Indian J. Plant Genet. Resour. 29(3): 340-342 (2016)
DOI 10.5958/0976-1926.2016.00062.0

Microbial Conservation Strategies and Methodologies: Status and Challenges

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Microorganisms, '*silent and unseen majority of life*', are one of the earth's greatest treasures around us because they have the largest genetic and metabolic diversity which can be protected and utilized for sustaining plant productivity, ecological balance and human life. Researchers are working on exploration and conservation of the microbial universe for the future applications. These microbial strains could be exploited for several biotechnological purposes in industry, medical, pharmaceutical and agriculture sectors. Today, almost no branch of science e.g., engineering, agriculture, or medicine is untouched with microbial interventions. If we go back in the history of microbial ecology, Beijerinck (1913) quoted a most famous maxim in microbiology "*everything is everywhere, but, the environment selects*", suggests (Baas Becking, 1934) suggesting that microorganisms do not exhibit any biogeographical pattern and, hence, these are ubiquitous and cosmopolitan. However, some microbes are inhabitant to specific environments therefore; there is no question of extinction of microorganisms. However, this is not absolutely factual and some microbes do face real threats of their continued existence due to habitat and climatic aberrations. Contrary to first part of the maxim '*everything is everywhere*', microbes do exhibit biogeographical pattern. For example (1) a group of bacteria inhabiting leaf surface indeed appear ubiquitous but some microbes exhibit localized endemism and show absence of panmixis (Gerstein and Moore, 2011); (2) many pathogens have well-defined distribution in animals, humans and crops. Second part of the maxim '*the environment selects*', means some microorganisms live in very specialized habitats and loss of habitat may cause extinction of the associated microorganisms. Curtis (2006) issued a call to arms for microbial ecologists – it's time to start thinking big and requires worthy attention to conserve microbes at risk of extinction. Therefore, the conservation of microbial diversity in the environment has been realised by scientists and researchers through understanding and application of rRNA gene barcoding and use of operational taxonomic units (OTUs) coupled with next generation sequencing (NGS). It is high time to address on the knowledge gaps and communication barriers that currently lags amongst microbiologists, microbial ecologists and conservation biologists while undertaking microbial conservation. With this background, here we will highlight strategies of microbial conservation and challenges associated with it.

Strategies of Microbial Conservation

Innumerable microbial species are supposed to exist, however only 1-10% species are characterised, preserved and utilised for various purposes. In order to fully utilise the genetic resources of the microbes which are still uncharacterised, we need to conserve microorganisms in their habitats. Microbial conservation strategies applied "*in situ*", "*ex situ*" and "*in-factory*" form of conservation. "*In situ*" ('on site', 'in place') conservation links the microbes in their natural habitats and is the most appropriate way of conserving viable populations in their ecosystem and natural habitats. "*Ex situ*" (off site) conservation preserves and maintains the distinct wild/isolated/cultivated species and their genetic resources in artificial media and are taxonomically well described. "*In-factory*" form of conservation is an intermediate form of conservation and mainly used by the agro-industrial sectors.

a. In situ Conservation

In situ conservation methods have the potential for long-term preservation of ecosystems, species, and populations under conditions of continuing adaptations. *In situ* conservation is essential in places whose microbiome had not been adequately inventoried. *In*

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situ conservation is a set of conservation techniques involving the designation, management and monitoring of biodiversity within the context of the ecosystem in which it is found. *In situ* management approaches can either be targeted at populations of selected species (species-centred), or whole ecosystems (Heywood and Dulloo, 2005). Traditionally, protected areas have been seen as the cornerstone of *in situ* conservation. Conservation approaches that are more adaptable to individual situations and applicable beyond protected areas, are being increasingly applied. Convention on Biological Diversity (CBD), a treaty declared in June 1992 at the Rio de Janerio Earth Summit, recognized the importance of conservation of biodiversity, however microbial diversity were not rightly addressed in CBD and therefore, in subsequent years GW Griffith advocated Global Strategies for Microbial Conservation (GSMC) (Griffith, 2012). Extinction of specific microbes inhabiting in specific habitats particularly those which are on the verge of extinction are need to be conserved on priority basis. The following are some specific examples which would highlight the challenges and importance of *in situ* conservation of habitats for conserving associated microbes for future application.

1. In tropics, soil bacterial diversity is maximum with neutral soil pH, and lower in acidic and alkali soils, make these areas more like desert for soil bacteria, whereas grassland and desert soils have much higher bacterial diversity than tropical rainforest soils. Soil texture, chemistry, moisture, and a multitude of other factors vary with depth, providing unique, vertically-stratified habitats for soil-dwelling organisms. Several studies have shown that soil organisms vary with soil depth, from fungi or bacteria (Parker, 2010). These examples show the relationships between soil diversity and soil functions, as well as the biogeographical patterns that are poorly understood and pose a challenge in *in situ* conservation.

2. Some novel anaerobic fungi specific to the hindgut have been reported from critically endangered animals like Somali wild ass and red Kangroo (Liggenstoffer *et al*., 2010). Recently, a new anaerobic fungus *(Oontomyces anksri* gen. nov., sp. nov.) from the digestive tract of the Indian camel (*Camelus dromedarius*), has been reported and raising the possibility that this fungus may be specific to camelids (Dagar *et al*., 2015). In case of plant, *Goodyera pubescens*, an evergreen terrestrial orchid, interacts with the same fungal partner during periods of modest environmental variation but is able to switch to a different fungal partner under extreme environmental perturbations (McCormick *et al.,* 2006). These are some examples where environment selects a particular type of microbes which may extinct on extinction of larger partners and advocate habitat conservation.

3. Some of the extreme ecosystems such as desert soil crust, hydrothermal vent, hypersaline areas, hot springs, cryoconite hole on glacier and unusual habitat do support occurrence of specific type of microbes that need to be conserved.

b. *Ex situ* Conservation

Microorganisms are being conserved in laboratories using specialized methods for their present and future application. At an international level, the World Federation for Culture Collections (WFCC) which is a federation of the International Union of Microbiological Societies (IUMS) and a commission of the International Union of Biological Sciences (IUBS) with responsibility for the promotion and development of collections of cultures of microorganisms and cultured cells. There are many microbial resource centres for conservation in many countries of the world, where microbes are collected, identified, characterised and conserved as per the guidelines of best practice of the Organisation for Economic Development and Cooperation (OECD). Further, the aims of Budapest Treaty is to conserve the patented microbes in the bioresource centres (repository) like DSMZ, KACC, MTCC, MCC etc. The microbes of specific nature can be protected and utilized in sustainable way in these repositories. National Agriculturally Important Microbial Culture Collection (NAIMCC), ICAR-NBAIM is a Designated Repository (DR) recognised by National Biodiversity Authority (NBA), Government of India, for conservation of agriculturally important microbial wealth of the country.

In *ex situ* methods of conservation, the microbial activities are reduced or stopped by imposing conditions. There are several methods employed for the preservation and maintenance of microorganisms, for example, sub-culturing, preservation on agar beads, use of mineral oils, silica gel storage, spray-drying, fluidized bed drying, cryopreservation, lyophilization (freeze-drying), L-drying, desiccation, induced anhydrobiosis, sterile distilled water and gelatin discs. Preservation by cryopreservation and lyophilization is considered as the most valuable and widespread methods available to achieve long-term, stable storage of microorganisms. Some microorganisms are recalcitrant either to lyophilisation or cryopreservation and have a greater challenge for preservation of such microorganisms through different approaches. *Ex situ* conservation is rarely enough to save a species from extinction.

c. *In-factory Conservation*

I*n-factory* method of microbial conservation means keeping them in normal conditions of use. Two different ways of conservation can be implemented: *dynamic* and *static conservation. Dynamic conservation* does not impose significant restrictions 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 conditions such as to avoid any kind of changes.

Conclusion

Despite of the fact that microorganisms play a pivotal role in ecosystem function and human life, a large proportion of these '*tiny life form*' have been basically ignored so far. In general different microorganisms often require special preservation methods for ensuring their optimal viability, storage, and purity. For protection point of view and to minimize the possibility of cultures being lost or contaminated therefore it is suggested to preserve the strains by at least two different procedures. Of which one should be based on freeze-drying (lyophilization) or storage in liquid nitrogen (cryopreservation) where applicable; these are the best methods for minimizing the risks of mutation or genetic change. Despite of above established protocols, only some culturable microbes have been preserved in some repositories located in different parts of the world. It is likely that there could be large gaps in understandings on methodology followed in preservation, knowledge of the distribution and abundance of microorganisms. A comprehensive strategy should focus on the conservation of microorganisms in their habitats with extreme environmental conditions and endangered plants and animals harbouring specific microbes. Hence, global strategies for conservation of hidden microbial treasure must come from microbiologist, researchers, and research institutes and must be supported by government as a national policy to sustain life on the planet earth. It is worth quoting tenet of Tom Curtis which indicates importance of conservation of nitrifying bacteria '*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*'. Subsequently, recent discoveries of two processes namely, anammox (anaerobic ammonium oxidation) and comammox (one step aerobic ammonium oxidation by single bacterium; Nitrospira) are evidence of continued ignorance of many microbial processes. This ignorance argue for maximizing conservation of microorganisms and also a challenge of what and how to conserve in this dynamic and rapidly evolving microbial world.

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