

Chapter 1

The Contribution of Microbial Biotechnology for Achieving Sustainable Development



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Abstract Microbes are requisite constituent of biotic diversity that maintain sustainable ecosystem. They are chief customs of life which have progressed into environmentally, metabolically and genetically diverse species. In ecosystem, microbial diversity strives to comprehend innumerable metabolic courses to maintain resolute integrity for sustainable ecology. Utility of microbial communities has better indulgent of the bio-network. Until now, only 0.1–10% of microbial species are recognized and, the rest being uncultured, inhabit noteworthy niches in biomes and are accountable for several loom based on molecular genetics, systems and synthetic biology, genomics, proteomics and metagenomics. Exploring biotechnological applications and understanding their mechanism of alteration permit the progress on the circumstances necessary for various microbial applications with stare to sustainable development, community structure and environmental processes. Most appreciated tools for investigating the microbial resistance to antibiotics and search for new antimicrobials can be done using molecular techniques. Therefore, currently, metagenomics and meta-proteomics studies have been utilized effectively to get novel microbes as well as their by-products from uncultured microorganisms. Microbes can be used for a variety of biotechnological appliance such as food products, therapeutic protein, recombinant microbes, vaccine and diagnostic tool. Even though microbe's inventorying and cataloguing are discouraging tasks, requiring skills and creativity but imparting considerable pecuniary import.

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1.1 Introduction

The richness, variability and complexity among the living organisms are designated under the term biodiversity or biological diversity. It is determined by different plants, animals and microbes in natural ecosystems. Biodiversity is classified in terms of three fundamental levels, viz. genetic, species and ecosystem diversity.

Biodiversity of the microbial world has focused on the evolution of all forms of life on earth. Microbial diversity covers a wide range of inconsistency between prokaryotes, eukaryotes and viruses in each possible habitat on the planet. They are found in almost every nook and cranny yet in that environment where all forms of life cannot exist (Vitorino and Bessa 2018). They thrive in extreme conditions, viz. sites of hydrothermal vent, hot springs, ocean, sea ice, hypersaline environment and extreme pH and temperature that are unfavourable for survival. These organisms are called as extremophiles that flourish in environments which are lethal for survival of other living beings (Rampelotto 2013).

Microbes are incredibly minute and constitute maximum proportion among living beings on the globe. However, particularly small portion of this huge variety has been searched for the development of microbial diversity. It has been reported that greater part of microbes cannot be cultured in laboratories (Sharma et al. 2018). It is a crucial part of microbial rallies to craft these organisms and those isolated microbes accessible to the research community. Microorganisms can be cultured for conservation and utilization after they are isolated from their native environment. Microbial activities make earth liveable and have limitless commercial applications principally in the field of life science (Arrigo 2005).

Gases such as oxygen and nitrogen which are the result of microbial activities make habitable climate. They occupy significant part in remediation of harmful chemical compounds (Kostka et al. 2011). Microorganisms also offer primary and secondary metabolites which have potent antimicrobials, immunosuppressants and anti-inflammatory and antitumour properties (Challis and Hopwood 2003). More than 104 metabolites produced from microbes have been explored for these compounds in the last decades. Bioplastics of microbial origin are promising substitute to chemical-based plastics, and these possess medical importance (Verlindin et al. 2007). Microbial diversity studies can be classified on the basis of culture-dependent (culturable) and culture-independent (unculturable) methods.

1.2 Assortment of Nonculturable and Culturable Microbes

1.2.1 Culture-Noncontingent and Culture-Contingent Method

The word ‘unculturable microbes’ designates microbes that have hitherto been cultured in vitro on non-natural media (Hugenholtz et al. 1998). This method involves mining of DNA from the environmental illustration and afterwards, examined through molecular biology-based methods, or it involved another method for unculturable microbes in vitro by mimicking natural environment for the cultivation of unculturable microbes which show resistance to grow on cultivation media (Vartoukian et al. 2010).

1.2.2 Intents for ‘Unculturability’

In the history of science, the microbial life endurance was recognized from more than 300 years ago (Girio et al. 2010). It was found that some microbes have yet not been known by cultural analysis. This might be owing to the piece of evidence that low occurrence and sluggish growers of microbes have been ignored. Moreover, in traditional biochemical identification methods, many characteristics of microbes are overlapped which makes their identification difficult (Schmeisser et al. 2007).

In contrast, certain microbes require particular nutrients for their fastidious growth. They had reported various substances and growth requirements of microbes of marshy sediments and found that microbes are specific to particular cultivation method. Therefore, only certain groups of microbes are identified on the basis of traditional method; however, the rest remain unidentified. In a mixed population, growth of a specific group of microorganisms is suppressed by the microbial product of other organism in the medium (Tamaki et al. 2005). Culture-independent approach has been extensively used to study microbes in different habitat (Yashiro et al. 2016).

1.2.3 Perception of Nonculturability

Metagenomics study revealed the genetics of uncultured microbes which aims to know microbial environment as well as enhance biotechnological aspects. It is well known that uncultured microbes are known for their novel compounds which are yet to be discovered (Schmeisser et al. 2007). Some microorganisms are described on the basis of special habitat in spite of the microbial ubiquity theory, due to which their distribution is more restricted, as they tend to be giving rarer results in intricacy to culture these species in the samples.

Alain and Querellou (2009) found 30 cultured groups among 100 phyla all the way through phylogenetic analysis. Till date, only a small proportion of microbes (0.1–10%) have been cultivated of this vast diversity (Leadbetter 2003). Molecular studies make uncultivated microbes—oligotrophs and fastidious organisms to grow. The difficulty faced in cultivation of organisms includes slow or late growth of microbes on nutrient-rich media, lack of knowledge about novel media formulation and inefficiency of individuals, trained in the field of microbiology (Leadbetter 2003). This can be overcome by exploring microbes with well-equipped approaches and knowledge about microbes (Gest 2001).

The effect and response of microbial diversity to long-term environmental change are not properly understood, and it is also not clear that how much local microbial communities have impact on the environment (Tripp et al. 2008). *Pelagibacter ubique* SAR11 is widely distributed among heterotrophs cultured using sea water which was ameliorated with traces of phosphorus and ammonium ions. Many bacterial strains have to be cultured by maintaining solidifying agents in natural media, for example—Acidobacteria incubates for longer duration (Kuske et al. 2002). *Nitrosopumilus maritimus* is the first mesophile that belongs to Crenarchaea which is abundant in nature (Stevenson et al. 2004).

Cultivation of microbes is a very tedious task incorporated with many complexities. For the inhibition of microbial growth on Petri dish, many growth factors are responsible such as nutritional shock (Overmann 2006). Therefore, it is very imperative to discern the difficulties faced during the cultivation of new microorganisms. Although there is significant progress in the development of cultivation techniques, meticulous strategies are required for new media formulation. It is impossible to culture samples of all habitats; therefore, microbiologists are encouraged to explore novel microbes especially in extreme conditions. Different investigation showed that different microbes are able to colonize in cold environment of the planet from north to south poles.

Microorganisms are divided into two groups—fast growers (r-strategist) and slow growers (k-strategist) according to the microbial growth pattern and their potential of survivability (Overmann 2006). Nutritional shock is one of the parameter responsible for the growth inhibition or lethal to microbial cell. Apart from nutritional shock, in short duration, excessive growth of fast growers inhibits the growth of slow grower's types of microbes. Sometimes, undesired microbial growth also inhibits the growth of desired microbes in absence of inhibitory compounds. As a result of which, only few microbes are cultivated in the Petri dishes (Zengler 2009).

Even though all present acquaintance on the variety of microorganisms, it is believed that investigations in unexplored sites may result via additional evidence. Broad and unidentified speciations, mainly in the bacteria and archaea domains, are still unexplored. Usually, for the cultivation of bacteria and fungi, antifungal and antibacterial antibiotics are used, respectively. Environmental samples are serially diluted, and, by plating different dilutions, broad range of microbes are obtained (Tripp et al. 2008). Using traditional cultivation strategies, relevant but slow-growing microorganisms were not yet cultured and are then known as unculturable microbes. Due to several complications in cultivation of microbes, many endeavours have been made for new microorganism cultivation (Gest 2008).

The biotic interactions provide nutrients to the plants, increase soil fertility and showed adverse effects on pathogens but essential for the sustainability of natural ecosystems. Till now, various types of nutrient-rich media have been used for fastidious organism cultivation over slow growers (Koch 1997) and may be repressed by substrate-rich conventional media. For the cultivation of oligotrophs, the incubation period has increased; as a result of which, fastidious organisms progressively die off in mixed cultures. Davis et al. (2005) isolated most rare species after 12 weeks. Similarly, results have been reported for the segregation of strains from SAR11 clade after 24 weeks (Song et al. 2009). Many microbes require chemicals for their growth. For instance, *Abiotrophia* and *Granulicatella* required pyridoxal or L-cysteine for their augmentation; on the other hand, *Tannerella* is nutritionally dependent on N-acetyl muramic acid for their growth.

Another method is mimicking the natural environment in laboratory conditions for the culture of as-yet-uncultivated organisms. Kaeberlein et al. (2002) have intended a diffusion chamber for the marine bacteria that were precedently uncultivated. These organisms are completely dependent on other bacteria for their existence in a media. Since the mid-1980s, molecular biology practices have been focussed on microbial diversity and their ecology in their natural habitat. Molecular approaches revealed the molecular sequences of many uncultivated microbes which have many potential applications. An additional pioneering method resembling natural environment involves microcolony development of uncultured soil bacteria on soil substrate membrane system (Ferrari et al. 2008). This method involved viability staining and micromanipulation techniques for the detection and isolation of live microcolonies (Ferrari and Gillings 2009). However, colony hybridization method involves the isolation of colony containing a plasmid from a mixed microbial population (Salama et al. 1993). Culturing of the uncultured member of phylum utilizes this approach for research. Synergistetes had been isolated from dental plaque samples (Vartoukian et al. 2010). Flow cytometry and cell sorting (FACS) is a method that has also been used for the cultivation of cultured as-yet-uncultivated organisms (Zengler et al. 2002).

Genomic analysis of cultured as-yet-uncultivated organisms helps in identifying these organisms as well as gives some more information about the organism which will help in cultivation of previously uncultivated microbes in the vicinity of prospect (Tripp et al. 2008). Ghosh et al. (2010) used cultivation-independent molecular approach to study microbial diversity in the mangrove sediment of the Sundarbans, India. Proteobacteria (alpha, beta, gamma and delta), Flexibacteria (CFB group), Actinobacteria, Acidobacteria, Chloroflexi, Firmicutes, Planctomycetes and Gemmatimonadetes were the major divisions of detected bacterial phyla. Several reports suggested that *Bacillus* is an efficient tissue colonizer in different plants including *Coffea arabica* L., sunflower, cotton, potato, strawberry, *Panax notoginseng* and citrus plants (Vega et al. 2005). *Microbacterium* sp. was indigenous to plants such as maize, rice and wheat (Rijavec et al. 2007). Genus *Pseudomonas* is an extensively disseminated plant-associated bacterium reported activity of growth promotion in plants such as alfalfa (Gagné et al. 1987), clover (Sturz et al. 1997), potato (Reiter et al. 2002) and pea. Some minor groups such as Enterobacteriaceae,

Moraxellaceae, Xanthomonadaceae and Burkholderiaceae were also observed from proteobacterial phylum. Shivaji et al. (2004) recognized bacterial community including culturable bacteria from soil in the vicinity of Lake Zub, Schirmacher Oasis, Antarctica, has its place in the genera such as *Pseudomonas*, *Sphingobacterium*, *Arthrobacter*, *Micrococcus*, *Brevundimonas*, *Rhodococcus* and *Microbacterium*. Jiang et al. (2006) used culture-dependent and culture-independent techniques to examine microbial assortment. It was reported that gram-positive bacteria were predominant among the bacterial strains isolated from Lake Chaka. Analyzed bacterial diversity includes *Aeromonas hydrophila*, *Escherichia coli*, *Chryseomonas luteola*, *Chromobacterium violaceum*, *Pseudomonas aeruginosa*, *Serratia rubidaea*, *Klebsiella pneumoniae* and *Enterobacter cloacae* in mangrove soil at Tanjung Lumpur. They have also investigated their resistance against several antibiotics.

1.3 Microbes Role in Habitat/Environment

The environment is an essential perception because microbes are greatly affected by the atmosphere. Microorganisms are involved in many biogeochemical processes in different habitat. They are richest repertoire and considered as pillars of existence in nature. On earth, more than four billion years ago, microbes have been evolved and play numerous and important roles for maintaining sustainable biosphere that includes nutrient (elemental) cycling and detoxification of hazardous compounds present in the atmosphere. The microbial world is a treasure in itself and covers broad range of discrepancy of microbes among all types of microorganisms (bacteria, archaea, eukaryotes and viruses) in each possible habitat and is linked with plants and human on the planet. They are proficient in exploiting broad spectrum of energy sources and inhabitant of different environments like normal as well as extreme hot mainsprings, hydrothermal vent sites, drought, ocean and sea, polar ice, hypersaline and extremes pH that is lethal, or in other environments that are unfavourable for survival. Microorganisms have become an important part of the natural elemental cycle and played significant roles in biogeochemical cycles and converted the oxidized forms of molecules into reduced forms. Unicellular and filamentous cyanobacteria are mainly accountable for the fixation of nitrogen. Microbial study in different surroundings has confirmed that assessment of metabolically effective group is the key to explain microbial activities (Baldrian et al. 2012).

1.3.1 Role in Terrestrial Ecosystem

This type of ecosystem is surrounded by forests, cropping systems and grazing lands. Soil acts as source of micro- as well as macronutrients. These nutrients are necessary for the plants, insects, protozoa, nematodes, worms and microbial growth (Staben et al. 1997). This biological diversity is responsible for the formation,

maintenance and degradation of soil. Among this vast community, microbes constitute the major proportion and are versatile in their action. Bacterial community counts approximately 10^8 – 10^9 cell g^{-1} dry weight of soil in surface of the soil microscopically, while fungi can be contemporary up to numerous metres of hyphae in g^{-1} of soil. Plant actions also augmented microbes in the soil. Microbes are associated with plants via roots (rhizosphere and rhizoplane) and leaves (phyllosphere and phylloplane). Rhizosphere acts as a reservoir for microbial diversity (Singh et al. 2019). Some may induce resistance or suppress the development of plant pathogens (Lanteigne et al. 2012) and exhibit positive as well as negative impact on plant growth. Microorganisms are versatile in nature and play significant role in increasing soil fertility. Microbial action contributes to nutrients cycling in soil such as carbon, nitrogen, sulphur, iron and manganese cycles. They act as biofertilizers and fix atmospheric nitrogen, phosphorus, and sulphur and other elements which are unavailable for plants and finally contribute to plant's nutrition (Yadav and Saxena 2018). The degradation of hydrocarbons and dead and decayed plants and animal matter along with its involvement in the formation of humus is an important role played by bacterial community in the soil. Actinomycetes imparts soils their characteristic earthy odour by producing a compound called eosin by *Streptomyces* species.

1.3.2 Role in Mangrove Ecosystems

High load of biological diversity belonging to plants, animals and microorganisms occurs at mangrove forests occurring at the border of terrestrial and marine environment. Mangroves cover nearly 70% of the world's tropical and subtropical coastal regions, which are identified to be highly fecund ecosystems of huge ecological value. These ecosystems are highly productive all over the world despite they are fragile and sparsely distributed. In this habitat, microbes transform nutrients and detoxify pollution-causing agents, and as biocontrol of pests, a unique environment harbouring diverse groups of microbes such as bacteria, fungi, cyanobacteria, microalgae, macroalgae and protists is provided by them. They are abundantly nitrogen and phosphorus deficient (Holguin et al. 1999).

Amid the microbial distribution, bacteria and fungi represent the foremost proportion after algae and protozoa. The most common bacteria are sulphate reducers belonging to the genera *Desulfovibrio*, *Desulfotomaculum*, *Desulfosarcina* and *Desulfococcus*, nitrogen fixers and methane producers (genera *Azospirillum*, *Azotobacter*, *Rhizobium*, *Clostridium*, *Klebsiella*, *Methanococcoides methylutens*), phosphate solubilizers (genera *Bacillus*, *Paenibacillus*, *Xanthobacter*, *Vibrio proteolyticus*, *Enterobacter*, *Kluyvera*, *Chryseomonas* and *Pseudomonas*) and photosynthetic anoxygenic bacteria (genera *Chloronema*, *Chromatium*, *Beggiatoa*, *Thiopedia*, *Leucothoe* bacteria) (Das et al. 2009). Moreover, fungi, such as ligninolytic, cellulolytic, pectinolytic, amylolytic and proteolytic fungi, as well as actinomycetes are present in mangrove ecosystems. Among the algae, Chlorophyta,

Chrysophyta, Phaeophyta, Rhodophyta and Cyanophyta are the dominant groups of the mangrove ecosystem. They harbour unique microbial composition which contains major source of therapeutic enzymes, antimicrobial and antitumour agents, insecticides, etc.

1.3.3 Role in Aquatic Environment

Aquatic habitat classified into fresh water, marine and both. Fresh water consists of lakes and rivers. Open ocean, coral reefs, and intertidal zones constitute the marine environment. Ecosystems that are considered both marine and freshwater system is composed of estuaries and salt marshes. Microbes are widespread, well-adapted in fresh water and involved in diverse biogeochemical processes, such as petrification of organic compounds; nutrients can be remineralized in maintenance of water ecosystem (Newton and McLellan 2015). They have its place to the group of photosynthetic oxygenic and anoxygenic organisms that include bacteria, algae and cyanobacteria. Further 70% of the earth is roofed by ocean, and microbes are accounted for more than 98% of ocean biomass. Marine microbes are called as ‘the canary in the coal mine’. The marine microbial diversity constitutes microalgae, bacteria and archaea, fungi and viruses (Fuhrman and Noble 1995). The Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 (PhTAC125) is studied for their enormously fast growth and its wide temperature array from -2.5 to 25 °C (Wilkins et al. 2013). They offer a tremendous biodiversity and potential for drug sighting and delivery of novel marine-derived products in therapeutic claims. They play diverse role in the marine environment such as in food chain, transformation of nutrients and sustaining marine ecosystem for the survival of marine organisms.

1.3.4 Role in Extreme Environments

On the basis of stability, environment can be classified into two types—normal and extreme environment. Stable condition can be considered as ‘normal’, while in extreme environment, organisms experience dramatic changes. The inhabitant organisms of extreme ecology are known as extremophiles. High and low temperature classify the nature of microbe’s adaptability for extremophiles; these factors are the basis of their classification (thermophiles and psychrophiles), high salt concentration, high and low pH (acidophiles and alkaliphiles) and low water activity (a_w). The microbial product produces from extremophiles are of immense importance. Many information on microbial diversity from thrilling environments, for instance, low temperature (Yadav 2015), high temperature, saline soil, drought, acidic soil and alkaline soil, have been reported. In an unstable environment, the costs to survive in stress condition may increase for some organisms, while most will probably die off. Extreme environments are well known for novel microbial diversity.

Microbes at high temperature make a hydrophobic environment for their survival (Acharya and Chaudhary 2012). Complicated zig-zag structure of proteins provides microbial cells to withstand denaturation and proteolysis.

1.3.5 Role in Saline Environment

Microorganisms are widely distributed in hypersaline environment from solar salt-erns to deep salt mines (Selvarajan et al. 2017). Most of the Indian saline ecosystems, such as Sambhar Lake in Rajasthan, Chilika Lake in Odisha, the Great Rann of Kutch in Gujarat and Lonar Lake in Maharashtra, are known for novel and potential applications. In this environment, microbes play vital character in the remineralization of organic matter (Joshi et al. 2008). The microorganisms in saline environments that have been isolated and identified mainly belong to the family Halobacteriaceae. Halophilic microbes have been described from different phylum including Actinobacteria, Bacteroides, Euryarchaeota, Firmicutes, Proteobacteria and Spirochaetes (Yadav and Saxena 2018).

1.3.6 Role in Cold Environment

Cold environments cover the largest region on the earth. The term ‘psychrophiles’ is used for the microbe that are living/inhabitant in cold condition. In India, microbes are widely distributed and explored in the Himalayan region. Psychrophiles are potential sources for production of extracellular proteins. Polyhydroxyalkanoates (PHAs) are chiefly produced by psychrophiles which increase survivability in stress conditions (Tribelli and López 2018). It includes diverse groups of microorganisms, i.e. archaea, bacteria and fungi. had reported many species from high-altitude and low-temperature environments of Indian Himalayan region belonging to genera *Aurantimonas*, *Bacillus*, *Disemia* and *Paenibacillus*.

1.3.7 Role in Drought Environment

The desert microbiota potentially is known for their efficiency in maintaining the harmony of recycling of different nutrients and ecological balance as well as for the development of soil structure. In rain-fed conditions, microorganisms that are tolerated in drought environment have been secluded and characterized for plant growth promoters (PGP) and have its place to the family Halobacteriaceae and genera such as *Haloarcula argentinensis*, *Halobacterium* sp., *Halococcus hamelinensis*, *Haloferax alexandrinus*, *Haloferax larsenii*, *Haloferax volcanii*, *Halolamina pelagic*, *Halostagnicola kamekurae*, *Haloterrigena thermotolerans*, *Natrinema* sp. and *Nanoarchaeum mannanilyticum*.

1.3.8 *Role of Microbes in Human Health*

The human body is immensely colonized with microbes in different tissues and body parts. Approximately, thousands of different bacterial species exist side-by-side together in the intestinal tract of human. Among various organs of human, the digestive tract heavily occupied with enormous bacteria represents the dominant genera *Lactobacillus* sp., *Escherichia coli*, *Klebsiella* sp. and *Proteus* sp. that perform various roles in metabolic processes of substrates, build up defence mechanism against various infections, synthesize vitamins and various cofactors for their development, support in degradation of fats and polysaccharides and also have antioxidant properties of foodstuffs which in turn enhance the nutritional value (Odonkor and Ampofo 2013). Probiotics (live microorganisms) predominately belonging to the genera *Lactobacillus* and *Bifidobacterium* are dietary supplements added to the foodstuff impacting their nutritional and therapeutic value (Kumar et al. 2012). Important roles played by microbes in the gut are energy generation, production of cellular constituents and processing of nutrients (metabolism). In certain circumstances, microbiota may result in diverse health issues such as diarrhoea, human gastritis, typhoid, gastroenteritis, bacterial vaginosis, chronic peptic ulcers, urinary tract infections and gastric adenocarcinoma (Peris-Bondia et al. 2011).

1.4 Potential Applications

Divergence among microbes is imperative for the endurance of all life forms and offers enormous reservoirs that exploit for human welfare. They have become reservoirs of many substances. Microbes have been used in beer, wine, acetic acid, cheese and yoghurt production and involved in many industries, viz. baking, leather, paper pulp and textile industries (Acharya and Chaudhary 2012). Methanogens play a significant role in the biogas production; however, psychrophiles are being subjugated in biodiesel production (Bernard et al. 2012). We have summarized the application of microbial biotechnology to maintain the sustainable development of the ecosystem in Fig. 1.1.

1.4.1 *Environmental Applications (Bioremediation)*

Grouping of diverse technologies, such as designed biosensors for assessing the level of contamination, mining of the large number of polluted spots and designing of geohydrobiological engineering models, via polishing the spots with microbe-assisted flora (Pilon-Smits 2005), is the most competent and cost-effective way of bioremediation. Bioremediation has capability to fix polluted environments. For the retrieval of degraded lands, the integrative attempt might provide an evidence to be

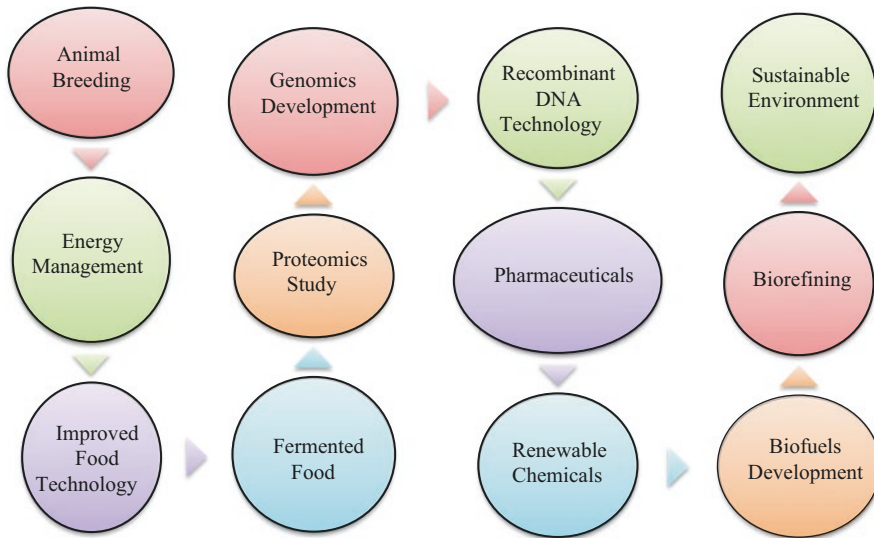


Fig. 1.1 Application of microbial biotechnology to maintain the sustainable development of the ecosystem

one of the pre-eminent ecological practices. Microbes actively participate in the removal of toxic compounds and oil biodegradation. Microbial-based biosensors provide application in monitoring of toxic compounds.

1.4.2 *In Industry (Novel Biotechnological and Pharmaceutical Products)*

Soil has proven to be the major resource of microbes from where they are extracted and used in industries, food processing and production, biocontrol agents, advancement of biocides, drugs and other natural products. Microorganisms can occur naturally or even through human ingenuity, i.e. it can be genetically engineered. Mangrove ecosystem has comprehended many biotechnological importances.

1.4.3 *Enzymes*

The enzymes have many advantages over chemical-based industries due to its high efficiency and negligible substrate loss (Acharya and Chaudhary 2012). Microbes have a varied array of enzymatic activities and are proficient in catalyzing numerous biochemical reactions with novel enzymes. Microbes in marine environment offer microbial proteins which have therapeutic importance for human welfare. Enzymes

contributing to sustainable development of industries such as lipases, proteases, cellulases and amylases showed numerous potential in detergent industry; amylases, cellulases and catalases are used in textile industry, amylases and pullulanases in starch and proteases and lipases in leather industry (Acharya and Chaudhary 2012). Cellulases have gained interest worldwide because of its potential role in the production of transportation of fuel and also are considered as third largest industrial enzyme globally. Mostly, fungi and bacteria have been exploited for cellulase production (Acharya and Chaudhary 2012). Halophilic bacteria produce hydrolytic enzymes which have economic importance (Ventosa and Nieto 1995).

Halococcus asparaginase was reported in mangrove habitat and assessed various properties of oxidative stress-related enzymes such as oxidase, peroxidase and catalase from gram-negative bacteria in mangrove ecosystem of Bhitarkanika. In Brazil, bacteria isolated from mangrove were found to generate different biocatalysts, starch hydrolyzing enzyme, amylases; proteolytic enzymes, proteases; and ester lipid hydrolytic enzyme, esterase and lipase, extracellularly (Das et al. 2009). Husain et al. (2016, 2017) documented chemotherapeutic enzymes (asparaginase, arginase and arginine deiminase) isolated from rhizospheric soil, and endophytic bacteria revealed that *Aspergillus niger* isolated from mangrove ecosystem can generate an enzyme xylanase which can withstand high temperature and pH and carry out biobleaching of paper pulp. Carbonic anhydrase (CA) is a biocatalyst exploited for the sequestration of carbon dioxide. In fresh water, Sharma et al. (2018) optimized various factors for increasing CA production from the genera *Enterobacter* sp. and *Aeromonas* sp. and purified and compared *P. fragi* CA, *M. lylae* CA and *M. luteus* 2 CA against commercial bovine carbonic anhydrase (BCA). In food industry, enzymes play a vital role to process food.

1.4.4 Biosurfactants

Biosurfactants exhibit several therapeutic significances such as antibacterial, antifungal, antiviral and anticoagulation properties. High surface and emulsifying activity of microbial molecules are categorized as biosurfactants or bio-emulsifiers. Due to lower toxicity, mild production conditions, environmental compatibility and higher biodegradability, biosurfactants have gained interest to a large extent as compared to chemical surfactants (Mulligan et al. 2011). All these biosurfactant properties have driven their importance in protecting the environment and have been utilized in many industries, viz. food, cosmetics, biopesticides and pharmaceuticals. On the basis of microbial origin of biosurfactants and their chemical configuration characteristics, biosurfactants can be classified as glycolipids, phospholipids, lipopeptides and polymeric surfactants. The most common biosurfactants among these four groups assessed are glycolipids and lipopeptides produced by *P. aeruginosa* and *B. subtilis*, respectively (Pornsunthorntawee et al. 2008). Properties of biosurfactants, i.e. degradation of substances, less toxic and efficient at low/high pH or temperature, make them more valuable than chemical surfactants and display prominent

eco-friendly compatibility by enhancing bioremediation efficiency. Mulligan et al. (2011) reported and assessed the promising biosurfactant production from *Leucobacter komagotae* 183 strain isolated from mangrove ecosystem of Thailand.

1.5 Medical Importance (Antimicrobial Substances)

The necessity for variety and expansion of novel classes of antimicrobial agents is growing due to resistance shown against several antibiotics by diverse groups of bacteria, fungi and other microorganisms that causes severe complications in repression of contagious diseases. Many documents have reported antifungal substances from mangrove ecosystem. Two despidones (auranticins A and B) that exhibit antimicrobial activity were generated from fungus *Preussia aurantiaca* (Poch and Gloer 1991). In mangrove environment, isolated *Aigialus parvus* BCC-5311 synthesized aigialomycins A–E, resorcylic macrolides and hypothemycin. Few studies reported a novel compound—enniatin G—extracted from *Fusarium* sp. which showed antibiotic, antitumour, phytotoxic and insecticidal activity. Lin et al. (2008) described an actinomycete *Streptomyces* sp. that strongly constrains the growth of gram-negative as well as gram-positive bacteria.

1.5.1 Bio-mediated Compounds

Study revealed that plants, fungi, bacteria and actinomycetes were found to produce bioactive compounds. Actinomycetes act as promising candidate for the treatment of diabetes and neurodegenerative diseases and are likely to be the rich cause for the detection of antitumour and anti-inflammatory compounds after few genetic modifications. Microorganisms living in the mangrove ecosystems are considered as a natural ‘hotspot’ for producing novel and superior drugs. It was reported that 2000 microbes, viz. fungi, bacteria and actinomycetes, that have potential to synthesize secondary metabolites were also having anticancer, antitumour and anti-inflammatory properties. *Streptomyces albidoflavus* isolated from the Pichavaram mangrove that exhibited antitumour properties was reported by Marine algae are the key source of phycocolloids such as agar, carrageenan and alginate (Shanmugam and Mody 1999). They were also reported to have anticomplementary, anti-mutagenic, blood anticoagulant, antiviral, hypolipidemic, hypoglycaemic, immunomodulating, anti-inflammatory and antitumour activities.

Microorganisms are in diverse form and a vital constituent of biotic diversity that has played a momentous role in origin of life on the earth and in maintaining ecology of various habitats. They are the abundant janitors across the globe occurring in all climatic regions, including Arctic, Antarctic, deep within rocks and oceanic hot vents. The extensive genetic variation encompasses the spectrum of variability among various species. Microbial diversity comprises broad group of microbes that

are useful for food production and global environmental protection as well as have many applications: for example, as immunosuppressants; as antimicrobial and anti-proliferative drugs; as immunomodulators; as anthelmintics in pharma industries; as a fermentation product in food industries; as food processing agents, antiparasitic agents and biopesticides in agricultural region; as a microbial product intended for manufacturing organic compounds, vitamins, amino acids, biocatalyst and bioconversion agents; as detergents by chemical industries; as bioenergy and bioremediation agents in environmental industries using biotechnological approaches to clean up the contaminated sites, in recycling process of nutrients and in maintenance of ecosystem health in biosphere.

The traditional techniques for cultivating microbes and advanced culture-independent methods might be deliberated as a principal approach to understand how microbes live as well as their role in extreme habitats. The studies of microbial diversity pave a healthier thought of the role and purpose of microbial communities in terrestrial, aquatic and marine environments and a better understanding of the consequences of extinction of plant and animal species and of trepidations on ecosystem. Therefore, microbial communities are excellent models for studying and examining fundamental biological interactions for maintainable ecology of plants and animals and improved dimensions to uphold water quality and soil fertility.

1.6 Conclusion

The usage of microbial enzymes is dispersed in several fields such as in preparing enantiomer, pure drugs from racemic mixture; for the production of robust drugs, as a therapeutic agent; etc., while pathogenic microbes causing disease to humans, plants and animals pose a threat to health, food safety and security. Recent advances in molecular genetics are currently gaining attention which is being supplemented by culture-dependent analysis. Diversity among microbes can be used to monitor and predict the changes in the environment as microbes are the major sources that have been involved in sustainable development. The ecosystem may function as a key parameter that controls various global cycles (nitrogen, carbon, sulphur, phosphorus and heavy metal cycles) by maintaining the dynamic equilibrium and integrity of our planet. The extensive industrial development has led to the exploitation of diverse forms of microbial communities by gradual changes in existing biotic and abiotic factors. Therefore, metagenomics is useful in exploiting unidentified microorganisms in various environments to reveal genomic content of new species and biomarkers for detecting several metabolic activities; it also delivers innovative techniques to obtain products from microorganisms without culturing them in laboratories and can be helpful in understanding complexity within microbial communities. The inability of traditional culturing techniques has shown the diversity of microorganisms and also that the species assortment in terrestrial and aquatic habitats is far superior than expected. The mainstream of microbial diversity (>90%) remains to be revealed. The extensive genetic variation encompasses the spectrum of alteration among various species. Nevertheless, the information regarding microbial physiology and genetics is crucial for transcriptomic and gene-level studies.

1.7 Future Perspectives

Most assorted group of organisms found in any form of environment are microbes. Till date, investigation has focused on those microbes that are culturable; however, an affluence of evidence is now being collected from unculturable microbes. A grander consideration of the microbial world can benefit ecological organization. These organisms are the root for revolutions that empower life to endure; thus, acquaintance of their interfaces, characters and tasks is vivacious to our understanding. We are in the middle-of-the-road to explore, identify, conserve and use microbes to support mankind specifically and ecology. Marine organisms thrive not just in the surface of ocean but also in addition in the lower and deep profundities from coastal to the offshore regions of a particular habitat. This community of microbes is still unknown and might have evolved with many mysteries which are still to be answered. Plant microbiome-based solutions could achieve a change in perspective of their role in health and illness and have significant results for biocontrol and medical problem. Targeted microbiome engineering for crops is an upcoming inclination. Biodiversity should be a biomarker for these microbiome cadences. Higher plant-associated miscellany can be achieved not only through the enactment of biological control agents which shifts the microbiome but also by the solicitation of microbial consortia. Half-life of gut microbes could be managed through probiotics, and estrogen replacement therapy can be benefited by long-term users through altering properties of estrogen without increasing the risk of reproductive cancers. Microbiota populations have been found in the human skin, mucosal membranes, and gut where they can influence several disorders, such as diabetes, obesity, cancer and colitis. Thus, a multiomics approach to identify and sequence different microbial populations in the body can provide valuable information. Gut bacteria markers can be called as ‘smoking gun’ for liver disease as they can spot the early stages of liver disease by releasing chemical compounds produced by the bacteria in our gut. Microbiologists are finding new-fangled ways to sightsee the new places and new biotechnological intervention in the hunt of new medicine and new techniques to help mankind. Some longstanding mysteries about microbial diversity are their diversity and stability in various ecosystems which can solve many queries about evolution and could also help to understand the future and make it easy.

References

- Acharya S, Chaudhary A (2012) Bioprospecting thermophiles for cellulase production. *Braz J Microbiol* 43:844–856
- Alain K, Querellou J (2009) Cultivating the uncultured: limits, advances and future challenges. *Extremophiles* 4:583–594
- Arrigo K (2005) Marine microorganisms and global nutrient cycles. *Nature* 437:349–355
- Baldrian P, Kolařík M, Stursová M, Kopecký J, Valášková V, Větrovský T, Zifčáková L, Snajdr J, Rídl J, Vlček C, Voříšková J (2012) Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J* 6(2):248–258

- Bernard L, Chapuis-Lardy L, Razafimbelo T, Razafindrakoto M, Pablo AL, Legname E, Poulain J, Brüls T, O'Donohue M, Brauman A, Chotte JL, Blanchart E (2012) Endogeic earthworms shape bacterial functional communities and affect organic matter mineralization in a tropical soil. *ISME J* 6(1):213–222
- Challis G, Hopwood DA (2003) Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proc Natl Acad Sci U S A* 2:14555–14561
- Das P, Mukherjee S, Sen R (2009) Substrate dependent production of extracellular biosurfactant by a marine bacterium. *Bioresour Technol* 100:1015–1019
- Davis KE, Joseph SJ, Janssen PH (2005) Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. *Appl Environ Microbiol* 71:826–834
- Ferrari B, Gillings MR (2009) Cultivation of fastidious bacteria by viability staining and micromanipulation in a soil substrate membrane system. *Appl Environ Microbiol* 75:3352–3354
- Ferrari B, Winsley T, Gillings M, Binnerup S (2008) Cultivating previously uncultured soil bacteria using a soil substrate membrane system. *Nat Protoc* 3:1261–1269
- Fuhrman JA, Noble RT (1995) Viruses and protists cause similar bacterial mortality in coastal seawater. *Limnol Oceanogr* 40(7):1236–1242
- Gagné S, Richard C, Rousseau H, Antoun H (1987) Xylem-residing bacteria in alfalfa roots. *Can J Microbiol* 33(11):996–1000
- Gest H (2001) Evolution of knowledge encapsulated in scientific definitions. *Persp Biol Med* 44:556–564
- Gest H (2008; posting date) The modern myth of “unculturable” bacteria/scotoma of contemporary microbiology. <http://hdl.handle.net/2022/3149>
- Ghosh K, Roy M, Kar N, Ringø E (2010) Gastrointestinal bacteria in rohu, *Labeo rohita* (Actinopterygii: Cypriniformes: Cyprinidae): scanning electron microscopy and bacteriological study. *Acta Ichthyol Piscatoria* 40:129–135
- Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Lukasik R (2010) Hemicelluloses for fuel ethanol: a review. *Bioresour Technol* 13:4775–4800
- Holguin G, Bashan Y, Mendoza-Salgado RA, Amador E, Toledo G, Vazquez P, Amador A (1999) La Microbiología de los manglares. Bosques en la frontera entre el mar y la tierra. *Ciencia Desarrollo* 144:26–35
- Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* 180:4765–4774
- Husain I, Sharma A, Kumar S, Malik F (2016) Purification and characterization of glutaminase free asparaginase from *Pseudomonas otitidis*: induce apoptosis in human leukemia MOLT-4 cells. *Biochimie* 121:38
- Husain I, Bala K, Wani A, Makhdoomi U, Malik F, Sharma A (2017) Arginase purified from endophytic *Pseudomonas aeruginosa* IH2: induce apoptosis through both cell cycle arrest and MMP loss in human leukemic HL-60 cells. *Chem Biol Interact* 274:35–49
- Jiang HL, Tay STL, Maszenan AM, Tay JH (2006) Physiological traits of bacterial strains isolated from phenol-degrading aerobic granules. *FEMS Microbiol Ecol*
- Joshi AA, Kanekar PP, Kelkar AS, Shouche YS, Vani AA, Borgave SB, Sarnaik SS (2008) Cultivable bacterial diversity of alkaline Lonar Lake, India. *Microb Ecol* 55:163–172
- Kaerberlein T, Lewis K, Epstein SS (2002) Isolating ‘uncultivable’ microorganisms in pure culture in a simulated natural environment. *Science* 296:1127–1129
- Koch AL (1997) Microbial physiology and ecology of slow growth. *Microbiol Mol Biol Rev* 61:305–318
- Kostka JE, Prakash O, Overholt W, Green S, Freyer G, Canion A, Delgado J, Norton N, Huettel M (2011) Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Appl Environ Microbiol* 77:7962–7797
- Kumar S, Narayani S, Shankar T, Pandian V (2012) Optimization of cultural conditions for exopolysaccharides production by *Frateuria aurantia*. *Int J Appl Biol Pharmaceut Technol* 3:133–143

- Kuske C, Ticknor LO, Miller ME, Dunbar JM, Davis JA, Barns SM, Belnap J (2002) Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Appl Environ Microbiol* 68:1854–1863
- Lanteigne C, Gadkar VJ, Wallon T, Novinscak A, Filion M (2012) Production of DAPG and HCN by *Pseudomonas* sp. LBUM300 contributes to the biological control of bacterial canker of tomato. *Phytopathology* 102:967–973
- Leadbetter JR (2003) Cultivation of recalcitrant microbes: cells are alive, well and revealing their secrets in the 21st century laboratory. *Curr Opin Microbiol* 6(3):274–281
- Lin DX, Wang ET, Tang H, Han TX, He YR, Guan SH, Chen WX (2008) *Shinella kummerowiae* sp. nov., a symbiotic bacterium isolated from root nodules of the herbal legume *Kummerowia stipulacea*. *Int J Syst Evol Microbiol* 58(Pt 6):1409–1413
- Mulligan C, Fischer M, Thomas GH (2011) Tripartite ATP-independent periplasmic (TRAP) transporters in bacteria and archaea. *FEMS Microbiol Rev*
- Newton RJ, McLellan SL (2015) A unique assemblage of cosmopolitan freshwater bacteria and higher community diversity differentiate an urbanized estuary from oligotrophic Lake Michigan. *Front Microbiol* 6:1028
- Odonkor S, Ampofo JK (2013) *Escherichia coli* as an indicator of bacteriological quality of water. *Microbiol Res* 4:5–11
- Overmann J (2006) Principal of enrichment, isolation, cultivation and preservation of prokaryotes. *Prokaryotes* 1:80–136
- Peris-Bondia F, Latorre A, Artacho A, Moya A, D’Auria G (2011) The active human gut microbiota differs from the total microbiota. *PLoS One*:e22448
- Pilon-Smits E (2005) Phytoremediation. *Annu Rev Plant Biol* 56:15–39
- Poch GK, Gloer JB (1991) Auranticins A and B: two new depsidones from a mangrove isolate of the fungus *Preussia aurantiaca*. *J Nat Prod* 54(1):213–217
- Pornsunthorntawe O, Wongpanit P, Chavadej S, Abe M, Rujiravanit R (2008) Structural and physicochemical characterization of crude biosurfactant produced by *Pseudomonas aeruginosa* SP4 isolated from petroleum-contaminated soil. *Bioresour Technol* 99:1589–1595
- Rampelotto PH (2013) Extremophiles and extreme environments. *Life (Basel)* 3(3):482–485
- Reiter B, Pfeifer U, Schwab H, Sessitsch A (2002) Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. *Appl Environ Microbiol* 68(5):2261–2268
- Rijavec T, Lapanje A, Dermastia M, Rupnik M (2007) Isolation of bacterial endophytes from germinated maize kernels. *Can J Microbiol* 53(6):802–808
- Salama MS, Sandine WE, Giovannoni SJ (1993) Isolation of *Lactococcus lactis* subsp. *cremoris* from nature by colony hybridization with rRNA probes. *Appl Environ Microbiol* 59:3941–3945
- Schmeisser C, Steele H, Streit WR (2007) Metagenomics, biotechnology with non-culturable microbes. *Appl Microbiol Biotechnol* 75(5):955–962
- Selvarajan R, Sibanda T, Tekere M, Hand N, Taylor SM (2017) Diversity analysis and bioresource characterization of halophilic bacteria isolated from a South African Saltpan. *Molecules* 22(4)
- Shanmugam MH, Mody K (1999) Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. *Curr Sci* 79
- Sharma A, Pal PB, Rehman MB, Sharma J (2018) Monitoring of phenolic compounds and surfactants in waste water mixing with Narmada river at Jabalpur, (India). *Int J Adv Res* 6(4):97–102
- Shivaji S, Reddy G, Aduri P, Kuty R, Ravensschlag K (2004) Bacterial diversity of a soil sample from Schirmacher Oasis, Antarctica. *Cell Mol Biol (Noisy-le-Grand, France)* 50:525–536
- Singh D, Raina TK, Kumar A, Singh J, Prasad R (2019) Plant microbiome: a reservoir of novel genes and metabolites. *Plant Gene*. <https://doi.org/10.1016/j.plgene.2019.100177>
- Song J, Oh HM, Cho JC (2009) Improved culturability of SAR11 strains in dilution-to-extinction culturing from the East Sea, West Pacific Ocean. *FEMS Microbiol Lett* 295:141–147
- Staben ML, Bezdicke DF, Fauci MF, Smith JL (1997) Assessment of soil quality in conservation reserve program and wheat-fallow soils. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America

- Stevenson BS, Eichorst SA, Wertz JT, Schmidt TM, Breznak JA (2004) New strategies for cultivation and detection of previously uncultured microbe. *Appl Environ Microbiol* 70:4748–4755
- Sturz AV, Christie BR, Matheson G, Nowak J (1997) Biodiversity of endophytic bacteria which colonizer clover nodules, roots, stem and foliage and their influence on host growth. *Biol Fertile Soils* 25:13–19
- Tamaki H, Sekiguchi Y, Hanada S, Nakamura K, Nomura N, Matsumura M, Kamagata Y (2005) Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques. *Appl Environ Microbiol* 71(4):2162–2169
- Tribelli PM, López NI (2018) Reporting key features in cold-adapted bacteria. *Life (Basel)* 8(1):8
- Tripp HJ, Kitner JB, Schwalbach MS, Dacey JW, Wilhelm LJ, Giovannoni SJ (2008) SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature* 452:741–744
- Vartoukian SR, Palmer RM, Wade WG (2010) Cultivation of a Synergistetes strain representing a previously uncultivated lineage. *Environ Microbiol* 12:916–928
- Vega FE, Pava-Ripoll M, Posada F, Buyer JS (2005) Endophytic bacteria in *Coffea arabica* L. *J Basic Microbiol*
- Ventosa A, Nieto J (1995) Biotechnological applications and potentialities of halophilic microorganisms. *World J Microbiol Biotechnol* 11:85–94
- Verlindin RA, Hill DJ, Kenward MA, Williams CD, Radecka I (2007) Bacterial synthesis of biodegradable polyhydroxyalkanoates. *J Appl Microbiol* 102:1437–1449
- Vitorino LC, Bessa LA (2018) Microbial diversity: the gap between the estimated and the known. Laboratory of Agricultural Microbiology, Goiano Federal Institute, Rio Verde Campus, Rio Verde GO 75901-970, Brazil
- Wilkins D, Seville EV, Stephen R, Rintoul SR, Lauro FM, Cavicchioli R (2013) Advection shapes Southern Ocean microbial assemblages independent of distance and environment effects. *Nat Commun* 4:2457
- Yadav AN (2015) Bacterial diversity of cold deserts and mining of genes for low temperature tolerance. <https://doi.org/10.13140/RG.2.1.2948.1283/2>
- Yadav AN, Saxena AK (2018) Biodiversity and biotechnological applications of halophilic microbes for sustainable agriculture. *J Appl Biol Biotechnol*
- Yashiro E, Figueroa EP, Buri A, Spangenberg JE, Adatte T, Hirzel HN et al (2016) Local environmental factors drive divergent grassland soil bacterial communities in the western Swiss Alps. *Appl Environ Microbiol* 82:6303–6316
- Zengler K (2009) Central role of the cell in microbial ecology. *Microbiol Mol Biol Rev* 73:712–729
- Zengler K, Toledo C, Rappe M, Elkins J, Mathur EJ, Short JM, Keller M (2002) Cultivating the uncultured. *Proc Natl Acad Sci USA* 99:15681–15686