Chapter 1
Microbial Genetic Resources: Status, Conservation, and Access and Benefit-Sharing Regulations

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Abstract Majority of the biomass and biodiversity of life on the earth are accounted by microbes, and so far about 10% of the earth's microbial diversity has been characterized. They play a significant role in biogeochemical cycles and extend various ecosystem services. Many microorganisms are rich and serve as untapped reservoirs of metabolic products and, hence, they are potentially important for scientific, industrial and economic purposes. The uninterrupted availability of such microbes for modern scientific security and their ultimate utilization for academia and industry are of paramount importance. Despite countless facts about the role of microorganisms in the biosphere, they have largely been ignored by conservation efforts and never considered part of conservation biology and thus leave number of questions unanswered. Notwithstanding, there are many factors including climate change and habitat destruction affect microbial structure and diversity calls for a consistent environmental ethics for parallel support and protection of microbes and their long-term conservation. It is needless to mention that microbial resources play important roles in developing bio-economy. However, long-term success in conservation strategies requires thorough understanding of basic biology of microorganisms and

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their application through state-of-the-art modern tools leading to longer viability and unaltered genome of microbes. In this regard, specialized training and laboratory infrastructures are required to be extended for significant contributions in protection and successful conservation of microbial gene pool in repositories and natural habitats. Therefore, conservation biologists now are bound to realize that the microbial system on which our livelihoods depend is at a risk of extinction, and this requires serious attention to ensure their sustainability in nature for continuous biogeochemical processes, diversity and abundance. In this way, we are becoming more concerned with the broader aspects of microbial conservation. This review attempts to bring together various aspects with regard to the status of conserved microbes, conservation strategies, methodologies and challenges. Further, this chapter will also appraise about the regulatory mechanisms on sharing of microorganisms at global level under the ambit of Nagoya Protocol of Convention of Biological Diversity (CBD) and Biological Diversity (BD) Act 2002 and Rules 2004 of India.

1.1 Introduction

Diversity of microorganisms is dominant and cosmopolitan on this planet. They are the earth's greatest treasures possessing largest genetic and metabolic diversity that are utilized for improving productivity and sustaining life on earth. Some specific microbes are hostile to their characteristic environments, and it has been assumed that existence of all life forms in the biosphere depends on microorganisms. Microbes belong to all important domains of life like bacteria, archaea and eukarya as well as the viruses. Despite huge importance, $<0.1\%$ of microbial species of the total microorganisms have been characterized, preserved and utilized for various purposes (Alain and Querellou 2009). Every microbial species has its own microbiome, and researchers explore microbial universe and their genetic traits for exploitation for biotechnological purposes in various fields of food industry, medical, pharmaceutical and agriculture (Bull 2004; Challis 2008; Cockell and Jones 2009). These microbes significantly contribute to the global economy through multibillion-dollar biotechnology industry. Apart from its industrial applications, conventional use of microorganisms in taxonomic studies, as standard/reference strains for diagnostic purposes, commercial production of metabolic product, or in biological transformation are also required (Martin 1964). Almost every branch of science uses processes and products of microbial origin. However, it is now well understood that ex situ conservation of microorganisms is essential for ensuring that a source of living cells is readily available for scientific scrutiny of pure and applied nature. Moreover, they need protection from climate change, habitat destruction and various other factors since microorganisms isolated from environmental samples cannot always be recovered. However, even if fresh isolates are obtained, they may lack the desired properties originally expressed. As such importance of microorganisms in biogeochemical processes and industries is well understood by researchers and other professional customers from a scientific and industrial perspective.

However, in order to fully utilize the genetic resources of the uncharacterized microbes, we also need to develop suitable strategy to conserve different microorganisms in their habitats. Anthropogenic interventions and changes in ecosystems are the major threats to existence of many microbes that leads to new challenges of retrieving descriptive data on microorganisms for consideration as part of conservation biology (Sharma et al. 2016) and its impact on ecosystem functioning. With the advent of modern molecular approaches, richness of microbial diversity is increasingly evident and is becoming part of conservation of global genetic resources. The conservation of microbial diversity, their cells or replicable parts (e.g. genomes, plasmids, viruses, cDNAs) (Arora et al. 2005) in the environment has been realized by researchers by understanding and application of rRNA gene barcoding (detection of uncultured and unseen microbes) and use of operational taxonomic units (OTUs) as species representative with the help of next-generation sequencing (NGS) (Sharma et al. 2016).

Recent years have witnessed awareness in conservation of microbial gene pool manyfold, and conservation biologist have realized that microbes are at risk of extinction, and this requires serious attention for their long-term protection (Curtis 2006). There is a widespread concern worldwide for conservation of microorganisms. In this regard, bio-resource centres/culture collections play vital roles in maintaining these microbial gene pools making them available to others for scientific scrutiny and use it for supporting research and development programmes. Basically, resource collections/culture collections are of different types. Some of them are small private collection, while others serve as large service collections, with differing policies and holdings. Some are very small collections holding specific organisms used in individual research activity. Public service collections perform several tasks and serve as custodians of ex situ genetic resources. Functioning of these bio-resource centres is redefined and influenced by several regulations and therefore plays key roles in the conservation strategies (Kirsop and Hawksworth 1994).

World Data Centre for Microorganisms (WDCM) records about 758 microbial culture collections distributed across 76 countries. Most of the bio-resource centres follow operational guidelines of World Federation for Culture Collections (WFCC) and best practices of the Organisation for Economic Development and Cooperation (OECD) (DSTI/STP/BIO (2007)9/REV1). Microbial resource centres (MRCs) collect the microbes and/or microbiomes of high standards for long-term storage and supply of the authentic microbial strains as reference material to researches of academia and industry. They serve as an integral part of a broader activity of protecting and maintaining the ecosystem which helps in stabilizing global environment in order to ensure the availability of earth's biological resources to future generations. With these backgrounds, the present chapter highlights status of conservation of microbial resources and their global sharing following the regulations of access and benefit-sharing mechanisms under the ambit of Nagoya Protocol of CBD with special reference to 'Biological Diversity (BD) Act 2002 and Rules 2004' of India.

1.2 Microbial Resources Centres (MRCs)

Genetic resource can be defined as any material of biological origin containing functional unit of heredity, whereas 'microbial genetic resource' can be defined as any microbial strain that is authenticated, taxonomically defined, physiologically characterized, quality controlled and well documented. Alternatively, microbial genetic materials of actual and potential value are defined as microbial genetic resources. Microbial resource centres (MRCs) play a dynamic role not only in collections and conservation in controlled conditions, but also in sustainable utilization of genetic resources for various applications by academic, research and industry (Table 1.1). They are also necessary for the supply of authentic/reference cultures needed for regulatory compliance to health and trade (Sly 2010). MRCs broadly comply with the framework of Convention on Biological Diversity (CBD) that is implemented to support the conservation and utilization of biodiversity and recognizes the principles of fair and equitable benefit sharing (OECD 2001, 2007; Sly 2010). MRCs ensure long-term maintenance of living microbial material and its replicable parts (genomes, plasmids, viruses, cDNAs) (OECD 2001; Overmann 2015) and their sustainable use and management of related information of molecular and physiological data (Arora et al. 2005; Sigler 2004). They preserve and provide authenticated (technically and legally fit-for-use) genetically stable (consistent quality) microbial and cell cultures. Similarly, MRCs provide access to information on cultures and their characteristics for industrial and scientific research and testing.

Table 1.1 Some important international microbial resource centres/microbial culture collections

Acronym	WDCM no.	Collection name	Country
ATCC	WDCM 1	American Type Culture Collection	USA
JCM	WDCM 567	Japan Collection of Microorganisms	Japan
CBS	WDCM 133	Westerdijk Fungal Biodiversity Institute (Formerly, Centraalbureauvoor Schimmelcultures, Filamentous Fungi and Yeast Collection)	Netherlands
CGMCC	WDCM 550	China General Microbiological Culture Collection Center	China
KCTC	WDCM597	KCTC Korean Collection for Type Cultures	Korea (Rep. of)
CBMAI	WDCM823	Brazilian Collection of Microorganisms from the Environment and Industry	Brazil
IEKC, SSI	WDCM158	The International *Escherichia* and *Klebsiella* Centre (WHO)	Denmark
TISTR	WDCM383	TISTR Culture Collection Bangkok MIRCEN	Thailand
DSMZ	WDCM274	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH	Germany
LMG or BCCM/ LMG	WDCM296	Belgian Coordinated Collections of Microorganisms/ LMG Bacteria Collection	Belgium

Source: http://www.wfcc.info/ccinfo

Besides, identification and characterization of species new to science is also undertaken by MRCs. Other important cultures resulting from research and technological perspectives are also accessioned and conserved for supporting basic researches and applications in industry. MRCs also maintain extensive information regarding individual strain and in the form of databases and thus provide access to information on culture characteristics, morphological and physiological characteristics and DNA sequences. The MRCs are the centres of taxonomic expertise and play a vital role in capacity building through hands-on training. Based on requirements, MRCs are of different types such as safe depository to researchers and small and large industries and adhere to the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the 'Purpose of Patent Procedure' (Winter and Adam 2001). Many MRCs act as service collection to suffice the requirements of basic and applied research, while others serve as private and speciality microbial collections holding specific group of microorganisms. In a nutshell, MRCs meet *the high standards of quality and expertise demanded by the international community of scientists and industry for the delivery of biological information and materials* (OECD 2001). Advancement in information technology (Internet, PC clusters, cloud computing) and sequencing technology (next-generation sequencers, third- and fourth-generation sequencers) helped MRCs to wisely enrich the strain information and resources for delivery of quality research materials to future generation.

1.3 Scenario of Microbial Conservation at Global and Indian Context

1.3.1 Global Scenario

Convention on Biological Diversity (CBD) is a legally binding agreement on biodiversity under the United Nations (UN). As per standard text, negotiations started late in 1987, and the final text was endorsed in Rio de Janeiro, Brazil, in the year 1992 and enforced in December 1993. The World Federation for Culture Collections (WFCC) is a multidisciplinary commission of the International Union of Biological Sciences (IUBS) and a federation within the International Union of Microbiological Societies (IUMS). Its aim is to promote and support the establishment of culture collections and related services, to provide liaison and set up an information network between the collection centres and their users to ensure the long-term perpetuation of important collections in the world. The WFCC pioneered the development of an international database on culture resources worldwide. World Data Center for Microorganisms (WDCM) (http://www.wfcc.info/ccinfo/) maintains the database of culture collections. World directory of culture collections (Sixth Version, 2014) has records of nearly 758 culture collections from 76 countries or regions and holds a total of 2,963,856 microbes. Out of these, 1,221,657 are bacteria, 814,082 are fungi, 38,002 are viruses and 32,125 are cell lines (http://www.wfcc.

info/ccinfo/; accessed on 01 August 2018). The records also contain data on the organization, management, services and scientific interests of these collections. About 402 collections produce catalogue of holdings, and 6406 people work in these culture collections. There are 32 collections in India holding 197,723 cultures. Each of these records is linked to a second record containing the list of species held. The WDCM database forms an important information resource of all microbiological activities and also acts as a nodal agency for managing data activities among WFCC members. The Culture Collections Information Worldwide (CCINFO) is a database management system of culture collections in the world. It includes CCINFO and STRAIN. The CCINFO is a world directory of all registered culture collections, and STRAIN includes list of holdings from registered culture collections (http://www.wfcc.info/ccinfo/). Another important initiative took place to create a robust, reliable and user-friendly WFCC Global Catalogue of Microorganisms (GCM) to help culture collections to manage, disseminate and share the information related to their holdings. It also provides a uniform interface for the scientific and industrial communities to access the comprehensive microbial resource information from any corner of the world. Presently, 120 culture collections from 46 countries and regions have joined the campaign (http://gcm.wfcc.info/overview/; accessed on 01 August 2018).

1.3.2 Indian Scenario

Basic research is considered the backbone to applied discipline. It is a fact that 'taxonomy' as a science has seen some dynamic change and has made significant advances in the past and thereby supported the studies on biodiversity. Considering biodiversity-rich country, proper authentication of the strain is a very important requirement for academic interest and also from the point of protecting intellectual property rights (IPR) on the process and products developed through international patenting. Maintenance of these authentic microbial cultures in a pure state in microbial germplasm bank is a strategic requirement for developing innovative technological processes and products. In addition, awareness of the principles of convention on biological diversity, national regulation governing genetic resources and international patent laws is essential to protect our microbial-based intellectual property rights (IPR) in the global context. As per WDCM updates, 32 microbial culture collections exist in India (Table 1.2). Maharashtra state tops the list with a maximum of 09 numbers of culture collections followed by Uttar Pradesh (04), Delhi (03), Chandigarh (02), Gujarat (02), West Bengal (02), Telangana (02), Tamil Nadu (02), Jammu and Kashmir (01), Haryana (01), Rajasthan (01), Goa (01) and Kerala (01) (Fig. 1.1). Developing sustainable utilization strategies of Indian microbial wealth under the framework of CBD can play important roles in generating bio-economy (http://www.wfcc.info/ccinfo/).

Table 1.2 Major microbial collections in India

Acronym	WDCM no.	Collection name
ABRC	WDCM 912	Anaerobic Bacterial Resource Centre, University of Hyderabad, Hyderabad, Telangana
AYL	WDCM 934	Whylabs Resources Centre for Microorganisms, Hyderabad, Telangana
BAB	WDCM 1058	Bank A Bug, Gujarat Biodiversity Gene Bank, Gandhinagar, Gujarat
BDU	WDCM 976	National Facility for Marine Cyanobacteria, Tiruchirappalli, Tamil Nadu
BT	WDCM 1036	*Bacillus thuringiensis*, M.S. Swaminathan Research Foundation Chennai, Tamil Nadu
CCBB	WDCM 1169	Mushrooms Biodiversity – Western Regional Centre TERI, Belapur, Maharashtra
CCDMBI	WDCM 119	Culture Collection, Department of Microbiology, Bose Institute, Kolkata, West Bengal
CIPDE	WDCM 462	Collection of Insect Pathogens, Department of Entomology, Parbhani, Maharashtra
CM	WDCM 1033	*Chroococcus minor*, Shri Jagdish Prasad Jhabarmal Tibrewala, Jhunjhunu, Rajasthan
DBV	WDCM 173	Division of Standardisation, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh
DMSRDE	WDCM 166	Defence Materials and Stores Research and Development Establishment Culture Collection, DRDO, Kanpur, Uttar Pradesh
DUM	WDCM 40	Mycological Herbarium, Department of Botany, Delhi University, Delhi
EntoPatho	WDCM 1013	Entomopathogen, M.S. University of Baroda, Vadodara, Gujarat
GCC	WDCM 1165	Global Collection of Cyanobacteria, Varanasi, Uttar Pradesh
GFCC	WDCM 946	Goa University Fungus Culture Collection and Research Unit, Goa University, Panaji, Goa
ITCC	WDCM 430	Indian Type Culture Collection, ICAR-Indian Agricultural Research Institute, New Delhi
MCC	WDCM 930	National Centre for Microbial Resources (Formerly Microbial Culture Collection), NCCS, Pune, Maharashtra
MCM	WDCM 561	MACS Collection of Microorganisms, Agharkar Research Institute, Pune, Maharashtra
MPKV	WDCM 448	Biological Nitrogen Fixation Project College of Agriculture, Rahuri, Maharashtra
MRCJ	WDCM 1117	Col. Sir R. N. Chopra, Microbial Resource Center, CSIR-IIIM, Jammu and Kashmir
MTCC	WDCM 773	Microbial Type Culture Collection and Gene Bank, CSIR-IMTECH, Chandigarh, Union Territory
NAIMCC	WDCM 1060	National Agriculturally Important Microbial Culture Collection, ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, Uttar Pradesh
NCCPF	WDCM 1118	National Culture Collection of Pathogenic Fungi, PGIMER, Chandigarh, Union Territory
NCDC	WDCM 775	National Collection of Dairy Cultures, ICAR-National Dairy Research Institute, Karnal, Haryana

(continued)

Table 1.2 (continued)

Acronym	WDCM no.	Collection name
NCIM	WDCM 3	National Collection of Industrial Microorganisms, CSIR-National Chemical Laboratory, Pune, Maharashtra
NFCCI	WDCM 932	National Fungal Culture Collection of India, ARI, Pune, Maharashtra
NIICC	WDCM 961	NII Microbial Culture Collection, National Institute for Interdisciplinary Science and Technology, Trivandrum, Kerala
NMCC	WDCM 972	North Maharashtra Microbial Culture Collection Centre, North Maharashtra University, Jalgaon, Maharashtra
NTCCI	WDCM 107	Culture Collection, Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore, Karnataka
UMFFTD	WDCM 562	Food and Fermentation Technology Division, University of Mumbai, Mumbai, Maharashtra
VBCCA	WDCM 931	Vishva-Bharati Culture Collection of Algae, Visva Bharati Central University, Santiniketan, West Bengal
VPCI	WDCM 497	Fungal Culture Collection, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi

Sources: www.wfcc.info/ccinfo/collection/coll_by_country/i/91/

1.4 Strategies and Methodologies for Microbial Conservation

MRCs follow various strategies for conserving microbial diversity. Generally, conservation strategies include 'in situ', 'ex situ' and 'in factory' forms of conservation. As a principle, 'in situ' ('on site', 'in place') approach links to conservation in their natural habitats and are considered as the most appropriate way of conserving viable populations of microbes in their natural ecosystem and natural habitats (CBD 1992). The storage and culturing of microorganisms in artificial/culture collections (ex situ) have made enormous contributions to the existing microbial conservation efforts. The systematic link between field conservation efforts and the preservation of important microbial species from such environments would increase the value of culture collections. A system of well-organized MRCs play a distinctive role in preservation and maintenance of distinct wild/isolated/cultivated species\strains and their genetic populations on artificial media. Being taxonomically well described, they provide remarkable research openings on the components of microbial genetic diversity. 'In factory' form of conservation is an intermediate form of conservation, mainly used by the agro-industrial sector (Sharma et al. 2016).

1.4.1 In Situ Conservation Methods

In situ conservation refers to conserving microbial species in their natural habitats by long-term preservation of ecosystems, species and populations under conditions of continuing adaptations. It also protects associated animals together with microbes,

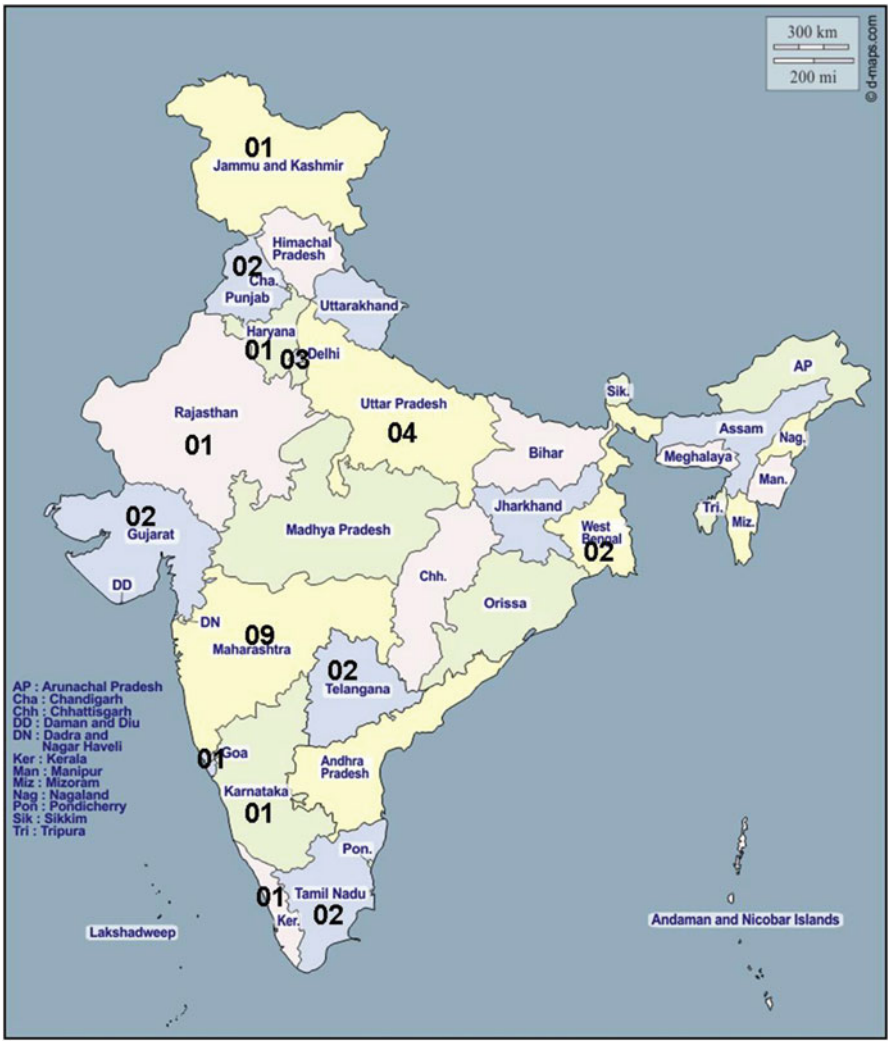

Fig. 1.1 Distribution of microbial culture collections in different states of India

thereby enabling free energy flow. Generally, in situ conservation is considered essential in places where microbiome has not been adequately inventoried. In the new era, creation of wildlife parks, reserved forests, biosphere reserves, eco-parks, etc. where diversity of microbial forms can thrive in various habitats and in natural associations with their plant and animal hosts may lead up to some extent destruction of these natural habitats. There are large numbers of microbial species that are yet to be discovered (Alain and Querellou 2009; Stewart 2012), to determine their complex interactions and critical roles in ecological processes. Bacteria that can be grown in the laboratory are only a small fraction of the total diversity that exists in nature (Stewart 2012). Plethora of microorganisms is known to be non-cultivable and hence

can be conserved by in situ conservation. At all levels of bacterial phylogeny, uncultured clades are found and play a critical role in recycling of elements, synthesizing novel natural products and impacting the surrounding environment and associated organisms (Stewart 2012). Over the past 30 years, traditional cultivation methods have failed to grow ecologically and phylogenetically more relevant microorganisms (slow growers, oligotrophs, fastidious and recalcitrant microorganisms) in the laboratory. The repeated failures reported in cultivating some phylogenetic microbial lineages are major challenge in microbial ecology. A coordinated methodology to discover and understand microbial diversity and communities is required along with conservation of world's microbial diversity through concerted efforts. In situ management can either be targeted at populations of selected species (species-centric approach) or whole ecosystems (ecosystem-based approaches) (Heywood and Dulloo 2005).

Information on 16S rRNA gene barcoding is exceptionally helpful in decoding certain evolutionary lineage and flow of genetic information in bacteria, but sequences alone cannot direct its in situ expression. Ex situ conservation is required to support in situ conservation as measures to achieve the conservation targets. However, it is challenging to judge the usefulness of in situ conservation approach in the absence of data and appropriate statistics on the extent of microbial diversity. Protected areas are usually considered as the cornerstone of in situ conservation that are more adaptable to individual situations (CBD 1992). Historically, in situ conservation was the preferred approach over ex situ conservation (Lacy 2010). In situ measures are perceived as more holistic in their approach and allow the conservation of processes or habitats (e.g. soil microbial processes, evolutionary processes, specific ecosystems or species with highly specialized needs). Convention on Biological Diversity (CBD) recognizes complementary role of both in situ and ex situ conservation strategies. Article 8 and 9 of the CBD set the guidelines for the use of in situ and ex situ measures, respectively. In particular, the CBD specifies that ex situ facilities and techniques should predominantly be implemented for the purpose of complementing and supporting in situ measures (FAO 2010). There are some specific examples that highlights the importance of in situ conservation of habitats and associated microbes such as conservation of microbial biodiversity of agricultural interest (soil fertility, crop nutrition, biocontrol, bio-fertilizers) and food security (Tables 1.3, 1.4, 1.5, and 1.6). Extreme ecosystems with wide range of modern and ancient geological environments such as caves, desert soils, fresh and marine water, hot springs, hydrothermal vent, hypersaline areas, cryoconite hole on glacier, unique niches and unusual habitat (digestive tract, rocks, and caves, etc.) do support the occurrence of specific type of microbes that need to be conserved (Sharma et al. 2016) in their habitats. Ecosystem and habitat preservation are in infancy stage require serious attention to start preservation of microorganisms at ecosystem or habitat level to save them from extinction.

Table 1.3 Microbial resources contribute to agroecological and food security

Nitrogen (N_2) fixation: (e.g. *Cyanobacteria*, *Rhizobium* and *Frankia*)
Plant growth promoting rhizobacteria (PGPR): (e.g. *Bacillus*, *Pseudomonas, Streptomyces*, *Azosprillum* sp., and *Bradyrhizobium* sp., as Biofertilizer and production of phytohormones, ACC deaminase, phosphate solubilisation, etc.)
Plant endosymbionts (e.g. *Rhizobium* for biological nitrogen fixation)
Biocontrol agents (e.g. *Bacillus* sp. as pathogens of weeds, fungi, insects through production of antibiotics, siderophores, lytic enzymes)
Inocula to restore soil health and nutrient release (e.g. *Pseudomonas* sp. as phosphate solubilizer)
Source of genes for plant improvement (e.g. insect resistance)
Rhizosphere bacteria for disease suppression
Crop improvement
Sustainable agriculture
Land reclamation
Reference cultures for food safety testing, quarantine and trade
Reference cultures for animal and plant disease testing
Enzymes for food improvement and processing
Cultures for food fermentations and nutritional supplements
Innovative biodiscovery
Locust and other insect pests (*Metarhizium anisopliae*, *Beauveria bassiana, Isaria fumosorosea,* and *Verticillium lecanii*)
Insect pests belonging to Lepidoptera, Diptera, and Coleoptera (*Bacillus thuringiensis*)

Table 1.4 Microbial resources contribute to industrial and nutritional security

Acetobacter as preservation and fermentation
Arthrobacter as preservation and fermentation
Bacillus as thermo stable enzyme production, fermentation and preservation
Brevibacterium as preservation and fermentation
Clostridium as fermentation and preservation
Enterococcus as fermentation and preservation
Lactobacillus as fermentation and preservation
Lactococcus as fermentation and preservation
Leuconostoc as fermentation and preservation
Listeria as fermentation and preservation
Microbacterium as fermentation and preservation
Micrococci as fermentation and preservation
Pediococcus as fermentation and preservation
Streptococcus as fermentation and preservation

1.4.2 Ex Situ Conservation Methods

There are many approaches for microbial conservation which have been modified from time to time by individual microbial resource centres based on feasibility and availability of resources. The microbial conservation could be achieved either by

Table 1.5 Microbial resources contribute to various pharmaceutical products

Ethanol—*Escherichia coli*
Butanol—*Saccharomyces* sp.
Bioethanol—*Zymononas mobilis*
Battery active material (MnO_2)—*Rhizobium* sp.
Bioinsecticide (Mosquitocidal)—*Bacillus sphaericus*
Phage based biosorbent kit—*Salmonella enteritidis* (with lux genes)
Recombinant polypeptide—*Streptomyces* sp.
D-Mannitol/D-Fructose—*Leuconostoc*
Riboflavin—Engineered *Bacillus subtilis*
Biomining (Uranium, Copper)—*Bacillus* sp., *Pseudomonas* sp.
Deacetoxycephalosporin C—*Acremonium chrysogenum* (genetically engineered)
Evernimicin—*Micromonospora carbonacea*
Penicillin—*Penicillium* spp.
Cephalosporins—*Cephalosporium* spp.
Cyclosporin—*Tolypocladium* spp.
Tetracycline, actinomycine, and adrimycine—*Streptomyces* spp.
Polymyxin B and bacitracin—*Bacillus* spp.

Table 1.6 Microbial resources contribute to various enzymes production for industrial and research application

Cellulase/Hemicellulase—*Volvariella volvacea*
Ligninolytic enzymes—*Coriolus versicolor*
Laundry detergent (alkaline cellulase/protease)—Alkaliphile microorganisms
Cyclomaltodextrin gluanotransferase—Alkaliphile microorganisms (for cyclodextrin production)
Extracellular lipase—*Pseudomonas aeruginosa*
Keratinase—*Bacillus licheniformis*
Alpha-galactosidase—*Aspergillus awamori* and *A. oryzae*
Amylase—*Bacillus circulans*
Extracellular deoxyribonuclease—*Streptomyces thermonitrificans*
Beta-xylosidase (thermostable)—*Thermomonospora curvata*
GTP cyclohydrolase II/3, 4-DH-2-B-4-PS—*Bacillus subtilis*
L-Aspartate beta-decarboxylase—*Alcaligenes faecalis*
Creatininase—*E.coli*
Proteinase—*Tritirachium album*
Serine hydroxymethyltransferase—*E. coli*

detention of the cell vitality to minimum (hypobiosis state) or subjecting the microorganisms to drying, freezing at low temperatures and lyophilization (an anabiotic state) (Uzunova-Doneva and Donev 2005). Several methods are used for ex situ conservation of microorganisms (Gorman and Adley 2004; Berner and Viernstein 2006; Morgan et al. 2006; Smith et al. 2008; Winters and Winn 2010;

Fig. 1.2 Preservation of microorganisms through lyophilization and cryopreservation

Fu and Chen 2011; Garcia 2011; García-García et al. 2014; Mutlu et al. 2015), for example, repeated subcultivation, preservation on agar beads (refrigerator temperatures 5 °C), storage in sterile soil, mineral oils (preventing dehydration and slows down metabolic activity by reducing oxygen availability), silica gel storage, spray-drying, fluidized bed drying, freezing (ultralow temperatures), cryopreservation, lyophilization (freeze-drying), liquid-drying, desiccation, induced anhydrobiosis, vitrification (a non-equilibrium, ultra-rapid cooling technique), sterile distilled water, water-salt solutions, and gelatin discs. Among these methods, cryopreservation and lyophilization (Fig. 1.2) have been considered most valuable, widespread and reliable methods for long-term and stable storage of important microorganisms (Smith and Ryan 2012; Singh and Baghela 2017).

1.4.3 In Factory Conservation

In factory method of microbial conservation means keeping them in normal conditions for practical use. As a principle, two different ways of conservation strategy can be executed: dynamic and static conservation. Dynamic conservation does not impose much restriction on the use of strains, except for the introduction or mixing with cultures of different origin. Static conservation is very restrictive and tries to maintain strains under its original conditions and avoid any kind of changes.

1.5 Preservation of Whole Microbial Community

After successful preservation of pure cultures in culture collections (ex situ) and laboratories, researchers are now trying to preserve whole microbiomes that are involved in certain activities to be useful for biotechnological purposes in agriculture and industry Efforts have been made to preserve selected specific microbial communities. For example, (1) Rothrock et al. (2011) developed a simple preservation protocol for the long-term storage and reactivation of the anammox biomass in which anammox biomass was first frozen in liquid nitrogen (−196 °C) followed by lyophilization in skimmed milk media without glycerol. This is the first report of the successful reactivation of anammox biomass preserved via sub-zero freezing and/or lyophilization. (2) Kerckhof et al. (2014) evaluated the effect of different cryoprotectants on preservation of community structure and functions of several microbiomes. They demonstrated that the use of DMSO, tryptone and soy broth preserved the better functionality than unpreserved cells. (3) Tatangelo et al. (2014) have evaluated the effect of different preservation methods on assessment of bacterial community structure on soil and water samples. (4) Yu et al. (2015) preserved whole microbial communities involved in degradation of switch grass with DMSO and glycerol and showed that samples preserved with DMSO and glycerol experience a consistent shift in community composition though dominant microorganisms were retained in the active community. Despite shifts in the community with storage, the samples were active upon revival under thermophilic and high-solid conditions. (5) Weißbecker et al. (2017) collected soil samples from grasslands of Germany and immediately freeze-dried and stored at 4 °C that allowed preservation of microbial community for 7 days and thus enabled transportation of samples across the continents.

1.6 Preservation of Rust Fungi

Rust fungi are a well-known and economically important group of plant pathogens which are comprised of more than 120 genera and 6000 species. *Puccinia graminis*, the causal agent of stem rust, is well-known for causing devastating epidemics in most of the wheat-grown regions of the world. A new highly virulent strain (Ug99) threatened wheat production worldwide without affecting India as most of the wheat varieties are resistant to Ug99. Similarly, epidemics of poplar leaf rust, *Melampsora* spp., have been a major constraint to the development of bioenergy programmes based on domesticated poplars which resulted in the lack of durable host resistance. Rust fungi are obligate biotrophic parasites with a complex life cycle often including two phylogenetically unrelated hosts. They have evolved specialized structures, haustoria, formed within the host tissue to efficiently acquire nutrients and suppress host defence responses (Duplessis et al. 2011). Preservation of rust fungi was demonstrated by Ryan and Ellison (2003) wherein they reported preservation of *Puccinia spegazzinii*, identified as potential biological control agent to the invasive weed *Mikania micrantha* (Asteraceae). The embedded teliospores and delicate basidiospores of this microcyclic rust are not amenable to direct plunge freezing. In situ cryopreservation technique was the only way out for the long-term cryopreservation of the ten isolates tested. Material from either petiole or stem tissue remained viable after cryopreservation, determined by the ability of the material to produce basidiospores, but infection of the host plant by these basidiospores produced from previously cryopreserved teliospores, embedded in leaf petioles, was not achieved. Garcia et al. (2007) developed a method for long-term preservation of uredospores of *Puccinia melanocephala*, causal organisms of rust in sugarcane. The best results were obtained when uredospores, collected using a vacuum pump from naturally rusted leaves, were dehydrated in silica gel followed by storage at −20 and −80 °C. Testing even after 1 year of storage, uredospores induced rust symptoms on inoculated plants at levels of severity which is adequate to start screening test for sugarcane resistance to rust. Salustiano et al. (2008) evaluated the viability and infectivity of urediniospores of *Puccinia psidii* multiplied on *Syzygium jambos* after preservation in different conditions, like liquid nitrogen (−196 °C), deep freezer (−80 °C), refrigerator (5 °C), BOD incubator (25 °C) and as herbarium specimen (25 °C) for 150 days. The viability and infectivity of urediniospores stored in BOD and as herbarium were not successful, while the urediniospores stored in deep freezer, liquid nitrogen and refrigerator maintained their viability and infectivity for 150 days. Tibolla et al. (2012) reported preservation methods of urediniospores of *Puccinia kuehnii*, in which leaves with symptoms of orange rust were harvested from the sugarcane cultivar SP89 1115 and urediniospores were extracted from the leaves with the aid of a vacuum pump. Dehydration method in silica gel followed by storage at −80 °C was found to allow viability of urediniospores (1.2%) after 180 days.

Table 1.7 Some fungi and bacteria possessing new processes discovered in recent past

Bacteria	Processes	References
Candidatus *Brocadia caroliniensis*	First anammox bacteria identified	Kuenen and Jetten (2001)
Fusarium oxysporum MT-811	Dissimilatory reduction of nitrate to ammonium noticed in this fungus under anaerobic conditions	Zhou et al. (2002)
Saccharomyces cerevisiae CEN.PK 102-5B	Demonstrates an alternative metabolic pathway for butanol and isobutanol production in the yeast *S. cerevisiae*, using glycine as a substrate	Branduardi et al. (2013)
Methylomicrobium alcaliphilum 20Z	Demonstrates that methane assimilation is coupled with a highly efficient pyrophosphate-mediated glycolytic pathway, which is under oxygen limitation participates in a novel form of fermentation-based methanotrophy	Kalyuzhnaya et al. (2013)
Pseudomonas deceptionensis M1T, *Rhizobium leguminosarum* J391 and *Bradyrhizobium diazoefficiens* USDA 110	Microbial catabolism of the marine osmolyte dimethylsulphoniopropionate (DMSP) is thought to be the major biological process generating dimethyl sulphide (DMS). Reporting the discovery and characterization of the first gene, *mddA*, for DMSP-independent DMS production by bacteria. This gene, *mddA*, encodes amethyltransferase that methylatesmethanethiol and generates DMS	Carrion et al. (2014)
Actobacterium woodii	Novel mode of lactate metabolisms involving bifurcating lactate dehydrogenase (LDH) solve low energy substrate lactate in anaerobes without cytochrome, quinines or other membrane-soluble electron carriers and give rationale for the presence of the Rnf complex in the anaerobes	Weghoff et al. (2014)
Candidatus Nitrospira inopinata	Discovery of Comammox: One step nitrification by this single bacterium	Daims et al. (2015) van Kessel et al. (2015) Pinto et al. (2015) Camejo et al. (2017)
Nitrospira moscoviensis	*N. moscoviensis* possesses genes encoding for a urease and cleaves urea to ammonia and CO_2. Ureolysis was not reported earlier in nitrite oxidising bacteria (NOB) but later on discovered ureolytic process in *N. moscoviensis* to supply ammonium to ammonium oxidizing bacteria (AOB) lacking urease	Koch et al. (2015)

(continued)

Table 1.7 (continued)

Bacteria	Processes	References
Clostridium thermocellum DSM1313-derived strains	Demonstrates that *C. thermocellum*, a cellulose degrading bacterium, can fix CO_2 while growing predominantly on cellobiose	Xiong et al. (2016)
Bacteroides thetaiotaomicron	A gut bacterium degrades the most structurally complex glycan, known containing 13 different sugars and 21 distinct glycosidic linkages, utilizing previously undiscovered enzyme families and novel catalytic activities	Ndeh et al. (2017)
Candidatus *Thiolava veneris*	A novel bacterium, discovered from volcanic eruption sites of the Tagoro, Canary Archiplego, Spain, utilizes both organic and inorganic carbon released from volcanic degassing	Danovaro et al. (2017)

1.7 Conservation of Microbes Possessing Novel Processes/Traits

Conservation initiatives have evolved since the late twentieth century from an initial focus on protection of pristine areas and particular (charismatic) species of animals and plants to a more holistic ecosystem-based approach (Salafsky et al. 2002). This planet is home of innumerable microbes with huge metabolic diversity, which operate a number of known and unknown processes and thereby sustaining life on the earth. Present decade has witnessed a number of novel processes discovered in different niches governed by the existing microorganisms or in the newly identified microbes (Table 1.7). Such processes were not known earlier, and were an enigma for the researchers on how the ecosystems are sustained in different niches where human mind cannot predict such processes based on existing knowledge. The microbial resource centres are preserving microbes ex situ following various methods on long-term basis. However, still many microbes require specific methods for long-term preservation and to maintain their viability. Despite the availability of established protocols, only some specific culturable microbes are preserved in some of the resource centres of the world. It is because of the large gap in knowledge of microbial world and methodologies to preserve them. Discovery of new processes/traits in microbes and their ex situ and in situ conservation is of a great challenge to researchers. Hence, comprehensive conservation strategies need focus on conservation of microorganisms in their habitats and ex situ too. In this context, once Tom Curtis (2006) said that '*if the last blue whale choked to death on the last panda, it would be disastrous but not the end of the world. But if we accidentally poisoned the last two species of ammonia oxidizers, that would be another matter. It could be happening now and we wouldn't even know*' emphasizing conservation of niches associated microbes particularly nitrifying bacteria for existence of this world (Fig. 1.3). In recent past, discoveries of many new processes, for example, anammox

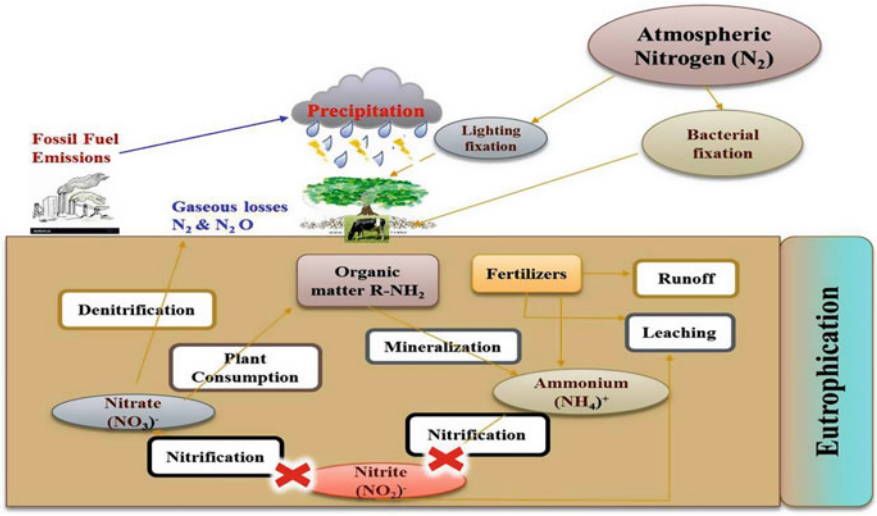

Fig. 1.3 Stopping nitrification jeopardise life on the earth (Source: Curtis 2006)

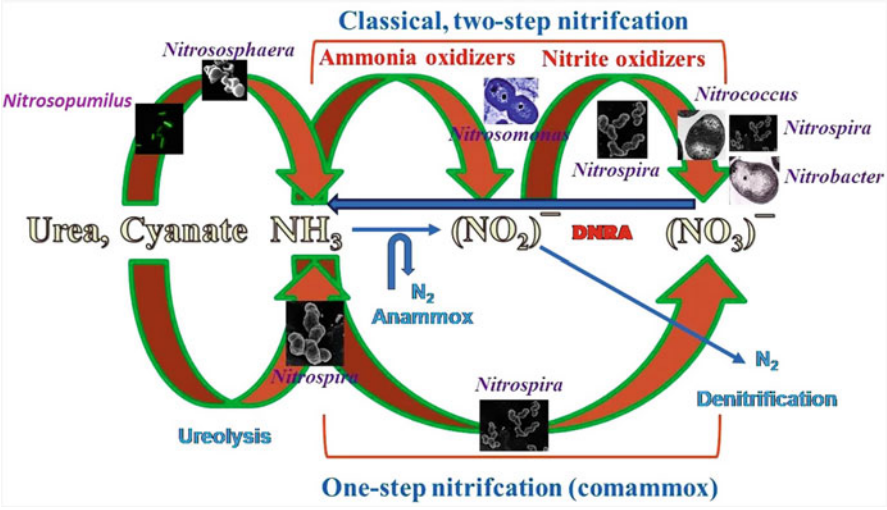

Fig. 1.4 Diagram depicting different processes of nitrogen cycles. *DNRA* Dissimilatory nitrate reduction to ammonium, *Anammox* Anaerobic ammonium oxidation (Sources: Daims et al. 2015; Koch et al. 2015; Lowson and Lucker 2018; Vaishnav et al. 2018)

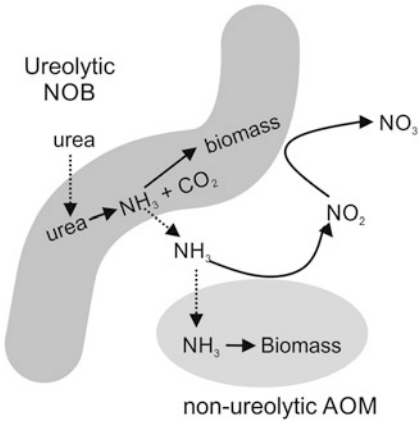

Fig. 1.5 Operation of ureolytic process by nitrite oxidising bacteria (NOB) and utilization of NOB's generated-ammonium by non-ureolytic ammonium oxidizing microorganisms (AOM): an example of reciprocal feeding (Source: Koch et al. 2015)

(anaerobic ammonium oxidation), ureolysis by nitrifying bacteria and comammox (one step aerobic ammonium oxidation by single bacterium; *Nitrospira*), DNRA (dissimilatory nitrate reduction to ammonium) and novel mode of lactate metabolisms in anaerobes, are evidence of continued occurrence of many microbial processes, which were consistently ignored knowingly and unknowingly for years (Figs. 1.4 and 1.5). Thus, ex situ preservation of such microbes is important for studying ecological processes and their uses in teaching, research, biotechnology and agriculture for future generation.

1.8 Biological Diversity Act 2002 and Rules 2004

Before Biological Diversity Act came in to existence, three legislations, namely, Indian Forest Act, 1927; Wildlife Protection Act, 1972; and Environment Protection Act, 1986, were being followed for protecting the environment and forest. The first attempt to bring the biodiversity into the legal framework was made by the introduction of the Biodiversity Bill, 2000, which was passed by both the houses of Parliament in December 2002. The objectives of the Act are (1) conservation of biological diversity, (2) sustainable use of its components and (3) fair and equitable sharing of the benefits arising out of utilization of genetic resources. Apart from these main objectives, the Act has also enforced some of the terms of CBD with inclusion of the following provisions: (1) to set up the National Biodiversity Authority (NBA), State Biodiversity Board (SBB) and Biodiversity Management Committees (BMCs), (2) to respect and protect knowledge of local community traditional knowledge related to biodiversity and (3) to conserve and develop areas of importance from the standpoint of biological diversity by declaring them biological diversity heritage sites.

The Biological Diversity Act, 2002, and Rules, 2004, are implemented by the National Biodiversity Authority (NBA) at the national level, State Biological Board

Table 1.8 Designated National Repositories (NDRs) of India for deposition of safe deposit of type strains, reference strains and samples of microbial resources accessed by foreign citizens

Name of the Institution for deposition of the microbial resources	Category of microbial resources
National Bureau of Agriculturally Important Microorganisms, Mau Nath Bhanjan, UP	Agriculturally important microorganisms (Bacteria, fungi, actinomycetes and cyanobacteria)
Institute of Microbial Technology, Chandigarh	Microorganisms for industrial use (actinobacteria, bacteria, fungi and yeasts)
National Institute of Virology, Pune, Maharashtra	Viruses
Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi	Fungi
National Centre for Microbial Resources (Formerly, Microbial Culture Collection), Pune, Maharastra	Microbes (bacteria and fungi including yeasts)

Source: National Biodiversity Authority, India; www.nbaindia.org

(SBB) at state level and Biodiversity Management Committees (BMCs) at local levels. Some of the major functions of these authorities are:

- To regulate activities, approve and advice the government of India on matters relating to the conservation of biodiversity, sustainable use of its components and equitable sharing of benefits.
- To grant approval under Sections 3, 4 and 6 of Biological Diversity Act, 2002.
- To notify areas of biodiversity importance as biodiversity heritage sites under this act and perform other functions as may be necessary to carry out the provisions of the Act.
- To take measures to protect biodiversity of the country as well as to oppose the grant of intellectual property rights to any country outside or any biological resources obtained from India.

The National Biodiversity Authority deals with the requests for access to the biological resources as well as transfer of information of traditional knowledge to foreign nationals, institutions and companies. Through this way, piracy of intellectual property rights in and around India is prevented, and it also saves the indigenous people from exploitation.

The NBA has declared Microbial-Designated National Repositories (M-DNRs) under 'Section 39' as an important aspect of infrastructure for biodiversity conservation. These M-DNRs provide various services such as preservation and maintenance of type and reference strains, genomes of organisms and information relating to heredity and function of microbial system (Table 1.8). Most recently, NBA has constituted a 'core expert group' for developing guidelines for identification of repositories for plant, animal and microbe under Section 39 of the BD Act 2002. This is the first ever approach to intensify identification of more repositories for India.

In another effort, NBA has developed Guidelines for Selection and Management of the Biodiversity Heritage Sites (BHS) under 'Section 37' of BD Act 2002 in order to strengthen the biodiversity conservation in traditionally managed areas and to stem the rapid loss of biodiversity in intensively managed areas, which need special attention (www.nbaindia.org/content/106/29/1/bhs.html; accessed on 01 August 2018). Such areas often represent a positive interface between nature, culture, society and technologies so that both conservation and livelihood security can be achieved. The BHS can be defined briefly as 'a well-defined areas that are unique, ecologically fragile ecosystems having rich biodiversity, high endemism, presence of rare and threatened species, keystone species, species of evolutionary significance, wild ancestors of domestic/cultivated species and fossils with or without long history of human association with them'. So far, 11 BHS were identified, (1) Nallur Tamarind Grove, Devanahalli, Bengaluru, Karnataka; (2) Hogrekan, Chikmagalur, Karnataka; (3) UAS, GKVK campus, Bengaluru, Karnataka; (4) Ambaraguda, Shimoga; (5) Glory of Allapalli, Gadchiroli, Maharashtra; (6) Tonglu, Darjeeling, West Bengal; (7) Dhotrey, Darjeeling, West Bengal; (8) Dialong Village, Tamenglong, Manipur; (9) Ammeenpur Lake, Sangareddy, Telangana; (10) Majuli, Majuli, Assam; and (11) Ghariyal Rehabilitation Centre, Lucknow, Uttar Pradesh have been selected to conserve flora and fauna of the respective areas. However, so far there is no area that has been selected which is rich in unique and rare microorganisms that can be utilized for many purposes of human benefit. The main drawback of this guideline is that it does not give any emphasis on selection of sites which are microbially diverse and rich.

1.9 Flaw in BD Act 2002

The major drawback in this act is that it does not give sufficient consideration to conservation; rather it lays more emphasis on preventing profit-sharing from the commercial use of the biological resources. It is true that the foundation of this act was laid to prevent biopiracy by the developed nations. However, the major aim of this act is to protect the biodiversity that required to be considered on priority basis. Therefore, revision in BD Act is highly needed.

1.10 Nagoya Protocol on Access and Benefit Sharing

The CBD was signed by 150 government leaders at the Earth Summit, Rio de Janeiro, in 1992 that came into force in 1993. Article 3 of CBD has recognized that states have sovereign right to exploit their own biological resources and thus provider country has to control access of biological resources by other user countries. Article 15 of the Convention recognizes sovereign rights of provider country over bioresources expect a fair and equitable share of any benefits arising out of their utilization for commercial purposes from users. To facilitate these aims, each member country is required to enact legislation to manage and grant permission to

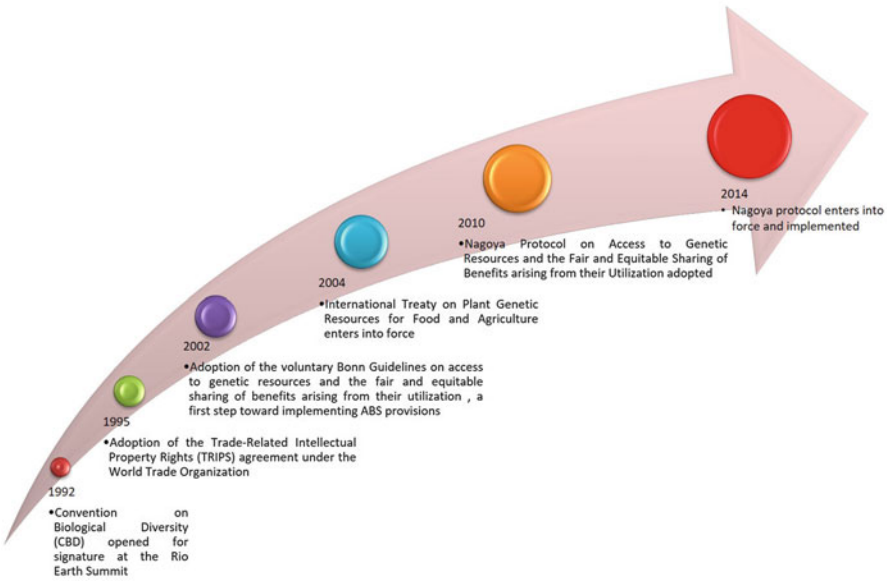

Fig. 1.6 Evolution of Nagoya Protocol on access and benefit sharing of genetic resources (Source: modified from Wynberg and Laird 2018)

the users to access samples from the country where bio-resource is available by seeking and receiving prior informed consent (PIC) and mutually agreed term (MAT) from competent authority of the country. To accomplish this goal, CBD has brought a supplementary global agreement called 'the Nagoya Protocol (NP) on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization' (https://www.cbd.int/convention/). This protocol provides a legal framework for the effective implementation of one of the three objectives of the CBD: the fair and equitable sharing of the benefits arising out of the utilization of genetic resources. The Protocol was adopted on 29 October 2010 in Nagoya (Japan) and come into force on 12 October 2014 (Fig. 1.6). Implementation of the CBD obligates each country to set up their own regulations and restrictions, which in turn resulted in an unmanageable multitude of national regulations. By designing common ways of organizing access to resources, the NP aimed at easing access to countries' genetic resources and ensuring that potential benefits arising from the use of these resources would be shared with provider countries. Importantly, the CBD and the NP are legal binding agreements, and countries that sign them are obligated to implement their provisions nationally. After NP has come into force, new national laws and regulations providing requirements for access to genetic resources are changing traditional views on collection, deposition and distribution of microorganisms (Fig. 1.7).

In order to implement NP globally, CBD had established a platform called 'Access and Benefit-sharing Clearing-House (ABS Clearing-House)' at Montreal, Canada, for exchanging information on access and benefit sharing under Article

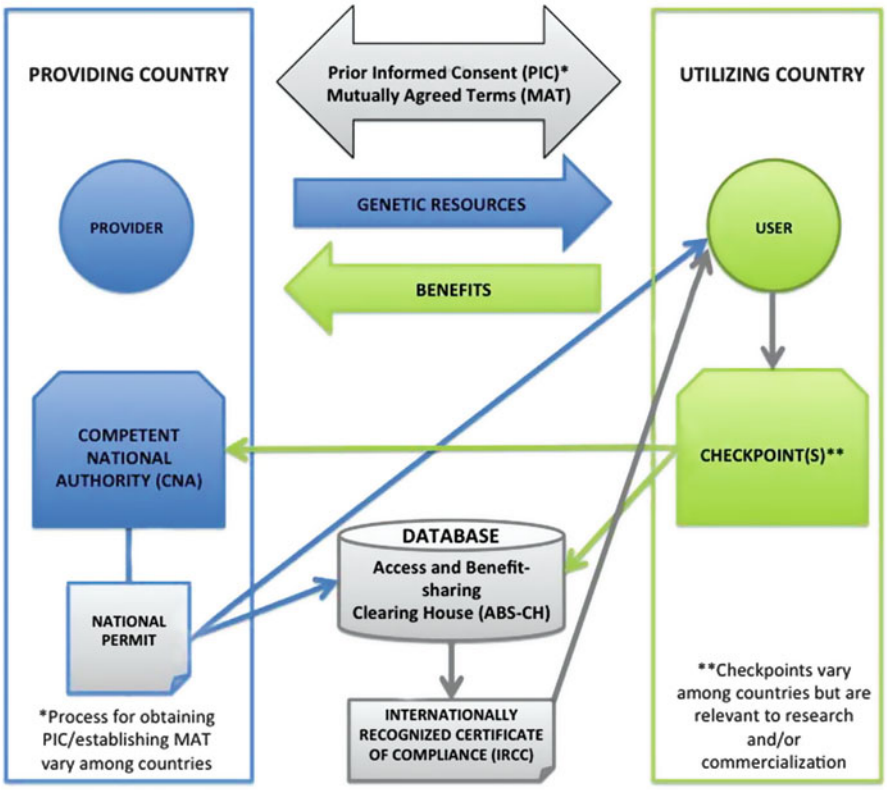

Fig. 1.7 Compliance mechanisms of access and benefit sharing of genetic resources under Nagoya Protocol

14 of the Protocol, as part of the Clearing-House of the Convention established under Article 18, paragraph 3 of the Convention (Fig. 1.8). The ABS Clearing-House is a key tool for enhancing legal certainty and transparency on procedures for access and benefit sharing and for monitoring the utilization of genetic resources along the value chain, involving internationally recognized certificate of compliance. By hosting relevant information regarding ABS, the ABS Clearing-House will offer opportunities for connecting users' and provider's country of genetic resources and associated traditional knowledge. Out of the 198 parties of CBD, 171 have established the National Focal Points (NFP) for ABS, 76 have formed Competent National Authority (CNA); 216 have brought legislative, administrative or policy measures (MSR); and 43 have set up national databases and websites (NDB) (https://absch.cbd.int/; accessed on 01 August 2018).

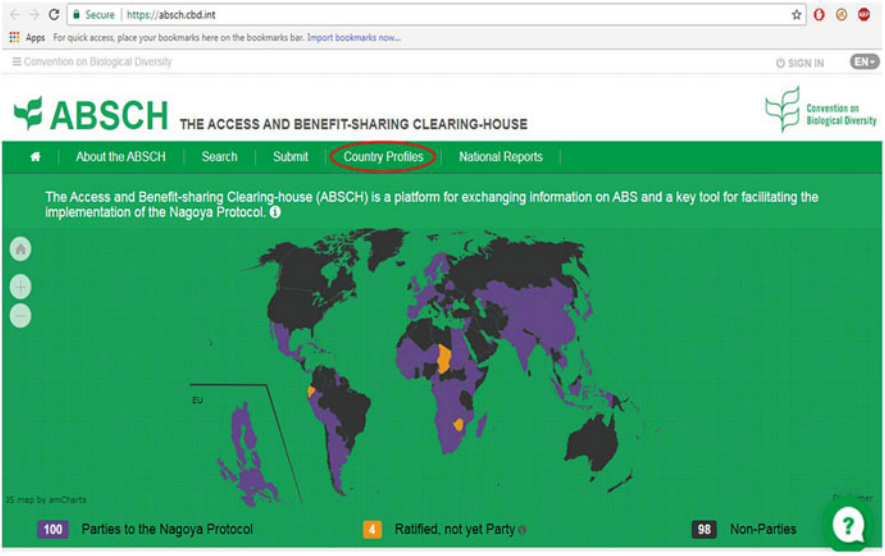

Fig. 1.8 A floor for exchange of information and facilitation to the party on ABS

1.11 View of Selected Stakeholders on 'DSI on Genetic Resources' in Relation to ABS Regulations

Digital sequence information (DSI) is considered an important information that originates by the analysis of the data contained in a digital file with precise order of nucleotides, amino acids or molecular structure of protein. The main function of these sequences is the storage and transmission of genetic information. The issue of digital sequence information on genetic resources emerged as a cross-cutting debatable issue during the 'Conference of the Parties 13 (COP 13) to the Convention on Biological Diversity' in Cancún, Mexico, 4–17 December 2016 (CBD/COP/DEC/XIII/16 dated 16 Dec. 2016). Representatives of 196 nations agreed during the conference that any potential implications of the use of digital sequence information on genetic resources in reference to three objectives of the CBD and the objective of the Nagoya Protocol will be considered at the next meetings of the governing bodies of the Convention and the Nagoya Protocol, in 2018 (COP 14 and COP-MOP 3). Based on the decision during this conference, the fact-finding committee on this issue recently drafted a document called 'The Emergence and Growth of Digital Sequence Information in Research and Development: Implication for the Conservation and Sustainable Use of Biodiversity, and Fair and Equitable Benefit Sharing' (https://www.cbd.int/abs/DSI-peer/Switzerland-FOAG2.pdf) submitted to Secretariat of CBD for comments and suggestions by international community/member party in order to clarify terminology and concept and to assess the extent and the terms and conditions of the use of digital sequence information in the context of CBD and

Nagoya Protocol (NP). In terms of ABS mechanisms, party to NP, researchers, societies and research institutions have their own views. For example:

(a) *India*: As per definition of utilization of genetic resources under NP, utilization of genetic resources is not confined to research and development on the tangible genetic resources, but is extended to activities over the genetic and biochemical composition of resources which is nothing but expression of gene sequence whether intangible (digital) or tangible. Synthesizing DNA from accessed digital information would therefore fall within the scope of utilization under NP. Such utilization of accessed information qualifies for the application of ABS regulatory framework even though there is no physical access of the genetic materials (http://www.cbd.int/abs/DSI-views/India-DSI.pdf).

(b) *Australia*: Digital sequence information (DSI) on genetic resources is not defined under the convention. For the purposes of this submission, Australia defines 'DSI on genetic resources' as electronically held sequence information, which represents biological composition of "genetic material" as defined under the Convention. Australia considers digital sequence information, physical genetic resources and material as distinct entities. To consider DSI on genetic resources, the CBD and NP should require to redefine "genetic material" that must include "functional unity of heredity" or "gene" (http://www.cbd.int/abs/DSI-views/Australia-DSI.pdf).

(c) *Brazil*: Brazilian Law 13.123/2015 defines genetic resources as the genetic information from plant, animal and microbial species or any other species, including substances originating from the metabolisms of these organisms. Therefore, the law of access and benefit sharing recognizes access to dematerialized genetic resources in its framework without the need for access of genetic samples as such (http://www.cbd.int/abs/DSI-views/Brazil-DSI.pdf).

(d) *Japan*: Believe that accumulation, open access and free use of DSI will facilitate development of science and will benefit conservation and sustainable use of biological diversity (http://www.cbd.int/abs/DSI-views/Japan-DSI.pdf).

(e) *USA*: As part of research best practices, DSI and GSD (genetic sequence data) are openly available via international data repositories such as GenBank and the International Nucleotide Sequence Database Collaboration, as well as in journals found in print and online. These repositories and journals further encourage collaboration by providing a free flow of GSD to both researchers and the general public. The open access and collaboration are the key benefits of GSD; regulation of access to and sharing of GSD would likely lead to a significant reduction in data sharing through these and other such mechanisms. GSD regulation could also force changes to procedures for information management within laboratories, with consequent costs and other negative implications for innovation. These dynamics would stifle research, which would then hinder activity to further CBD and Nagoya Protocol objectives (http://www.cbd.int/abs/DSI-views/USA-DSI.pdf).

(f) *Global Genome Biodiversity Network (GGBN)*: Sequence data is neither a genetic material, nor is it a genetic resource. GGBN and its members distinguish

between the genetic materials we hold and make accessible to the scientific community and the data describing this material. We note that it is essential to distinguish between these concepts and definitions. We also challenge the view that simple comparisons of sequence data for non-commercial uses can be considered for utilization; further, we are concerned that including digital sequence data under the scope of the Nagoya Protocol will have a severe limiting effect on essential global science by making data unavailable (http://www.cbd.int/abs/DSI-views/GGBN-DSI.pdf).

1.12 Microbial Commons and ABS Regulation

The concept of microbial commons deals with sharing of microbial resources at institutional levels for addressing the common problems of global concerns for direct benefits to the society, for example, (1) stem rust race 15B and Ug99, (2) microbes with probiotic properties for human health and (3) bioprospecting of microbes from colder regions for their utilization in ice cream for the purpose of regulating freezing points (Dedeurwaerdere 2010). A regulation either separately or under the ambit of ABS needs to be developed in order to facilitate sharing of microbial commons at global level.

1.13 ABS Regulation in India

In pursuance of the Nagoya Protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization to CBD and BD Act 2002, the National Biodiversity Authority (NBA) had made regulations called 'Guidelines on Access to Biological Resources and Associated Knowledge and Benefit Sharing Regulations, 2014' which was enforced on 21 November 2014 (Fig. 1.9). The following sections of BD Act 2002 are dealing with above said purposes.

Section 3 Deals with any foreign person seeking to access any biological resource and associated knowledge including traditional knowledge from India for their commercial utilization (excluding conventional breeding, traditional practices and other practices of agricultural sciences) shall have to take prior approval from the NBA before access to such bio-resources. For this purpose, Form I or Form I with Form A is required to be filled up with prescribed fee.

Section 4 Person who wants to transfer any biological entity or results of research relating to bio-resources to any foreign national has to apply and obtain prior approval from NBA. To this purpose, Form II is required to be filled up.

Section 6 Requires Indians to seek prior approval from NBA for applying intellectual property rights anywhere in the globe. To seek prior approval, an applicant has to fill up Form III.

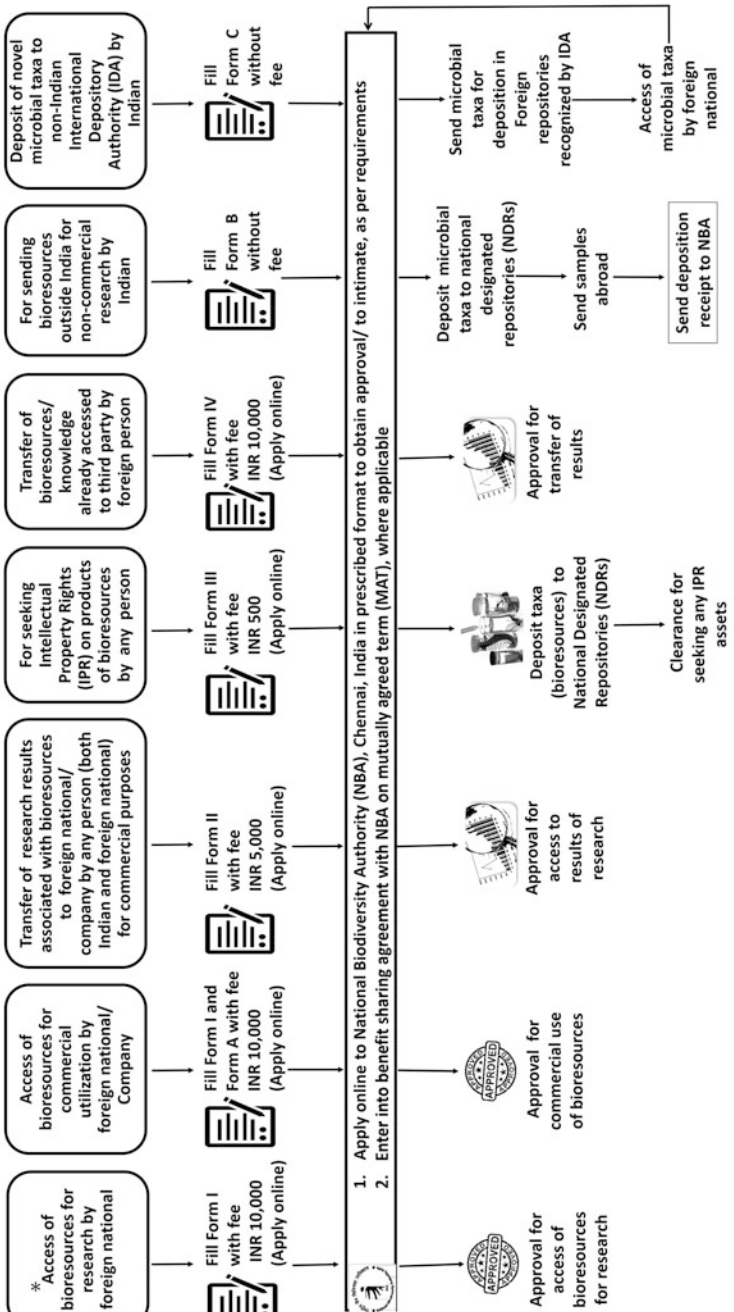

Fig. 1.9 Procedure for access and benefit sharing of bioresources in India (*Indian does not require approval of NBA for access of biological resources for research purpose

Section 7 A person wants to utilize the bio-resource would have to intimate the SBBs from state where the bio-resource is to be accessed. For intimation, an applicant needs to apply through Form IV. Indian person is not covered under above sections.

Section 40 Pursuant to Central Government notifying any item including biological resources normally traded as commodities under this section, the same becomes exempted from the purview of the Act. Currently six mushrooms are also under traded commodities in addition to other commodities of biological origin.

Form B To conduct non-commercial research or research for emergency purpose outside India by Indian researchers/government institutions under memorandum of understanding (MoU).

Form C To deposit the microorganism in non-Indian repository for claim of novel species. The Form C is for prior intimation purposes and to seek approval from NBA.

1.14 Problems Associated with Exchange of Microbial Genetic Resource for Taxonomic Purpose

In case of deposition of microbial culture in the non-Indian repository for claiming novel taxa, Indian researcher has to intimate NBA through 'Form C' in advance before sending cultures outside India. However, foreign researchers still need approval from the NBA before ordering strains of Indian origin from international repositories even for research purposes. Due to this regulatory enforcement by NBA, researchers and culture collections of India are now facing two serious problems: (1) type strains deposited in Indian culture collections are not recognised as valid deposit for publishing in certain journals since they are not available to researchers of foreign countries, and (2) foreign culture collections refuse to accept microbial cultures from Indian researchers to deposit because of the existing clause of seeking 'prior approval' to its further distribution for scientific scrutiny from NBA.

In order to facilitate compliance with the requirements of the NBA and to help researchers performing taxonomical characterization, the concerned Indian culture collections holding the particular strains may be authorized to take the initiative to share cultures worldwide by securing permission from NBA, instead of the researchers, having to seek that permission themselves. The existing regulations must be streamlined in order to facilitate taxonomic work in India.

1.15 Biosafety

Biosafety has become a major concern for several countries creating numerous activities to counter risk assessment, legislation and emergency response. The goal is to implement measures to protect against malicious use of microorganisms, their products, information and technology transfer. The issue to promote biosafety awareness was discussed in the second meeting of the Conference of Parties (CoP) held in November 1995 and established an Open-ended Ad Hoc Working Group on Biosafety. The working group was entrusted to prepare a draft protocol on biosafety focusing on transboundary movement of any living modified organism (LMO) resulting from modern biotechnology considered to have adverse effect on the conservation and sustainable utilization of biological diversity. After several years of negotiations, the Cartagena Protocol on Biosafety was finalized and adopted in Montreal on 29 January 2000 at an extraordinary meeting of the CoPs. Finally, protocol has been considered as important step forward that provides an international regulatory framework to reconcile the respective requirement of trade and environmental protection with respect to a rapidly growing global biotechnology industry. Cartagena Protocol thus creates an enabling environment to the environmentally sound application of biotechnology, making it possible to derive maximum benefit while minimizing the possible risks to the environment and to human health. Different articles (texts and annexes) of protocol states specific requirements to be complied by the party (Cartagena Protocol on Biosafety 2000). In this regard article 8(g) states that Parties should take up at national level, while Article 19, paragraph 3, sets the stage for the development of an international legal binding instrument to address the issue of biosafety.

1.16 Conclusion

Microbes have established their credentials over the years as promising goldmines for various applications. Despite countless facts about the role of microorganisms in the biosphere, they have been largely ignored and never considered in a real sense as part of conservation biology. However, careful examination and concerted efforts are required for conservation of microbial resources on sustained basis. In this regard the uses of conventional as well as modern tools play very important roles in order to minimize the loss of microbial diversity. Similarly, efforts are required to make the awareness about underlying causes of microbial diversity loss and their impact on various fields that include society, environment and agriculture with a view to reduce direct pressures on microbial diversity and to promote sustainable use. It has also been realized to improve the status of microbial diversity by safeguarding ecosystems and genetic diversity and enhance the benefits to all from microbial diversity and ecosystem services. Declining interests in teaching taxonomy and systematics is alarming and drawing serious attention of capacity building to future generation.

Besides, microbial resource centres are likely to have an increasingly important role in taxonomy research training, and these need to be recognized and funded accordingly. A national strategic plan is required to be developed for protecting microbial habitats that will help in meeting large gaps in our knowledge of the distribution and abundance of microbes. Besides, endangered microbial habitats of extreme environments need sound strategies for conservation on high priority basis. Overall, a road map of action plan with long-term financial provision by government and industry is essential for achieving the microbial conservation to an acceptable level. This review emphasizes the current status of conserved microbes, its conservation strategies, methodologies and regulatory framework at global level on conservation and sharing of microorganisms.

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