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MINI-REVIEW

Microbial cultivation and the role of microbial resource centers in the omics era

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Abstract Despite tremendous advances in microbial ecology over the past two decades, traditional cultivation methods have failed to grow ecologically more relevant microorganisms in the laboratory, leading to a predominance of weed-like species in the world's culture collections. In this review, we highlight the gap between culture-based and culture-independent methods of microbial diversity analysis, especially in investigations of slow growers, oligotrophs, and fastidious and recalcitrant microorganisms. Furthermore, we emphasize the importance of microbial cultivation and the acquisition of the cultivation-based phenotypic data for the testing of hypotheses arising from genomics and proteomics approaches. Technical difficulties in cultivating novel microorganisms and how modern approaches have helped to overcome these limitations are highlighted. After cultivation, adequate preservation without changes in genotypic and phenotypic features of these microorganisms is necessary for future research and training. Hence, the contribution of microbial resource centers in the handling, preservation, and distribution of this novel diversity is discussed. Finally, we explore the concept of microbial patenting and requisite guidelines of the "Budapest Treaty" for establishment of an International Depositary Authority.

Keywords Cultivation · Preservation · Microbial resource centers · Budapest Treaty

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Introduction

The influence of microorganisms on human life can be summarized succinctly. We need to breathe and to eat. We need clean water and clean energy, and we do not want to die of terrible diseases. All of these societal requirements are intimately intertwined with the capabilities of microorganisms. Microorganisms provide critical ecosystem services that keep our planet habitable, and their economic potential is limitless, especially in the areas of biotechnology and bioprospecting (Fig. 1). The majority of the gases that make up breathable air in Earth's atmosphere (nitrogen, oxygen) are generated by microorganisms (Walker 1980; Arrigo 2005). Microbes predominate over the global cycling of nutrients and the production of greenhouse gases, both of which act to regulate Earth's climate. The removal of harmful chemicals such as excess nitrogen fertilizers from aquatic environments is largely mediated by microbial processes. Microorganisms drive bioremediation and waste treatment strategies (Rawlings and Silver 1995; Arrigo 2005; Zaidi et al. 2009; Green et al. 2010; Lal et al. 2010; Singh et al. 2010; Kostka et al. 2011) and serve as a promising source for sustainable or renewable bioenergy in the form of biogas, bioethanol, biodiesel, and microbial fuel cells (Endy 2005; Lovely 2006; Gírio et al. 2010). Microbes have, for long, comprised a natural source of primary and secondary metabolites such as antimicrobials, growth hormones, immunosuppressants, natural herbicides, anti-inflammatories and antitumor compounds, organic acids, and vitamins (Challis and Hopewood 2003; Senni et al. 2011). To date, $>10^4$ different kinds of microbially generated metabolites have been discovered. Microbial-based bioplastics (polyhydroxybutyrate and polyhydroxyalkanoates) are emerging as a better alternative to petrochemical-based plastics as well as for biomedical applications like bone fixation and drug delivery (Chen and Wu 2005; Endy 2005; Verlindin et al. 2007). Microbial enzymes, especially those tolerant of extreme

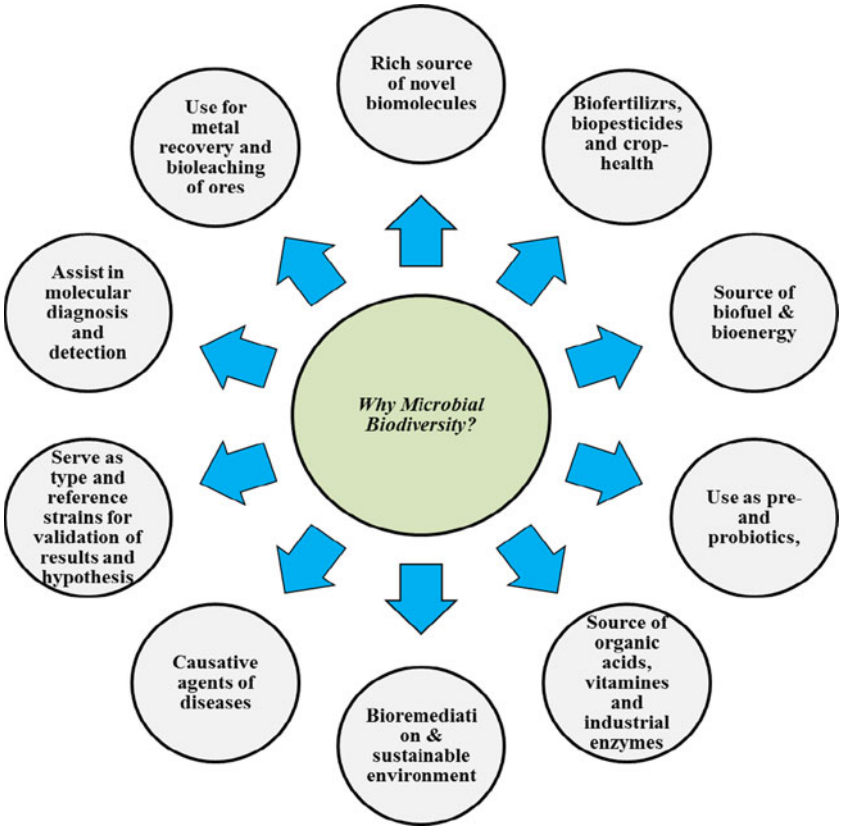

Fig. 1 Figure represents application of culturable diversity in different areas of research and development including agriculture, bioenergy, industries, ecosystem services, and development of novel therapeutics

conditions, are extensively used in industry for degradation of complex organics (Ogawa and Shimizu 2002) and in biotechnological applications (Gupta et al. 2002; Bouzas et al. 2006; Saeki et al. 2007; Unsworth et al. 2007). Lastly, the microbiome is emerging as an integral component to human health through promotion of digestion, protection of the host from establishment of pathogenic microorganisms, and homeostasis of host immune system. Microbes are the natural source of clinical compounds and work as pro- and prebiotics to improve the gut health (Colwell 1997; Lomax and Calder 2009). Despite their beneficial activities, microbes continue to cause devastating diseases in plants, animals, and humans that serve as an economic burden and create risk for human health and hygiene (Lupp 2007).

Though the development of high-throughput genetic sequencing and omics-based approaches have revolutionized microbiology, further developments in biotechnology and environmental research must be anchored by corresponding developments in the study of pure cultures. The metabolic potential of microbes in the laboratory or in ecosystem function can only be truly verified in studies of cultivated organisms. Thus, the isolation, characterization, and preservation of novel microbes are a requisite for the future growth of science and technology. This review article discusses the importance of microbial cultivation in current perspective when most of the microbiologists are moving toward omics. We highlight the loopholes of traditional cultivation approaches and give clues for the cultivation of not-yet cultured microbes. In contrast to recent articles on advances in cultivation approaches and culture resource centers (Alain and Querellou 2009; Emerson and Wilson 2009; Stackebrandt 2011; Heylen et al. 2012; Pham and Kim 2012; Stewart 2012), we focus on the concept of non-culturabilty and microbial preservation in the context of microbial resource centers (MRCs) and microbial patenting.

Is cultivation still relevant?

With the development of massively parallel sequencing technologies, conducting omics studies has become streamlined and inexpensive (Petrosino et al. 2009; Metzker 2010), and microbiologists are moving toward molecular techniques at the expense of more tedious cultivation-based approaches (Palleroni 1997; Gest 2001; Rappe et al. 2002; Stevenson et al. 2004; Giovannoni and Stingl 2007). Now the question arises: Is cultivation still relevant in the era of omics? The answer is undoubtedly affirmative. Though a vast amount of microbial diversity has been revealed since the advent of omics-based approaches, a huge knowledge gap remains between assessment of genomic potential and the assignment of function to genes or proteins (Wiebe 1998; Zengler et al. 2002; Giovannoni and Stingl 2007; Cardenas and Tiedje 2008). Metagenomics has successfully expanded

our view of microbial diversity and metabolic potential. However, the assembly and annotation of sequence information remains a daunting challenge, especially in highly diverse ecosystems. Despite tremendous progress in transcriptomics and proteomics, the physiology and metabolism of specific microbial groups cannot be determined based solely on omics data in complex ecosystems. Thus, it becomes increasingly important to cultivate and preserve representative organisms in the face of ever-expanding sequence-based estimates of microbial diversity (Palleroni 1997; Gest 2001; Rappe et al. 2002; Keller and Zengler 2004; Stevenson et al. 2004; Giovannoni and Stingl 2007).

Cultivation-based approaches can acquire rare microorganisms that are undetected by molecular methods and allow for the verification and testing of hypotheses of metabolic potential determined by the metagenomic data (Leadbetter 2003; Giovannoni and Stingl 2007; Bent and Forney 2008; Zengler 2009; Green et al. 2010). Cultivation and purification also provide new genome sequences that assist in designing of better primers and probes for the refinement of molecular detection methods. History shows that the majority of advances in basic and applied microbial science including physiology, biochemistry, genetics, medicine, diagnostics, and biotechnology are founded in studies of pure cultures. Even the interpretation of current sequence databases that serve as the basis for culture-independent studies is dependent on cultured microorganisms. The importance of reference strains in the study of physiology and functionality is well-documented (Janssen et al. 2010; Heylen et al. 2012). In the absence of reference strains, the authentication and cross-verification of traits or phenotype is not possible, and results may be called into question. Cultivation-based approaches not only provide reference strains for study of physiology, genetics, pathogenicity, and adaptation but broaden our view in the area of basic research and gives new organisms for novel metabolites, enzymes of industrial application. It bears repeating that studies of pure cultures serve as the backbone of molecular biology, microbial physiology, and the biotechnological revolution. Therefore, any discussion promoting the investigation of microbial diversity based solely on molecular approaches and excluding the concept of in vitro cultivation is premature, unjustified, and incomplete.

Concept of non-culturability

The gap between known microbial phyla and their culturable representatives is now clearly visible. An enormous diversity of not-yet cultured microorganisms is present in nature (Zengler 2009). Estimates show that out of 100 phyla established through phylogenetic analysis, only 30 contain cultured representatives (Alain and Querella 2009), further substantiating the concept of great plate count anomaly. Only a minor fraction (1–10 %) of available microbial diversity has been cultured (Amann 2000; Leadbetter 2003; Alain and Querellou 2009). The efficacy of molecular approaches and the challenges of cultivating ecologically relevant microorganisms have given birth to the labeling of oligotrophs and fastidious and recalcitrant organisms as “unculturable” or “nonculturable” (Amann 2000; Gest 2001; Leadbetter 2003; Giovannani et al. 2007; Alain and Querellou 2009). Potential reasons for the reluctance of researchers to pursue cultivation include the lack of growth of many microorganisms on nutrient-rich common laboratory media, a lack of interest and desire for new media formulation and optimization, and a paucity of individuals properly trained in studies of microbial nutrition and physiology. The challenge is to bring these recalcitrant microorganisms into the laboratory for future exploration. With well-designed strategies, hard work, patience, and a thorough knowledge of microbial physiology, representatives from a much longer list of phyla should be acquired (Gest 2001; Kamagata and Tamaki 2005).

Despite the daunting task, the desire and excitement for cultivation of recalcitrant and fastidious microorganisms remain (Fry 2000; Kaeberlein et al. 2002; Leadbetter 2003; Davis et al. 2005; Giovannoni and Stingle 2007; Dorit 2008; Tripp et al. 2008). For example, the most abundant heterotroph on the planet, *Pelagibacter ubique* SAR-11, was successfully cultivated and isolated 10 years ago using sterile sea water amended with low concentration of phosphorus and ammonium (Rappe et al. 2002). More recently, a number of *Acidobacteria* strains, which have been poorly represented in culture collections, were isolated by simply adjusting the solidifying agents, using natural carbon substrates and a longer incubation time (Kuske et al. 2002). The first mesophilic member of the Crenarchaea, *Nitrosopumilus maritimus*, now shown to be ubiquitously distributed worldwide, was also brought into cultivation within the past 10 years. These examples clearly demonstrate that the cultivation of novel microorganisms is alive and well (Fry 2000; Stevenson et al. 2004).

Loopholes of cultivation approaches

The subject of microbial cultivation appears simple on the surface, but a closer look reveals a multitude of complexities. Many factors, such as nutritional shock or substrate-accelerated death, inhibit the growth of newly acquired microorganisms on laboratory Petri dishes (Leadbetter 2003; Overmann 2006; Stevenson et al. 2004). We need to understand these complexities before attempting to cultivate novel or recalcitrant microorganisms.

Despite the major advances in cultivation described above, microbiologists often avoid the painstaking procedure of new media formulation and do not take sufficient care during sampling, transportation, and storage of the samples used as inocula. Furthermore, researchers do not show sufficient patience during incubation to acquire slow-growing microorganisms. Other pitfalls include a lack of patience to acquire slow-growing microorganisms, the use of nondiagnostic or complex culture media, and a lack of consideration of the physicochemical conditions of sampling sites (Gordon et al. 1993; Leadbetter 2003; Davis et al. 2005; Overmann 2006; Donachie et al. 2007; Giovannoni and Stingl 2007; Giovannoni et al. 2007; Cardnas and Tiedje 2008; Kim et al. 2008; Gest 2008; Alain and Querellou 2009; Zengler 2009). For these reasons, the majority of current cultivated microorganisms belong to fast-growing weed-like taxa such as the metabolically versatile *Gammaproteobacteria*.

Based on the growth pattern and survival potential, all the microorganisms are classified in two different categories: fast-growing weed-type of microorganisms, generally known as r-strategist, and slow-growing, ecologically more relevant k-strategist (Overmann 2006). While most natural habitats are oligotrophic, the transition of microorganisms from their oligotrophic natural habitat to nutrient-rich laboratory media inhibits cell growth or even kills them due to nutritional shock. Other than nutritional shock, overgrowth of fast-growing weed-type r-strategist and short incubation time (generally 48–72 h) generally deselects ecologically more valuable, slow-growing k-strategist type microorganisms. Most microbiologists generally use nutrient-rich complex media like nutrient agar, Luria-agar, and tryptic soy agar for cultivation work. Such media only support the growth of fast-growing weed-type of microorganisms while inhibiting the growth of slow-growing oligotrophic bacteria due to nutritional shock, thereby allowing the least cultivable diversity to be harvested (Leadbetter 2003; Stevension et al. 2004; Overmann 2006; Alain and Querellou 2009; Zengler 2009). In addition, disruption of inter- and intracellular communications as a consequence of cell separation or isolation during the process of in vitro cultivation induces the tendency of recalcitrance in microbial cells. Rapid growth of undesired microorganisms in the absence of inhibitory chemicals in culture medium generally suppress the growth of desired microorganisms and selects only a narrow range of microbial population in the plates. Several factors including the competition for niche and nutrients, production of inhibitory chemicals like bacteriocin and secondary metabolites, and accumulation of toxins are responsible for above phenomenon. Use of antifungal antibiotics for isolation of bacteria and antibacterial antibiotics for fungal isolation is a common practice in microbiology. Serial dilution of the samples and plating from different dilutions is another way to get the wide range of phylotypes. Serial dilutions restrict the growth of less populated fast growers at higher dilutions consequently provide more space and time for the emergence of dominant but slow-growing microorganisms.

Furthermore, the inability to simulate the conditions of natural habitats like nutritional environment, syntrophic interaction of coculture, symbiosis, and signaling in in vitro condition are the major factors responsible for low culturability of microorganisms (Gordon et al. 1993; Huber et al. 1995; Ohno et al. 1999; Rappe et al. 2002; Davis et al. 2005; Tyson and Banfield 2005; Giovannoni et al. 2007; Hughes and Sperandio 2008; Nichols et al. 2008; Tripp et al. 2008). The lack of technical advances in the field of cultivation and sensitive detection methods for low cell yield of microorganisms are also responsible to a certain extent. Use of limited range of electron donors and acceptors combinations, application of narrow set of culture conditions (temperatures, pH, salinity, pressure) during cultivation, and finally, lack of patience and desire to formulate new media skipped several culturable microorganisms to enter into the laboratories. A diagrammatic representation of these factors is given in Fig. 2.

Modern approaches of microbial cultivation

Using traditional cultivation strategies, we often miss the ecologically more relevant but slow-growing microorganisms in the culture and which are then termed non-culturable (Gest 2008). However, given the many complexities of microbial cultivation, several efforts for cultivation of novel microorganisms have been made (Table 1). These include novel media formulation and optimization, enrichment for specific group of microorganisms, cultivation mimicking the natural conditions using simulated environment, use of oligotrophic media and extinction to the dilution approach, development of in situ cultivation strategy to enhance the syntrophic interaction, and single-cell isolation using micromanipulator or tweezers (Huber et al. 1995; Frohlich and Konig 2000; Kaeberlein et al. 2002; Zengler et al. 2002; Leadbetter 2003; Hahn et al. 2004; Stevenson et al. 2004; Davis et al. 2005; Giovannoni and Stingl 2007; Giovannoni et al. 2007; Cardnas and Tiedje 2008; Gest 2008; Kim et al. 2008; Alain and Querellou 2009; Zengler 2009).

Up to some extent, the innovative approaches of cultivation brought the previously uncultured microorganisms in laboratory Petri dishes and increased the proportion of novel microorganisms substantially. The addition of signaling molecules like autoinducers and homoserine lactone in the culture medium increased the culturabilty of some bacteria (Bruns et al. 2002; Overmann 2006; Hughes and Sperandio 2008). Zengler et al. (2002) discovered the high-throughput method for cultivation of previously uncultured microorganisms using

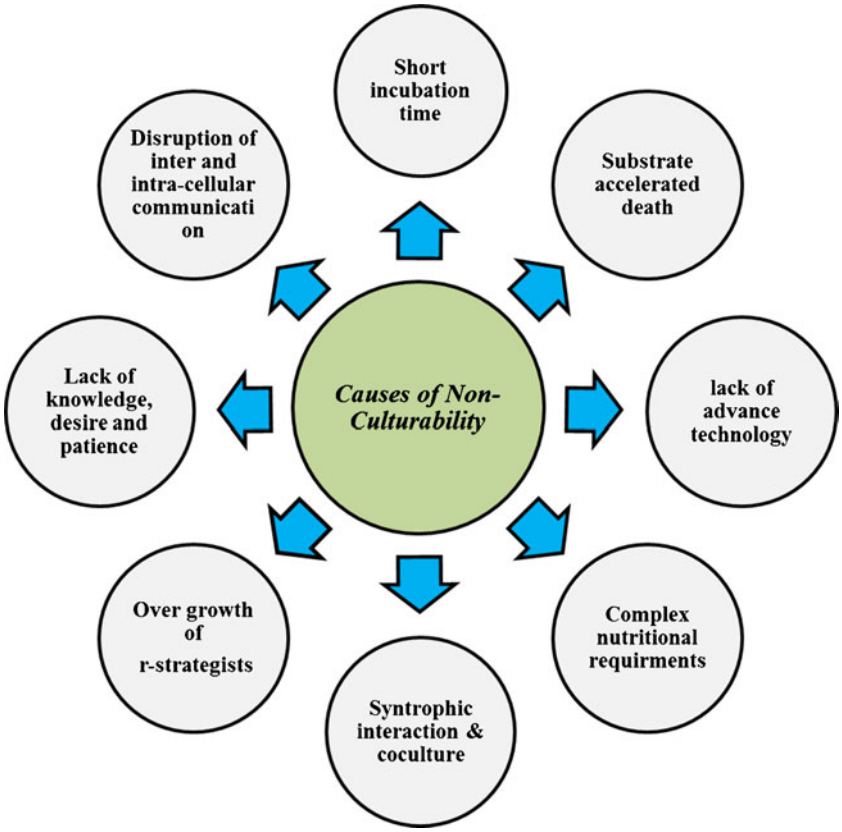

Fig. 2 Diagram shows the root cause of non-culturabilty that inhibits growth of microorganisms in laboratory Petri dishes

single-cell encapsulation inside gel microdroplets followed by growth under continuous flow of low-nutrient medium. Using the above method, they demonstrated several-fold higher culturability of microorganisms than previously used traditional

Table 1 Approaches for cultivation of uncultured diversity

Cultivation strategies	Methods	References
Simulated natural environment	Combined use of polycarbonate membrane, soil extract, and viable staining (soil substrate membrane system)	Ferarri et al. 2008; Ferrari and Gillings 2009
Oligotrophic condition and extinction to the dilution of the sample	Filtered marine water with extra source of nitrogen and phosphorous used as cultivation medium and extinction to the dilution of the samples	Connon and Giovannoni 2002; Rappe et al. 2002
Single cell separation and oligotrophic environment	High-throughput cultivation using microgel-droplet en capsulation and continuous flow of low-nutrient medium	Zengler et al. 2002
Extension of growth substrate, electron acceptors, inoculum size, and incubation time	Used wide sets of electron donors and acceptors. Expanded the range of incubation temperatures, increased inoculum size, and extended the incubation time of plates	Joseph et al. 2003; Davis et al. 2005; Köpke et al. 2005; Song et al. 2009
Simulated natural environment	Provided simulated natural environment using diffusion chamber	Kaeberlein et al. 2002
Single-cell separation	Used optical tweezers for single-cell separation and cultivation	Huber et al. 1995; Frohlich and Konig 2000
Filtration–acclimatization method (FAM)	Removed the fast growers, then gradually acclimated the cells to complex medium conditions	Hahn et al. 2004
High-throughput cultivation and screening	Used micro-Petri dish (a million-well growth chip) for the culture and high-throughput screening of microorganisms	Ingham et al. 2007
Inclusion of additional nutritional requirements and signaling molecules in culture medium	Co-culture, addition of culture supernatant, growth-promoting factors	Hughes and Sperandio 2008; Kim et al. 2008; Nichols et al. 2008; Ohno et al. 1999; Tripp et al. 2008

culture methods. Invention of diffusion chamber method by Kaeberlein et al. (2002) for the growth of novel microorganisms under simulated environment successfully cultivated several marine microorganisms previously uncultured using traditional approach. Furthermore, based on the concept of simulated natural environment, Ferrari et al. (2008) developed protocols for cultivation of terrestrial recalcitrant microorganisms. They used polycarbonate membrane as solid support and soil slurry as a source of carbon and natural component required for the growth of microcolonies.

Importance of microbial preservation and role of microbial resource centers

Now, it is evident that using only isolation and characterization of novel microbes is not enough, but preservation of the isolated strains without changes in phenotypic and genotypic features is mandatory for future reference, research, and new discoveries in the microbiology (Prakash et al. 2012). The work on isolation and characterization seems incomplete until the cultures are adequately preserved. Therefore, it is important that, after growing novel microbes, researchers should devise appropriate preservation protocol(s) suitable for them. Data from past indicate that most of the researchers do not bother about deposition of reference strains in public collections. In addition, several other factors, including the retirement of the employee, termination of projects, reduced funding, diversion of interest of researchers, and students moving out after the completion of their academic programs, result in a loss of such important microbes. Therefore, apart from preserving the cultures in their own laboratory, researchers should deposit them in public collection of two different countries to ensure its future accessibility for reference, research, and application (Ward et al. 2001; Coenye and Vandamme 2004; Field and Hughes 2005; Labeda and Oren 2008).

Benefit of microbial preservation and role of MRCs in ex situ preservation of microbial diversity are the topic of hot discussion (Ward et al. 2001; Emerson and Wilson 2009; Janssens et al. 2010; Heylen et al. 2012). Microbial depositories work as a knowledge hub for life science and backbone of biotech industries (Stern 2004; Cypess 2003; Janssens et al. 2010). Besides providing a home for ex situ preservation of microorganisms, MRCs also play an important role in development of protocols related to long-term preservation, checking the viability and authenticity of preserved cultures, providing training in the areas related to microbial handling, biosafety, and biosecurity, and offering reference strains to the scientific community for quality control and molecular biology research (Janssens et al. 2010; Stackebrandt 2011; Heylen et al. 2012).

In the past, microbiologists lost countless number of valuable cultures due to lack of microbial depositories. Later, the scientific community realized the role of microbial culture collections for collection, maintenance, distribution, and preparation of effective database of microorganisms for teaching, research, and industrial applications. Many countries are now trying to establish good culture collections or Biological Resource Centers (BRCs) or MRCs with well-equipped infrastructures, hiring the diverse range of expertise for ex situ preservation of its native biodiversity for future research, reference, and applications. We have compiled a list of some of world's well-established culture collections in order to provide an overview of their holdings and services to the readers (Table 2).

Culture collections not only provide the platform for the preservation of valuable gene pool of microorganisms for the future generations but also play a crucial role to support the field of microbial taxonomy, ecology, biodiversity, preservation, and genomics by their inherent research activities in above-mentioned areas (Malik and Claus 1987; Kamagata et al. 1997; Emersion and Wilson 2009; Stackebrandt 2011). Therefore, it is advisable to the curators of MRCs that they should maintain their scientific as well as service interest together, because both are inseparable and interdependent. The research and development section of collection center should improve the quality of service by providing up-to-date protocols and techniques, while provision of good services will, in turn, attract customers and generate revenue, thereby financially strengthening the collection. In his letter to the editor of *International Journal of Systematic and Evolutionary Microbiology (IJSEM)*, Stackebrandt (2011) emphasized the importance of culture collections and their role in deposition of strains for future generations. The decision of International Committee on Systematic of Prokaryotes for deposition of type strains in two publicly accessible culture collections prior to publishing them in *IJSEM* is appreciable. Stackebrandt (2011) also highlighted several other aspects including the importance of networking among the MRCs in order to share the information, resources, and transfer of microorganisms from endangered academic collections to well-established public collection in order to protect the previously stored valuable cultures like extremophiles, recalcitrant, under-represented, and fastidious types of microorganisms. He also recommended the use of new techniques for characterization of microorganisms and encouraged the curators for hiring of new expertise for the expansion of nature of MRCs to protect wide range of biodiversity. Apart from handling the well-representative phyla like *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*, MRCs should also focus on under-represented taxa including archaea, extremophiles, oligotrophs, fastidious, recalcitrant anaerobe, phototrophs, mycoplasmas, chlamydiae, verrucomicrobiae, Planctomycetes,

Table 2 List of the famous microbial culture collections of the world with their nature of holdings and types of services

Culture collections	Acronym	Types of holdings	URL	Country	Services
Agricultural Research Service Culture Collection	NRRL	Bacteria, fungi, yeast,	http://nrrl.ncaur.usda.gov/	USA	Deposit and distribution
American Type Culture Collection	ATCC	Bacteria, viruses, fungi, yeast	http://www.atcc.org	USA	GD, SD, IDA mycoplasma testing, cell authentication, DNA, RNA supply from ATCC strains
Belgian Coordinated Collections of Microorganisms/lmg Bacteria Collection	LMG	Fungi, yeasts, plasmids , DNA libraries, bacteria, mycobacteria, polar Cyanobacteria	http://bccm.belspo.be/index.php	Belgium	GD, SD, IDA identification and supply
Belgian Coordinated Collections of Microorganisms	BCCM/IHEM	Biomedical fungi and yeast	http://bccm.belspo.be/about/ihem.php	Belgium	GD, SD, IDA supply cultures and identification
Czech Collection of Microorganisms	CCM	Bacteria (aerobic), fungi, yeast, viruses, archaea	http://sci.muni.cz/ccm/index.html	Czech	GD, SD, IDA identification and supply
Microbial Type Culture Collection and Gene Bank	MTCC	Bacteria, fungi, yeast, *Actinomycetes*, Cynobacteria	http://mtcc.imtech.res.in	India	GD, SD, IDA identification and supply
Microbial Culture Collection	MCC	Bacteria, fungi, yeast, *Actinomycetes*	http://www.nccs.res.in/	India	GD, SD, IDA identification and supply
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH	DSMZ	Bacteria fungi, yeast, viruses, archaea, plasmid	http://www.dsmz.de	Germany	GD, SD, IDA identification and supply strains and DNA
Japan Collection of Microorganisms	JCM	Bacteria, fungi, yeast, *Actinomycetes*, archaea	http://www.jcm.riken.jp/	Japan	GD, supply of DNA and strains
NITE Biological Resource Center	NBRC	Bacteria, Archaea, yeasts, filamentous fungi, bacteriophages, algae	http://www.nbrc.nite.go.jp/e/index.html	Japan	GD, SD, IDA supply cultures and identification
Institute for Fermentation, Osaka	IFO	Bacteria, fungi, yeast, animal cell line, bacteriophage	http://www.ifo.or.jp/index_e.html	Japan	GD, SD, IDA supply cultures and identification
KCTC Korean Collection for Type Cultures	KCTC	Bacteria fungi, yeast, *Actinomycetes*	http://www.brc.re.kr/English/ekctc.aspx	Korea	GD, SD, IDA supply of DNA and strains
The Netherlands Culture Collection of Bacteria	NCCB,	Bacteria, bacteriophages, Plasmids	http://www.cbs.knaw.nl/nccb	Netherland	GD, SD, IDA supply of DNA and strains, identification
Collection de L'Institut Pasteur	CIP	Bacteria, plasmid, spores	http://www.crbip.pasteur.fr	France	GD, deposit, supply
All-Russian Collection of Microorganisms	VKM	Bacteria, fungi, yeast, archaea	http://www.vkm.ru	Russia	GD, SD, IDA supply strains, identification
CABI Genetic Resource Collection	IMI	Bacteria, fungi, yeast, extremophile	http://www.cabi.org	UK	GD, SD, IDA supply strains, identification
National Collections of Industrial Food and Marine Bacteria	NCIMB	Bacteria, phages, plasmids	http://www.ncimb.co.uk	UK	GD, SD, IDA supply strains, identification
National Collection of Type Cultures	NCTC	Bacteria, mycoplasma	UKNCC http://www.ukncc.co.uk/	UK	GD, SD, IDA supply cultures, DNA, RNA and mycoplasma test
National Collection of Plant Pathogenic Bacteria	NCPPB	Bacteria	http://www.ncppb.com/	UK	Deposit, supply, identification
Culture Collection, University of Goteborg	CCUG	Bacteria, fungi, yeast	http://www.ccug.se/	Sweden	GD, SD, IDA supply cultures, DNA, RNA and identification
	ACBR	Fungi, yeast	http://www.biotec.boku.ac.at/acbr.html	Austria	Deposit, supply identification

Table 2 (continued)

Culture collections	Acronym	Types of holdings	URL	Country	Services
Austrian Center of Biological Resources and Applied Mycology					
Brazilian Collection of Microorganisms from the Environment and Industry	CBMAI	Bacteria, fungi, yeast	http://www.cpqba.unicamp.br/cbmai	Brazil	GD, SD, supply cultures, and identification
Salmonella Genetic Stock Centre	SGSC	Bacteria, plasmid, virus	http://www.ucalgary.ca/□kesander	Canada	Deposit and supply
The International *Escherichia* and *Klebsiella* Centre (WHO)	IEKC, SSI	Bacteria	http://www.ssi.dk/sw1397.asp	Denmark	Supply, deposit identification, training for identification, sero- and virulence typing
Industrial Yeasts Collection	DBVPG	Yeast	http://www.agr.unipg.it/dbvpg	Italy	GD, SD, IDA supply cultures, and identification
Coleccion Espanola de Cultivos Tipo	CECT	Bacteria, fungi, yeast	http://www.cect.org	Spain	GD, SD, IDA supply cultures, and identification

Data collected from the Website of WFCC and respective culture collections

GD general deposit, *SD* safe deposit, *IDA* patent deposit

Chloroflexi, *Acidobacteria*, and pathogens (Stackebrandt 2011). Similarly, Emersion and Wilson (2009) and Ward et al. (2001) also emphasized the importance of BRCs and highlighted the need for establishment of BRCs with wide range of technical expertise as well as ecosystem-level preservation facility in order to preserve natural diversity.

Microbial patenting and Budapest Treaty for deposit of microorganisms

The cultivation, purification, characterization, and the bioprospecting of novel microbes are time-consuming and challenging. Because of the amount of efforts involved, it is imperative that researchers working in the area of cultivation and bioprospecting should, in addition, have the basic knowledge about intellectual property right (IPR) and microbial patenting, to protect their discovery and ensuing benefits from such discoveries. A patent not only protects the discovery and interest of the discoverer but also encourages the growth and innovation in the area of science and technology. In order to provide readers with a glimpse of microbial issues related to IPR, an overview of microbial patenting, and the role of the Budapest Treaty and International Depositary Authority (IDA) in the patenting of microbes is discussed below.

According to the Convention on Biological Diversity, the country of origin of biological materials is entitled to supreme rights, but the rules that address its patenting and IPR vary from country to country. Patenting is a method of protection of IPR, with organism(s) of concern, to the discoverer up to a certain period of time. The first microbial-based patent was granted in 1873. Over 100 years later, in 1980, after hearing the case of AM Chakrabarty, the US Supreme Court for the first time passed a law that live microorganisms are patentable. Law for microbial patenting is more or less similar worldwide with a few exceptions. In order to obtain microbial patent(s), the culture must be deposited into an IDA recognized under the Budapest Treaty, and a registration number of the deposition must be quoted in a patent application form during the time of patent filing. It is also mandatory that, during the time of deposition in a culture collection (IDA), ownership of IPR, if any, must be addressed clearly and a complete written disclosure of the invention must be provided. Wild organism(s) isolated from nature as such and descriptions of novel species are not patentable because these are not related to an invention but rather are considered as a natural resource. However, organisms isolated using a special procedure or that demonstrate a novel aspect are patentable. Genetically modified organisms, microbial products, a microbial process, or the new use of an existing product(s) generally fall under the category of an invention and are patentable. Novelty,

inventiveness or non-obviousness, and utility are some basic aspects that determine the patentability of an organism (Fritze 1994; Kelley and Smith 1997; Sekar and Kandavel 2004).

The Budapest Treaty is an international treaty for the deposition of microorganisms for patent purposes (http://www.cnpat.com/worldlaw/treaty/budapest_en.htm). The treaty is controlled by the World Intellectual Property Organization (http://www.wipo.org/) and was signed in Budapest, Hungary, on April 25, 1977, entered in force on August 9, 1980, and amended in September 26, 1980. The Budapest Treaty recognizes IDAs and sets minimum standards, requirements, and guidelines for the deposit of microorganisms. There is no clear-cut definition of microorganisms under the Budapest Treaty. These can include bacteria, fungi, yeast, eukaryotes, nucleic acids, algae, plants, spores and expression vectors, plant tissue culture, animal cell lines, etc. Any IDA established under the Budapest Treaty must have the following features: (1) continuous and independent existence; (2) adequate staff, facilities, and expertise for maintaining and testing the viability of deposited materials in a manner to ensure its future viability and uncontaminated state; (3) sufficient safety requirements in order to minimize the risk of losing the deposited materials; (4) impartiality; (5) expeditious sample furnishing; and (6) the ability to promptly notify a depositor about its inability to furnish the sample with adequate reasons (Fritze 1994; Kelley and Smith 1997; Sekar and Kandavel 2004).

Conclusions

Although the use of culture-independent methods has opened an expansive window into microbial diversity, it has simultaneously overshadowed cultivation efforts and generated a wide gap between culture-independent and culture-based databases. Molecular techniques alone cannot reveal the function or physiology of microorganisms, either in the laboratory or in nature. The exclusive employment of omics-based approaches for exploration of microbial diversity can lead to dissatisfaction and a lack of success (Morales and Holben 2011). Conversely, most of the development in microbial science and technology is based on availability of pure cultures. Unfortunately, culture-based databases are lacking, with most of the culture collections of the world predominated by weed-like microorganisms. Traditional cultivation approaches often fail to acquire ecologically relevant organisms, either due to lack of appropriate cultivation technique or lack of expertise in formulating new media for growing recalcitrant microorganisms. Therefore, in order to grow and advance the field, adequate knowledge of microbiology in terms of microbial nutrition, in situ geochemical conditions should be promoted along with excitement and interest in cultivation. Otherwise, cultivation-based microbiology may become a lost art, and we will lose the knowledge of those with master ability in the field. Furthermore, we should think differently

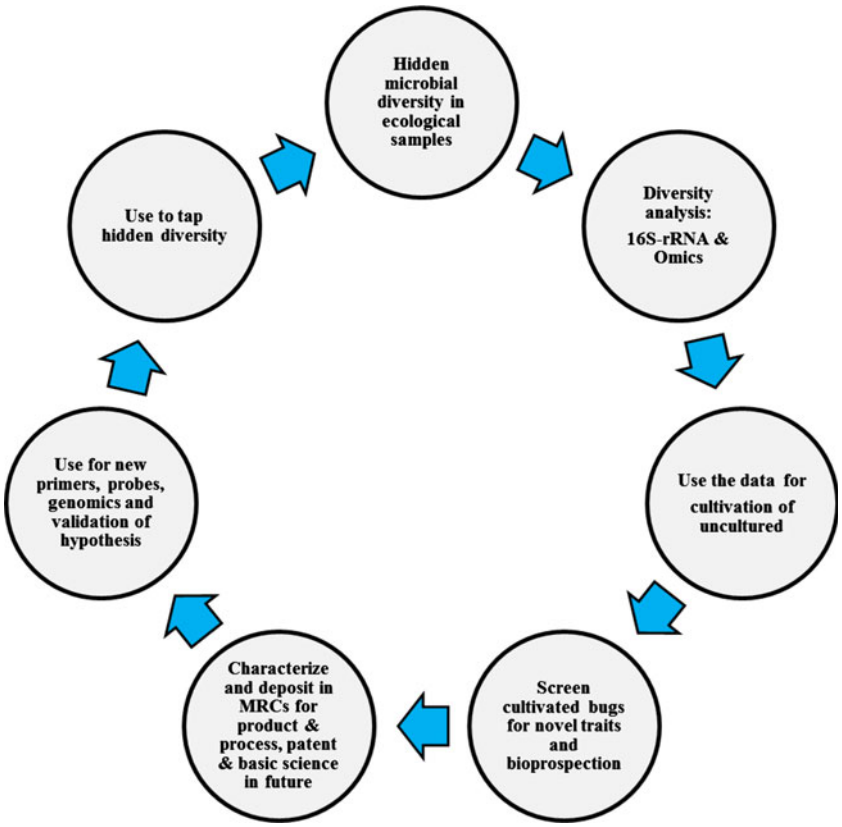

Fig. 3 Cyclic representation of basic steps of microbial diversity research. Culture-independent and cultivation-based studies are interdependent, equally valuable, and support each other to trace out the novel microorganisms

when designing new cultivation protocols and be mindful of potential pitfalls.

Along with the reluctance of researchers to pursue cultivation, several other factors including the time-consuming nature of cultivation and related physiological research, and the limited time span of academic programs (BS, MS, and PhD) and tendency of researchers to compete with peers in terms of productivity are also responsible to some extent for diminishing cultivation work. Neither cultivation-based nor molecular approaches alone are sufficient to profile microbial diversity adequately. Therefore, researchers in microbial ecology and community profiling should apply a polyphasic strategy that closely couples cultivation and molecular techniques. Genomics can be used as a foundation for hypothesis generation and in formulating cultivation protocols. Acquisition of novel organisms will lead to the discovery of new metabolic pathways and the design of more effective primers and probes to tap the hidden diversity (Giovannoni and Stingl 2007; Song et al. 2009; Stewart 2012; Zengler and Palsson 2012; Fig. 3). Such a polyphasic approach may begin with in situ characterization using next-generation sequencing to depict community structure. Subsequently, hypotheses generated from omics data can be employed to optimize cultivation protocols for the acquisition of ecologically prominent/relevant but not-yet-cultured microorganisms (Fig. 3). After novel cultures are obtained, it will be critical for researchers to optimize storage and cultivation protocols. Finally, cultivated microbes may be used as model systems for the phenotypic and genotypic testing of omics-based hypotheses (Huber et al. 2002; Lewis et al. 2012; Pham and Kim 2012; Stewart 2012).

Mere cultivation of novel microorganisms is not enough. Adequate preservation of germplasms for academia, research, and bio-prospection is equally important. The scientific community must promote the establishment of BRCs with well-equipped infrastructure and a wide range of expertise in order to adequately protect and characterize microbial diversity for future generations and commercial exploitation. It is also recommended that national governments provide sufficient funding to support BRCs and secure their long-term interests.

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