD	Convention on Biological Diversity
DMSO	Dimethyl sulpfoxide
DSMZ	Germany's Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
CCFC	Canadian Collection of Fungal Cultures
FAME	Fatty Acid Methyl Ester
gyrB	gyrase beta subunit
hsp	heat shock protein
ICAR	Indian Council of Agricultural Research
ICSP	International Committee on Systematics of Prokaryotes
IJSEM	International Journal of Systematic and Evolutionary Microbiology
IPR	Intellectual Property Rights
ITS	Internal transcribed spacer region
MGRC	Microbial Germplasm Registration Committee
MLST	Multilocus sequence typing
MTA	Material Transfer Agreement
NARS	National Agricultural Research System
NBAIM	National Bureau of Agriculturally Important Microorganisms
NGB	National Gene bank
NMR	Nuclear magnetic resonance
NRC	National Research Centre
OTU	Operational Taxonomic Unit
PC	Project Co-ordinator
PCR	Polymerase chain reaction
PD	Project Director

recA	recombinase A
RFLP	Restriction fragment length polymorphisms
rpoB	RNA polymerase beta subunit
SMTA	Standard material transfer agreement
SNP	Single nucleotide polymorphisms
SSRs	Simple sequence repeats
WTO	World Health Organization
GPS	Geographical positioning system

GLOSSARY

Accession number: A unique identifier assigned to an accession, when it is registered with a gene bank. This number is never assigned again to another accession even after loss of the accession.

Acid-fast staining: A staining procedure that differentiates between bacteria based on their ability to retain a dye when washed with an acid alcohol solution.

Archaea: The domain that contains procaryotes with isoprenoid glycerol diether or diglycerol tetraether lipids in their membranes and archaeal rRNA (among many differences).

Bacteria: Living organisms, microscopic in size, which usually consist of a single cell. Most bacteria use organic matter for their food and produce waste products as a result of their life processes.

Biochemical markers: Biochemical markers are the genetic markers and can be classified on the basis of protein/isozymes

Biological diversity: All the variability among microorganisms from all sources and the ecological complexes of which they are part and includes diversity within species or between species and of ecosystems**.**

Characterization: Characterization is the description of highly heritable traits for distinguishing the microbial accessions from each other**.** It involves recording of qualitative traits based on standard descriptors and descriptors' states.

Collector: A specialist who explore, survey and collects microbial germplasm in the form of soil, plants, seeds, water, and propagule etc. and records related information on diversity distribution, use, environmental features, etc.

Conservation: The practice that permits the perpetuation of a microbial resource.

Cryopreservation: Storage of cells, tissues organs or organisms at ultra-low temperatures usually below -100°C.

Denaturing gradient gel electrophoresis (DGGE): A method that separates PCR-amplified rDNA according to differences in sequence G-C content, based on differential mobility through a DNA-denaturing gel.

Documentation: Correct and orderly presentation of recorded data on microbial germplasm accessions in a standard format.

Evaluation: Process of assessing the true potential of microbial germplasm accessions through quantitative traits that are influenced by environmental factors

Fatty acid methyl esters (FAME): Culture-independent lipid biomarker assays in which the nature and distribution of various membrane lipids are used to construct the phylogeny and metabolic activity profiles for a microbial community.

Freeze drying (lyophilization): A dehydration process used for long-term preservation of microorganisms which makes the material more convenient for storage and transport. Freeze drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas. The addition of lyoprotectants, typically polyhydroxy compounds such as sugars (mono-, di-, and polysaccharides), polyalcohols, and their derivatives supports the viability of organisms.

Fungus: An organism of the kingdom Fungi lacking chlorophyll and feeding on organic matter; ranging from unicellular or multicellular organisms to spore-bearing syncytia

Gene bank: A type of bio-repository which preserves genetic material

Genomics: Genomics is the study of the genomes of organisms includes intensive efforts to determine the entire DNA sequence and genetic mapping.

Genotyping: The process of identifying the genetic make-up of an organism by using molecular methods (DNA sequence level).

Germplasm exchange: Mutual give and take of germplasm or microbial genetic resources from all available sources.

Import permit: An official document that allows the import of any resource into the country from outside

***In vitro* conservation:** Maintenance of germplasm, in a relatively stable form, under more or less defined nutrient conditions, in an artificial environment**.**

Intellectual property rights: Intellectual Property Rights (IPR) are legal right that are conferred to the owner of an intellectual creation. The IPR are granted by means of protection through appropriate legislation, based on the type of creation, that generally include patents, copyright, trademark, industrial designs, geographical indications, trade secrets, protection of layout design of integrated circuits and protection of new plant varieties. The Intellectual Property Right protection entitles, the owner of the Intellectual Property or his assignee the exclusive right to fully utilize the invention/creation for commercial gain generally for a fixed period of time.

L-drying: Liquid drying is a useful alternative method of vacuum-drying for the preservation of bacteria that are particularly sensitive to the initial freezing stage of the normal lyophilization process. The intrinsic feature of this process is that cultures are prevented from freezing; drying occurs direct from the liquid phase.

Liquid nitrogen: It is the liquid produced industrially by fractional distillation of liquid air. It boils at -1960C and freezes at -2100C. Being a cryogenic fluid, it causes rapid frost-bites and when insulated from ambient heat, it can be stored and transported in a vacuum flask.

Material transfer agreement: A Material Transfer Agreement (MTA) is a contractual arrangement, executed on a bilateral basis that governs the transfer of genetic resources between two organizations. It provides an opportunity for the provider to negotiate a share of the benefits derived from the use of the genetic resources. The MTA may specify the permitted or prohibited uses of the genetic resources provided, including whether or not it may be commercialized.

Metagenomics: The study of genetic material recovered directly from environmental samples. Also referred as environmental genomics, ecogenomics or community genomics

Microbial characterization: The use of colony growth, cellular morphology, differential staining, and key diagnostic features to characterize a laboratory isolate for trending and investigative purposes without identification.

Microbial classification: The arrangement of microorganisms into taxonomic groups based on their similarities and relationships.

Microbial identification: The determination of which broad group (e.g., bacteria, yeast, or mold) or narrow group (e.g., genus and/or species) to which a laboratory isolate belongs.

Mol %GC: The molecular percentage of guanine–cytosine range within the chromosomal DNA. [Note-The %GC + %AT = 100%]

Novel and unique Trait: Novel, unique, distinct and stable trait(s), which has been the basis for registration of germplasm.

Passport data: Gathering important observations on samples and their collection sites during execution of collecting mission

Phenotyping: The method of determining differences in the appearance of a microorganism at phenotypic level.

Phylogenetic species: A species consisting of many strains including the type of strain that shares at least 70% total genome DNA–DNA hybridization and less than $5^{o}\Delta Tm$ (difference in melting point of the hybrid).

Polyphasic taxonomy: Taxonomy that assembles and assimilates many levels of information from molecular, physiological, morphological, serological, or ecological sources to classify a microorganism.

Quality control: Quality control is involved in developing systems to ensure products or services are designed and produced to meet or exceed customer requirements.

Quality management: A method for ensuring that all the activities necessary to design, develop and implement a product or service are effective and efficient with respect to the system and its performance. Quality management consists of quality control, quality assurance and quality improvement. Quality management is focused not only on product quality, but also the means to achieve it.

Randomly amplified polymorphic DNA (RAPD): A sequence-independent PCR-based method that can be used to create a community profile based on its ability to generate a unique set of amplicons for each genome present in the sample.

Regeneration: Regeneration is the renewal of accession which will possess the same characteristics as the original population and is the most critical operation in genebank management.

Relatedness: The extent of relationship or similarity of two (or more) organisms on a Phylogenetic Tree or a Dendrogram.

Ribosomal intergenic spacer analysis (RISA): A PCR based technique that amplifies the prokaryotic ribosomal intergenic region, creating a community profile based on the species-specific length polymorphisms in this region.

rRNA Sequence: The DNA sequences that encode rRNA used in protein synthesis are highly conserved among microorganisms of a common ancestry. They are used to determine the phylogenetic distance between organisms and are useful in microbial taxonomy and identification.

Sampling: Drawing a part of the whole lot, i.e., a few individuals out of a population, mainly with an assumption that a sample represents the diversity available in the source population.

Strain typing: Strain typing is an integral part of epidemiological investigations in clinical and public health microbiology. Methods including pulsed-field gel electrophoresis, riboprinting, arbitrarily primed polymerized chain reaction, and whole genome ordered restriction or optical mapping can be used to demonstrate that microbial species are the same strain and most likely are from a common source.

Strain: A specific isolate of a species that is maintained in pure culture and is characterized. The type strain is representative of the species that provides a reference for the species based on its historic isolation, characterization, and deposition in recognized culture collections.

Taxon (plural: taxa): A formal taxonomic unit or category at any level in a classification (family, genus, species, etc.).

Taxonomy: Classification of microorganisms in hierarchical groups called taxa (singular taxon).

CONTENTS

Introduction

Microorganisms have come to stay as a cosmopolitan conglomerate of highly compatible organisms and are being considered as the pioneer colonizers of this planet. They abound in habitats with extremes of pH, temperature, water and salt stress. The versatility and importance of microbes in sustaining the life can be understood by the recognition of 'deep hot biosphere' with unique microbial–animal assemblages and nutrient dynamics. Microbes, bestowed with remarkable inherent physiological and functional diversity, have found application in agriculture, industry, medicine and environment. Much better known and exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture. India is home to billions of diverse microbes, many of them being unique. Besides the recognized hot spots like Western Ghats and Northeastern hill region, India is endowed with other rich biodiversity locales like the Bastar region inhabited by tribals, Andaman and Nicobar Islands, the mangrove forests of Sunderbans, wet evergreen rain forest of Kerala, playas of Rajasthan, Chilka lake in Orissa, Sonar Lake of Maharashtra, thermal springs in the Central Himalayan region, that are abode to large unexplored microbial diversity. In addition, the entire riverine system, the coastal shallow areas, the cold deserts of Leh and Ladakh and forest ecosystems provide other diversity-rich spots yet to be exploited and conserved. Understanding and exploring microbial diversity will lead to judicious and gainful utilization of this nature's treasure. Microbial Resource Centers (MRCs) are knowledge hubs that support innovation in the field of microbial biotechnology by facilitating acquisition of and access to existing research materials through worldwide network of centralized deposit and access services. The principle role of these centers is to provide authenticated strains of microbes to researchers as well as to act as a repository for newly discovered or industrially relevant ones.

It is recommended that: access to ex-situ microbial genetic resources should remain unimpeded for the purposes of scientific research, industrial application, education and health care. Scientists isolating microorganisms may have a variety of objectives. They may be involved in environmental, taxonomic, agricultural or biochemical research, or be interested in screening for novel products that may have commercial value. For the purposes of describing new taxa and to act as essential reference standards for future study and use, the conservation and accessibility of type strains (those on which the taxonomic description is based) and other representative isolates, is fundamental. Furthermore, the ex-situ conservation of all isolated microorganisms, studied and reported in the scientific literature, is also important if science is to progress. The uncertainties associated with re- isolation underline the need for deposit in a Culture Collection (CC), providing conservation skills, ready accessibility and the provision of a conserved and unique reference number. Without this, scientists would constantly need to conduct the skilled and expensive processes of

characterization and identification at the start of each new study. Careful documentation of all conserved material is essential. The operational guidelines or a voluntary code of conduct for the introduction of access and benefit sharing procedures is needed for the proper management of genetic resources. This document aims to provide the information relating to access to ex-situ microbial resources and to make recommendations on policy developments that are conceptually sound, scientifically feasible and operationally practicable.

Chapter 1

CHARACTERIZATION OF MICROBIAL GERMPLASM

A guideline on the applications of the methods most frequently used for the identification and characterization of microbial strains, communities, or consortia is essential for the analysis of microbial diversity. A subset of these methodologies is used to derive taxonomic information from a community sample; these rely on the identification of key subspecies of biomolecules that differ slightly but characteristically between species, genera, and higher biological groupings.

The purpose of this document is to provide guidance on acceptable scientific methodologies and techniques for the identification and characterization of microorganisms intended for use for microbial diversity analysis. It focuses on the taxonomic identification of pure cultures of microorganisms like bacteria, archaea and fungi. It also enlists standard resources and reference materials on taxonomic classification and nomenclature of the above groups of microorganisms. Recommended standards for the characterization of a species/strain include information on the natural habitat, ecology and phenotypic properties including morphology, physiology and pigments and on genetic information and nucleic acid data.

1.1 Microbial Identification

Knowledge of the phenotypic, genotypic and biological characteristics of a microorganism is imperative in differentiating it from its pathogenic and/or toxigenic relatives or other microorganisms that are detrimental to the health of plants, animals, humans and the environment. As such, accurate identification of the active microorganism(s) is a fundamental component of all the microbial diversity analysis efforts along with the pathogenic and non-pathogenic entities. Furthermore, the conclusions pertaining to microbe-related product safety or its impacts on human health or the environment are valid only if the active microorganism(s) are correctly identified.

- Microbial identification method(s) chosen should be well-described in scientific literature and consistent with those currently used in the field of microbial identification and taxonomic classification and they must enable identification of the organisms to the genus and species and, if possible, strain level.
- It is recommended that researchers should adopt an integrated polyphasic approach that includes classical microbiological and phenotypic analysis along with molecular tools, to accurately identify microorganism(s).

- In case of new and novel species, identification based upon at least five strains/isolates (i) that have been demonstrated to be different by at least one molecular technique and (ii) that are not related temporally, geographically, or epidemiologically.
- The natural habitat and its geographical location of the proposed species/strain should be described in as much detail as possible. It should include the pH, temperature range, salinity and mineral salts composition. The geographical positioning system (GPS) positioning should also be included. If possible, information on the microbial abundance and distribution at the natural habitat should be included as well as knowledge of the occurrence in different habitats.
- For usual and rare species/ biotype identification, methods must differ (e.g., a PCR based system and a traditional method) and relevant phenotype(s) or genotype(s) used for identification and method(s) of detection (e.g., AP120E, septyl code, and identification probability, and/or percent similarity or divergence from a published 16S rDNA gene/ ITS rDNA genes sequence already in a database) must be reported.

1.1.1 Phenotypic analysis

Preliminary analysis in microbial identification often involves one or more phenotypic methods. Phenotypic methods are suitable for microorganisms that are culturable (i.e., can grow as pure culture on artificial media), have well-established growth parameters, and physiological and biochemical profiles. The following points need to be taken into consideration while performing phenotypic analysis of a species/strain.

- Media used for isolation and growth need to be described precisely in their composition and preparation, or references should be given. It is necessary that basic conditions for optimum growth (responses to pH, temperature, salinity, light and oxygen) are reported. They should be established before other phenotypic properties of the isolates are determined, in order to ensure that these tests are performed at the optima of these basic parameters.
- The properties of colony and cell morphology should be determined under optimum pH, temperature, light and salt concentration. The medium and culture conditions under which these properties were determined have to be described. Cell morphology should be studied by phase-contrast microscopy of exponentially growing liquid cultures. The shape and size, the staining behaviour in the Gram-stain, the type of cell division, any special morphological properties (presence of flagella, formation of cell aggregates, slime, capsules, cellular inclusions, spore formation),

motility of the cells, and other cellular inclusions and ultrastructural characteristics have to be reported.

1.1.2 Analysis of biochemical, physiological and metabolic characteristics

- The biochemical tests should perform on specific growth media, nutrients, chemicals or growth conditions to elicit an observable or measurable biochemical response from the microorganism for precise and accurate identification and characterization. These tests include: utilization of carbon and nitrogen sources, growth requirements (anaerobic or aerobic; temperature-optimum and range, pH optimum and range), preferred osmotic conditions, generation of fermentation products, production of enzymes, production of antimicrobial compounds, as well as sensitivity to metabolic inhibitors and antibiotics. Examples of recognized tests include: phenol red carbohydrate, catalase and oxidase tests, oxidation-fermentation tests, methyl red tests, Voges-Proskauer tests, nitrate reduction, starch hydrolysis, tryptophan hydrolysis, hydrogen sulfide production, citrate utilization, litmus milk reactions, etc. Several miniaturized and automated commercial systems are currently available with well-defined quality control procedures that allow for rapid identification of microorganisms.

1.1.3 Analysis of Fatty Acid Methyl Ester composition (FAME analysis)

- Microorganisms can be identified by analyzing the fatty acid profiles of whole cells or cell membranes using gas-liquid chromatography or mass spectrometry. Therefore, the data on the type, content, proportion and variation in the fatty acid profile should be used to identify and characterize the genus and species by comparing it against the fatty acid profiles of known organisms.
- The expression of microbial phenotypes is highly dependent on environmental variables (e.g., culture pH, temperature, selective vs non-selective media, depletion of nutrients, presence of stressors etc.), and thus, may introduce inconsistencies in the identification process. The phenotypic methods are only acceptable if the response criteria are sufficient to identify the microorganism with a high level of confidence and distinguish it from phylogenetically close relatives that potentially pose safety concerns. Also, the applicability of the method is based on the robustness of information in reference databases. As such, results from phenotypic methods may require supporting data from other methods to accurately identify a microorganism.

1.1.4 Molecular Methods

Development of molecular methods has greatly improved the ability to rapidly detect, identify and classify microorganisms and also establish the taxonomic relationship among closely related genera and species. Identification, using molecular methods, relies on the comparison of the nucleic acid sequences (DNA, RNA) or protein profiles of a microorganism with documented data on known organisms. The molecular methods are considered sensitive enough to allow detection of low concentrations of viable or non-viable microorganisms in both pure cultures and complex samples (e.g., soil, peat, water etc.).

- The description of a species or any new species needs to contain information on the G+C content of their DNA and the nucleic acid sequence of the 16S rRNA (more than 1200 bp), 18S rRNA, RNA polymerase beta subunit (*rpoB*), gyrase beta subunit (*gyrB*), recombinase A (*recA*), and heat shock protein (*hsp60*), ITS region and housekeeping genes. The DNA base composition may be determined by any of the commonly used techniques, such as ultracentrifugation in a CsCl gradient, thermal denaturation or HPLC of nucleotides after hydrolysis.
- The 16S rRNA nucleotide sequence is considered important to give information on the phylogenetic position of novel/new bacterial isolates. Sequences should be determined for representative strains included in the study. If a greater number of strains are available, alternative methods (e.g. genomic fingerprints) may be used to demonstrate the heterogeneity within the species and the differentiation from other species.
- Sequences need to be deposited with a recognized database [e.g. GenBank; Ribosomal Database Project (RDP); Europe's collection of nucleotide sequence data (EMBL); and Universal Protein Resource (UniProt) etc.]. The database records and publications must include correct statements of the identity of the source strain. The accession numbers must be given together with the species description. The sequences of molecules other than 16S rDNA [such as *puf, RNA polymerase beta subunit (rpoB), gyrase beta subunit (gyrB), recombinase A (recA), and heat shock protein (hsp60)* gene sequences or others] may provide important additional phylogenetic information and should be included in considerations whenever possible.
- Some of the limitations associated with genotypic methods also include: difficulties in differentiating between species that share identical and/or similar conserved region sequences, limited information on the quality of sequence data available in public databases and the complexity of taxonomic nomenclature overall. Considering the above, it is recommended to validate the results of genotypic microbial identification methods with data from other sources (e.g., morphological and/or phenotypic analysis).

- The discrimination of closely related species of the same genus should be demonstrated by DNA–DNA relatedness studies. DNA–DNA relatedness studies can be used to assess relationships only within narrow ranges of variation, because relatedness values fall to low levels (less than 30%) for phenotypically moderately different species. By and large, values of more than 70% DNA–DNA relatedness and differences in the denaturation temperatures of homo- and heteroduplexes of less than 5°C should be used as criteria for species recognition.
- Hybridization studies should include several strains of the newly proposed species, including the proposed type strain, as well as type strains of related species. DNA–DNA hybridization studies are required to ensure species identity when 16S rRNA , ITS rDNA or any other conserved gene sequences reveal similarities of more than 97% (sequence similarity of more than 97% does not necessarily mean identity at the species level).

1.1.4.1 Genotypic methods

- Genotypic microbial identification and characterization methods are theoretically more reliable because nucleic acid sequences are highly conserved in most microbial species. Applicable genotypic methods include DNA–DNA hybridization, PCR, 16S and 23S rRNA sequencing, multilocus sequence typing (MLST), pyrosequencing, DNA probes, and comparisons of restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP) or G+C content (%) in the genomic DNA with corresponding data on known organisms.
- DNA sequencing of the first 500 base pairs of the 16S rRNA sequence is useful for identification to the species level but may not provide sufficient power to resolve among closely related species or strains of the same species. In contrast, Southern hybridization of restriction endonuclease digests is powerful and can be effective in demonstrating differences between two strains. If the banding patterns appear identical, this shows only that restriction endonuclease has similar cleavage sites in that region of the two organisms. Demonstration that the two organisms are the same should include two or more different restriction endonuclease digests, each of which yields bands in the area of interest. All bands from the two organisms must be identical.
- Comparative sequence-based identification using the nuclear ribosomal internal transcribed spacer (ITS) region (ITS1, 5.8S rRNA, and ITS2) located between the nuclear small- and large-subunit rRNA genes should be employed for species complex-level identification of fungal species and for identification within some species complexes of *Fusarium*, *Colletotrichum*, *Phytophthora* etc. The ITS region

satisfies most of the aforementioned requirements of a universal marker since this region can be reliably amplified for most fungi, is conserved, is present as multiple copies in the fungal genome, yields sufficient taxonomic resolution for most fungi, and has the additional advantage that the GenBank), European Molecular Biology Laboratory nucleotide sequence database (http://www.ebi.ac.uk/embl/), and DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/) contain a large number of sequences from this locus, enabling a ready comparison of the sequence from an unknown isolate.

1.1.4.2 Protein based methods

- Serological methods such as Western blotting, Immuno-precipitation and Enzyme Linked Immuno-sorbant Assay (ELISA) use antibodies to detect specific proteins that are unique and/or characteristic of a microorganism. The applicability of serological methods is dependent on the availability, sensitivity and specificity of the antibodies used which can be polyclonal or monoclonal. There are commercial kits available for immuno-detection of several microorganisms. Protein based methods also include gel electrophoresis (SDS-PAGE, 2D-gels, etc.) that can separate cellular proteins on a defined matrix and identify microbial proteins of interest by comparing with microorganisms with known protein profiles. This method should be applied for the assessment of microbial communities in different habitats.

1.1.4.3 Genomic procedures

- More recently, complete profiling of the transcriptome, genome, proteome or metabolome have been used to identify and characterize organisms. Several modern technologies such as DNA and protein microarray analyses, mass spectral protein profiling, nuclear magnetic resonance (NMR) spectral analysis, *in-silico* microbial metabolome platforms can be used for the identification and characterization of microorganisms.
- The knowledge of the sensitivity and specificity of genomic tools and their application in microbial identification is rapidly evolving. However, challenges related to standardization of genomics methodologies (including optimization of protocols and bioinformatics tools for reliable data annotation, interpretation etc.) continue to hinder their applicability in safety (risk) assessment and regulatory decision making. The researchers can consider data generated by genomics-methods on a case-by-case basis. However, validation of genomics data using alternate methods is currently required to substantiate the identification and taxonomic classification of an active microorganism(s) in a supplement product.

1.1.5 Taxonomic Classification and Nomenclature

- The taxonomic identification of the microorganism(s) should be based on the currently used and internationally accepted taxonomic classification system. The description of the microorganism(s) in the product and its characteristics must correspond to the characteristics described in standard resources and/or references that are commonly used by the scientific community to validate taxonomic classification. These can include but are not limited to: textbooks such as the Bergey's Manual of Systematic Bacteriology; The Prokaryotes; Applied Microbial Systematics; Principles of fungal taxonomy etc.; online resources such as the Catalogue of Life; PubMed Taxonomy and UniProt Taxonomy etc.; and peer reviewed journals.
- The taxonomic name should follow the nomenclature code officially recognized by the International Committee on Systematics of Prokaryotes (ICSP). Applicants should verify the "Approved List of Bacterial Names" to ensure that the nomenclature is in accordance with the latest Validation List developed and updated by the International Journal of Systematic and Evolutionary Microbiology (IJSEM).
- Microbial taxonomic classification and nomenclature, particularly for bacteria, is in a constant state of flux as methodologies evolve to generate more reliable information to identify/classify and/or reclassify the current taxonomic scheme. Cross referencing more than one resource/reference will help in validating the current taxonomic designation and classification of a microorganism.

1.1.6 Microorganisms obtained from culture collections

- If the microorganism is obtained or purchased from a recognized International Culture Collection such as the American Type Culture Collection (ATCC), Germany's Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), USDA-ARS National *Rhizobium* Germplasm Collection (BRCC), or Canadian Collection of Fungal Cultures (CCFC) etc., then the authenticated information from those culture collections will be required to substantiate the taxonomic identification of the microorganism.

1.1.7 Characterization of environmental isolates

- For all environmental isolates, taxonomic designation should be based on a complementary, polyphasic approach that enables identification and substantiation of the microorganism up to species level, and if possible, up to strain level, and allows differentiation from closely related pathogenic and/or toxigenic species and/or strains. This is particularly important for microorganisms belonging to genera where

pathogenic and/or toxigenic species and strains are known to exist. For instance, strain level identification is required for microorganisms belonging to the genera *Bacillus*, in order to differentiate the microorganism from related phylogenetic kins that include pathogenic and/or toxigenic species (e.g., *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus cereus* etc.), and from potentially pathogenic and/or toxigenic strains within a particular species (e.g., *Bacillus licheniformis*, etc.).

Chapter-2

Exploration and collection of microbial Germplasm

Microbial germplasm represents living microbial entities (bacteria, actinomycetes, cyanobacterial, fungi, etc.) available in different habitats in its original life-form on the earth. These life forms and their communities are the most viable, dynamic and live unseen natural creatures that reflect the original state-of-the-art lively condition of every habitat including the soil, water, air, sediments as well as plants, animals including humans and microbes themselves (microbiome). During the course of evolution, the environmental pressure has given birth to a huge genetic diversity of microbial accessions on the earth. This diversity has evolved with better chemical, molecular and genetic strategies to overcome biotic and abiotic pressures and now, is being looked upon as a wealthy resource to overcome the problems of food security, ecological balance, agricultural upliftment, hunger and poverty and social livelihood.

Conservation, preservation and cataloguing of the world's diverse life forms are currently a priority area for governments and non-governmental organizations with major emphasis and focus in the agriculture, environment and societal benefits. As, bacteria and fungi are vitally important life forms on the earth for humankind not only because of their ubiquitous nature and cosmopolitan presence but due to their obvious role and direct involvement in the natural biogeochemical cycles, cycling of biologically important elements, minerals and nutrients, food web, environmental remediation, crop production or protection.

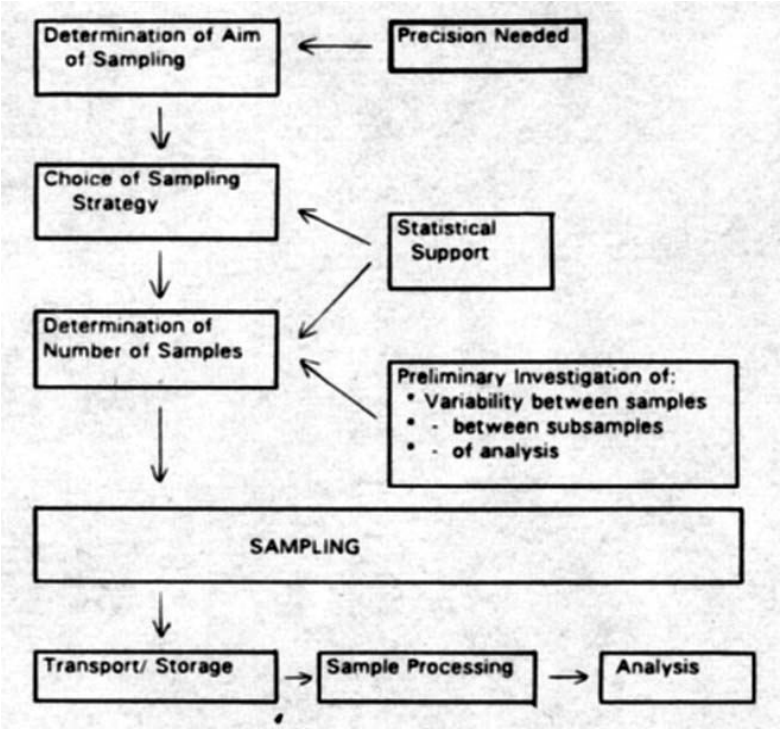
It is has been realized that the earth's microbial biomass is almost as great as that of plants itself. Biotechnological revolutions kept these vital organisms on top for the industrial and agricultural exploitation. For these reasons, the full extent of microbial diversity on the earth is now being realized as the most vital, rich and live. Moreover, looking into the vast potential of microbial communities lying in different habitats and their functional benefits to the agriculture and environment, acquisition of microbes from different habitats is an essential and most important task for any culture collection. This will not only strengthen the live microbial forms in the culture collection but add to different vital functions to the collections related to agriculture, environment, and human benefits.

2.1 Exploration for collection of germplasm

The management and exploitation of microbial diversity has an important role in sustainable development with the industrial and commercial application of microbial diversity. For this purpose, microbial germplasm (bacteria, fungi, actinomycetes,

cyanobacteria) can be collected and maintained through exploration surveys of different ecological niches in the country. For organizing exploration for collection of germplasm followings points need to be taken into consideration:

- Regular explorations are required to collect samples for the isolation and characterization of microbial accessions.
- A representative soil sample is essential when determining microbial parameters. Since soils are inherently very heterogeneous it is essential to determine what is to be sampled and devise a sampling strategy to determine what microbes are present as well as individual microbial populations within the soil ecosystem.
- While taking samples from a site, they should be viewed as a composite sample of varying micro-ecological environments.
- Sample storage is another concern when sampling for microbial biota. Therefore, one should store samples in polyethylene plastic bags. The important steps of sampling are given as a flow chart (Fig 1).



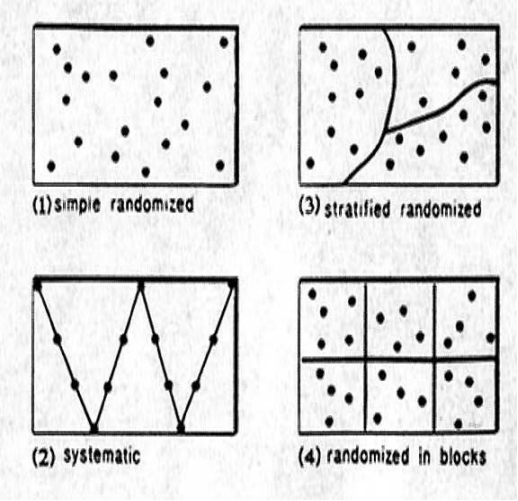


Fig 1: Flow chart showing important steps of sampling

- The major guiding factors taken into consideration during exploration survey may include:-
  - **i)** Select appropriate and conducive environmental conditions for exploration. Make sure about ecological niches by recording geographical location data, climatic conditions, height from the sea, boundaries of the sample collection sites, vegetation, abiotic conditions like soil and water salinity, acidity, alkalinity etc.
  - **ii)** Make arrangements for obtaining regulatory permissions from the concerned govt. authorities if required for the restricted areas.Make arrangements for the storage of samples collected.
  - iii) Make arrangements for the transportation to the laboratory as soon as possible after sample collection.
  - iv) Make arrangements for the on-site samples processing for the collection of metagenomic samples or degenerative samples.

2.1.1 Method

Methods involved for the microbial diversity analysis of a habitat are not usually generalized but vary from conditions to conditions and samples to samples. For the collection of soil samples usually routine soil collection method from a field or any other region is followed.

- Use replicates of 1 to 5 g of soil to measure diversity and then conclusions about the community.
- Sampling methodology of soil and other habitats should be done on a smaller scale with more samples per unit area to represent the diversity of microorganisms in the microhabitats.

- For the sample collection from the soil, organic matter should be broken down into its component parts. It is recommended that 1-5 g of this sample can be suspended in sterile water and poured on the media plates for the isolation of microbes.
- For the sampling of microbial populations, one should make sure to sterilize the equipment used to prevent cross contamination.
- Any specific health and safety issues relating to soil sample handling should be determined and appropriate protective measures be adopted.
- Soil for microbiological studies should be collected from the 0-23 cm depth for arable soils and 0-10 cm depth for grassland.
- If a large area of land is to be sampled, soil samples should be taken at intervals along the lines of a series of imaginary zig-zags or `W's across it. If the sampling area of a plot is restricted in some way, e.g. perhaps only the edges are permitted to be sampled, every effort should be made to ensure that the pooled soil samples give as representative a bulk soil sample as is possible.
- It is recommended that soils for use in experiments involving pesticide testing should not be collected when there has been no rainfall for 30 days. If a period of drought and a sampling date should coincide, the researcher may probably use his/her discretion as to whether or not to go ahead with the soil collection, depending on the nature of the experiment for which it is required.

2.1.2 Number of samples

Number of sampling sites, either ecoregions or microhabitats may vary according to the interest in the microbial diversity.

- Number of sampling of the soil or water for the isolation of microbial communities should be done in such a way that it should represent a wider perspective of that particular niche. In general, samples for microbial analysis require relatively small samples to be obtained at each site.
- For a relatively homogenous site on the macroscale, it is recommended to obtain 15 subsamples for one total composite sample over a 2 ha area and 15 sub samples for each of 5 total composites over a 10-20 ha area.
- It is important to collect physical data about the site because this may provide valuable knowledge when studying the microflora. The following items should at least be collected at each subsampling location:

    (i) Slope

    (ii) Aspect

(iii) Topographic

(iv) location

(v) Parent Material

(vi) Texture

(vii) Moisture status

(viii) Animal presence

(ix) Field History

(x) Bulk Density

(xi) Temperature range

(xii) Rainfall amounts

(xiii) Weather

2.1.3 Time of collection

Time of sample collection is very important in case of microbial isolation, identification and characterization.

- Samples from diverse ecoregions should be collected as per the requirement of the research protocols. It is suggested that for assessing periodical microbial communities in a particular habitat, continuous sampling at different time intervals is necessary.
- Samplings may be done throughout the year in order to map the changes in the microbial communities of a niche.
- In the extreme environments, sampling time may vary, but if one has to look into the microbial diversity of a cold desert or hot environment, it is advisable to collect samples in all the seasons and compare the diversity.

2.1.4 Sample size

- Sample size varies from 1-5 g in case of soil samples or rhizosphere soils of the field.
- In case of plant samples, leaves, tissues and other parts should be collected in appropriate numbers or on fresh weight basis.
- Water samples from the surface water bodies or sub surface should be collected in air tight glass vessels in a minimum volume of 100 ml and more.
- In the case of freshwater or marine water bodies, again geographical indicators should be recorded properly and again number of samples should be 01-10 in unit area of 10 to 100 m^2.

2.1.5 Storage

- Storage facility for the samples either soil, sediment or water should be provided at two stages; one at the time of sampling and then taking this to laboratory for the processing and second after processing.
- It is advised to collect the samples in dry, cool and moisture less or minimum moisture conditions and keep the filled poly bags or bottles in a cool place away from direct heat, sunlight and any kind of radiation.
- If possible samples should be processed immediately after the sample is collected but in the cases when it is far from a laboratory conditions, it should be brought to the lab conditions as soon as possible to avoid degradation of the plant material debris, moist soil or water. In the lab conditions before processing, the samples should be kept at 4^{o}C in a refrigerator or in a cold room especially designed for the storage. However, in any case the samples should be processed at the minimum delay because a delay will create changes in the microbial communities.

2.1.6 Transportation

- The samples collected should be brought immediately to the laboratory.
- All the samples should be transported in a sealed, cool and non-moisture conditions to reach to the laboratory in proper care and in contamination-free environment.

Chapter 3

Conservation & maintenance of microbial germplasm

Microbial communities have a significant role in existence of life on the earth and contribute the maximum biodiversity. The impact of the microbial world on plants is evident worldwide, as nitrogen fixation by rhizobia and other bacteria for promotion of plant growth. What is less appreciated, and less well understood, is the pervasive influence that other microbes have on plant health and growth; they enhance stress tolerance, provide disease resistance, aid nutrient availability and uptake and promote biodiversity. Similarly also causes diseases and result in economic losses. The plant microbe interactions have produced a large amount of knowledge and given insights into the mechanisms of these interactions, their application in biotechnology and agriculture has yet to be exploited. However, biological diversity has been reduced significantly by varied human activities (e.g. air and water pollution, clearing of tropical moist forests for agricultural purposes, global climate change, etc.). If a strain is lost, the recovery of that strain, or even of the same species, from the natural environment can be difficult and for practical purposes impossible, unless it is a common organism and has a specific host or environment. But then, even if the same species is re-isolated, the properties of the strain may be different due to considerable intraspecific variation that exists. It is, therefore, imperative that isolates are adequately preserved to maintain their integrity for future use in screening, genetic improvement and production of desirable end products.

Long-term preservation of microbial strains is essential for their in-depth study and for future exploitation. However, both the viability and the stability of living cells should be ensured during the preservation period. Maintenance of the fungi in certain culture media requires lot of care, because they are quickly consumed and require frequent sub culturing, demanding time and allowing contamination and decrease in virulence. Due to these difficulties, fungal strains are preserved for short term on sand, silica gel, dried host tissues and distilled water. Lyophilization and conservation in liquid nitrogen are for long term conservation.

Preservation methods for filamentous fungi vary depending on species of concern, type and degree of sporulation and resources available. Some low-cost methods of preservation, such as storage in distilled water and the silica gel, are good, but for short term. Spore- forming strains (with the exception of zoosporic fungi) can usually be freeze-dried

successfully. Similar success with non-sporulating strains is far less likely. Both types can be frozen and stored for long periods in liquid nitrogen or liquid nitrogen vapor.

3.1 Conservation Methods:

There are different methods of preservation of microbial cultures *ex-situ* as per the type of organism, availability of resources, size of collection and need. The primary methods of culture preservation are as continuous growth, drying and freezing.

- In continuous growth methods, cultures are grown on agar medium and typically used for short storage.
- Drying is the most useful method of preservation for culture that produces spores or other resting structures. Silica gel, Glass beads and Soil are substrata commonly used in drying.
- Freezing methods include, cryopreservation and low temperature storage, are versatile and widely applicable.
- Freeze-drying or lyophilization, the microbial cultures are frozen and then subsequently dried under vacuum. This method is highly successful for fungi that produce spores. Commonly used methods are cryopreservation and lyophilization for long term preservation and storage in mineral oil, water, silica gel, glycerol and slants for short term and needs revival continuously. Other than these, there are different methods which are specific for particular genus and group of organisms.

3.2. Storage in Silica gel

- Storage in the silica gel is a good option for short term storage of sporulating fungi where lyophilization or liquid nitrogen storage is not possible.

3.2.1 Storage in Water

- Another inexpensive and low-maintenance method for storing fungal cultures is in distilled water. Apparently, the water suppresses morphological changes in most fungi. The method has been used successfully to preserve oomycetes, basidiomycetes, ectomycorrhizal fungi, ascomycetes, hyphomycetes and plant pathogenic fungi.

3.2.3 Mineral Oil Storage

- Mineral oil is generally used to preserve filamentous fungi and yeast but can be applied successfully to bacteria. A wide range of fungi survives by this method, saprolegniaceae, water molds, species of *Aspergillus, Penicillium, Phytophthora* and *Pythium*. However, the fungi are sensitive to other techniques can be stored successfully in oils. It involves the covering of cultures with mineral oil to prevent the dehydration and slowdown the metabolic activity and growth through reduced oxygen tension.

- Pure and sporulating cultures on agar slants (30 ^{o}C to the horizontal in 30 ml universal bottles) are covered by 10mm sterile (achieved by autoclaving twice at 121^{0}C for 15 minutes) minerals oil (liquid paraffin or medicinal paraffin specific gravity 0.83 to 0.89). If oil overlay is more than 10 mm the fungi may not receive sufficient oxygen and may die. While, if the depth is less, exposed mycelium or agar on the sides of the container may allow moisture to evaporate and the cultures become dry out. Screw cap tubes are stored in a temperature controlled (15 to 18 ^{0}C) room.
- Preparation of mineral oil stock should be carried out in appropriate microbiological safety cabinet using aseptic techniques and good laboratory practice at all time.
- The cultures should be incubated at optimum temperature until suitable growth and sporulation.
- The maximum height of oil overlay should not be exceeding more than 10 mm.
- Store at 18 – 20^{0}C
- More than one subculture may require after retrieval as the growth rate may be reduced because of oil.
- Inoculate at least two screw cap tubes for each strain to be maintained and label one culture as reverse stock, the other as working stock.
- The reserve stock culture is used only when re- preservation becomes necessary when all the inoculum has been removed, when it is contaminated or when the shelf life expiry date set for the organism has been reached.

3.2.4 Cryopreservation

- Lowering the temperature of biological materials reduces the rate of metabolism. Below -70^{o}C very little metabolic activity takes place. However, recrystallization of ice can occur at temperature above – 139 ^{o}C and this can cause damage during storage. Consequently, the storage of microorganism at ultra- low temperature (below -139 ^{o}C) in or above liquid nitrogen is the best method. All the fungi can be cryopreserved but this method has special significance for those that do not sporulate in culture and fungi that have large and delicate spores that will not survive in freeze drying. It is also equally good for long term storage of bacteria
- Freezing and thawing can damage the living cells. To minimize the damage, chemical protectants, known as cryoprotectants, are used in most of the protocols. Cryoprotectants are of two types:
    - Penetrating agents such as glycerol, polyethylene glycol and dimethyl sulpfoxide (DMSO), which readily pass through the cell membrane and protect intracellularly and extracellularly.
    - Non penetrating agents such as sucrose, glucose, mannitol, sorbitol, dextran, polyvinyl-pyrrolidone, hydroxyl-ethyl-starch,etc., which exert their protection effect external to membrane.

- Gycerol and DMSO have been proved to be most effective for fungi. During freezing the bulk of the free water crystallizes, leaving a saturated solution of high osmotic value surrounding the still unfrozen cells, which dehydrate and shrink to obtain an osmotic equilibrium. The exposure of the cells to this solution should result in dehydration to such an extent that the freezing temperature is depressed sufficiently. The optimal cooling rate depends on the size of the cell, the thickness of the cell-wall and the cryoprotectant.
- Material is revived by warming it as fast as possible. Generally the cryovials are thawed in a water bath (revival 5 min. at 30°C). However, some species or groups may not survive 30°C. For example, Oomycota are thawed at 25° C.

3.5 Revival

Organisms are revived immediately after storage and after 5 years. For revival, vials are thawed in a water bath for five min at 30°C. (Some species or groups may not survive 30°C; for example, Oomycota are thawed at 25°C). The vials are rinsed in ethanol 70%, opened gently and placed on the suitable agar medium. Growth and identity of the 5 to 8 plugs per dish is checked by the specialists and these data are recorded in a logbook and in the stock control system, including eventual remarks. Changes in morphology are also noted in the database.

3.6 Sample Size

Minimum sample size required for preservation and multiplication of microorganisms may be equivalent to one inoculation.

3.7 Viability monitoring

Viability test of preserved microorganisms should be carried out periodically once in every 3 year.

3.8 Microbial Genomic Resources conservation

- Microbial genetic resources are the foundation for sustainable agriculture and global food security. Genetic diversity of microorganisms enables plants to adapt to new pest and diseases as well as to climatic and environmental changes in different ways (drought tolerance, plant growth promotion, biocontrol, pest and disease management, etc). Agro-biodiversity is the component of biodiversity that contributes to food and agriculture. Today, the world is losing genetic resources at unprecedented rates. The biological basis for food security is fast disappearing. Agricultural sustainability is therefore dependent on a strong conservation component. Biodiversity can either be maintained in or out of its natural environment, i.e. in or ex situ.
- An organism's genes are essentially its blueprints, and detailed strands of DNA in every living cell. By properly freezing this genetic material, we can preserve the blueprints for ages to come. On one hand, these efforts are very much useful for the researchers

to continue their research work without any hindrance. Each genetic material is preserved by at least two methods according to the type of genomic material, either under short term storage, or long term storage in Luria Broth (LB) freezing buffer at – 80 ^{0}C.

- Plasmids are the extra chromosomal, self-replicating, and double stranded closed and circular DNA molecules present in the bacterial cell. Plasmids contain sufficient genetic informations for their own replication. A number of host properties are specified by plasmids, such as antibiotic and heavy metal resistance, nitrogen fixation, pollutant degradation, bacteriocin and toxin product ioivcolicin factors and phages (Dahl *et al,* 1981). Naturally occurring plasmids can be modified by *in vitro* techniques. Cohen *et al.* (1973) for the first time reported the cloning DNA by using plasmid as vector. Bacterial glycerol stocks are important for long term storage of plasmids. Although plasmid DNA can be stored at -20 ^{o}C, but bacterial glycerol stocks are desirable for long term and in case of avoidance of cloning and transformation steps when more plasmid DNA is required. Bacteria on an LB agar plate can be stored at 4^{o}C for few weeks. For longer times bacteria can be stored in media containing 15% glycerol at –80°C for many years.
- Glycerol stocks preserve plasmid DNA and allow for quick production of additional plasmid. Fresh plasmid is produced by scraping the frozen glycerol stock with a sterile inoculating loop, and streaking a fresh agar plate containing the appropriate selection antibiotic. The plate is incubated overnight at 37°C, and a single colony is selected and grown for purification. Find protocols for generating glycerol stocks in Sambrook and Russell (2001).
- It is best to store purified DNA in nuclease-free buffer under slightly basic conditions, and in the presence of EDTA. EDTA inhibits nucleases by chelating divalent cations such as Mg^{2+} that are necessary for nuclease activity. Plasmids can be stored in aliquots at –20°C, in a freezer that is not frost-free, for up to one year. Repeated freeze-thaw cycles will degrade nucleic acids, and should be avoided. For storage longer than one year, storage at –70°C is recommended.
- The microorganisms conserved in the microbial resource centers should be periodically checked for its viability and genetic integrity may be checked through the amplification, sequencing and Comparison of the sequences of conserved genes, most commonly those encoding 16S rRNA. Among some taxa, variation within this gene does not allow confident species identification. Thus some other genes may also be used suchas RNA polymerase beta-subunit encoding gene (rpoB), rpoD, gyr, gac, tef, beta tublin, histone etc as a multilocus sequence typing approach. The microarray and gene chips developed commercially might be as an alternative tool for universal bacterial genotypic identification and integrity monitoring.

3.9. Regeneration/Rejuvenation/ Multiplication (Bacteria, Fungi, Actinomycetes and Cyanobacteria)

One of the remarkable attributes of microorganisms is the relatively short generation time, the time required for a microbial population to double in numbers. Therefore, the rejuvenation and multiplication is relatively less difficult provided the cultures are viable and uncontaminated. The generation time varies among the group of microorganisms The growth of a bacterial population can be expressed in various phases of a growth curve. The logarithms of the actual numbers in the population are plotted in the growth curve along the side axis, and the time is plotted at the base. Four phases of growth are recognized in the growth curve. In the first phase, called the lag phase, the population remains at the same number as the bacteria become accustomed to their new environment. Metabolic activity is taking place, and new cells are being produced to offset those that are dying. In the logarithmic phase, or log phase, bacterial growth occurs at its optimal level and the population doubles rapidly. This phase is represented by a straight line, and the population is at its metabolic peak. Research experiments are often performed at this time. During the next phase, the stationary phase, the reproduction of bacterial cells is offset by their death, and the population reaches a plateau. The reasons for bacterial death include the accumulation of waste, the lack of nutrients, and the unfavorable environmental conditions that may have developed. If the conditions are not altered, the population will enter its decline, or death phase. The bacteria die off rapidly, the curve turns downward, and the last cell in the population soon dies.

3.10 Microbial measurements

- In order to measure the number of bacteria in a population, various methods are applied such as: the plate count method, whereas sample of bacteria is diluted in saline solution, distilled water, or other holding fluid. Samples of the dilutions are then placed in Petri dishes with a growth medium and set aside to incubate. Following incubation, the count of colonies is taken and multiplied by the dilution factor represented by that plate. Generally, plates with between 30 and 300 colonies are selected for determining the final count, which is expressed as the number of bacteria per original ml of sample.
- Another measuring method is to determine the most probable number. This technique is often used to determine the number of bacteria in a sample of contaminated water. Water samples are added to numerous tubes of single-strength and double-strength lactose broth. If coliform bacteria (such as *E. coli*) are present, they will ferment the lactose and produce gas. Judging by the number of tubes that contain gas at the end of the test, one may approximate the original number of

bacteria in the water sample. Another evaluative method is by a direct microscopic count.

Chapter 4

Distribution and exchange of microbial germplasm

The National Bureau of Agriculturally Important Microorganisms (NBAIM), act as the nodal agency for all exchange of genetic resources of agriculturally important microorganisms for the purposes of research and conservation for sustainable use. Provided that the exchange/ export/ import of said microbial germplasm shall be decided on case-to-case basis in respect of the following categories of microbial germplasm:

- Microorganisms, which are used for human/ animal consumption. For example, different edible microorganisms, and fungi having therapeutic characteristics or microbial single cell protein.
- Microbes/ microbial formulations which are used as Biopesticides/ Mycoherbicides/ Biocontrol Agents, which can be exported or imported in the form of "Industrial Formulations".
- Active biomolecules derived from various microorganisms, and thereafter marketing the products in different countries. As most of the information in this regard is of proprietary in nature, this possibly cannot be available to academic/ government organizations.

4.1 Quarantine procedures for import of PGR

- Import Permit is an official document authorizing importation of a commodity in accordance with specified phytosanitary requirement.
- A risk analysis carried out on a case-to-case basis for the pathogenic/ non-pathogenic microbes
- In case an indentor/exporter is unable to comply with the requirements given in the Import Permit, they may seek permission for relaxation of the requirements under additional declarations.
- In case of quarantine for microbial samples in the form of active germplasm, the entire sample to be examined.
- Samples should be verified by a team of experts in Fungal Pathology and Bacteriology.
- For all the details regarding the rules applicable for the import/export of microbes, the plant protection and quarantine office should be contacted.
- All applications received by the Director, NBAIM for the exchange of germplasm should be forwarded for further consideration to DARE along with all the particulars, information including consent, if any and also the inputs by NBAIM.

4.2 Procedure

- DARE shall consult for the issuance of the export and the import permits the National Biodiversity Authority constituted under the Biological Diversity Act, The Director, NBAIM, while recommending to DARE for the exchange/export/import of microbial GRFA shall ensure execution of a Material Transfer Agreement (MTA) setting forth the conditions for such exchange, including that of the legal ownership/ IPR on research products or materials derived from research products, and granting of exclusive and nonexclusive rights of research products/materials/ royalty or benefit sharing, etc.

4.3 Quality of Materials

Bio-Safety and Biohazards: The NBAIM shall gather/ carefully examine/evaluate all relevant and necessary information pertaining to the biological safety of the microbial germplasm, which is subject to export/import.

Biological microbial agent hazards are relatively well defined, especially in the case of conventional disease-producing agents. Major exceptions to this general observation are oncogenic agents and "slow virus" infections.

Two major risk situations can be identified as follows:

a. the known microbial agents that are integral to scientific research or teaching, which must be very carefully imported/exported, and
b. the potentially harmful microbial agents that are endogenous to humans or animals or to animal tissues or fluids needs to be handled in desired contaminant level.

The possibility of cross-contamination is high, if bio-safety of exporting/ importing microorganisms is not taken seriously. The negligence in this part may also jeopardize the agricultural sector of the economy.

Special care for bio-safety and biohazards must be taken while exporting and importing the microorganisms including general microbes of significant importance and genetically modified microorganisms

6. The microorganisms, which could be used for biological weapons, shall not be exported or imported.

7. All requests made for the permission to exchange/export/import microbial germplasm samples must be accompanied with the following information:

Related information

These are some general information required for distribution and exchange of microorganisms;

Preliminary Information:

i. Scientific name and taxonomy.
ii. Usual strain, cultivar or other name.
iii. Phenotypic and genetic markers (if available).
iv. The degree of relatedness between other related groups.
v. The brief description of identification and detection techniques.
vi. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.
vii. The description of the geographic distribution and of the natural habitat of the organisms including information on natural predators, prey, parasites and competitors, symbionts and hosts.
viii. Possible ecological sites from where microorganisms were isolated.
ix. Verification of the genetic stability of the organisms and factors affecting that stability (if known).

4.4 Pathological, ecological and physiological traits

i. the classification of hazard concerning the protection of human health and the environment;
ii. the generation time in natural ecosystems and the sexual and asexual reproductive structure;
iii. information on survivability, including seasonability and the ability to form survival structures, including spores and sclerotia etc.;
iv. pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, ability to act as a carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms;
v. antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;
vi. involvement in environmental processes, including primary production, nutrient turnover, decomposition of organic matter and respiration.;
vii. if the organisms are pathogenic to humans who are immunocompetent ;
viii. host range and possibility of alteration; antibiotic resistance patterns,
ix. allergenicity, and availability of appropriate therapies;
x. the other product hazards.

c. Behaviour and characteristics of the organism and its ecological impact as carried out in simulated natural environments, such as microcosms, growth rooms and greenhouses.

d. The description of ecosystems to which the organisms could be disseminated.

e. The potential for excessive population increase of the organisms in the environment.

f. The known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens.

g. The known or predicted involvement of the organisms in biogeochemical processes.

h. Any other potential interactions of the organisms with the environment.

4.5 International Distribution

The following written assurance shall be mandatory before the export of microbial genetic resources, that:

a. the microorganism will be used exclusively for research purposes and any genetic modification research will not be undertaken without the written consent of Indian authorities.

b. commercial exploitation of the exported microbial genetic resources shall not be done in the form received or of its derivatives except for after entering into a benefit/royalty sharing agreement.

8. It shall be mandatory to maintain all relevant and necessary records for exchange/ export/ import of microbial genetic resources at the NBAIM in the form of an electronic database as well as hard copy.

9. In all cases where any plant material is used as carrier for sending any specific microorganism, the same shall be forwarded by the Director, NBAIM to the Directorate of Plant Quarantine and Storage, Ministry of Agriculture, Government of India, New Delhi or the NBPGR, New Delhi for taking the quarantine measures.

Chapter 5

Registration of microbial Germplasm

5.1 Definitions for the purpose of Registration:

(i) References to a "registration" shall be construed as references to registration for microorganisms/ inventions, inventors' certificates, utility certificates, utility models, inventors' certificates of addition, and utility certificates of addition.

(ii) "Deposit of a microorganism" means the transmittal of a microorganism to NBAIM or other national depositary authority, which receives and accepts it, or the storage of such a microorganism by the national depositary authority.

(iii) "Publication for the purposes of registration" means the official publication, or the official laying open for public inspection, of a registration application.

(iv) "Depositary institution" means an institution which provides for the receipt, acceptance and storage of microorganisms and the furnishing of samples thereof.

(v) "Depositor" means the natural person or legal entity transmitting a microorganism to NBAIM or National Depositary Authority, which receives and accepts it.

(vi) For purposes of registration, a biological agent is defined as any organism or toxin regardless of biosafety level. All biosafety level agents must be registered.

(vii) The "Registration Authority" includes Director, NBAIM; a senior scientist from NBAIM and three experts (microbiologist / plant pathologist / virologist) from various ICAR Institutes / SAU's.

5.2 GUIDELINES FOR REGISTRATION OF MICROBIAL GERMPLASM

5.2.1. Microbial Germplasm to be registered

Germplasm of the microorganisms, including bacteria, fungi, cynobacteria, actinomycetes and viruses, which is unique, distinguish and stable, and has potential proven attributes of academic, scientific, agricultural and industrial or commercial value shall be registered.

5.2.2 Eligibility Criteria for Registration

The germplasm has complete passport data information, including taxonomy, geographical location, method of preservation, risk group and the uniqueness of the germplasm consists of:

1) Complete passport data that should include
    - Scientific name
    - Any other name or synonymous, if any
    - Phenotypic and genetic markers (if available)
    - The degree of relatedness with other related groups
    - The brief description of identification and detection techniques
    - The sensitivity, reliability in quantitative terms and specificity of detection and identification techniques
    - Verification of the genetic stability of the organisms and factors affecting that stability (if known)
    - The description of the geographic distribution and of the natural habitat of the organisms including information on natural predators, prey parasites and competitors, symbionts and hosts.
    - Possible ecological sites from where microorganisms were isolated

2) Authenticated taxonomic identity
3) Institutional or national identity
4) Information relating to the development and contribution.
5) The Depositor / Principal Investigator (PI) is responsible for completing a Biosafety registration form. This is needed to maintain registries of persons at risk for exposure to biological agents.

All claims concerning the material submitted for registration should accompany scientific evidence for uniqueness, novelty, reproducibility and value in the form of-

(i) Publication in standard peer reviewed journal (a copy of reprint to be submitted).

AND/ OR

(ii) Certified evaluation data for at least three years under AICRP trial/nursery tests supported with relevant extracts of the documents or verification by concerned PD/ PC or three location data under any other relevant system.

AND/ OR

(iii) Publication of information on potential value of proposed germplasm in institute annual report or any other such reports.

AND/ OR

(iv) Certificate of the validation test of the claimed attribute by any institution as per the advice of registration authority.

5.2.3. Microbial Germplasm Ineligible for Registration

(i) Microbial germplasm and genetic stock without accompanying documentary evidence for the claim made in the application.

(ii) Do not contain complete passport data, including authenticated taxonomic identity, isolation and sporulation requirements, institutional or national identity, geographical location of origin and all such information relating to the development and contribution, if any to the uniqueness of the germplasm.

(iii) Material for which any form of protection has been sought elsewhere.

(iv) The NBAIM accepts these microorganisms whether they are, or not, genetically modified. The NBAIM will not recognize plasmids as “kind of microorganism", but as part of the properties of an accepted microorganism.

(v) Microorganisms are not accepted where manipulation cells for physical containment levels as P3 or P4; exceptionally a microorganism of Group 3 may be accepted, or a microorganism of Group 2 may be refused, according to the evaluation of the risks as for the manipulation and for the storage of the microorganism in the environment of the NBAIM.

(vi) Microorganisms are not accepted where their properties are so exceptional that the NBAIM is technically not in a position to perform the tasks.

(vii) Microorganisms are not accepted where they are contaminated.

5.2.4 Microbial Germplasm Registration Committee

1. A committee would be constituted under the chairmanship of Deputy Director General (Crop Sciences), Indian Council of Agricultural Research for a maximum of three years period.

2. The Director NBAIM will be a permanent member and it would include a senior level scientist from NBAIM to function as Member Secretary.

3. It will have provision for adoption of need-based specialists of a group of microorganism with reference to the material under consideration, with the approval of the Chairman.

5.3. Nodal Agency

1. NBAIM, Maunath Bhanjan, U.P. will be the nodal agency for registration of germplasm. The application should be addressed to the Director, NBAIM, along with the microbial cultures or a certificate of submission of propagules with respective microorganism for conservation.

2. The Member Secretary, MGRC will duly acknowledge with date, the receipt of the application and of the microbial culture.

3. NBAIM will maintain a permanent register and database listing the germplasm materials approved by MGRC with details on unique traits and other related information.

5.4. Application Form

Application shall be made on the prescribed Proforma (Form A, Annexure I). The MGRC shall meet at least twice a year, with the concurrence of the Chairman, for consideration of applications and related matters.

5.5 Screening of Application(s) and their Consideration by the MGRC

1. The Member Secretary, MGRC, will screen the proposal(s) submitted on prescribed Proforma, as per the guidelines of the checklist at NBAIM (Annexure II).

2. Each proposal will be forwarded to the relevant Director, PD or PC for validation of information, particularly on uniqueness and novelty of the proposed germplasm.

3. After initial screening, the incomplete applications would be advised for appropriate revision.

4. The application in which the validation of the data is felt necessary, the applicant would be asked to produce a validation report from an appropriate institute, advised by the Director NBAIM. The revised application should accompany such report duly endorsed by the competent authority of the institute, advised for the validation.

5. The proposals complete in all respect along with the comments of relevant Director, PD or PC will be put up to the Registration Committee for consideration.

6. The MGRC will consider the proposal as early as possible and not later than one year.

7. The decision of the MGRC will be final.

5.6 Validity of Registration

- The period for validity of registration shall be five years after which the registered germplasm would be national sovereign property.

5.7 Notification of Registered Germplasm

All germplasm material approved for registration would be officially communicated to the applicants along with Registration Number. A certificate to this effect will also be issued to the applicant. A brief description of not more than one page (see annexure III for instructions) would be published in the ensuing issue of appropriate periodicals, such as:

1. Indian Journal of Microbiology – published by Association of Microbiologist of India, Delhi University, New Delhi – 110 012

2. NBAIM Annual Report, NBAIM, Maunath Bhanjan, UP. 275101

3. ICAR News - published by the Publication and Information Division, Krishi Anusandhan Bhavan, ICAR, New Delhi – 110 012

4. NBAIM Internet Website http://www.nbaim.org.in

5. ICAR Annual Report(s)

5.8. Conservation, Maintenance and Sustainable Utilization of Registered Germplasm

1. Registered germplasm will be conserved in the culture collection of NBAIM in mineral oil or liquid nitrogen or lyophilized.

2. The novel gene will be conserved in the gene bank of the NBAIM.

3. The institution associated with the development of the germplasm is also to be mandated with the maintenance of working stock of germplasm for supply to users.

5.9 De-registration

A registration may be repealed by the MGRC in case of false claim(s) or disputed IPR claim. Appeal for counter claim, if any, should reach the MGRC within a period of three months of the publication of brief note.

Chapter 6

Biosafety and security of microbial germplasm

In order to minimize the risks to important microbial genetic resources from fire, flooding, earthquakes, war or other catastrophes, collections should arrange to have duplicates of the most important and irreplaceable strains (and their associated documentation) securely housed in a different building, or ideally at a separate site. Also, microbes, like animals and plants do not get extinct or endangered but they lose important traits with time either by spontaneous mutations or loss of certain genes. Safety duplicates should be genetically identical to the base collection and are referred to as the secondary most original sample. Safety duplicates will include both the duplication of material and its related information, and will be deposited in a base collection at a different locations within country. The location will be chosen to minimize possible risks and provides the best possible storage facilities. Safety duplication is generally under a 'black-box' approach. Recommendations based on scientific evidence and principles are presented as best practices for safety duplication of microbial germplasm. Some general principles and criteria for safety duplication will be:

- Selection of location (three in number in which one will be at permafrost region) according to suitable environment, good security and low risk for the duplicate samples.
- Appropriate consideration will be taken into account towards risks to loss of genetic integrity within accessions and loss of accessions in selection of the location.
- Consideration of costs and practical arrangements.
- To maintain at least one duplicate of each accession as a safety back-up.

Conservation of fungi, bacteria, actinomycetes, cyanobacteria and genomic resources in safety duplicates will be done by as many methods as possible (not less than two) depending upon the type of microorganism/genomic resource like fungi will be preserved under mineral oil storage, or freeze-drying/ lyophilization or slants; bacteria, actinomycetes and yeast under or freeze-drying/ lyophilization or slants or in glycerol at – 80^{o} C and *Genomic Resources* in appropriate buffers at desired temperature/s.

6.1 Standards for security and personnel

6.1.1 Power backup

Power supply to various laboratories and culture collection unit must be stable and continuous. An alternative back-up generator with adequate fuel supply will be

preferred. Different power backups should also be used in different equipment power systems, security system and communication system.

6.1.2 Training

Adequate and periodical training of personnels involved in various activities like collection, characterization, maintenance, conservation and evaluation of microbial genomic resources will be done. Laboratory biosecurity training, complementary to laboratory biosafety training and commensurate with the roles, responsibilities and authorities of staff, should be provided to all those working at a facility, including maintenance and cleaning personnel, and to external first-responders and responsible staff involved in ensuring the security of the laboratory facility. Such training should help understand the need for protection of valuable microbiological materials and equipment and the rationale for the laboratory biosecurity measures adopted, and should include a review of relevant national policies and institution-specific procedures. Training should provide for protection, assurance and continuity of operations. Procedures describing the security roles, responsibilities and authority of personnel in the event of emergencies or security breaches should also be provided during training, as well as details of security risks judged not significant enough to warrant protection measures. The biorisk management plan should ensure that laboratory personnel and external partners (police, fire brigade, medical emergency personnel) participate actively in laboratory biosecurity drills and exercises, conducted at regular intervals, to revise emergency procedures and prepare personnel for emergencies. Training should also provide guidance on the implementation of codes of conduct and should help laboratory workers understand and discuss ethical issues. Training should also include the development of communication skills among partners, the improvement of productive collaboration, and the endorsement of confidentiality or of communication of pertinent information to and from employees and other relevant parties. Training should not be a one-time event. Training should be offered regularly and taken recurrently. It should represent an opportunity for personnels to refresh their memories and to learn about new developments and advances in different areas. Training is also important in providing occasions for discussions and bonding among staff members, and in strengthening of the team spirit among members of an institution.

6.1.3 Security system

Appropriate security measures should be undertaken for risks against untoward accidents like fire, thefts, electric short circuits, etc. The following systems should be installed at appropriate places:

6.1.3.1 Alarm Systems - Intrusion and Hold-up Systems: Doors to the Building / Floor are to be monitored via , the security system.

6.1.3.2 Access Controlled Doors: Access Control (electronic card based device) is to be installed on Entrance Doors to Building and Entrance Doors to the Suite/Floor.

6.2 Protective clothing

- Protective laboratory clothing (e.g., coats, gowns, smocks, or uniforms) designated for laboratory use should be worn to prevent contamination of personal clothing and must be worn when working at laboratory or when working with other hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering and laboratory clothing must not be taken home. Eye protection must be worn in the laboratory. Eye and face protection (goggles, mask, face shield, or other splatter guard) must be used when it is anticipated that splashes, sprays, splatters, or droplets of infectious or other hazardous materials may be generated and could contaminate the eyes, nose, or mouth (e.g., when RG2 microorganisms must be handled outside the BSC or containment device). This eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves that were used in laboratory work must not be worn outside the laboratory. Eye, face, and respiratory protection should be used in rooms containing infected animals or plants, as determined by the risk assessment.

6.3 Personal Hygiene and Dress:

- Wash hands prior to and following manipulations of organisms or cultures and whenever contamination is suspected. Wear personal protective equipment (PPE) to protect the worker and to prevent research materials from contamination. Change gloves when contaminated. Routinely clean lab coats or throw away disposable coats.

6.4 Environmental Protection process

- Environmental Protection process design requirements - includes wastewater and sewage treatment, noise protection, radiation protection, bio-security requirements. Attention should be paid to environmental protection and proper disposal of waste, ensuring the laboratory staffs, visiting scholars and the well operation of the surrounding facilities and the maintenance of construction academic atmosphere is very important. Therefore, it is necessary to control strictly the laboratory's hazardous materials, hazardous waste reserving and

processing, distributed storage facilities, wastewater discharge, solid waste management and recycling, gas distribution.

6.5 Complaint system of refrigeration

- Refrigeration standards and equipment should conform to Facilities efficient temperature for Genetic Conservation of microbial bodies. There should be trained personnel and available spare parts for repair and maintenance. Routine preventive maintenance should be carried out.

6.6 Monitoring devices

Various monitoring devices at appropriate places should be installed like:

- **Closed Circuit Television (CCTV)**: CCTV is to be sited at all Main Entrances to the building, installed to enable monitoring staff to recognize a person "a figure should occupy at least 50% of the screen height enabling viewers to say with a high degree of certainty whether or not an individual shown is the same as someone they have seen before". A monitor is required within the lobby to show the image of the camera on the outside of the unit, this is to enable staff to see that their exit route is clear.
- **Control and Indicating Devices** – Main Control Panel to be sited within the suite in a suitable location, remote keypad to be sited adjacent to the Main Entrance/Exit Door, setting of the system is to be via the door contact programmed to set on final door setting and unsetting of the system is to be actioned by a valid read of the Access Control Reader.
- **Level of Detection Devices** – Opening of and penetration through external doors and windows etc. plus trap protection as well as special consideration to high-risk items/areas.
- **Fire fighting system -** Fire precautions should be undertaken and appropriate fire fighting equipment tested periodically. Personnel must be trained to use this equipment. Installation of lightning conductor rod, alarm system and high temperature cut out for the cooling system is recommended. The main means of microbial laboratory fire fighting system is required. The special requirements of laboratory fire protection should be considered in accordance with relevant local standard laboratory settings and in accordance with the regional laboratory using standardized building set on fire for fire rating materials, setting fire dampers, automatic sprinkler system, fire signal system, flue gas duct detection system, fire hydrant and fire pump and other fire facilities.

References

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Bergey's Manual of Systematic Bacteriology, Second edition, volume 1 to 5, The Williams and Wilkins Company, Baltimore, 2001-2009

Bridge, PD, Spooner BM and Roberts, PJ (2004) Reliability and use of published sequence data. *New Phytologist* 161: 15.

Day JG. and Stacey G N (2007) Cryopreservation and Freeze-Drying Protocols. Humana Press Inc. Totowa, New Jersey pp. 347.

Kirsop, B. E. and Doyle, A. (1991) Maintenance of Micro-Organisms and Cultured Cells: A Manual of Laboratory Methods, 2nd edition, Academic, London, UK.

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Perry S F. (1998) Freeze-drying and cryopreservation of bacteria. Molecular Biotechnology 9 (1): 59-64

Ryan MJ, Smith D and Jeffries P (2000) A decision-based key to determine the most appropriate protocol for the preservation of fungi. *World J Microbiol Biotechnol* 16: 183-186.

Spiegelman D, Whissell G, and Greer C W (2005) A survey of the methods for the characterization of microbial consortia and communities. *Can. J. Microbiol.* **51**: 355–386.

Tan CS (1997). Preservation of fungi. *Cryptogamie Mycol* 18: 157-163.

Web site links

Biological Resource Centres – Underpinning the Future of Life Sciences and Biotechnology (http://oecdpublications.gfi-nb.com/cgi-bin/oecdbookshop.storefront).

OECD best Practice Guidelines for Biological resource Centres. www.wfcc.nig.ac.jp/Documents/OECD.pdf

Common Access to Biological Resources and Information (CABRI) guidelines (http://www.cabri.org)

International Standards Organisation (ISO)

http://www.iso.ch/iso/en/ISOOnline.frontpage.

UKNCC quality management system

(http://www.ukncc.co.uk).

World Federation for Culture Collections (WFCC) Guidelines for the establishment and operation of collections of microorganisms

(http://wdcm.nig.ac.jp/wfcc/index.html

Guidelines for registration of microbial germplasm

www.mgrportal.org.in

Annexure-I

PROCEDURE FOR SUBMISSION OF PROPOSAL/ GERMPLASM MATERIAL

1. Submission of Application and Material

1. All microbial germplasm proposed to be registered should be submitted to the following address:

   The Director
   National Bureau of Agriculturally Important Microorganisms
   Kusmaur, Kaithauli, Maunath Bhanjan, UP. 275101
   Phone: 0547-2530080;
   FAX: 0547-2530358
   Email: nbaimicar@gmail.com.

2. The material must be accompanied with properly filled Form-A (Annexure-I), duly signed by the applicant and the Head of the institution with official seal (15 copies each with attached documentary evidences to be submitted).

3. The Form-A must be accompanied by complete description of the germplasm material using standard taxonomic descriptors. It may include morphological characters (along with colony and microphotograph), biochemical and molecular characteristics.

4. A declaration to the effect that working-stock for supply to users would be maintained by the institution associated with the development of the material.

2. Guidelines for Submitting the Culturable Microorganisms

The microorganism that can be cultured on different media should be submitted as:

1. Two lyophilized tubes or three slants of fungi, bacteria, actinomycetes and cyanobacteria should be submitted.

2. In case of spore forming fungi, they should be preserved after sporulation or submitted on slants with details of growth and sporulation conditions.

3. The cultures should be viable and pure.

3. Guidelines for Submission of Obligate Microorganisms

These are the microorganisms remained associated with their living host and can not be cultured on the medium should be submitted as:

1. The obligate microorganism can be submitted to NBAIM, only in cases, where established protocols are available for their conservation using cryogenic or any other technology.

2. These microorganisms should be submitted with infected part of their host or in the form of resting structures / spores should be submitted with established conservation protocol.

3. In case of viruses it should be in the form of purified virus preparation or in any other established protocol for conservation like low temperature vacuum dried infected leaves.

Quarantine aspects:

The import of genetic resources shall be primarily as per the existing regulations laid down in plant quarantine order amended from time to time. The PPA of the Department of Agriculture and Co-operation is the authority to issue the import permit. However, the cases related to microorganisms should be viewed as

- No cultures of pathogenic nature shall be allowed for import.
- The genetically modified microorganisms shall not be permitted for import until proper justification is given and it is approved by statutory bodies.

Documentation

Passport Data for Submission of Microbial Cultures

Name of Depositor:	
Designation:	
Address:	
Phone/Fax:	

E-mail:	

Microorganism's details

Name of Microorganism					
TypeofMicroorganism	Fungus	Bacterium	Actinomycetos	Cynobacterium	Other

Isolation details

Source of Isolation	Plant	Animal	Insect	Soil	Water	Other
Name of host						
Variety						
Isolated from which part						
Isolated by with date						

Geographical origin

Longitude & Latitude	
Place/Village	
District	
State	

Growth and maintenance

Growth & maintenance	Growth	Sporulation
Media		
Temperature		
Incubation time		
Other		

Identification details

Morphological description	Image
Chemotaxonomic description	Image

Physiological description	Image
Molecular description (with NCBI/EMBL/DDBJ Accession number, if any)	Image

Economic importance (provide details)

Agriculturally	
Industrially	
Medicinally	
Pathogenic details	
(virulence/aggressiveness)	
Other	

Other

Deposited in form of	
Deposited in other collection with no.	
IPR/Patent information	
Any other	

Signature

Date

Attach separate sheet, if necessary. Photocopies of this form may be used. Attach separate sheet for each isolate. Tick appropriate box.

Annexure II

Form A

Application for Registration of Microbial Germplasm

(To be submitted to The Director, NBAIM, Maunath Bhanjan, UP. 275101)

Please refer to guidelines for filling the application form appended and Codes

1. Application status (Code)

New	Revised

2. Type of microorganism

Fungi	Bacteria	Actinomyces
Cynobacteria	Virus	Other

New	Revised

3. Taxonomic name

4. Biological status of the material to be registered

RE	MU	GP		Other

5. Alternate identity

6. Criteria for registration (Unique feature(s))

I.
II.
III.

7. Quantity deposited (Ac

8. Value referred to (code)

Scientific	Academic	**Commercial**	Other

9. Basis of eligibility (code)

AR	PR	CT	Other

10. Validity test suggested

Yes	No

For Use of NBAIM

(i) Application numb

(ii) Date of application

(iii) Whether new or revised

(iv) If revised, date of 1st application

(v) If validation test suggested, whether report attached

(vi) Action taken

a. Forwarded for registration
b. Sent for validation
c. Incomplete, sent for revision

(vii) Whether registered or rejected

Yes	No

(viii) Date of registration or rejection

(ix) Registration Number

(x) Notified on

(xi) Remarks

11. Particulars of the scientist(s)/person(s) who developed germplasm/genetic stock

Name (Dr./Ms/Mr.)

Designation

Address

Telephone Fax E-mail

(Please attach separate sheet for additional name(s) and address(es) of co-authors (Persons responsible)

12. Name and address of the corresponding person (Developer / Depositor)

Name (Dr./Ms/Mr.)

Designation

Address

Telephone Fax E-mail

13. Passport information of germplasm

Accession numbe Other identity

Source | Plant | Animal | Soil | Insect | Other

Place / geographical area of is

Growth and maintenance

Culture media for grov and sporulation

Temperature for growth and sporulation

Time for sporulation any other

Importance of microorgi | Agricultural | Industrial | Biotechnological | | Other

Nature of microorganisn | Plant pathogenic | Animal / human pathogenic | Hazardous | Risk to environment | Unknown | Other

Gene identified (if a

14. Salient characteristics morphological, biochemical and molecular (if necessary attach details in separate sheet)

1. 2.

15. Additional information / remarks (if any)

16. Recommendation of Institute's Germplasm Identification Committee

UNDERTAKING

1. *I/We undertake to ensure deposition of genetic material for long term conservation of the aforesaid germplasm/genetic stock at the National Genebank, NBAIM and also its sustainable use by maintaining appropriate quantity of Active/Working Collection and providing access as appropriate on prior informed consent and on mutually agreed terms. I/We also agree to provide any further information or data pertaining to the description and unique characteristics to the ICAR/NBAIM in a transparent manner.*

COUNTERSIGNED BY HEAD OF INSTITUTE

Full Name
Designation & Address
(Stamp)

SIGNATURES OF THE DEPOSITOR

Name
Designation & Address

Guidelines for filling Form A and description of Codes

1. Use capital letters or write legibly. All items are self-explanatory. Give minimal explanation for a particular item in “Remarks” (Item 16), wherever needed.

2. Be to the point for Item 6, give only the most salient features, traits or alleles, considered suitable for consideration of registration.

3. On the other hand, give detailed description of traits and characteristics of the material in Item 14. Use separate sheet if needed.

Give main taxonomic and morphological characteristics in description. Include isozyme or DNA profile or other chemical/biochemical characteristics, if available.

4. Use codes for filling in Item 1, 4, 7 [actual], 8, 9 and 13. In case of the code "Other" fill in specific details in Item 15.

5. Give name(s) of all persons associated with development of the material in Item 11. Use separate sheet and fill in additional names along with designation, address and phone/fax/email, etc. beginning with S. No. 2 on new sheet, in the same format

6. In item 13 you must provide basic passport information that should include National Identity accession number given by NBAIM or other Identity Number allotted and maintained in other culture collection. In case of non-availability of national identity, NBAIM will provide a unique accession number, based on passport data provided.

7. Give particulars of developer(s) in Item 11 over and that of corresponding person in Item 12 as the applicant and developer may not be always the same as the first person responsible for development of the material.

8. Undertaking to the effect ensuring long term conservation and maintenance of active material for facilitating access and sustainable use has been given, which may be read and implied before putting signatures.

Codes for filling information in Col. 1, 4, 7 [actual], 8, 9 and 13

Item 1	N = New; R = Revised
Item 4	RE = Recombinant ; MU = Mutant ; GP = Germplasm
Item 7	SL = Slant; LY = Lyophilised; MO = Mineral oil; GY = Glycerol stock; SB = Saline buffer; CP = Culture plate; OT = Other (specify)
Item 8	SC = Scientific; AC = Academic; CM = Commercial
Item 9	AR = Institute Annual Report; PR = Published with peer review; CT = All India Co-coordinated trail data; OT = Other (specify)

Annexure III

CHECK-LIST FOR SCREENING OF APPLICATIONS

The Member Secretary, MGRC at NBAIM shall screen all applications and make recommendations to the MGRC for *inter alia* the following points:

Whether this is a new application?	Yes ☐ No ☐
The application is revised one?	Yes ☐ No ☐
Whether same or similar material has been registered earlier?	Yes ☐ No ☐
Whether unique or distinguishing characteristics of potential value merit consideration for registration?	Yes ☐ No ☐
Whether documentary evidence or data is provided in support of the claim on potential value of germplasm?	Yes ☐ No ☐
State any other economic potential value of germplasm, if possible.	
NBAIM viewpoint about the candidate germplasm.	
Whether applicant, institution, university, or centre has given a commitment for maintenance and supply of germplasm for use?	Yes ☐ No ☐
Whether appropriate size of germplasm sample for long-term storage at National Genebank or for conservation and maintenance?	Yes ☐ No ☐
Whether detailed address of the corresponding person is given?	Yes ☐ No ☐
Whether appropriate institutional authority has duly endorsed the application?	Yes ☐ No ☐

Annexure IV

Uniqueness of Agriculturally Important Microorganisms:

A. Uniqueness for Academic / research
    1. Morphological
    2. Biochemical
    3. Genetic

B. Uniqueness for utilization in agriculture
    1. Nitrogen fixer
        i. Symbiotic
        ii. Non Symbiotic
    2. Plant Growth Promoter
        i. P solublizer
        ii. Sidrophore producer
        iii. IAA producer
        iv. ACC deaminase producer
        v. Ammonia producer
        vi. Any other ……..
    3. Biocontrol agent
        i. Plant diseases
        ii. Insects
        iii. Nematodes
        iv. Weeds
        v. Any other ……..
    4. Bioremediation
        i. Agro waste decomposer
        ii. Pesticide remediation
        iii. Heavy metal remediation
        iv. Any other ……..
    5. Edible fungi
        i. Mushroom
        ii. Yeast
        iii. Any other ……..
    6. Mycorrhizae
    7. Any other ……..

C. Uniqueness other than in agriculture i.e. industry, medical, environment etc.

……

D. Uniqueness other then above mentioned, if any

……

Note:

- The uniqueness of the organism should be supported with proper documentary evidence.
- Attach separate sheet, if required.

Annexure V

Passport Data Form

Form for Submission of Microbial Cultures/Passport Data

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो

National Bureau of Agriculturally Important Microorganisms (NBAIM)

For NBAIM use only
Our Accession Number:
Registration Number:
Microbe Code:
Scientific Staff Code:

Name of the depositor Last First Middle	**Designation** **Affiliation** **Phone** **E-mail** **Fax No.**
Name of the microorganisms	**Type of culture** ☐ Bacterium ☐ Virus ☐ Fungus ☐ Actinomycetes ☐ Other
Details of source of culture Place of isolation (habitat, crop, plant or animals etc.) Collection date Collected by District & State	
Details of isolation Isolated by (person and address) Isolation date Specimen isolated from (eg.leaf, stem, root, soil, egg mass, insect, etc.)	

Growth and maintainance Medium of growth Medium for sporulation Optimum temperature for growth Incubation time Subculture period Special requirements for growth & sporulation, if any	
Identified by	
Geographical origin	
A brief description or distinctive features of the microorganisms	
Any record on RFLP/RAPD pattern or unique markers	
Whether deposited miroorganisms are	☐ Agriculturally Important, if yes, please provide details/published materials. ☐ Industrial/Biotechnological importance ☐ Any Other
Taxonomic data	☐ Morphological ☐ Chemotaxonomic/Immunological ☐ Molecular (DNA fingerprint/Sequence analysis) (Enclose available documents)
Microorganisms is deposited in	☐ Lyophilized form (in ampoules) ☐ In Slants/Petri plates ☐ Any Other
Nature	☐ Plant pathogenic ☐ Pathogenic to animals/humans ☐ Hazardous ☐ Risk to environment ☐ Unknown ☐ Any Other
IPR/Patent information, if any	Group ☐☐☐☐
Provide accession number, if deposited elsewhere	
Any Other information	
Signature and date	

- Attach separate sheet, if necessary.
- Photocopies of this form may be used.
- Attach separate form for each isolate.
- Tick appropriate Box

Address:
National Bureau of Agriculturally Important Microorganisms
Kusmaur, P. Box No. 36, Mau Nath Bhanjan
Uttar Pradesh 275 101 - INDIA
Ph: 0547-2530302, Fax: 0547-2530700
Email: nbaim2000@yahoo.com, nbaimmau@sify.com
Website: http://www.nbaim.org.in/nbaim/index.htm