Rhizosphere Biology

Reeta Goel
Ravindra Soni
Deep Chandra Suyal *Editors*

Microbiological Advancements for Higher Altitude Agro-Ecosystems & Sustainability

Springer

Rhizosphere Biology

Series Editor

Anil K. Sharma
Biological Sciences, CBSH, G. B. Pant University of Agriculture & Technology,
Pantnagar, Uttarakhand, India

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Editors
Reeta Goel
Department of Microbiology
Govind Ballabh Pant University
Pantnagar, Uttarakhand, India

Ravindra Soni
Department of Agricultural Microbiology
Indira Gandhi Krishi Vishwavidyalaya
Raipur, Chhattisgarh, India

Deep Chandra Suyal
Department of Microbiology
Eternal University
Sirmaur, Himachal Pradesh, India

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Dedicated to

THE HIMALAYAS

Preface

Higher altitude agro-ecosystems are the most neglected crop-lands due to several geographical and climatic constraints, considering soil infertility and land degradation. In present scenario, soil management strategies are mainly dependent on inorganic chemical-based fertilizers and therefore cannot be recommended for these agro-ecosystems. Nevertheless, they are also referred as “water tower” for freshwater supply to low lying areas and, thus, any anthropogenic contamination on them can disperse throughout the riverfront. In this perspective, implication of the bio-inoculants at high altitude marginal lands could be a promising approach for agricultural sustainability. However, identification, characterization, and documentation of native micro-flora with reference to local environmental conditions and natural resources are inevitable for the development of agricultural strategies.

This book consists of 23 chapters including challenges and opportunities, microbial interactions, and nutrient dynamics at higher altitudes. It also describes the microbiological advancements and their scope for attaining agricultural sustainability, *viz.* agriculturally important genes and enzymes, rhizosphere modeling and engineering, genetically engineered bio-inoculants, next-generation technologies, omics and nano-based technologies, etc. Moreover, traditional agricultural practices employed at higher altitudes have also been revealed along with the recent trends.

In the perspective of the global food demand, sustainable agricultural policies involving uncultivable and/or neglected lands became the need of this hour. However, till date, very few attempts have been made on the cold-adapted bio-inoculants and their exploration. Therefore, this book is an effort to enlist and document the various aspects of related microbiomes and their advancement towards the high altitude farming and agricultural sustainability.

The editors gracefully acknowledge the help, suggestions, and encouragement made by their colleagues and well-wishers. Nonetheless, the editors are grateful to all the authors who have contributed to this book. They are really thankful for their timely response, cooperation, and patience. Unfortunately, each and every aspect could not be added in this book due to predefined page limit. However, they can be included in the next book of this series.

Suggestions for the improvement of the book will be highly appreciated and incorporated in the subsequent editions. Finally, senior editor would like to acknowledge the motivation, commitment, and sincerity of both the co-editors because without their efforts this book would not have taken its present status.

Pantnagar, Uttarakhand, India — Reeta Goel
Raipur, Chhattisgarh, India — Ravindra Soni
Sirmaur, Himachal Pradesh, India — Deep Chandra Suyal

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About the Editors

Reeta Goel is a Professor and Head of the Department of Microbiology, G. B. Pant University of Agriculture and Technology, Uttarakhand, India. She has also served as a visiting scientist at various institutes in the USA and has more than 35 years of teaching and research experience in the area of plant–microbe interactions, microbial ecology, biodegradation, and metagenomics, especially with reference to higher altitudes. Moreover, she has worked extensively in the field of genomics and proteomics of cold-adapted microorganisms. She has published over 100 research articles, book chapters, and edited books. She holds three patents including one U.S. Patent and three are filed. She has received numerous National and International awards for her contributions to science and technology.

Ravindra Soni is an Assistant Professor at the Department of Agricultural Microbiology, IGKV, Raipur, Chhattisgarh, India. He has more than 10 years of teaching and research experience in the field of agricultural microbiology, microbial ecology, molecular biology and biotechnology, especially with reference to higher altitudes. He has published several research articles and chapters in respected international journals and books. He has filed one patent.

Deep Chandra Suyal is an Assistant Professor at the Department of Microbiology, Eternal University, Baru Sahib, Sirmaur, Himachal Pradesh (India). He has been actively engaged in research for 9 years and has experience in the fields of agricultural microbiology, molecular biology and biotechnology, and metagenomics. He is currently investigating the genomics and proteomics of cold-adapted microorganisms. He has published numerous articles in respected international journals and filed three patents.

High Altitude Agro-ecosystems: Challenges and Opportunities

1

Anil Kumar Shankhwar, Prashansa Tamta, Rashmi Paliwal, and R. K. Srivastava

Abstract

The high altitude agro-ecosystem is very delicate and fragile due to its topography and water scarcity. It triggers the disruption to the soil resilience between nutrient retention and loss, after the weathering of parent material. The topographic challenges of undulated and sloppy land coupled with natural calamities like landslides, soil erosion, and earthquakes hamper the water storage and management strategies. The most prominent adversity in high altitude is water scacity that restricted hill agriculture to seasonal rain-fed agriculture. The plantation of tree in arable land has contributed the vital impact on soil properties to cater the microbial growth. The shallow soil with poor soil health is also affected by the phenolic compounds. The technologies available currently for hill-specific agriculture are not effectively leading towards traditional farming. The high altitude is the land of enriched agro-biodiversity with unique agro-ecosystem blessed with traditional organic farming and utilizing the virtues of trees maximally. Tree-oriented technologies are the heart of hill agro-ecosystem and play vital role like the water recharging, agroforestry, and sustainable management of hill. The implementation of indigenous technologies and knowledge of local people and

A. K. Shankhwar (✉)
Centre for Environmental Science and Engineering, Indian Institute of Technology Kanpur, Kanpur, India

P. Tamta
University School of Environment Management, GGS Indraprastha University, New Delhi, India

R. Paliwal
Institute of Environmental Studies, Kurukshetra University, Kurukshetra, Haryana, India

R. K. Srivastava
Department of Environmental Science, G. B. Pant University of Agriculture and Technology, Pantnagar, India

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their perception is must. Therefore, the chapter presents the different agro-ecosystem of higher altitudes of India, challenges occurred, and the opportunities.

Keywords
Agriculture · High altitude agro-ecosystems · Agrobiodiversity · Hill farming · Climate change

1.1 Introduction

The high altitude agro-ecosystem (Hal-agro-ecosystem) is one of the most unique, fragile and pristine ecosystem that needs extensively conservation. The Hal-agro-ecosystem is essentially important as a resource to supply the numerous ecosystems and the source of many rivers in India and adjoining countries. The undulated, undefined landscapes are one of the most fragile ecosystems. A wide human interventions in region of Hal-agro-ecosystem has hammered the biodiversity and converted to monoculture land system. In the simplest way of understanding, the monoculture practices, will promote a single plant population on soil system that will nourish and intake some sort of preferable nutrient from the soil systems for their growth and development. In turn during the process of the lifecycle, it will definitely add and harness some particular nutrients to the soil of Hal-agro-ecosystem. In the monoculture practice, the nutrient cycling process will be shifted or manipulated as the monoculture plant species will harness certain typical nutrients from the soil by its vital activities for nutrient cycling.

The tendency of water to move downward by the gravitational pull in hilly terrain escalates the discharge of precipitated water tremendously as runoff. This discharge of water in hilly terrain is the source of rivulets. However, the low temperature in high hill creates the temperate climate to grow the trees but the scarcity of water makes it adverse situation for survival of plants. Hence the high altitude agro-ecosystem is very delicate and fragile due to its topography and water scarcity. The runoff of water dissolves out the uppermost fertile soil layers and makes it inferior in nutritive values. Apart from the nutrient loss, the runoff triggers many other changes like low percolation, low water holding capacity of soil, thinning of the soil depth, lowering the soil humus, influencing the soil pH, soil Eh, soil buffering capacity, and many more which ultimately disrupt the soil resilience. The inherent challenges of hill soil are of undulated and sloppy land coupled with natural calamities like landslides, soil erosion, and earthquakes. The conservation practices in these topographic regimes is not well managed so far and require site-specific management strategies as well as water storage. The water storage strategy is mingling of management strategies in hill. The water scarcity is the most prominent adversity in high altitude that results to rain-fed agriculture. The rain-fed agriculture is solely season depended and restricted to very narrow period of time.

The leaf litter decomposition is a vital activity that affects/limits the regeneration in natural forest. The littered leaves in forest is piled up in layers and prevent the seed contact with soil and moisture, results hindrance in seed germination in general. Oak tree is considered water conserving tree species; however, chirpine is water non-conserving species, which results in water loss in hills. As soon as the water scarcity increases, it also affects the tree diversity and dynamics. It will promote the growth and spread of the chirpine and will reduce the oak forest. The water scarcity coupled with the forest fire is directly responsible for reduction of oak from forest. In the chirpine dominated forest the needles of leaf litter affect the seed germination more drastically as the chirpine needle requires the more time in decomposition of leaf litter into humus due to the presence of phenolic compounds. The phenolic compounds have allelopathic and/or pathogenic traits that negatively effect on roots of vegetation (Sherrod and Domsch 1970). The leaf litter deposited in the form of slippery mat in undulated sloppy land regimes is caused detrimental to seed dispersal in high altitude agro-ecosystem (Hal-agro-ecosystem) by sliping down and rendering to contact the soil. In ancient times, the chirpine resins were used to apply for the torch. The phenolic compounds are the root cause for spreading forest fire. Thus, the chirpine needles are injurious to soil health in many ways and negatively impact plant diversity. The soil thinning makes adverse situation for propagation of newly recruits in the hilly soil as the seeds fail to get the proper soil, humus, and moisture for its germination. The ultimate effect of this soil thinning also affects the biodiversity of the region. Therefore, it requires the longer time for humus formation. Humus forms the top fertile soil and it supplies the nutrients to the soil also. Moreover, the phenolic compound also triggers the forest fire, the most burning issue in hill. Another interesting fact in this regard is the chirpine trees, which are fire-resistant in nature and the only species to survive during forest fire. Hence the cumulative effect of chirpine on forest with time reduces the oak and other water conserving tree species. Hence the ecological conditions of hilly area are dramatically different from the conditions prevailing in non-hilly areas and require all the strategies as per the problems and prospects of hilly areas.

1.1.1 Characteristics of High Altitude

In India, the high altitude prevails in the central and foothills of Himalaya. The hilly terrain is pristine as well as fragile ecosystem. The fragile ecosystem is subjected to high conservation strategies; hence, at least 66% forest cover is mandatory to hilly regions. The Indian Himalaya is rich in distribution of floral biodiversity and characteristics from dry deciduous in foothills to alpine meadows at above timberline (Kumar and Thakur 2008). As per the tree dominancy, broadly the soil of Hal-agro-ecosystems may be categourised into two of following: (1) Oak-based forest soil and (2) Chirpine-based forest soil. The oak-based Hal-agro-ecosystem is progressive, constructive, ecofriendly, and sustainable in many ways. However, the chirpine-based Hal-agro-ecosystem is degradative, destructive and unsustainable ecosystem.

1.1.2 Farming System in High Altitude

The farming system of hilly terrain is not influenced with the trend of farming due to its remote location and this is why hill farming system in India is based on organic farming at several places. The farming system also practices to cultivate the old-fashioned food grain traditionally especially small millets like mandua, ragi, kodo, sava, etc. The farming system is indispensably pre-dominated by trees. The traditional agro-forestry system is very common and tree at bunds is more prominent in Hal-agro-ecosystem. On the basis of dominant tree species, the Hal-agro-ecosystem may be divided into oak and chirpine ecosystem (Fig. 1.1).

1.1.3 Prominent Agro-ecosystem

The agricultural practices at high altitude are traditional and tree-dominating which is essentially based on perennial fruit bearing trees. In the high altitude agro-ecosystems (Hal-agro-ecosystem), as per the altitude a wide variety of horticulture trees are grown, namely apple, plum, peach, pear, malta, akharot, and apricot. However, chirpine, oak, and sal are the main dominating trees in Hal-agro-ecosystem. The prominent crops in Hal-agro-ecosystem are cash crops. A farmer's perception is that if they will stop to cultivate the cash crop the economic stability will be misbalanced (Bijalwan et al. 2018).

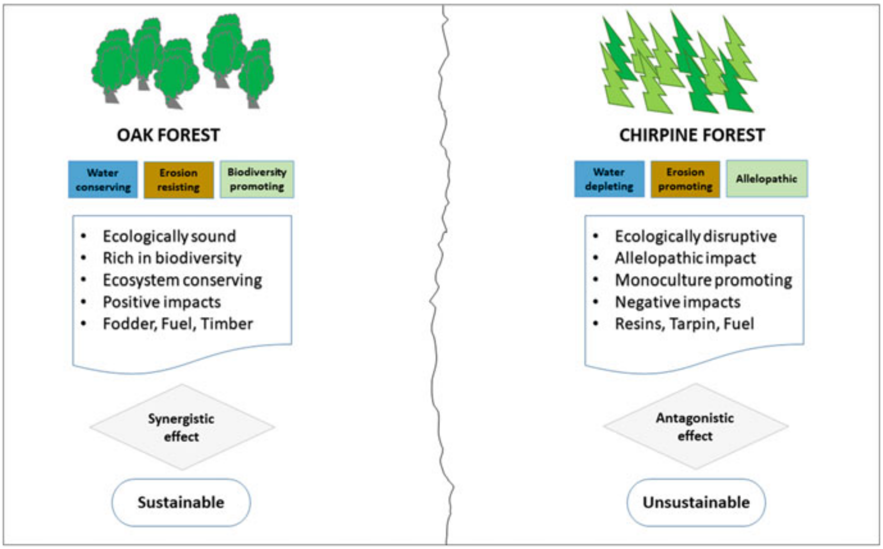

Fig. 1.1 Oak and chir based Hal-agro-ecosystem

1.1.4 Challenges and Opportunities

The Hal-agro-ecosystem of Himalayan constituency prevails tree-based ecosystem. The functions of Hal-agro-ecosystem are as self-governing support system for the livelihoods, livestock, and local value chain. The environment as abiotic constituent attributes the production of Hal-agro-ecosystem in forest of Kumaun Himalaya (Adhikari et al. 1991; Singh et al. 1994). Apart from the steep slopes, the factors are such as elevation, temperature, rainfall, moisture, pressure, sunshine, humidity, cloudiness, and soil nutrients. Adequate numbers of research findings (Kroeger et al. 2018; Khan et al. 2018; da C Jesus et al. 2009; Xun et al. 2018) admitted that physicochemical properties of soil directly affect the microbial composition of soil and its diversity as well.

The vegetation in Hal-agro-ecosystem designs the ecological niche and vice versa. The composition of soil biota (microflora, microfauna, mesofauna, and macrofauna) and its matrices interact to each other in complex way. These complex interrelations trigger the nutrient cycles of the ecosystem through conditioning the environment to attract the specific microbes. The soil biota interacts in Hal-agro-ecosystem to regulate the flow of nutrients from organic matter (OM) by stimulating the microbes for metabolic reactions for nutrient turnover. The supply of nutrient also mediates the vegetation growth of ecosystem, in turn more OM is generated. The dynamics of nutrient in an ecosystem also influence the soil physical properties and ultimately structure (Altieri 1999; Hendrix et al. 1990; Monika et al. 2017).

The decomposition of plant residue release nutrient to return back and ensure the continuous supply of nutrients to plants and its consumers, thus contributing to ecosystem dynamics (Wardle 2004). The positive response between the ecosystem accelerates the plant growth and more leaf to decompose, finally controlled the rate of decomposition (Aerts 1997; Cornwell et al. 2008; Bontti et al. 2009) by the attributes of Hal-agro-ecosystem soil regimes, including soil physicochemical properties, decomposer communities, and microclimatic conditions created by established plants (Fig. 1.2).

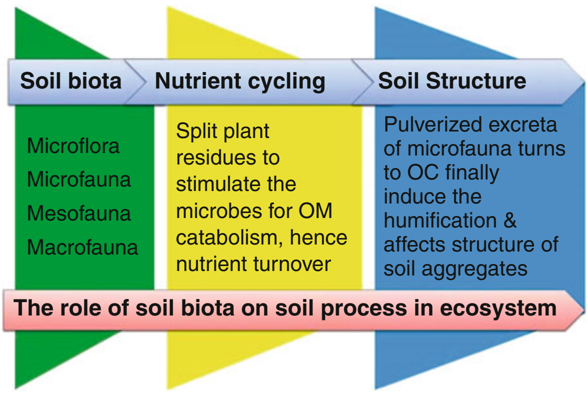

Fig. 1.2 Role of soil biota in soil nutrient dynamics in ecosystem

1.1.4.1 Soil Health

The assessment of any soil ecosystem is based on some certain soil quality parameters (SQPs), and the SQPs are dynamic in nature and variable in short time of duration. The indicative SQPs responsible for the soil health are as follows: soil depth, soil bulk density, water holding capacity, porosity, infiltration rate, pH, EC, SOC, CEC, available N, P and K, microbial biomass, mineralizable nitrogen and dehydrogenase enzymatic activity. These SQPs are collectively used for soil quality index (Prasad et al. 2017). Broadly, the soil depth in Hal-agro-ecosystem is thin and has less SOC and low water holding capacity due to the sloppy terrain. The unavailability of water is a most prominent issue in Hal-agro-ecosystem.

1.1.4.2 Soil–Water Interaction (SWI)

The soil–water interaction (SWI) is the outcome of various components, viz. cations exchange capacity (CEC), concentration effects, primary mineral decomposition or parent material, water flow rate, and addition of acid or base in soil. Mainly, the SWIs are interplay of the types of soil (in different land characteristics) and its hydrological pathway. Ultimately, the nutrient constituent of water is also determined through the SWIs. For proper SWI, the higher contact time is desirable and it plays the crucial role and in high altitude.

Hal-agro-ecosystem in Himalayan terrain is unique and outcome of the following parameters: (1) Altitude plays a significant role in the forest productivity as the number of trees per unit area and total basal cover decreases with increasing altitude; (2) Soil parameters like the bulk density, particle density, particle size, and texture changes with altitude; (3) Forest type is also change with rainfall, temperature, altitude, and latitude, and it influences the amount of soil organic matter; (4) The non-coniferous tree absorbs more carbon than conifers due to shape of leaves; (5) Highly fragile ecosystem of Hal-agro-ecosystem in Himalaya required intensive management inputs as compared to the ecosystem at lower elevations; and (6) Hal-agro-ecosystem reveals lesser capability of soil to stabilize soil organic matter (Singh et al. 2012).

1.1.5 Small Landholding

In India, only 13% farmers hold more than 2 ha agricultural land, majority with 31% landholdings falls under 0.01–0.40 ha, 20% with 1.0–2.0 ha, 30% with 0.41–1.0 ha, and even 6% farmers have $<0.01\%$ landholding (NAFIS, 2016–2017). The figures reflect that small landholding of agricultural land exerts pressure and ultimately forces farmers to disillusion from agriculture practices. Since a majority of the landholdings are under small or marginal farming system which is impractical to handle and may lead towards land degradation especially in hilly terrains. Therefore, the integrated farming system will reduce the costs significantly through economies of scale (Table 1.1).

Table 1.1 State-wise average landholding of household in hilly states (lands in hectare)

S.N.	States	Agricultural households	Non-agricultural households
1.	Uttarakhand	0.63	0.10
2.	Himachal Pradesh	0.47	0.16
3.	Jammu	0.44	0.23
4.	**India**	**1.00**	**0.13**

Source: NAFIS, 2016–2017

1.1.6 Tools and Farming Implements

The farming system is supported by improved tools and implements to enhance efficiency of agricultural practice and ultimately production (Sarkar et al. 2015). The traditional farming systems exist in hill that supports the livelihoods of hill farmers with the small landholdings. Actually, the farming practice is women and youth centric in India and improved tools and implements are desirable as the system is input intensive.

In the hill, the fields are undulated, small, and in form of the strips. The agriculture system depends on non-mechanized and tradition tools and implements. The agricultural implements specific to agriculture in hill areas are not available and need to be developed, as the available tractors and power tillers are not feasible to use in hilly location due to undulated land structure. Thus the farming system is owing to female farmers without proper mechanized farming in hilly states of India. For improving the productivity of land and hill farmers, the hill and female farmers oriented agricultural tools and implements are essentially required to design.

1.1.7 Mountain Agriculture and Climate Change

The Hal-agro-ecosystem vested by ecological attributes and results in the production of unique crops like apple, akharot, kiwi, yarsagumba, and so on. As per the changing environmental conditions and rising temperature, erratic rainfall and skipped snowfall consequently hamper the agricultural production. The consequence is like chilling requirement in apples, altitudinal and specific environment of yarsagumba, weather condition of akharot, etc. The climate change may affect these crops due to changes in niche area of cultivation. The tree-based Hal-agro-ecosystem contributes as a carbon depositor (Pan et al. 2011) and is helpful for CO_2 mitigation. The leaf abscission followed by its decomposition enhance the organic carbon to the Hal-agro-ecosystem and it is beneficial to the soil by lowering the *C*/*N* ratio (Monika et al. 2017). However, the system is input intensive, and the addition of organic carbon with higher *C*/*N* ratio waste is undesirable and needs to be avoided (Jeet et al. 2014).

The role of an ecosystem for sequestering carbon is dependent to the type of vegetation. In this regard, the stored carbon of plant biomass of sal and oak dominated forest (80–92%) is compared with the chirpine and chirpine dominated (~50%) and found significantly higher in sal and oak forest (Jina et al. 2008).

1.2 Opportunities in Hill Farming

1.2.1 Ecological Niche Area of Cultivation

The rajma of Kinnaur (Himachal Pradesh) is well known and got fame as geographical indication. The study on Kinnaur rajma revealed that the selection of local landraces of rajma and its wild relatives is more useful to accelerate and enabled the genetic breeding programs for improvement of rajma (Kumar et al. 2009). The ecological niche may provide the economic benefits due to the uniqueness of produce and this ultimately uniqueness may be subjected to the environmental attributes (Boxes 1.1 and 1.2).

Box 1.1: Oak Tasar Silk

The geographic diversified landscape of India bestowed it with natural diversity and enabled the nation for rearing of all the four types of commercially important cultivars i.e., tropical Tasar, temperate Tasar, Eri, and Muga. Out of these cultivars, Tasar culture have been broadly experienced in plain (Jharkhand, Madhya Pradesh, Chhattisgarh, Orissa, and Uttar Pradesh) as well as hilly (Uttarakhand, northeastern, and sub-Himalayan belts of India) areas traditionally. Due to several unique reasons (ethnic, aesthetic, and eco-friendly), Tasar silk has a constant demand at local and global market. The production of Tasar silk is about 1.5% of total production and contributed to 16.5% silk of non-mulberry production in India. The invaluable contribution of forest and/or wasteland is the production of wild silk moth farming and to provide the perennial income as an added advantage. The Tasar silk moth is directly linked with the Oak tree as it survives on it. The Oak tree has many importance Hal-agro-ecosystem (Fig. 1.1) including the hosting the Tasar silk. The Tasar Oak cultivation in India provides ample opportunity for silk industry in India and generate a huge foreign revenue. Thus the rearing of Tasar will not only be supportive in reviving the Oak forests but would also become a good source for income generation in Hal-agro-ecosystem.

Box 1.2: Chilling Requirement in Apple cultivation

The chilling requirement is the need of apple to be in snowfall for good fruit-setting. A definite amount of cold temperature is needed for breaking the endodormancy at budding stage and is called as the chilling requirement (CR). Thus the cold temperature or snowfall is the limiting factor for apple cultivation. The apple production of any particular year is directly correlated to the snowfall of the year. In the present era of climate change, the skipping of

(continued)

Box 1.2 (continued)
snowfall affects the production of apple. Thus, for the apple cultivation the cold climate of Hal-agro-ecosystem is very crucial. But nowadays, due to climate change, the snowfall is also skipped and erratic rainfall is common that reduces the yield of apple.

The development of livelihood in Hal-agro-ecosystem is more important as other source of income generation in this region is inefficient. The migration is common in the Uttarakhand state; moreover, the economy of Uttarakhand is known as money order economy. Thus for the sustaining of the humans in tough terrain of Hal-agro-ecosystem, the site-specific management strategies coupled with mechanical marketing and tools are essentially needed. By virtue of Hal-agro-ecosystem is traditional, unique, and diverse, it has ample opportunity for conservation, resilience, and ecofriendly coupled with economically sound. The Hal-agro-ecosystem is conservation-centric region and renowned for ecotourism-based activity. Therefore, the concept of floriculture with the aim of ecological and aesthetic viewpoints is introduced by some farmers as eco-floricultural for economic return (Bijalwan et al. 2017). Hal-agro-ecosystem of northeastern India is mainly rain-fed with low agricultural productivity, which influences the livelihood security of hill farmers (Kumaresan et al. 2010).

1.2.2 Organic Farming Practices in Hill

Organic farming is an age-old concept with new challenges for farmers in current era with seven billion populations. Returning to organic farming has significance in India, for maintaining the soil health and adding nutritive quality to farm produces due to vibrant and resilience properties. The virtue of nature-based Hal-agro-ecosystem has inherent avenue for organic farming, and the nutraceutical properties of organic farming-grown produces added the economically viability along with the soil health management. In contrast, conventional farming is fully dependent to the application of synthetic chemicals and fertilizers and aims only at the enhancement of the crop production.

Hal-agro-ecosystem is the ecosystem that has its own limitations due to its location and considered as fragile and susceptible system. Thus it has a need of a sustainable approach of farming to tackle the food insecurities and loss of soil. The organic farming system relies on sustainable farming that securely employed in mountainous region efficient in agricultural sector without sacrificing natural attributes. It is basically an agriculture practice devoid the use of synthetic chemical and fertilizers in farming systems. This system retains the traditional farming practice including the crop rotation, recycling of nutrients of organic residues, animal dung, green manure, and agricultural wastage and leftovers. It also conveys the means of biological control of insect, pest, and disease. The system inputs are

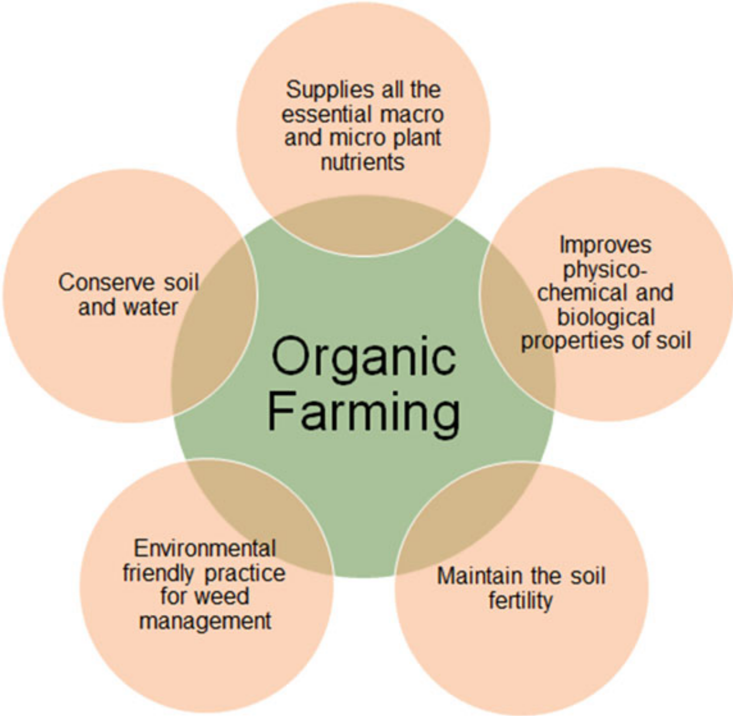

Fig. 1.3 Advantages of organic farming

nature based and therefore contain the benefits of nutritive produce with good soil health. It is an excellent example of integrated farming.

Organic farming practices cultivate many advantages (Fig. 1.3). It promotes the mixed cropping and application of manure, biofertilizers, etc. for soil management. It enhances the soil microbial and macro-faunal (earthworms) activities, thereby treating soil as living creature. As a part of life, people in high altitude traditionally depend upon the organic farming practices and manage the application of indigenous knowledge for maintaining the soil and agriculture. However, the industrial and institutional interventions have now successfully shifted the interests of farmers towards the fast agricultural development and replacement of organic practices with the inorganic one for rapid growth.

The hilly area requires retaining at least 66% forest for conserving the hilly environment and hence the technologies in this area are needed to be in coincidence with tree-based technology. Organic farming is based on conservation and sustainable to the environment. The system has self-replenish capacity due to recycling of nutrients of different types of wastage. Thus the organic farming system provides nutrients, soil safeguard, prevent land deteriorations, integrated waste management and scenic beauty. The most important aspect of organic farming is its easy to practice, farmers' friendly practices that strengthening the financial security to farmers. It is an integrated approach for the management of farming system, strengthening livelihoods, promoting the cow-bearing and traditional agriculture system. Thus it is one in all strategy of farming system, especially in Hal-agro-ecosystem. As in hilly areas the landholding is small and marginal as presented in Table 1.1. The agriculture system is basically as home-garden and other traditional farming system. Several studies have reported that organic farming is being successfully practiced in diverse climate, particularly in rain-fed, mountain, and hilly landforms of the nation. The nutracuticals are being widely grown over the Hal-agro-ecosystem due to the organic farming and these will avoid the ill impacts of chemical fertilizers and pesticides. These products are highly specialized with good economic returns and grown organically like herbs, medicinal plants, etc.

1.2.2.1 Challenges in Organic Farming Practices in Hill

The disillusion from agriculture as well as migration from hills is growing problem that affects hill farming. Several problems facing in hills owe to the migration to metro and other cities. Socioeconomic studies concluded that for better school and other facility the population from hill move towards cities. The Hal-agro-ecosystem in hilly region is uniquely favor some specific crops, like *Apple, Tasar* silk, etc. In present time, the climate change prevails that affects the apple cultivation. Likewise, the monoculture of chirpine reduces tree biodiversity as well as it eliminates the oak forest which is essential for the *Tasar* silk rearing. The water unavailability is the most frequently occurring situation in high hill regions. The proper water conservation strategies are required for sustenance of Hal-agro-ecosystem in India. Insufficient technologies specific to the hill farming system are also contributing significantly for disillusion from the agriculture.

1.3 Conclusions

In nutshell, the farming system in Hal-agro-ecosystem is very crucial for the sustaining the livelihood in high hill area. The hilly terrains are undulated landscapes, deficient not only in nutrients but also in basic needs of cultivations too. Being at remote area, old-fashioned agricultural practices are commonly being practiced. The other problems facing in high hill is water unavailability and rain-fed pattern of agriculture with small landholdings. Apart from this the less purchasing power, migration and economically unstable livelihood in Uttarakhand and monoculture of Apple based farming in Himachal is common. Hal-agro-ecosystem has very tough terrain but it is also full of opportunities that are specifically for may practice in high hill only. These opportunities are the Tasar silk rearing, organic farming, Apple cultivation, and many more. Thus the pristine Hal-agro-ecosystem is needed to conserve for the conservation of nature.

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2 An Overview of Indian Agriculture with Focus on Challenges and Opportunities in North East

Sachin Kumar Vaid

Abstract

Present day Indian agriculture is mainly based on small and marginal land holdings with high level of dependency on monsoon rains. Since farming is full of uncertainties at every stage, operating such small or marginal land holdings is not at all a profitable venture for farmers and thus the share of agriculture in Indian economy has progressively declined to less than 15%. Population is expanding at an alarming rate and by 2050 it is estimated that India will be home to 1.7 billion people. This population explosion will bring with it increased food and water demand along with urbanization. To feed the mouth of teeming billions in the upcoming years, it is essential to maintain equilibrium in food production and its consumption rates. However, the degrading soil quality of major growing areas of India is a matter of serious concern and if the supply will depend only on yield growth, India will face significant deficit in its agriculture production in the forthcoming years. Increase in area and productivity of crops, diversification towards high value crops as well as uplifting the backwardness in agriculture development are the need of the hour. One such agriculturally underexploited region is the North Eastern Region (NER) of India which is endowed with rich organic soils, plenty of water and favourable climate. Even though the agricultural potential of the region has not been unlocked due to the lack of appropriate technologies/planning, difficult topography, established markets, transport facilities and many more. Thus uplifting the level of agriculture in NER will surely help in reducing the demand-supply gap which would arise in near future and will help in elevating the rural farm incomes (main occupation of livelihood) thereby improving the socio-economic development in the region.

S. K. Vaid (✉)
Regional Centre of Organic Farming, Imphal, Manipur, India

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2.1 Evolution of Agriculture in India

Before the discovery of agriculture, early hominids used to live the life of a hunter and wander's here and there in search of food. At that time they made tools for hunting purpose from the then available resources such as pebbles. Farming started first in Middle East and in Indian subcontinent around 9000–12,000 years back (SubbaRao 2003). The crops cultivated firstly in the Indian subcontinent were wheat, barley and jujube. Firstly domesticated animals were dogs which might help the early hominids in hunting of wild animals (Gupta 2004) followed by the domestication of sheep and goat. Although traditional farming in India started much later, it laid the foundation of Indus Valley Civilization which offered man a sedentary lifestyle instead of a nomadic one and leads to the formation of villages and towns. The farmers of Indus Valley Civilization used bullock driven plough and established well managed irrigation channels for agricultural operations. They grew rice, peas, sesame, cotton and dates. Many of the written records of our Vedic literature compiled in famous treatise Arthashastra, Brihatsamhita and Agnipurana reveal that Indian farmers were well aware about climatology, plant physiology, soil types, mixed cropping, crop protection, ploughing, fallowing, irrigation and about use of different types of manures in agriculture (SubbaRao 2003). According to Indian Sanskrit text named Bhumivargaha, our ancestors had classified the agricultural land into 12 categories and the divisions were as follows: urvara (fertile), ushara (barren), maru (desert), aprahata (fallow), shadvala (grassy), pankikala (muddy), jalaprayah (watery), kachchaha (contiguous to water), sharkara (consist of pebbles and pieces of limestone), sharkaravati (sandy), nadimatruka (riverfed) and devamatruka (rainfed) (Kaushik and Jitender 2017). One of the traditional practices known as shifting cultivation or locally famous as jhum can be traced back to Neolithic period, i.e., around 8000 BC is still being practiced by different tribes in the hilly regions of North East India on a significant area.

2.2 Importance of Agriculture for India

Agriculture is the backbone of India since ancient times, as this sector continues to be the mainstay of the Indian population. The reason behind it is the living of the majority of Indian population approximately 70% in rural, remote and backward areas of the country. As a result masses are backward in terms of literacy and socio-economic development. When these people migrate to urban areas in search of other means of livelihood options they end up with lower level jobs such as construction labourer, plumber, carpenter, etc. Further the higher cost of living associated with urban cities makes it difficult for them to sustain their lives. Hence agriculture has been one of the largest employment sectors in India till date. According to Kapur (2018), employment share of agriculture was 82% in 1950–1951 and 72% in 2001. During the early 1950s the share of agriculture sector to the country's national income was 55% which declined to 25% in the early 2000s. Presently, the contribution from the agriculture sector in country's GDP is around 14% but still the dependency of the population on agriculture is very high. At present, approximately 58.4% of the Indian workforce is engaged in agriculture sector either as cultivators or as agricultural labourers (Kapur 2018). With an area of 3.288 million km^2, India is the seventh largest country in the world. Geographically, it is having second largest arable area in the world and having 127 diverse agro-climatic zones. Due to such strengths, India is globally second largest producer of rice, wheat, fish, fruits and vegetables. It is first in the world with respect to prawn production and in its export worldwide (Agricultural Situation in India 2018). Fishery sector alone in India is providing employment and source of livelihood to more than 15 million people of the country. Thus agriculture and its allied sectors are still playing a vital role in Indian economy even in this new millennium. At present population of India is approaching nearly 1.3 billion and thus the food demand is also rising. According to Chand (2017), since 1965–2015 Indian population has multiplied at the rate of 2.55 times while food production in the country has multiplied by 3.7 times. It means our country has recorded an increase of 45% in per person food production after the adoption of green revolution. This made India not only self-sufficient with respect to feeding its own population but also made our country capable of exporting different food commodities worldwide. Unfortunately due to the continuous increase in our country's population, the present rate of food grain production is not sufficient enough to meet the food demands of the new mouths which would be added in near future. Many of the authors in their articles have pointed out that food grain demand of the nation will reach 355 million tonnes by 2030 in comparison to the demand of approximately 250 million tonnes as reported for the year 2016. By 2050, another approximately 400 million new mouths would be added to the Indian population. It means current rate of cereal production should be doubled to at least 4.2% in order to maintain pace with the growing population (Guruswamy 2017) and that could be possible only by sealing the existing loop holes at different levels in Indian agriculture.

2.3 Current Challenges in Indian Agriculture

India is considered as the agricultural bowl of the world which is cleared from the statistics that we are the largest producer of milk, pulses and spices globally (World Bank 2012). In addition, India is the second largest producer of rice, wheat, fish, fruits and vegetables worldwide. India's contribution in world's fish production is nearly 6.2% (Agricultural Situation in India 2018). Despite all this, still challenges are existing in Indian agriculture. Due to the massive population burst in the country, the agricultural lands have been continuously shrinking and are converting into the concrete jungles. Since 2004–2005, nearly 10 Lakh Hectares of agricultural land has been diverted for non-agricultural uses (Chand 2017). The aftermath of this population expansion has resulted in two things: First, reduction in the size of farmlands and second continuous rise in food demand. Thus Indian agriculture at present is mainly characterized by the existence of small and fragmented land holdings available with farmers. According to a report, national average of marginal farmers in India is 68. Apart from it, even after 72 years of India's independence more than 60% of the agricultural lands in the country are rainfed, thus causing a major portion of country's crop productivity to be monsoon dependent. In addition, unavailability of quality and certified seeds to farmers is another big factor behind low productivity and production of crops. According to reports, except for wheat all other crops grown in our country have productivity below world average and are much lower in comparison to agriculturally advanced countries. A significant variation in crop productivity has been recorded among different states of our own country. As reported by Chand (2017), per hectare productivity data compilation of 487 districts (covering 94% of net sown area) of India for all crops taken together is Rs 56,510 obtained under largely irrigated conditions in comparison to Rs 35,352 obtained under rainfed conditions. Districts or states of India having same level of irrigation facilities even showed large variation in per hectare productivity which could be due to the poor or low adoption of advance agricultural technologies. All the above factors are the major culprits behind the agrarian distress in the country which is further boosted by the uncertainties associated with prices of agricultural commodities. There are many reasons behind the lack of better price realization by the farmers for their producers, one such factor is bumper production of a particular crop. This happens when most of the farmers growing the same crop leading to its bumper production which in turn severely brings down the market prices of that crop in particular. Other reasons are wrong government policies such as ban on agricultural exports, lack of post-harvest processing units, storage and transportation facilities, well maintained highways, well established markets, inappropriate procurement policies and finally lack of awareness among the farmers regarding adoption of good agricultural and post-harvest processing practices leading to border rejections of different export quality agricultural commodities. All these factors are responsible for the inadequate and intermittent flow of income to the farmers. It has been observed that in most of the cases and time, incomes from agriculture sector where it is principal occupation are inadequate for running a farm family through the year. This can be realized from the data gathered by National Sample Survey Office (NSSO), Govt. of India for the year 2011–2012. It reveals that more than one-fifth of the rural households with agriculture as principal occupation are below poverty line. Among all the states of India, it is Jharkhand which is leading in having maximum number of farm households below poverty line with a percentage of 45.3 followed by Odisha and Bihar with 32.1% and 28.4%, respectively. The national average of farm households falling in the category of below poverty line is 22.5%. On the other side, around 0.5% of farm households are below poverty line in the state of Punjab which is considered to be having least number of farm households in this category as per NSSO data. Thus in the light of the existing scenario, operating small and marginal land holdings is not at all viable and agriculture is presently not a profitable business. Uncertainties associated with agriculture sector forcing more and more cultivators to leave this profession and the same may cause loss of charm regarding this profession among individuals of younger age group.

2.4 North Eastern States of India: An introduction

The North Eastern Region of India comprises of eight states viz., Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. Among all the eight states, it is Assam which is located at the centre and thus considered as heart of North East India. In the vicinity of the North Eastern region, are present the hills of tropical rain forests. Nearly 70% of the North Eastern region is covered with hills and mostly are found in states such as Arunachal Pradesh, Meghalaya, Mizoram, Nagaland and Sikkim. However, half of Tripura, one-fifth of Assam and nine-tenth of Manipur are mountainous (NEDFi Databank 2019a). Mighty river Brahmaputra is dividing this North Eastern region into north and south. North Eastern states of India share international border with different countries. States like Assam, Meghalaya, Mizoram and Tripura share international borders with Bangladesh. While parts of Arunachal Pradesh, Nagaland, Mizoram and Manipur touch the international border of Myanmar. On the other side, the international borders of Sikkim are flanked by countries such as Bhutan and China. North East India is connected to mainland of Indian subcontinent via a narrow land corridor referred as “Siliguri Neck” or “Chicken’s Neck”. The North Eastern region of India is sprawled in an area of 2.62 Lakh km^2, which is nearly 7.9% of total geographical area and 3.4% of total cultivable area of the country. Unfortunately, food grain production of this region accounts only 2.8% in comparison to the total food grain production of the nation (Babu et al. 2015). This indicates that the region is yet to be explored systematically for adequate agricultural production. In 2014, the population of the region has been recorded as 47.9 million.

2.4.1 Arunachal Pradesh

Area wise Arunachal Pradesh is the largest state of North East India with an area of 83,743 km^2, which is 2.55% of the country’s total geographical area. The state geography is a mix of lush green forests, deep river valleys and beautiful plateaus. Himalayan ranges are moving from north to south in the state and dividing it into countless river valleys. Kameng, Subansiri, Siang, Lohit and Tirap are the main river valleys of the state. Climatic conditions are highly variable with hot and humid accompanied by heavy rainfall to occurrence of snowfall as one moves northwards towards higher altitudes. Tropical rain forests are mainly abundant in eastern side of the state while alpines are common in northern parts. According to India State of Forest Report 2017a, the land use pattern of Arunachal Pradesh indicates that the area available for utilization is 7239 Thousand Hectares and forest cover is 6732

Thousand Hectares which is approximately 93.01% of the state's reported area for land utilization. Net sown area of the state is reported as 225 Thousand Hectares while area under culturable wasteland is 63 Thousand Hectares. According to 2011 Census, state's total population was reported as 1.38 million with nearly 77.06% living in rural areas. Agriculture is the main occupation with 70% population dependence on it. Cereals, pulses, oilseeds, fruits, spices and tea are the commonly grown crops of the state. Pineapple, apple, banana, kiwi, citrus, orange and walnuts are among the commonly grown fruits. State climate is suitable for spices such as large cardamom, black pepper and ginger. However, ginger, turmeric, potato, chilly, sugarcane, vegetables and oilseeds are among the main commercial crops of the state.

2.4.2 Assam

In ancient times Assam was famous with the name of Kamarupa. It was ruled by many dynasties such as Varmanas, Salstambhas and KamarupaPalas. Ahoms ruled over Assam for nearly 600 years. According to India State of Forest Report 2017b, Assam covers an area of 78,438 km^2 which is nearly 2.39% of the country's total geographical area. An area of 1853 Thousand Hectares, i.e., around 23.62% of the state's total geographical area is under forest cover. Nearly 31.43% of state's land is not available for cultivation. Culturable wasteland of the state accounts to 144 thousand hectares. The main forest types are bamboo forests, tropical rain forests, deciduous forests and wetlands. The state is blessed with rivers Brahmaputra and Barak. According to 2011 Census, state population was reported as 3.12 Crores and around 85% of it lives in rural areas of Assam. Agriculture is providing employment to nearly 53% of the rural population. As per the Agricultural Census 2010–2011, state was reported to have 27.20 Lakh operational holdings which cover an area of 29.99 Lakh hectares. Around 67 and 18.25% of these land holdings of the state were having area less than 1 ha and in between 1 ha and 2 ha, respectively (NEDFi Databank 2019b). Climate of the state is subtropical and ideal for growing the crops such as tea, coffee, spices, pulses, cereals, fruits, vegetables, fibre crops and rubber. Commonly grown fibre crops are jute, cotton and mesta. A wide variety of horticultural crops can be seen growing in the state such as Assam lemon, litchi, jackfruit, pineapple, orange, papaya and banana. Chillies, turmeric and ginger are considered as the main commercial crops of the state. However rice, wheat and maize are the important cereal crops of the state.

2.4.3 Manipur

Manipur is an oval shaped valley surrounded by hills. The state sprawled in an area of 22,327 km^2 which is 0.68% of the total geographical area of the country. Geographically around 10% of the state is plain while rest 90% is occupied by hills. The land use pattern of the state indicates that area available for land utilization is 2111 Thousand Hectares. Around 78.68% area of the state's total geographical area, i.e., 1699 Thousand Hectares is covered by forests. Net sown area in Manipur is 377 Thousand Hectares while area not available for land cultivation and culturable wasteland are 27 and 1 Thousand Hectares, respectively. State climate is tropical with average annual rainfall ranging from 1250 to 2700 mm (India State of Forest Report 2017c). As per 2011 Census, state is having 2.86 million populations of which 70% reside in rural areas. Mainly grown fruits and vegetables are pineapple, orange, lemon, papaya, banana, passion fruit, beans, cabbage, cauliflower, potato and pea. Commercial crops of the state are cotton, kabrangchak, oilseeds and sugarcane. State has dedicated maximum area in 2015–2016, for the production of cereals with a percent value of 67.17 while 5.5% for pulses and only 0.34% for oilseeds. Rest of the 26.94% area was used for growing cotton, sugarcane and other crops (NEDFi Databank 2019c).

2.4.4 Meghalaya

Meghalaya is spread in an area of 22,429 km^2. Northern side of the state is surrounded by Brahmaputra valley while southern side by Surma valley of Bangladesh. Capital of the state is Shillong and is famous as "Scotland of the East". According to 2011 Census, state population is recorded as 29.67 Lakhs (Statistical Handbook Meghalaya 2017). State climate is highly variable and depends on the altitude. Jaintia and Khasi hills have pleasant temperature throughout the year while plains of Garo hills are hot and humid in summers. Meghalaya is also known for its highest average annual rainfall and bears the tag of one of the wettest places on earth. A village named "Mawsynram" located in Khasi Hills district is famous for receiving highest rainfalls in India which is recorded to be around 11,690 mm (Bose and Saxena 2001). According to India State of Forest Reports 2017d, area available for land utilization is 2241 Thousand Hectares out of which 946 Thousand Hectares or nearly 42% is under forest cover. The net sown area is reported as 285 Thousand Hectares which is 12.74% of the total available area. Nearly 85% of the state population lives in rural areas, thus agriculture is the principal occupation of the state. Meghalayan soils are derived from gneissic complex material and are dark brown to dark reddish brown in colour. They are rich in organic carbon but deficient in phosphorus; however, potassium availability varies from medium to low. Most of the soils present on higher altitudes receiving heavy rainfall are strongly acidic with pH values lying in between 4.5 and 5.0 while rest of the soil is having pH in between 5.0 and 6.0. Due to strong acidity, these soils are also found deficient in molybdenum and boron thus not suitable for intensive farming (Department of Agriculture, Meghalaya 2019). The main crops are rice, wheat, maize, mustard, soybean, jute, cotton, chillies, turmeric, areca nut, potato, sweet potato, tapioca, tobacco and ginger while the commonly grown horticultural crops are pineapple, banana, papaya and citrus fruits (Statistical Handbook Meghalaya 2017).

2.4.5 Mizoram

Mizoram is spread in an area of 21,081 km^2 which accounts 0.64% of the country's total geographical area. Geographically state is composed of rugged steep hills with interspersed valleys. Climate of the state varies from moist tropical to moist subtropical. Range of average annual rainfall is 2160–3500 mm. State accommodates 1.09 million people according to 2011 Census in which rural and urban areas are occupied by 47.89 and 52.11% population, respectively. The land use pattern of the state reveals that area available for land utilization accounts to 2093 Thousand Hectares while area under forest is 1585 Thousand Hectares (India State of Forest Report 2017e). The net sown area of Mizoram is 114 Thousand Hectares which is nearly 5.4% of the total geographic area of the state. The area not available for land cultivation is 100 Thousand Hectares while culturable wasteland is 7 Thousand Hectares. Agriculture is providing employment to nearly 60% of the state's population. Jhumming and terrace are the main cultivation patterns in the state. Major crops grown are rice and maize while horticultural crops include banana, pineapple, orange, passion fruit, chillies and ginger, etc.

2.4.6 Nagaland

Nagaland is one of the hilly states of North East India, covering an area of 16,579 km^2 which constitutes only 0.5% of the total geographical area of the country. The main river of the state is Barak River. Average annual rainfall and temperature of the state vary from 1800 to nearly 2500 mm and in between 21 and 40 °C, respectively. According to the India State of Forest Report 2017f, the land use pattern of the state indicates that the land available for utilization is 1652 Thousand Hectares and around 863 Thousand Hectare area is under forest cover. Land which is not available for cultivation accounts nearly 95 Thousand Hectares. However, the net sown area and culturable wasteland of the state are reported as 380 and 70 Thousand Hectares, respectively. According to 2011 Census, Nagaland is home to 0.16% people of India's population with a value of 1.98 million. Most of the population nearly 71% is residing in villages and have dependency on agriculture as their principal source of livelihood. Due to topographical constraint, jhum/shifting cultivation is predominant in state. Major commercial crops of the state are sugarcane, cotton, jute, tea, ramie, mesta, tapioca, colocasia, yam, ginger and potato. However, most of the area under cultivation is occupied by rice. Numerous horticultural crops can also be seen growing in the state. Some of these are apple, pear, plum, peach, orange, lemon, papaya, banana, pineapple, kiwi and passion fruit, etc. (NEDFi Databank 2019d).

2.4.7 Sikkim

Sikkim gathers special attention because it is declared as the India's first fully organic state and has won the "Oscar for best policies" conferred by Food and

Agriculture Organization after beating 51 nominated policies from 25 countries (FAO in India 2018). Sikkim is a hilly state of North East India with an area of 7096 km^2 which is just 0.22% of country's total geographical area. Chumbi valley of Tibet and Kingdom of Bhutan are present in the Eastern side of the State while Western side is flanked by Nepal. From North to South, the state stretches over a distance of 112 km while 64 km from East to West (NEDFi Databank 2019e). Climate of the state varies from subtropical to temperate and average annual rainfall distribution ranges from 2700 to 3200 mm (India State of Forest Report 2017g). The temperature of the state can be as low as sub-zero in winters and can reach up to 28 °C in summers. Main river of Sikkim is Teesta. According to 2011 Census, state bears a population of 0.61 million which is only 0.05% of the country's population. Majority of the state population nearly 75% is living in rural areas while rest 25% are residing in urban areas. According to land use pattern, reporting area for land utilization accounts for 443 Thousand Hectares out of which around 75.85% is under forest cover. Culturable wasteland is reported as 4 Thousand Hectares while net sown area as 77 Thousand Hectares. The soil texture varies from loamy sand to silty clay loam with pH range in between 4.3 and 6.4. Most of the agricultural fields fall under tropical, subtropical and alpine zones. Historically, it is the Bhutia people who started first growing crops on flat piece of land scattered throughout the state while settled agriculture was done by migrated Nepali immigrants. Buckwheat, finger millet, maize, rice are the main cereal crops while apple, pineapple, banana, jackfruit, passion fruit, guava and oranges are the main horticultural crops of the state. Sikkim has largest area under large cardamom cultivation and is also the largest producer in the country. In addition ginger and turmeric are also grown extensively in Sikkim. State is also home to variety of vegetables such as broccoli, brinjal, onion, tomato, carrot, pumpkin, etc. grown at high altitudes.

2.4.8 Tripura

State covers an area of 10,492 km^2 and shares its northern, western and south eastern borders with Bangladesh. However, its eastern side is flanked by Assam and Mizoram states of India. Around 60% of the state, i.e., 6294 km^2 area is under forest cover (ENVIS Centre 2019). There are two main types of forests found in Tripura. They are evergreen forest and moist deciduous forest. According to land utilization pattern, net sown area in the state is around 255 Thousand Hectares while gross cropped area is nearly 474 Thousand Hectares. Tripura boasts of its highest cropping intensity which accounts nearly 186% and is also highest among all the eight north eastern states. The main crops grown here include rice, wheat, maize, pulses, sugarcane, cotton, jute and mesta while mango, banana, citrus fruits and papaya are the common horticultural crops. Temperature in the state ranges in between 10 and 35 °C while average annual rainfall is nearly 2200 mm. As per the 2011 Census, approximate population of the state is 36.74 Lakhs and a major portion nearly 74% of it is residing in rural areas. Thus states dependency is high on agriculture. Around 82% land holdings in the state are categorized as marginal land holdings with area less than 1 Hectare while 13% fall under the category of small land holdings with area in between 1 and 2 ha (Agriculture Scenario of Tripura 2019). About 60% of the gross cropped area of state is under food grain crops and 21% under horticultural crops. Rice is the main staple crop here and its productivity is higher than national average. According to a report of DoA 2017, the rice productivity of Tripura is reported as 3060 kg/ha and the national average as 2404 kg/ha (DAC&FW 2017).

2.5 Hill Agriculture of North East India: Prevailing Scenario and Challenges

We all are aware that Green Revolution was mainly achieved and confined to North Western parts of our country, despite the fact that North Eastern region of India is blessed with rich organic high fertile soils, receives plenty of rainfall on yearly basis, home to wide variety of flora–fauna and harbours vast deposition of natural reserves such as of petroleum, hydel and forest, etc. even though the rate of agriculture growth in this region is still far behind the rest of the country. As we have already pointed out the fact in earlier paragraphs that majority of this region's population roughly more than 70% is living in rural areas thus their dependency on agriculture is very high and the fact that prevailing backwardness in agricultural growth in North Eastern region is severely affecting the socio-economic development of the residents and hence the region is home to high proportion of population falling below the poverty line. This can be evident from the Human Development Index (HDI) report which reveals that all the eight North Eastern states except Sikkim have performed poorly and fall under the category of least developed states of the entire country. HDI values reported for Arunachal Pradesh, Assam, Meghalaya, Mizoram, Manipur, Nagaland and Tripura were 0.321, 0.243, 0.286, 0.323, 0.262, 0.275 and 0.262, respectively (Bhagowati 2012). From these HDI values, we can easily conclude that Assam is least developed followed by Manipur and Tripura which are slightly better on the scale of Human Development Index in comparison to Assam. However, it is Sikkim which manages to retain itself in the range of medium level of human development according to Human Development Indices. Geographically, the North Eastern region of India occupies an area of 2.62 Lakh km^2, which is roughly 8.0% of country's total area, out of it approximately 53.4% is under mixed deciduous and evergreen forests. More than 70% area of North Eastern region is mountainous with steep slopes while nearly 30% occupied by Brahmaputra valley (Baruah et al. 2014). Net sown area of this region has been reported as 4534 Thousand Hectares which was just 3.21% of country's total net sown area for the year 2015. However, North Eastern region accounts nearly 9.3% of country's total barren and unculturable land with 1593 Thousand Hectares under this category while country's culturable wasteland in this region was estimated to be around 4.7% with a value of 590 Thousand Hectares (North Eastern Council Secretariat 2015). As shown in Table 2.1, maximum barren and unculturable lands have been reported for Assam which accounts nearly 15.2% of State's total geographical area. On the other hand,

Table 2.1 Pattern of land utilization in north east (2013–2014) (1000 ha)

Name of state	Geographical area	Forest cover	Barren and unculturable land	Culturable wasteland	Net sown area	Total cropped area	Area under irrigation by sources (2014–2015)
Arunachal Pradesh	8374	6732	39	63	225	296	56
Assam	7844	1853	1192	144	2820	4100	296
Manipur	2233	1699	1	1	377	377	69
Meghalaya	2243	946	131	391	286	343	81
Mizoram	2108	1585	8	7	114	114	16
Nagaland	1658	863	2	70	380	499	97
Sikkim	710	336	-	4	77	147	12
Tripura	1049	629	-	3	255	474	79

Source: Pattern of Land Utilization, Ministry of Agriculture & Farmers' Welfare, 2013–2014, Irrigation-Statistical Year Book India 2018, Directorate of Economics & Statistics, Ministry of Agriculture; http://mospi.nic.in/statistical-year-book-india/2018/181 culturable wasteland was highest in Meghalaya which was roughly 17.4% of its total geographical area while least in Manipur with 1 Thousand Hectare area, i.e., approximately 0.04% of State's total geographical area. Table 2.1 also depicts that among all the eight north eastern states, net sown area was reported highest in Assam with a value of 2820 Thousand Hectares which is around 36% of State's total geographical area followed by Tripura and Nagaland. Net sown area reported for these states was 255 and 380 Thousand Hectares, respectively, and it was 22.9 and 16.9% of respective State's total geographical area. Hills and mountains are most prominent in Arunachal Pradesh, Meghalaya, Mizoram, Nagaland and Sikkim while parts of Assam, Manipur and Tripura are also having flat topography and it is one of the reasons behind highest net sown area found in Assam followed by Tripura, among the eight North Eastern states. North East India was home to around 47.9 million people in 2014 and their total food grain requirement for that year was reported as 8444.7 Thousand Tonnes while the production recorded from the region was 8232.8 Thousand Tonnes, which depicts a net deficit of 2.51% (Roy et al. 2015). Data on food grain production from North East during 2015–2016 and 2016–2017 is shown in Table 2.2. This region reported 7654 Thousand Tonnes of food grain production for the year 2016–2017 while utilizing an area of 4071.4 Thousand Hectares. The area dedicated for food grain production in North East was 3.2% of the country's total area under food grains while production was merely 2.8% of country's total food grain production. Thus average yield of food grain production of this region was found to be 1837.3 kg/ha which was quite low in comparison to the national yield that averaged around 2129 kg/ha. Similarly, area utilized for vegetable production was 558.92 Thousand Hectares which was 5.5% of the country's total area under vegetables for the year 2015–2016 and production reported was 6239.92 Thousand Tonnes which was just 3.7% of the country's total vegetable production (Table 2.2). As far as the fruit production is concerned, North East contributed nearly 5.4% to country's total fruit production while utilizing an area of 484.96 Thousand Hectares, roughly 7.7% of country's total area under fruits. This indicates that despite the existence of highly fertile soils, the agriculture potential of North East has yet to be unlocked systematically. A strong reason behind it is the existing backwardness in agriculture because mostly the kind of agriculture being practiced in this region is subsistence farming, so farmers are not using nutrients in appropriate quantities and as per the need of their fields nor they are serious about the use of proper plant protection measures, irrigation facilities and farm machinery, etc. In addition they are growing mostly traditional varieties; according to a data high yielding varieties of rice were growing only on 56% of area in North East in comparison to 74% at national level (Babu et al. 2015). Apart from it, most of the farmers are taking only one crop on per year basis and keeping their lands as fallow in the second half of the year, thus the low cropping intensity is also indicating towards the under exploited agricultural potential of North East. As revealed in Table 2.1, the net sown area under irrigation by different sources in North East is just 15.6%, which indicates a very high dependency of agriculture on monsoon rains. The rate of N,P,K based fertilizer consumption in North Eastern region for the year 2017–2018 has been reported as 295.61 Thousand Tonnes while on per Hectare basis, it turns out to be 61.77 kg/ha (Table 2.3). The amount of fertilizer used in

Table 2.2 Area utilized for production of food grains, vegetables and fruits in north east

Name of state	Area under food grains (*'000 ha*) (2016–2017)	Total food grain production (*'000 Tonne*) (2016–2017)	Yield of food grain production (*kg/ha*) (2016–2017)	Area under vegetables (*'000 ha*) (2015–2016)	Total vegetable production (*'000 Tonne*) (2015–2016)	Area under fruits (*'000 ha*) (2015–2016)	Total fruit production (*'000 Tonne*) (2015–2016)
Arunachal Pradesh	203.5	343.3	1687	4.0	33.01	66.21	306.27
Assam	2667	4952.5	1857	317.59	3821.71	145.71	2077.77
Manipur	304.2	525.1	1726	34.36	316.51	51.12	467.76
Meghalaya	140.9	260.1	1846	47.50	494.88	36.59	395.40
Mizoram	46.2	75.2	1629	45.21	179.02	55.01	330.28
Nagaland	329.5	536.9	1629	43.53	494.61	37.05	374.13
Sikkim	62.7	101.3	1616	20.25	106.94	17.53	23.48
Tripura	317.4	859.6	2709	46.48	793.24	75.74	854.05
NER total	4071.4	7654	1837.3	558.92	6239.92	484.96	4829.14
All India	129,231.2	275,111.7	2129	10,106.29	169,063.93	6301	90,183

Source: Department of Agriculture, Cooperation and Farmers Welfare, Govt. of India Horticultural Statistics at a Glance 2017

Table 2.3 Zone-wise consumption of fertilizers in terms of nutrients (N,P,K)

Name of zone	Years 2015–2016	2016–2017	2017–2018
South Zone	6177.58 (179.46)	5788.00 (166.43)	5724.28 (160.34)
West Zone	8382.02 (93.85)	8426.41 (90.38)	8560.03 (92.48)
North Zone	7906.44 (174.47)	7805.66 (170.18)	8087.75 (175.62)
East Zone	4001.47 (140.90)	3653.88 (125.07)	3923.23 (137.15)
North East Zone	**285.09 (39.01)**	**275.2 (38.41)**	**295.61 (61.77)**
All India	26752.61 (130.66)	25949.15 (123.41)	26590.9 (128.02)

Source: Pocket Book of Agricultural Statistics 2018, Directorate of Economics & Statistics, DAC&FW, Ministry of Agriculture & Farmers Welfare, GOI

2017–2018 in North Eastern region was around 20.41 Thousand Tonnes more in comparison to the rate of fertilizer application reported for previous year. In 2016–2017, N,P,K fertilizer was applied in North East at the rate of 38.41 kg/ha. However, the total N,P,K fertilizer consumed at National level for the year 2017–2018 has been reported as 26590.9 Thousand Tonnes with around 128.02 kg being used on per Hectare basis at all India level. From the above statistics, it can be corroborated that the amount of N,P,K fertilizer consumption in North Eastern zone is merely 1.11% in comparison to its total consumption at all India level while the maximum consumption has been reported from West zone of the country with 8560.03 Thousand Tonnes or nearly 32.2% of the country's total consumption.

2.6 Opportunities and Initiatives

2.6.1 Enhancement in Agricultural Productivity of North East

The production of food grains for the year 2016–2017 while fruits and vegetables for the year 2015–2016 from North Eastern region has been reported as 2.8%, 3.7%, and 5.4% respectively, which is quite low in comparison to their production at all India basis (Table 2.1). The agricultural productivity of North East can be increased in two ways, i.e., either by increasing the area under crops or through increase in productivity of different crops grown in the region. As revealed in the land use pattern of all eight North Eastern states, a significant amount of land is classified as culturable wasteland, fallow lands and current fallows. These pieces of lands can be put under proper use for growing different agricultural crops as per climate suitability and it will certainly give a boost to the total agricultural output of the region. On the other hand, productivity of the crops in the region can be increased by the adoption of improved agricultural technologies which are specific to hills. Use of good quality seeds particularly of high yielding varieties that are pest and disease resistant as well as can tolerate flood conditions must be preferred over the use of traditional varieties. Optimum and crop specific nutrient application as well as availability of irrigation facility to crops throughout the year is another important factor in increasing crop productivity of this region. In addition, farmers of North East should change their perception of subsistence farming to intensive farming. However, more focused R&D is required with respect to the development of topographically compatible farm equipment, flood resistant/short duration crops and agricultural practices that are cost effective and can be conveniently used in hill agricultural operations. Easy access of quality seeds/seedlings and package of practices specially designed for North Eastern region to every single farmer will greatly help in elevating the level of agriculture in this region. Establishment of green houses will be a boon in areas prone to high rainfall conditions in North East. Area under shifting cultivation or jhum cultivation in north east accounts nearly 8500 km^2 but the data is not reliable as different agencies have different estimates on it (MoSPI 2014). Since the population has increased in North East which has created pressure on lands for production and thus reduced the land fallow cycle to less than 5 years is one of the leading reasons responsible for soil erosion. Instead of shifting cultivation the farmers should be encouraged for the establishment of home gardens and to promote it. State governments should come up with dedicated policies, guidelines and schemes (Pant et al. 2018). This will help in improving the food and nutritional security as well as in uplifting the incomes of especially women farmers apart from reducing the dependency of tribal population on it. To enhance the area under irrigation, central government has started an initiative in the form of *Pradhan Mantri Krishi Sinchayee Yojana*; on the other hand *Soil Health Card Scheme* was launched to create awareness among the farmers about the nutritional status of their fields, so that they can use different fertilizers in optimum quantities. This initiative will not only increase the crop productivity in an eco-friendly manner, but it will also help in significantly cutting down the cost of agricultural production which in turn will significantly influence the net profit of farmers. Under Soil Health Card Scheme around 2,297,688 soil health cards have been distributed to the farmers of North East till mid 2018 (Table 2.4).

2.6.2 Increases in Crop Intensity and Diversification

Crops can be taken twice a year in India from the same piece of land due to the existence of two crop growing seasons, i.e., kharif and rabi, which is also a common practice being extensively followed by the major agricultural states of India. However, it is unfortunate to note that a significant portion of arable lands in North East remains fallow during the rabi season, which might be due to the lack of proper irrigation facilities as well as improved technologies. Despite the fact that North Eastern region of India is blessed with nearly seven river basins in addition to the

Table 2.4 Status of Soil Health Card Scheme in North East as on 30.06.2018

Name of state	Numbers of Soil Health Cards Distributed (Cycle1 + Cycle2)
Arunachal Pradesh	20,532
Assam	1,300,901
Manipur	129,522
Meghalaya	366,475
Mizoram	20,177
Nagaland	207,079
Sikkim	46,000
Tripura	207,002
NER total	**2,297,688**

Source: Pocket Book of Agricultural Statistics 2018, Directorate of Economics & Statistics, DAC&FW, Ministry of Agriculture & Farmers Welfare, GOI

receiving of average annual rainfall of 2000 mm, some parts of the states like Meghalaya, Mizoram, Nagaland, Tripura and Manipur face water shortage problems during winters which indicates that the water resource potential of the region is not utilized efficiently. The percentage of net irrigated area to net cultivated area is also very less for different North Eastern states. Central or State schemes should be more focused on the establishment of more and more rain water harvesting reservoirs, construction of bore wells, etc. throughout North Eastern region as well as on revival of springs, utilization of local rivers and their tributaries for irrigation purposes. With the availability of irrigation water round the year, rabi season in North Eastern states can be efficiently utilized for growing diverse crops such as oilseeds, pulses and vegetables (Report of Working Group on Agricultural Development in Eastern and North Eastern India, Planning Commission 2001). The regions ideal climate and soil conditions are also supporting and favouring the growth of various indigenous as well as exotic varieties of fruits, spices and medicinal plants, etc. which are not growing elsewhere in the country. Some of these include passion fruit, dragon fruit, kiwi, pineapples, large cardamom, Nadia ginger (low fibre), Lakadong turmeric, King chilli, etc. These high value crops if grown on large scale by adopting good agricultural practices or through organic farming and with proper value addition will have an ample scope for export in international market. This will significantly enhance the farmer's income as well as improve the socio-economic status of the region.

2.6.3 Development of North East as Organic Hub

As we all are aware that the North Eastern region of India remains untouched by the modern agricultural practices especially in terms of use of different agrochemicals, thus this region bears the tag of organic by default which in turn brings it into the limelight of being developed as organic hub of India. In this initiative, the very first step was taken up by Sikkim after imposing bans on the use of different agrochemicals and declared the state as first organic state of India. Development of North East as organic hub is actually the need of the hour. Overuse of chemical fertilizers and pesticides in major growing states of India as part of intensive agriculture has caused degradation and loss of soil fertility. This could be due to the accumulation of unnecessary chemicals into the soil which in turn retarded the growth as well as killed many of the beneficial soil microorganisms responsible for nutrient cycling and maintenance of soil structure. Apart from it, non-judicious use of agrochemicals has also severely disturbed and deteriorated the underground water tables as well as nearby aquatic bodies. Traces of these unwanted chemicals can often be detected in some of the export quality food commodities where they used to cross the maximum residue limits (MRL) permitted by most of the importing countries. Such sanitary and phytosanitary (SPS) issues are detrimental to the international trade by Indian exporters. The top agricultural commodities being exported from India include basmati rice, non-basmati rice, cereal preparations (biscuits, pasta, cornflakes, etc.), processed items (jams, jellies, sauces, juice, ketchups, sauces, dried soups) and fresh vegetables. Lack of awareness about the adoption of good agricultural practices (GAP) as well as post-harvest processing by farmers and other stakeholders are major setbacks of India's global agricultural trade. Some of the earlier examples include ban on Indian export of mangoes, grapes and egg plants due to fruit flies or thrips infestation while others include use of adulterants such as carcinogenic dye malachite green in small cardamom or green peas to make them look more eye catchy. According to International Trade Statistics 2015, India's share in global agricultural export for the year 2014 was only 2.5% while Europe followed by USA was the leading exporters in the world. Europe also leads the tally of top importing countries in the world followed by China. On the other hand, import of agricultural commodities to India accounts nearly 1.5% in 2014 (Indian Agri Exports 2016). Agricultural exports account nearly 10% of the total exports from India and thereby has generated handsome revenue worth of USD 6.2 billion in 2015–2016. North Eastern region of India if utilized properly for agricultural production can significantly uplift the share of India's contribution in global agricultural exports. Besides, it will curb the expenses of the Indian government incurred on medical facilities of its citizens which contract many diseases after the consumption of substandard food items as well as it will significantly strengthen the food security of the nation. To promote organic agriculture at all India level a scheme named *Paramparagat Krishi Vikas Yojana* (PKVY) has been started by the Indian government while for promotion of organic farming in North East a special scheme has been designed and initiated with the name *Mission Organic Value Chain Development* (MOVCDNER) (Agricultural Situation in India 2018). The aim of the scheme is to develop certified organic produce in a value chain mode by linking growers with consumers and to support the development of entire value chain starting from inputs, seeds, certification to creation of facilities for collection, aggregation, processing, marketing and brand building initiative (MOVCD 2018). The target of this scheme is to cover 50,000 ha of North East under organic farming. Apart from supporting and strengthening the international trade of organic agricultural produce, Government of India has also focused on maintaining transparency in domestic trade of organic agricultural commodities by initiating a certification system, viz, *Participatory Guarantee System* (PGS) which is free of cost for farmers of the entire country. A total of 356,942 certificates have been generated till date under PGS scheme for 325174.15 ha area offered under organic farming (PGS-India 2019).

2.6.4 Establishment of Markets

Establishment of markets is an important aspect for augmenting the agriculture production of the region, as this will provide an assurance to farmers that their produce will be sold timely and will fetch a good price for them. But unfortunately, this important fact has always been overlooked by the authorities, which resulted in severe scarcity of well-established markets in North East. Since most of the region is rural area hence the few big markets are only concentrated and limited to major cities of the eight North Eastern states. Further, roads connecting rural areas to state capitals are so pathetic that carrying perishable agricultural produce is a headache as it often suffers bruising while transportation. Bruised agricultural perishables face problems of microbial infections and thus shortening of shelf life which significantly reduces their market demand. To strengthen the agriculture in North East, state governments should come up with schemes on establishing good markets and connecting them to surplus areas by construction of well-maintained roads/highways throughout the states. Government should provide land and build some infrastructure in rural as well as city areas which can be utilized efficiently by the local farmers for selling their produces. Special "Organic Huts" should be constructed in entire North Eastern region for exclusive sale of organically grown agricultural items. To facilitate the domestic as well as international export of agricultural produce from the topographically difficult terrains of North East, facility of airlifting can be utilized efficiently. Procurement policies should be prepared by the state governments for the surplus agricultural commodities of the region with provisions of proper storage of procured items so as to avoid post-harvest losses in addition to creation of value addition facilities. For use on difficult terrains, mobile cold storage vans can be utilized for safe collection and transportation of perishables to cold store go-downs/warehouses. To decrease the involvement of middleman and to increase the transparency in the sale of agricultural commodities by direct linking of farmers with buyers, Government of India has started an initiative of electronic National Agriculture Market (e-NAM) (SFAC 2019). It is an electronic trading portal which connects Agricultural Produce Marketing Committee (APMC) mandis and offers the advantage of real time price discovery as per demand and supply statistics, nationwide market access, various buy and sell trade offers as well as information on arrival of commodities to both sellers and buyers. The e-NAM concept has significantly reduced the imposition of multiple mandi charges levied on farmers which in turn cause escalation of commodity prices for consumers. Presently, e-NAM portal is operational in 16 states and 2 Union Territories. North Eastern states may also join or design strategies on the use of such e-portals. To bring transparency in auction process of different agriculture commodities, Spices Board and Metal Scrap Trade Corporation Ltd. (MSTC) has launched e-auction platforms. One such platform is e-Rashtriya Kisan Agri Mandi (e-RaKAM). Apart from it, to promote the sale of PGS certified organic produce, Ministry of Agriculture and Farmer's Welfare has recently launched a dedicated online portal with the name "JaivikKheti". This portal is a complete online trading platform where buyers, sellers and input suppliers can register themselves for sale and purchase of organic products (JaivikKheti 2019). Presently 1702 farmers from different North Eastern states have been registered on this portal.

2.6.5 Allied Sector Development

North Eastern states are highly prone to floods due to the torrential monsoon rains every year which increases the level of water many folds above normal in mighty rivers such as Brahmaputra and Barack in addition to their numerous tributaries. Topography of the North East as well as human intervention which includes encroachment of river banks and wetlands are probably responsible for recurrence of these floods year after year. Rivers and their tributaries are flowing downstream through the North Eastern states which significantly speeds up the flow of these rivers which in turn causing havoc by submerging islands, villages, agricultural fields and by damaging public and private infrastructure, roads, bridges, etc. Apart from it, frequent rainfalls causing land degradation by promoting the incidences of landslides, (Sangomla 2018) erosion of riverbanks, acidification and siltation of fertile lands. A possible and partial explanation behind the acidification of soils receiving frequent and high rainfalls depends on the leaching of alkaline elements such as calcium, magnesium and potassium present in the soil by rain water and leaving behind acidic elements such as hydrogen, aluminium and manganese to replace the bases (Morse 2019). Factors like soil erosion (either by rivers or through landslides), mountainous terrains and enormous forest cover leading to availability of small and marginal land holdings are the major obstacles in the establishment of settled agriculture in North East. Thus, the only dependence of North Eastern population on agriculture is detrimental with respect to food and socio-economic security in the region. Existences of varied agro-climatic conditions are among the major strengths of the region which favours the growth of genetically diverse species of economically important plants like rubber, bamboo, spices, orchids, medicinal plants and timber, etc. States like Tripura, Mizoram and Assam are the major producers of rubber in North East, thus offering enormous opportunities for establishment of rubber processing industries (Indian Council of Food and Agriculture 2017). Similarly, bamboo has many commercial uses such as in the manufacturing of laminated furniture, floorings and mattings, construction of traditional houses, high quality yarn/fabrics, in making of bamboo chopsticks and various other handicrafts. In addition, bamboo shoots and bamboo vinegar are also very popular items. About two-third of country's bamboo production is recorded from North East (Manu 2012). Thus, bamboo based industries can be a key driving force in generating rural employment and in reducing poverty to a significant level. Agro-processing industries for value addition of spices or related to use of medicinal plants such as pharmaceuticals if established will play a vital role in improving the socio-economic status of the region. Tourism industry can also flourish in North East due to the mesmerizing beauty of its mountains, rivers, valleys as well as presence of wildlife sanctuaries, national parks and numerous pilgrimage sites. The region is blessed with ideal climate (except in rainy season) particularly in the summer months when the rest of the country will be burned from the scorching heat of sun, the temperature remains ambient in North East India. The region is flanked by countries like Bangladesh, Bhutan, China, Myanmar and thus is home to different tribes varying from each other in traditions/cultures, food habits, folk dances, music, etc. altogether making it an ideal place to visit for globetrotters. The location of the region is also favourable for developing various adventurous sports activities such as mountaineering, bungee jumping, paragliding or river rafting, etc. which can significantly attract the attention of tourists from around the globe. In addition the region has immense potential for sericulture as silk weaving was practiced since ancient times and thus blessed with artistic weavers. North East is specifically known for its golden muga silk which is not found anywhere else in the country. Assam is the only state of India producing four varieties of silk (Unni et al. 2009) and eri silk is among one of them. Muga and eri are produced by non-mulberry silkworms. Sericulture is a cottage based industry with enormous contribution scope in rural poverty alleviation. If focused and more dedicated research is carried on host plant improvement, control of silkworm diseases and on establishing domestic as well as international trade linkages, this sector can create huge employment opportunities for the local youth. A significant portion of North Eastern population is dependent on non-vegetarian diet which includes the consumption of fish, eggs, poultry and pork. Demand for pork in North East was estimated nearly 3 Lakh Tonnes while region achieved slightly more than half of its production, i.e., 1.7 Lakh Tonnes, thus the demand-supply gap of 1.3 Lakh Tonnes was fulfilled by importing from outside the region (North Eastern Council Regional Plan 2017). This indicates that the region is having huge potential for the development of fishery, poultry and piggery sector.

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Sari System: A Traditional Cropping Pattern of the Uttarakhand Himalaya

3

Satish Chandra, Dinesh Chandra, Rajkumar Pandey, Arun Kumar Khajuria, Vivek Kumar, and Pankaj Bhatt

Abstract

The advent of agriculture changed the pathway of human evolution. Agriculture was invented in the different cradle sites and dispersed subsequently in different parts of the globe. Ethnic people of different areas practised agriculture differently. They cultivated crops suited for their areas and developed different agriculture practices as per the climatic conditions. The Himalayan region is endowed with enormous biodiversity including various wild relatives and landraces. People of the Uttarakhand Himalaya developed a special practice of agriculture known as Sari system. In this, people cultivate crops on a 2-year rotation. In the first year, during Rabi season, wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and lentil (*Lens culinaris*) are sown. While in the Kharif season, rice

S. Chandra (✉)
Department of Botany, Government Degree College Tiuni, Dehradun, Uttarakhand, India

D. Chandra
Department of Biological Sciences, CBSH, G. B. Pant University Agriculture and Technology, Pantnagar, Uttarakhand, India

R. Pandey
Department of Microbiology, CBSH, G. B. Pant University Agriculture and Technology, Pantnagar, Uttarakhand, India

A. K. Khajuria
Department of Botany, Government Degree College Kathua, Kathua, Jammu and Kashmir, India

V. Kumar
Department of Botany, Government P.G. College, Champawat, Uttarakhand, India

P. Bhatt
State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangdong Province Key Laboratory of Microbial Signals and Disease Control, Integrative Microbiology Research Centre, South China Agricultural University, Guangzhou, People's Republic of China

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(*Oryza sativa*) and barnyard millet (*Echinochloa frumentacea*) are cultivated as mixed crop. In the next Kharif season, nearly 12 or more crops (including finger millet, amaranth and legumes) are cultivated in the intermixed pattern. In this practice, culm of millets and stem of amaranth provide support to the growing legumes, and in exchange, legumes fix atmospheric N_2 and provide nourishment to other crops. Apart from this, the traditional crop rotation system also helps in the efficient use of rainwater, improve soil health and increase crop productivity. This system also acts as insurance for small landholding farmers in case of failure of a particular crop. In such conditions, other crops growing in an intermixed manner compensate the economic loss of the farmers. Outmigration of people from villages and monoculture of cash crops are major threats to this traditional practice. The present chapter is focused on different aspects of the Sari system.

Keywords
Agriculture · Barahnaja · Livelihood · Sar · Traditional knowledge · Traditional practices

3.1 Introduction

The invention of agriculture changed the pathway of human evolution. The barbaric hunter-gatherer human started vegetarian diet and settled into various cradle sites. Various wild plants were domesticated and various methods were developed for harvesting useful plant parts. Eleven sites around the globe are considered the centre of origin of various crop plants, and surprisingly, these represent almost all continents except Australia and Antarctica. Agriculture was gradually dispersed to different parts of the world. It not only changed the food habits of the human but also changed the surrounding ecology. Different plant and animal species were favoured for domestication by humans and subsequently their population increased. While other species either changed their phenological patterns as per the changing environment or migrated to other places. Thereafter, human started selecting the preferable phenotype of plant species among the available ones and also started cultivation of various plant species singly and in groups. In this experimental phase, people used various combinations of crops for better yield in specific climatic conditions. People also developed various practices for maximum utilization of plants and other available resources. These practices gave birth to various traditional agricultural systems. Different ethnic groups around the globe use different traditional systems of agriculture. These systems are suited for the environmental conditions of the particular locality (Martin and Sauerborn 2013; Thrall et al. 2010).

Himalayan region is famous for its picturesque valleys and serene beauty. Terrace farming is the lifeline of the Himalayan people. People cultivate various varieties of cereals, millets, pulses, oil seeds and pseudo-cereals. Most of the varieties grown in the Himalayan areas are landraces adapted to the local environmental conditions (Singh 2019). People also developed various indigenous practices for the utilization of the available resources (Singh and Rana 2019). Such practice includes field

Table 3.1 Agro-climatic zones of the Uttarakhand state (Source: SAP (2012–2017))

S. No.	Zone	Elevation meters (above sea level)	Region and area	Major crops
1.	Zone A	Up to 1000	Terai, Shivalik Hills and Valleys	Rice, wheat, sugarcane, lentil, chickpea, rapeseed mustard, mango, litchi, etc.
2.	Zone B	1000–1500	Mid hills south aspect	Rice, finger millet, wheat, potato, tomato, peas, cauliflower, pulses, peach, and plums
3.	Zone C	1500–2400	Temperate zone	Amaranth, finger millet, French-beans, Cole crops, potato, peas, peaches, plums, pear, and apple
4.	Zone D	>2400	Sub-alpine to alpine	Amaranth, buckwheat, peas, cauliflower, cabbage, apple, potato, and various aromatic and medicinal plants

preparation, seed sowing, weed eradication methods, selection of crops as per the suitability of the environmental conditions, pest management, harvesting methods and storage of the seeds. Indigenous people usually cultivate various crops in an intermixed or alternate manner, so that the soil health and crop productivity can be maintained simultaneously. Uttarakhand state is one of the mountainous states of India and famous for its huge biodiversity. Various threatened and endemic flora and fauna species are flourishing in the Uttarakhand. The state also houses various wild relatives of crops and landraces. Various agro-climatic zones of the state are depicted in Table 3.1. People of the state developed various practices of agriculture such as Sari system, Baranaja, integrated pest management practices, use of cow dung and shaded plant leaves as compost, different methods of field preparation for the different crops and different methods of the rouging and weeding (Kumari et al. 2009; Maikhuri et al. 1997). In the present chapter, authors compile information from their own field observations, meeting with local people and secondary sources.

3.2 Sari System

On the basis of elevation, terrace farms in Uttarakhand are broadly categorized into two groups. The high hills areas are known as *Danda* and comparatively low elevated hill areas are called *Gangarh*. Meteorologically *Danda* area receives more rainfall and snowfall than the *Gangarh*. Consequently, *Gangarh* areas are rainfed. On the basis of the available irrigation facilities, farms are also categorized into two groups: irrigated fields (locally known as *Sera*) and non-irrigated fields (locally known as *Ukhad/Usar*). Irrigated farming is limited only to valley areas, while the rest of the hilly areas practise *Ukhad* system. In the Ukhad system, people follow the *Sari* system of cropping for crop rotation. *Sari* or *Sar* is part of terrace farms which is being used for cropping during a particular season. Two Saris are generally designated on the basis of their

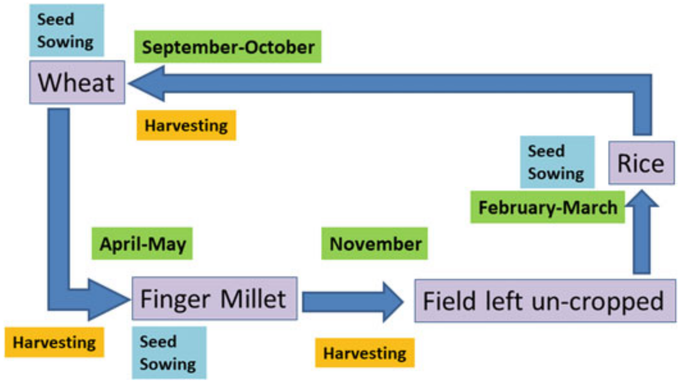

Fig. 3.1 Biannual crop rotation system of a *Sari* (dominant crops of the season are shown)

presence around the village. Terrace farms present above the village known as *Malli Sari* and below the village known as *Talli Sari*. Similarly, terrace farms present in the left side of the village are known as *Walli Sari* and right side of the village, *Palli Sari*. In a village, either *Talli-Malli* or *Walli-Palli* categorization of Sari is followed. During a particular year, if *Talli Sari* is used for rice farming, then *Malli Sari* would be used for the farming of millets, legumes and amaranth. However, in the subsequent year, the opposite pattern is followed (Pande et al. 2016; Ravera et al. 2019; Sati 2005, 2009; Sen 2015). Crop rotation of 2-year basis of a particular *Sari* is depicted in Fig. 3.1, and local people practising traditional practices is depicted in Fig. 3.2. Different traditional practices from the preparation of field to post-harvesting of the different crop are discussed below in detail.

3.2.1 Rabi Season (Wheat Cropping)

For wheat cropping, practices performed by the local people are discussed below:

3.2.1.1 Field Preparation

Walls of the terrace fields in the hilly areas usually fall due to heavy rains in the rainy season. Fallen and damaged walls (locally known as *Pagar*) are usually reconstructed before ploughing of the field. Shrubs and herbs growing on the walls of terrace fields and boundary walls are either uprooted or trimmed by the people before ploughing. Gravels and stones are handpicked from the field and disposed outside the field areas.

3.2.1.2 Ploughing

After preparing the fields, the farmers go for ploughing. Usually, people start ploughing on an auspicious day. Traditionally, it is decided by learned old men/women or priest (*Panditji*). For wheat, ploughing is done twice. Ploughing is

Fig. 3.2 (**a**) An area showing different *Sari* system (green terrace fields (wheat cropping) constitutes *Walli Sari* and un-cropped fields constitutes *Palli Sari*; (**b**) After initial ploughing, women are preparing field for seed sowing; (**c**) First author ploughing the field; (**d**) Traditional plough; (**e**) Farmer applying wooden plank in the field; (**f**) Women practising roguing of the rice field; (**g**) Finger millet field with associated barahnaja; (**h**) Monkey damaging rice crop

carried out by traditional plough and bullocks. The initial ploughing is locally known as *ban* and the subsequent ploughing is known as *bootan*. After initial farming, some large aggregates of the soil are formed. These soil aggregates are known as *dhika* and are dilapidated with the help of the wooden mace (*dhikaru*). After the initial ploughing, organic manure (made up of dung and remains of fodder) is applied in the field and left for 1–2 weeks. Thereafter, seeds of wheat (*Triticum aestivum*)/ barley (*Hordeum vulgare*), lentil (*Lens culinaris*), gram (*Cicer arietinum*), pea (*Pisum sativum*) and bhangjeera (*Perilla frutescens*) are sown by broadcasting method, followed by second ploughing. Finally, wooden plank (*jol*) is applied over the field. Usually mustard (*Brassica* spp.) is sown at the last by the broadcasting method. Generally pea, gram, lentil and mustard are sown intermixed with wheat or barley. Cropping of wheat and barley is considered as mutually exclusive for a particular field. Bhangjeera (*Perilla frutescens*) is sown on the upper corners (*dhiswal*) of the fields.

3.2.1.3 Roguing and Weeding

Roguing and weeding are done after 1.5–2 months of seed sowing with the help of traditional hoe (*kudal*). Usually no labourers are engaged in the wheat fields until harvesting of the crop.

3.2.1.4 Harvesting of Crop

Harvesting of crop is initiated on the auspicious day. The crop of wheat/barley is harvested by cutting the culm of plants close to ground by sickle (*dranti*) in March–April. Thereafter, bundles (*gaduli*) of plants are formed and fertile spike is separated from plants. In a season, mustard is harvested in the beginning, followed by barley. Thereafter, wheat (*Triticum aestivum*), lentil (*Lens culinaris*), gram (*Cicer arietinum*) and pea (*Pisum sativum*) are harvested simultaneously. Thrashing involves the separation of the grain from the spike and is generally done manually or under the feet of bullocks. For the lentil, gram, mustard and pea, harvesting is almost similar as of wheat. After 1–2 weeks of sun drying of lentil, gram, mustard and pea bundles, these are beaten with wooden sticks (*lattha*) for threshing. Further, seeds are separated, and the left over is used as fodder. It is important to note here that bhangjeera (*Perilla frutescens*) is not harvested in this season. This crop takes a complete year to mature and is harvested in the next season along with millets.

3.2.2 Kharif Season (Finger Millet Cropping)

Cropping of finger millet is the main component of Sari system and is also from the traditional *Branaja/Barah-anaja* (12 crops) system. Approximately 12 crops along with finger millet are grown in an intermix manner. Finger millet sown with pulses: urad/kali dal (*Vigna mungo*), lobia/sonta (*Vigna unguiculata*), naurangi/rains (*Vigna umbellata*), tor (*Cajanus cajan* var. *flavus*), soya bean/bhat (*Glycine max* 'Soyabean'), kalabhat (*Glycine max* 'kalabhat'), horse gram/gahat (*Macrotyloma uniflorum*); amaranth/chua/marchhu (*Amaranthus caudatus*), Japanese barnyard millet/jhangora (*Echinochloa frumentacea*), jakhya (*Cleome viscosa*) and foxtail millet/koni (*Setaria italica)*. Cropping of these crops starts immediately after the harvesting of wheat. Various practices performed by the local people during this season are discussed below:

3.2.2.1 Field Preparation

Practices of field preparation are similar to the practices of wheat cropping.

3.2.2.2 Ploughing

After preparing the fields, farmers start ploughing. Generally for this season, people do not follow much astrology to start ploughing. Ploughing is started 2–3 weeks after the harvesting of the wheat. Ploughing is done once during this season, and seeds of millets, pulses, amaranth, etc. are sown by broadcasting method. Some pulses, i.e. tor (*Cajanus cajan* var. *flavus*), soya bean/bhat (*Glycine max* 'Soyabean'), kalabhat (*Glycine max* 'Kala bhat') and horse gram/gahat

(*Macrotyloma uniflorum*) are sown near the lower edges of terrace farms (*tirwal*); while urad/kali dal (*Vigna mungo*), lobia/sonta (*Vigna unguiculata*), naurangi/rains (*Vigna umbellata*) and millets are sown in the remaining part of the field. Ploughing of this season is locally known as *nalchhad*. Finger millet is sown twice, before and after ploughing. In this season, not much labourers are engaged in the fields until rouging and weeding.

3.2.2.3 Roguing and Weeding

Roguing and weeding are done twice during this season. After 1 month of seed sowing, the initial one is conducted by traditional harrow (*maiya*) and the subsequent one is done by hoe (*kudal*) after 2 months of seed sowing. Traditional harrow is pulled by man/woman or bullocks. Roguing and weeding by hoe is tedious and labour-intensive process and is done by women. To prevent lodging in the millets, upper leaves are trimmed just before the flowering. This practice is locally known as *patyon*.

3.2.2.4 Harvesting of Crop

Harvesting of crop is initiated on any day baring Tuesday and Saturday. Finger millet is harvested first, followed by Japanese barnyard millet, urad (*Vigna mungo*), lobia (*Vigna unguiculata*), rains (*Vigna umbellata*), gahat (*Macrotyloma uniflorum*), amaranth (*Amaranthus caudatus*) and jakhya (*Cleome viscosa*). Tor (*Cajanus cajan* var. *flavus*), soya bean (*Glycine max* 'Soyabean') and kalabhat (*Glycine max* 'Kala bhat') are harvested at the last. The millet crops (finger millet, foxtail millet and Japanese barnyard millet) are harvested by cutting spike close to inflorescence base by sickle (*dranti*) in September–October. Pulses except *Cajanus cajan* var. *flavus* are harvested by cutting the stem of plants close to the ground by sickle. Whereas, *Cajanus cajan* var. *flavus*, amaranth (*Amaranthus* caudatus) and jakhya (*Cleome viscosa*) are harvested by cutting inflorescence and forming bundles. Crops of bhangjeera (*Perilla frutescens*) sown in the previous wheat season are also harvested during this season. Separated fertile spikes of millets are kept on open space for 2–3 weeks for sun drying, and thereafter, threshing is carried out by bullocks, or spikes are beaten by a wooden stick. Seeds from the inflorescence of *Amaranthus* caudatus, *Perilla frutescens* and *Cleome viscosa* are separated by braying with the foot. Bundles of pulses are also kept in open spaces for 2–3 weeks for sun drying, and thereafter, seeds are separated by beating bundles with wooden sticks.

As discussed earlier, this cropping season is known for the diversity of crops. It is traditionally known as *Baranaja/Bara-Anaja* (12 crops) season, because nearly 12 crops are sown in an inter-mixed manner. This season is one of the main components of the *Sari* system. In this system, finger millet is considered as main crop by the farmers (Kahane et al. 2013; Singh and Jardhari 2001; Zhardhari 2000). It is important to note here that the crops selected by farmers for this season form a unique combination. Different species of pulses are cropped with different millet species. Pulses are important source of nitrogen for the soil and important source of vegetable protein for the inhabitants. Further, different species of millets are important source of minerals and vitamins of the locals (Pandey 2010). Thus, this intermixed cropping fulfils the nutrient requirements of the local people. *Baranaja* is practised mainly in rainfed areas, and under such environment, this system provides good productivity. Different pulse species fix atmospheric nitrogen into the soil which is used by different millets. In exchange, millets provide support to the pulses and increase their productivity.

3.2.3 Kharif Season (Rice Cropping)

Cropping of rice is important in the hilly areas of the Uttarakhand. Practices of cultivation and harvesting of rice are tedious and labour intensive. Practices followed by the local people for rice cropping are discussed below:

3.2.3.1 Field Preparation

Practices are similar as that of wheat cropping.

3.2.3.2 Ploughing

After preparing the fields, farmers apply organic manure (dung and fodder remains) in the fields and thereafter start ploughing on an auspicious day. Fields are kept uncropped for 3 months after harvesting of finger millet crops. Thus ploughing is started nearly 3 months after the harvesting of the previous crop. For rice cropping, ploughing is done thrice. The initial ploughing is locally known as *ban* and subsequent ploughing is known as *dudyon* and *bootan*, respectively. After initial farming, some large aggregates of soil (*dhika*) appear in the field, and these are dilapidated with the help of the wooden mace (*dhikaru*). Further, root debris of the previous crops is collected by women with the help of hoe, and the practice is known as *kamaun*. The second ploughing (*dudyon*) is started after 2–3 weeks of the initial ploughing. To increase porosity of the soil, wooden plank (*jol*) is applied in the field after second ploughing. Final ploughing (*bootan*) is started on an auspicious day usually after 1–2 weeks of the second ploughing. Seeds of rice/satti (*Oryza sativa*), foxtail millet/koni (*Setaria italica)*, Japanese barnyard millet/jhangora (*Echinochloa frumentacea*), tor (*Cajanus cajan* var. *flavus*), soya bean/bhat (*Glycine max* 'Soyabean') and sesame/til (*Sesamum indicum*) are sown by broadcasting method. Generally, *Setaria italica, Cajanus cajan* var. *flavus, Glycine max* 'Soyabean' and *Echinochloa frumentacea* are sown towards the lower edges (*tirwal*) of the field. *Oryza sativa* and *Sesamum indicum* are sown in the rest of the field. After the final ploughing, soil is levelled by traditional hoe and special wooden plank (*chhapulu*) by women, and the practice is known as *Dhan*. Rice farming is tedious and requires enormous manpower.

3.2.3.3 Roguing and Weeding

Generally, practices of roguing and weeding are conducted five times in rice cropping. The initial one is carried out with traditional harrow (*maiya*) after 1 month of seed sowing, followed by three times rouging and weeding with a traditional hoe. After initial practice by harrow, the subsequent ones are done after a gap of 2–3 weeks. The last rouging and weeding is done by hands, just before the blossom of the crop. Roguing and weeding practised by traditional hoe locally is known as *gudai* and practised by hands is known as *nirai*. Upper leaves are trimmed just before flowering to prevent lodging in the rice, and the practice is locally known as *patyon*.

3.2.3.4 Harvesting of Crop

Harvesting of the crop started on the auspicious day. The spikes of *Setaria italica* are harvested first, followed by *Oryza sativa, Echinochloa frumentacea, Cajanus cajan* var. *flavus, Glycine max* 'Soyabean' and *Sesamum indicum.* Harvesting of *Oryza sativa* is complicated. First the culms are cut near ground by sickle, and bundles are formed. Further, these bundles are arranged in a dome-shaped structure in the field (known as *kwanku*). Formation of *kwanku* is an art, and local women are expert in it. Generally, 7–8 days after *kwanku* formation, rice grains are separated from the plant by braying through human feet. Variation at this step is also reported in different parts of Uttarakhand. The practices of harvesting and threshing of *Setaria italica, Echinochloa frumentacea, Cajanus cajan* var. *flavus* and *Glycine max* 'Soyabean' are similar as discussed in the earlier section. *Sesamum indicum* is harvested by cutting inflorescence and forming bundles. These bundles are sun-dried for 2–3 weeks, and the seeds are further separated by beating bundles with wooden sticks.

3.2.4 The Rainy Season (Zaid Season)

It is a short duration season (June–September) between Rabi and Kharif seasons. Maize (*Zea mays*) and various vegetables of family Cucurbitaceae are grown during this season. Crops of this season are usually grown in kitchen garden and some fields near the human settlement. Whereas, the main agriculture fields are not involved in cropping during this season.

3.3 Sari System and Diversity of Agricultural Crops

The *Sari* system is characterized by the rotation of crops in different seasons in a part of terrace farms. It also provides vertical distribution of crops in different hill elevations to support and maintain agro-biodiversity of the locality (Sati 2009). Crops being cultivated in different elevation of hills, i.e. higher Himalaya, mid-elevation and the valleys, vary significantly. *Sari* system is an important on-farm conservation system for the landraces of the area. Many landraces of Kharif, Rabi and Zaid seasons are cultivated by people since ages; the list of crop species of lower and middle Himalaya area is provided in Table 3.2. Apart from these, various species of vegetables (cucurbits, mustards, etc.) are also cultivated by local people in the kitchen garden and fields nearby houses. These species are not included in the purview of Sari system in the present chapter.

In higher elevation areas, ogal (*Fagopyrum tataricum*) and different varieties of French bean (*Phaseolus vulgaris*) are the main crops along with wheat and rice (Sati 2005). In Munsiyari and Dharchula areas of district Pithoragarh and Niti and Mana areas of district Chamoli, an indigenous *Hordeum* known as uva (*Hordeum himalayens*) is also cultivated. There is a considerable decrease in the cultivation area of this species since last one to two decades (Joshi and Palni 2005). Potato is also an important cash crop grown in the area. Potato is sown in April–May in the areas near to the alpine zone. Whereas, in the Mid-Himalayan (2000–3000 m.a.s.l.) areas, it is sown in February to March. After harvesting potato, people sow rice, mirch (*Capsicum annuum*) or Zaid crops in the field.

3.4 Crop Diversity and Economic Sustainability

Agriculture is one of the main sources of economy for Uttarakhand state. The state holds 5,992,604 ha land, and nearly 11.5% area (698,413 ha) is used for the cultivation of different crops. More than three-fourths population of the state directly depends on agriculture for their livelihood. There are nearly 15.81 million cultivators and 0.403 million agriculture-based labourers. Various other people are also engaged in different activities related to agriculture-based products for their livelihood in the state. Furthermore, most of the farmers are marginal and sub-marginal land holders. Uttarakhand holds nearly 912,000 operational land holdings, among these 73.68% of the operational holdings are of less than 1 ha (marginal holdings), 17.21% are 1–2 ha (small holdings), 8.99% are 2–10 ha (semi-medium and medium holdings) and 0.11% are >10 ha (large holdings) (Bird's Eye View of Uttarakhand Economy 2016–2017). Wheat occupies the maximum area of cultivation (341,429 ha) in the state, followed by rice (245,655 ha) and finger millet (107,175 ha). Area of cultivation of some other major crops is: barley (21,663 ha), black gram (14,569 ha), horse gram (13,297 ha), mustard (13,129 ha), soya bean (11,585 ha), lentil (10,221 ha), black soya bean (5839 ha), French bean (5743 ha) and sesame (2087 ha) (Sankhiyiki Diary Uttarakhand 2016–2017).

As discussed earlier, crop diversity is one of the important characteristics of agriculture system of the hilly areas. Diverse landraces are the result of various trials and errors of traditional agricultural practices. These landraces evolved over years and adapted themselves in the hilly environment. Economy of the area also evolved over years around the landraces. Sari system is one the sustainable economic model of agriculture. It provides different seasons for different crops and also provides enough gaps between crops, so that the fields can maintain and regain fertility. However, people also apply organic manure and practise different agricultural practices from time to time to increase the fertility of the soil. In the case of failure of a particular crop, due to either environmental conditions or diseases, other crops

Table 3.2 Crops grown under Sari system

S. No.	Season	Local name	Scientific name	Cultivation status (1970 onward, based on observation of local people)	
				Earlier	Present
1.	Rabi	Wheat/ Gehun	*Triticum aestivum*	There were various landraces of this species	Number of landraces have been reduced due to farming of high yielding variety
2.		Barley/Jau	*Hordeum vulgare*	Cultivated area was enough	Cultivated area has been reduced significantly
3.		Lentil/ Masur	*Lens culinaris*	Cultivated area was enough	Cultivated area has been reduced
4.		Gram/ Chana	*Cicer arietinum*	Cultivated primarily in Gangad area	Cultivated area has been reduced
5.		Pea/Matar	*Pisum sativum*	Cultivated area was enough	Cultivated area has been reduced
6.		Perilla/ Bhangjeera	*Perilla frutescens*	Cultivated area was enough	Cultivated area has been reduced significantly
7.		Rai/ Mustard	*Brassica nigra*	Cultivated primarily in kitchen garden and fields nearby houses	Cultivated primarily in kitchen garden and fields nearby houses
8.		Indian Mustard/ Gharia	*Brasssica juncea*	Cultivated area was enough	Cultivated area has been reduced
9.		Indian Rape/Radu	*Brassica campestris* var. *toria*	Cultivated area was enough	Cultivated area has been reduced
10.	Kharif	Black Gram/ Urad/Kali Dal	*Vigna mungo*	Cultivated area was enough	Cultivated area has been reduced
11.		Lobia/ Sonta	*Vigna unguiculata*	Cultivated area was enough	Cultivated area has been reduced
12.		Naurangi/ Rains	*Vigna umbellata*	Cultivated area was enough	Cultivated area has been reduced
13.		Tor	*Cajanus cajan* var. *flavus*	Cultivated area was enough	Cultivated area has been reduced
14.		Soyabean/ Bhat	*Glycine max* "Soyabean"	Cultivation was less	Cultivation increased during 80s-90s but reduced thereafter
15.		Black Soyabean/ Kala Bhat	*Glycine max* "Kala bhat"	Cultivated area was enough	Cultivated area has been reduced
16.		Horsegram/ Gahat	*Macrotyloma uniflorum*	Cultivated area was enough	Cultivated area has been reduced

(continued)

Table 3.2 (continued)

S. No.	Season	Local name	Scientific name	Cultivation status (1970 onward, based on observation of local people)	
				Earlier	Present
17.		Amaranth/ Chua/ Marchhu	*Amaranthus caudatus*	Cultivated area was enough	Cultivated area has been reduced significantly
18.		Japanese Barnyard Millet/ Jhangora	*Echinochloa frumentacea*	Cultivated area was enough	Cultivated area has been reduced
19.		Jakhya	*Cleome viscosa*	Cultivated area was enough	Cultivated area has been reduced
20.		Foxtail Millet/Koni	*Setaria italic*	Cultivated area was enough	Cultivated area has been reduced significantly
21.		Finger Millet/ Mandua/ Koda	*Eleusine coracana*	Cultivated area was enough	Cultivated area has been reduced
22.		Rice/Dhan/ Satti	*Oryza sativa*	There were various landraces of this species	Number of landraces have been reduced due to farming of high yielding variety
23.		Sesame/Til	*Sesamum indicum*	Cultivated area was enough	Cultivated area has been reduced significantly
24.	Zaid	Maize/ Bhutta/ Mungari	*Zea mays*	Cultivated primarily in kitchen garden and fields nearby houses	Cultivated area has been reduced

growing in the fields provide an alternative to the farmers. Thus, this farming can be viewed as a traditional risk management strategy of the farmers. Sometimes inadequate rain during rainy season drastically reduce the productivity of the rice, but the rainfed crops (millets and pulses) growing in the next sari balance the situation with handful productivity. Hence, farmers have some backup for their livelihood. Similarly, if a particular crop gets infected and fails in that season, under such circumstances also, other crops growing in the field compensate the loss of the cultivator. Due to such traditional approaches, famines are rare in these mountains. The traditional practice not only provides food to the inhabitants but also provides diversity of nutrients in different food crops. Millets are rich source of minerals and vitamins, rice and wheat are the main source of carbohydrate and protein (Chandra et al. 2016), and different pulses provide different dietary proteins and amino acids to the inhabitants (Sati 2012). Along with this, different seasonal vegetables, wild fruits, traditional beekeeping practices and dairying provide balanced diet to the local people (Goswami 2013; Negi et al. 2013).

3.5 Threats to the Sari System

The traditional agriculture system is confronting various threats at various levels. Major threat is outmigration of inhabitants. Since the inception of the state, development is unequal in the state. The four districts Haridwar, Udham Singh Nagar, Dehradun and Nainital became the centre of the industrial and agricultural advancement, and the rest of the nine districts were ignored. Industrial development in the aforementioned districts and various other cities of India (Delhi, Lucknow, etc.) provided various job opportunities to the skilled and non-skilled people. To grab these opportunities, people migrated from hilly areas to the plains. Further, educational and medical facilities are inadequate in the mountainous areas. Some people out-migrated to provide better education to their children and to enjoy the best medical facilities and other amenities of cities. Massive outmigration of people from hilly areas is also reflected in the 2011 census. The population growth rate of Uttarakhand (from 2001–2011) is 18.8%. However, the population growth rate of the districts Haridwar, Udham Singh Nagar, Dehradun and Nainital is 30.63%, 33.45%, 32.33% and 25.13%, respectively. This is higher than the average state population growth rate. Whereas, hilly districts such as Pauri Garhwal and Almora have shown negative growth rates of −1.41% and −1.28%, respectively. Other hilly districts Tehli Garhwal, Rudraprayag, Pithoragarh, Chamoli, Bageshwar, Uttarkashi and Champawat have shown population growth of 2.35%, 6.53%, 4.58%, 5.74%, 4.18%, 11.89% and 15.63%, respectively (Census 2011a, b). Continuous drifting of people from the hilly areas vacated the traditional villages, and the numbers of ghost villages are increasing in the state. As per the 2011 census, there were 1034 ghost villages in the state, and the number increased up to 1768 villages in 2018. On an average, nearly 138 people out-migrated from villages every day in the last 10 years. Among these, 33 never return to villages and 105 return periodically to villages for short intervals (Singh 2018). It is not only migration of people but their traditional knowledge is also migrating along with them. In the newly settled areas, people do not practise their traditional knowledge and practices. Consequently, this precious treasure of traditional knowledge is on the verge of extinction.

Due to the outmigration of the families, land fallowing is also increased. Many hectares of agricultural land have been left fallow over the years. Total numbers of farmers are also decreased by about 2.6% in the mountainous area of the state. However, in the plain areas of the state, the number of farmers increased by about 4% (Pathak et al. 2017). The average agriculture-based income is also less in Uttarakhand than the neighbour Himachal Pradesh. In Uttarakhand, monthly average income per agriculture household is INR 4701 (Indian national average, INR 4923); however, in Himachal Pradesh, it is INR 8777(Mamgain and Reddy 2015).

Outmigration is the root cause of various social and cultural changes that the area is witnessing. The social demography of the villages has been changed. Earlier farming was the main source of livelihood of the people, but presently jobs are the priorities and farming is considered as second class. Previously, farming was also a cooperative venture of the villagers. People used to participate in ploughing and other agricultural practices of one another. Earlier the whole community participated to encounter the problem of human–wildlife conflict. Especially, the monkeys and wild boars were kept away from the agricultural fields by community efforts. Presently, the population of the villages reduced considerably, and the community spirit is also weakened. Consequently, the human–wild life conflict is increasing. Agricultural crops are being destroyed by monkeys and wild boars. Fallow land of absentee families is acting as a good habitat of wild animals and various invasive plant species. Agriculture is closely interlinked with the animal husbandry. In recent one to two decades, dependency of the people on agriculture and livestock has been decreased. As a result of this, numbers of livestock in every village reduced significantly. It causes negative impact on traditional agricultural practices. Production of organic manure has been reduced and people are applying fertilizers to compensate the loss. It is also a serious challenge to the organic agriculture policy of the government of Uttarakhand (Pathak et al. 2017). In addition to this, people also started monoculture of several case crops such as ginger, garlic, soya bean, mango and potato. Due to this, the cultivation area of traditional crops such as sesame, barley and various millets has been reduced significantly (Sati 2012).

Water crises, loss of agricultural land, unavailability of proper irrigation system and loss of fertility in available agricultural land are the most important problems of the farmers of hills. Agriculture in Uttarakhand is very complex, and environmental heterogeneity and fragility have evolved subsistence production system. Disaster-prone environment of the state is also a considerable cause of hilly soil and field degradation. Whereas, poor crop productivity, crop destruction by wild animals, lack of knowledge about seeds and other inputs are other problems associated with agriculture systems of Uttarakhand. Currently, gradual reduction of fertility and productivity of soil was also reported by government of Uttarakhand but in limited extent. It is probably because of unsystematic use of chemicals and overexploitation of groundwater in some hilly regions. But the problem could be removed to an extent through the use of better management practices (SAP 2012–2017).

3.6 Proposed Plans for Improvement

Decrease in crop productivity along with outmigration is the major concern for the *Sari* system. The *Sari* system is one of the best methods to improve crop production and soil fertility. However, various constraints are limiting the crop productivity in the *Sari* system. Main constrains are poor soil health, inadequate irrigation facilities, farmers are less familiar with various scientific methods of cropping and unavailability of sufficient organic manure although chemical fertilizer and pesticide input are well-described methods for increase in crop productivity. But it is less considered due to various environmental and health hazardous. Considering all the above facts, Uttarakhand government has pushed the organic cropping system and the integrated pest management practices with a very high extent for the development of traditional agriculture system and thereby the farmers. Uttarakhand is the first and only state of India with elaborated organic farming policy (Maikhuri et al. 2015). The key objective of diversifying towards organic farming is to improve crop productivity, soil health and the price of the output, and thus the income of the farmers. Use of organic manure (farm yard manure, poultry manure, fish manures, sheep compost, etc.), use of organic cakes from neem, groundnut, castor, etc. and application of crop residues and leaves of bhang, gulmohar and peepal to soil are some effective methods to enhance crop productivity. Along with these, the use of leguminous crops for fixing atmospheric nitrogen and use of bio-fertilizers such as rhizobacteria and fungal spores can also be useful to enhance the crop yield (Maikhuri et al. 2015). In Uttarakhand hills, organic farming is prominent but unorganized. Therefore, the crop productivity is much lower than that required. Earlier, this was because of problems of inaccessibility to agrochemicals, lack of scientific knowledge and poor irrigation systems. To address these problems, various schemes such as The National project on organic farming, Paramparagat Krishi Vikas Yogana (PKVY) and Jaivik Mulya Shrankhala Vikas Mission project are implemented by the government.

The integrated pest management (IPM) uses all available pest control techniques and subsequent integration of appropriate measures for pest control. It is economically justified with reduced human and environmental risks. Various traditional IPM methods such as setting fire in the fields, spreading decomposed farm yard manure over field and broadcasting of common salt at the rate of 1 kg/nali (1 nali = 1/20 acre) are used for controlling white grab (*Anomala dimidiata*). Similarly, balls of urea and dry faecal material of horses or mules are used as rodent repellent. Further, mustard oil, turmeric, *Juglans regia* leaves, *Zanthoxylum armatum* leaves, etc. are used for the storage of seeds (Chandola et al. 2011). Therefore, scientific knowledge-based practices of field preparation, cropping, harvesting, pest management and storage needs to be encouraged for the sustainable farming in the hilly areas.

3.7 Conclusion

Sari system of agriculture acts as an umbrella for the conservation of various landraces of different crops. People cultivate different crops in different seasons by utilizing terrace farms of different *Sari*. Various traditional agricultural practices are also associated with this system. In recent past, outmigration hit hard the mountains of Uttarakhand, and consequently the traditional demography of villages has been changed. It adversely affects the traditional knowledge and various traditional practices of the mountains. Cultivated land under the *Sari* system has been reduced significantly over the last one to two decades. Traditional knowledge and practices act as raw materials for further scientific advancement. Thus, it is the need of hour to conserve this tradition of Himalaya. For this purpose, local people need to sensitize about the importance of their traditional knowledge and practices. Monoculture of crops cannot be avoided as it is directly associated with the income of farmers. But, people should be encouraged for the cultivation of traditional crops at least some parts of their land holdings. Seed banks need to be prepared at the village level for the conservation of the local varieties of the area. In this respect, Shri Vijay Zardhari did commendable work through Beej Bachao Abhiyan, and various other non-governmental organizations and societies are following the same path.

Government should promote on-farm conservation of local varieties and traditional practices through the ambitious organic agriculture policy.

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Factors Influencing Soil Ecosystem and Agricultural Productivity at Higher Altitudes

4

Narendra Kumar, Amit Kumar, Neha Jeena, Ranjeet Singh, and Hukum Singh

Abstract

Healthy soil ecosystem is a prerequisite for better agricultural productivity, which is governed by various local abiotic and biotic factors. Agricultural system at higher altitudes has unique characteristics and is entirely distinct from that at the lower altitude. The abiotic and biotic factors are the drivers of the soil ecosystem processes and functioning which improve plant growth and development, ultimately productivity. The key abiotic factors at higher altitudes consist of temperature, precipitation/rainfall pattern, wind profile, light intensity and duration, physiographic, etc.; and the key biotic factors are soil fauna and flora (microbes, fungi, protozoa, nematodes, etc.) influencing the soil ecosystem and agricultural productivity. These major biotic and abiotic factors interact with each other and influence the local agricultural system at higher altitude. The abiotic factors manipulate the microenvironment of soil microbial communities which eventually influence the activity of soil fauna and flora in the soil ecosystem that determines plant growth, resulting in agricultural productivity. Due to the course of these factors, decomposition pattern and rate in the ecosystem are altered, and the decomposition pattern/rate of crop residue has released the nutrients in the soil ecosystem which further are utilized by soil microbes and plants as the source of energy, resulting in increased soil productivity. In this perspective, this chapter explores the mystery of interrelationship of soil ecosystem functioning and various factors that govern the systematic agricultural productivity.

Keywords

Soil ecosystem · High altitude · Agriculture · Abiotic stress · Soil microbial communities

N. Kumar (✉) · A. Kumar · R. Singh · H. Singh
Forest Ecology and Climate Change Division, FRI, Dehradun, Uttarakhand, India

N. Jeena
Department of Biotechnology, Bhimtal Campus Kumaun University, Nainital, Uttarakhand, India

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4.1 Introduction

The present and the expected patterns of changes in the climatic condition are a key concern in various vicinity of socioeconomic activities, such as agriculture, forestry, soil ecosystem and are the foremost threat for biodiversity and ecosystem function at higher altitudes (Mendonca and Stott 2003). It is a consequence of greenhouse gas emission (e.g., CO_2, CH_4, and N_2O) which is responsible for atmospheric warming. CH_4 production by the process of fermentation as well as emitted from ruminant animals, stored manure (waste), and rice cultivation under flooded condition has been reported. Release of CO_2 from agriculture land results in the microbial decay or burning of plant residue and organic matter. Similarly, N_2O is a result of microbial transformation of nitrogen in soil manures. The emission of greenhouse gases from the agricultural fields is a complex process, but proper management of the fields can mitigate the problem. The soil ecosystem and agricultural productivity at high altitude are drastically affected by the local biotic and abiotic factors. For instance, tree cover is advantageous for the habitat of various herbaceous plants, lower plants, and animals; thus, the complex is ecosystem affected, and therefore, they need to be conserved and properly managed.

There are several biotic and abiotic factors that affect soil ecosystem and agricultural productivity at higher altitudes. The abiotic factors such as temperature, rainfall, and radiation are the major limiting factors that influence the soil ecosystem and agricultural productivity at higher altitudes. However, biotic factors include soil fauna (nematodes, termites, and earthworms, etc.), soil microbes (bacteria, fungi, etc.), and soil flora (leguminous tree, herbs, and shrubs, etc.). Both biotic and abiotic factors interact with each other and thus constitute an ecosystem (Fierer and Jackson 2006) (Fig. 4.1).

4.2 Abiotic/Climatic Factors

Abiotic/climatic factors are essential variables to decide the survival of soil ecosystem and agricultural productivity in the high altitudinal range of Himalaya. They may be extreme temperature, irradiation, water-logging, drought, inadequate mineral nutrients in the soil, rainfall, precipitation, etc. These stress drastically change the soil–plant–atmosphere continuum and responsible for the declining outcome of major crops. These are an integral element of climate change which is an intricate phenomenon with a generous range of irregular impacts on soil, environment, and agriculture. Extended exposure of these stress consequences altered physiology, metabolism, and damage to biomolecules, ultimately productivity.

4.2.1 Temperature

Global average surface temperature is increased by 0.80 °C since 1880, and it is predicted to rise >1.50 °C at the end of the twenty-first century (Středová et al. 2015).

Fig. 4.1 Factors affecting the ecosystem and agricultural productivity of the soil

Soil temperature is one of the determining factors which play a key role in estimating various biochemical and biophysical processes in soil. For instance, water and nutrient uptake by plant roots resolve rate of mineralization and soil organic matter decomposition, and numerous related plant growth, development mechanism, and processes (Williams et al. 2009). It also engaged in integrating the influence of changing the climate, soil, vegetation, and topography in high altitudinal region. Besides, at this range, distribution of plant and community composition are well managed (Tsui et al. 2004). Knowledge of soil temperature in terms of spatial and temporal variability is critical to know to change climate impact, but it was lacking in altitudinal ecosystems.

Besides, air temperature is also a determining factor; based on air temperature, slight significant uncertainty was found in predicting changing the climate on altitudinal soil ecosystem (van Pelt et al. 2010). It can overcome by gaining a better understanding of relative influences of altitude, aspect, climate, soil, and vegetation variables on the spatial and temporal variability of soil temperature and moisture. In addition, warmer air temperature can increase the growing season length and modify community composition of the plant in soil ecosystem and agriculture (Delgado-Baquerizo et al. 2016).

4.2.1.1 Disadvantages of Temperature in Soil Ecosystem and Agricultural Productivity

- Vapor pressure deficit (VPD) of the air increased, thereby increasing the transpiration rates.
- Habitat destruction.
- Low production and consumption.
- Uptake of minerals, nutrients, and water declined.
- Absorption of light energy declined.
- High evapotranspiration.
- Carbon exchange complex.
- Weathering.
- Solute transport.
- Ten soil decomposition
- Mineralization.
- Net primary productivity.
- Species distribution will be affected.
- Photosynthate production declined.
- Relative humidity decreased.
- Decomposition rate will be declined.
- Low agricultural productivity.

4.2.2 Wind Profile

Wind is considered as an important ecological factor in every ecosystem. In the case of the forest, wind is known to influence trees directly by damaging stems or uprooting or indirectly through modifying temperature and soil moisture. Affecting forest tree by wind depends on wind strength. Winds with a low intensity usually do minimal damage to forests and soil ecosystem; however, strong winds might cause severe damage. We can classify the wind damage to forests from fatal to negligible on the basis of their effect from regional level to community level *viz.* branch breakage defoliation, gap formation, etc. (Zhu et al. 2004). The wind is known to affect the snow distribution, temperature regulation, moisture, and evapotranspiration in an ecosystem.

Wind causes erosion in sandy soils of the arid region and modifies significantly (Zhu et al. 2004). In addition, soil particle separation and their relocation through wind speed are an energetic process of wind erosion, and it commences at the time period when the wind speed exceeds beyond the threshold value of soil resistance to erosion. There are several factors that influence the speed and extent of wind such as geological, anthropogenic, and climatic (van Pelt et al. 2010). Besides, it is a result of interactions of wind speed, surface roughness, rainfall, soil texture, soil aggregation, soil moisture, agricultural activities, vegetation cover, the plot area, as well as freezing and thawing cycles or freeze drying during winter seasons (Středová et al. 2015).

However, wind speed influences the growth rates and leaf shapes of the plant, but the suitable speed of wind leads to improve growth and primary productivity of plant while strong wind might damage plant functioning. It also takes away fine particles (e.g., silt and clay) of soil from soil surface first resulting in structural changes and reduction of moisture content and nutrient redistribution of the soil. In soil ecosystem, wind transfer and exchange of materials and energy in between atmosphere and land surface leads to change in soil micro-climate. For instance, with reducing wind, soil surface temperature will rise. In addition, wind can also alter moisture level and nutrient composition, thereby changing ecosystem structure and diversity.

There are several factors that decide the wind speed and intensity. Among them, the elevation is one of the factors that decide wind speed; with increasing elevation, the wind speed is also increased (Daly and Porporato 2005). Wind speed is one of the limiting factors for the agricultural productivity in high altitude which affects the growth by reducing tissue temperature, water status of the plant, stomatal functioning, and finally limiting photosynthesis (Daly and Porporato 2005). The wind is indispensable to all sorts of soil and forest ecosystems. It also contributes to the transportation of industrial pollutants, some of which can affect soil and tree health (Zhu et al. 2004).

4.2.2.1 Advantages of Wind in High Altitudinal Agriculture

- It enhances turbulence in the atmosphere, thereby increasing the supply of carbon dioxide which increases the rate of photosynthesis.
- It changes the hormonal balance in plants.
- It decreases GA3 content in shoot and root of rice crop with enhancing ethylene production.
- The concentration of nitrogen increases with wind speed.
- Disadvantages of wind on soil ecosystem and agricultural productivity.
- Evapotranspiration will be increased.
- Soil erosion.
- Crop lodging.
- Flowering will be disturbed.
- Soil surface dryness is increased.

4.2.3 Air Moisture Content

Change in land use, land cover, and the dynamics of water use are important issues to understand the global climate change and must be clearly understood to sustain certain ecosystems (Fischer and Sun 2001). Water resources are the limiting factors for soil ecosystem, agricultural productivity, and environmental health in diverse regions. Accumulation of water affects porosity, organic carbon, clay content, and bulk density in the soil. Moisture content and temperature of soil are the key variables involved in integrating the influence of climate, soil, vegetation, and topography in alpine regions. All these factors collectively contribute towards the microbial diversity and soil biological activity (Doran and Zeiss 2000; Griffiths et al. 2011). However, air moisture content limits the role of microbes in the soil (Gans et al. 2005; Roesch et al. 2007). Jung et al. (2010), reported that during the period of

1982–1997, global annual evapotranspiration increased by 7.1 mm per year. Due to this huge evapotranspiration, microclimate is affected which directly affects the soil ecosystem. The increase in relative humidity of air and a decrease in air temperature result in changes in the bacterial community structure in the soil. High-altitude ecosystems are reported more sensitive towards rainfall and show low water consumption as higher moisture in these soils causes higher transpiration rate (Wang et al. 2013, 2014).

Soil ecosystems are essentially indistinguishable. Even it is a lower portion of the biosphere in which detoxification of contaminants, recycling of nutrients, and production of radioactively active trace gases occur. Soil moisture thus exerts a significant control on the function of soil ecosystems (Sylvia et al. 1998). Soil moisture and temperature are controlled by a variety of factors, such as meteorological factors (e.g., radiation, rainfall, air temperature and humidity, wind, and snow cover), topography (e.g., altitude, slope, and aspect), soil physical properties (e.g., texture structure and porosity), and vegetation cover (Williams et al. 2009).

4.2.3.1 Disadvantages of Moisture Content in Soil Ecosystem and Agricultural Productivity

- Transpiration declined, thereby declining the uptake of nutrient and water throughout the plant.
- Affects the distribution of clouds and precipitation.
- Continuous wetness leads to disease incidence.
- Photosynthesis declined.
- Net productivity declined.
- Species disturbance.
- Microbial community affected.

4.2.4 Radiation

Solar radiation is a combination of electromagnetic radiation which is released by sun. The sun acts like a black body, emitting energy on the basis of Planck's law at a temperature of 6000 K. It ranges from infrared to ultraviolet. Radiations are of two types: longwave radiation and shortwave radiation. Shortwave radiation does not reach the earth surface because it is trapped by the surrounding atmospheric gases. Out of 100% radiation coming from the sun, only 22.5% radiation goes directly to the earth surface. This radiation is intercepted by the canopy as well as the other part of the earth, and the coming radiation and reflected radiation from the soil surface lead to determine crop development, soil ecosystem, and agricultural productivity (Daly and Porporato 2005).

Direct sunlight is highly injurious to most of the microbial population except algae but legume. Nitrogen fixing system which is driven by microbes is depended upon the light. However, the requirement of ATP for nitrogenase activity as well as the assimilating nitrogen suggests that photosynthesis and photosynthate distribution frequently affect the N_2 fixation. The insufficient light limits the supply of carbohydrates to the *Rhizobium*, but the light effects are not restricted to photosynthesis. Therefore, upper soil surface portion is usually sterile for microorganisms. High light intensity declines the biomass of soil microbes, whereas soil microbial structure (ratio of fungal biomass to bacterial biomass) is not affected by the light intensity. Composition of the soil ecosystem is highly drastically affected by both temperature and light intensity, but there are no changes due to the addition of nitrogen. Light intensity also has its role to determine the composition of the plant community (Daly and Porporato 2005).

4.2.5 Precipitation in Higher Altitudes

Precipitation in the high-altitude regions generally varies on small distances for a shorter time period (Anders et al. 2006) and is greatly influenced by the topography of the mountains (Roe et al. 2003; Bookhagen and Burbank 2006). It has been observed that annual precipitation in the Himalayas is 3000–4000 mm. In these areas, about 70–80% rainfall occurs during typical monsoon season (June to September). The valleys in the north of main Himalayan region (cold desert) receive generally less than 500 mm precipitation annually, and these areas receive most of the precipitation in winter and spring season (Singh et al. 2017). Moreover, the spatial and temporal variability of precipitation is reflected by the distribution of forest trees and other species in the region (Miehe et al. 2015). The intensity and temporal variability of precipitation of a region govern the development of soil and agricultural system (Istanbulluoglu and Bras 2006).

4.2.6 Impact on Soil Ecosystem

Precipitation is the main source of water in the soil which is also the connecting link between climatic and terrestrial water (Singh et al. 2017). Water moves within soil according to gradients in soil water potential which is mainly dominated by gravity in saturation condition and by capillary in dry conditions. The water may then be removed from the soil by evaporation and root uptake or be lost to deeper layers by drainage. The fluctuations in the rainfall within a season adversely affect the mountain soil and agro-ecosystems (Singh et al. 2014). The mountains are prone to natural calamities such as landslides and soil erosion and, therefore, need sustainable management to explore as well as conserve the natural flora and fauna. The traditional agricultural practices, i.e., mulching and terrace farming, have been proved beneficial for such fragile ecosystems (Sharma 2004).

4.2.7 Impact on Agriculture

The traditional agriculture practices are governed by the timing and average duration of rainfall in these regions. The major cropping systems are paddy–wheat and maize–wheat cropping along with the intercropping of oilseeds; pulses and millets are the major forms of the cropping pattern. Further, vegetables and rice crop required irrigation during their cultivation period. Moreover, high-altitude regions allowed only one crop to grow (only in summer) because in winter there is snow which makes agriculture unfeasible. In this region two short duration crops are grown, i.e., pea–buckwheat and pea–pea intercropped with potato (Rawat and Kharwal 2016).

The effect of precipitation is directly associated with crop productivity in the region. Sowing in Kharif season starts with the onset of monsoon. Poor monsoon severely affects crop production. In 2007–2008, about 50–60% of winter crops failed in the middle and lower Himalaya (600–1500 m) because of drought. The unseasonal rains and hailstorms could also cause complete crop failure. In higher altitudes of Himachal Pradesh and Kashmir, snowfall in late September 2018 destroyed the complete summer crops of potatoes, cauliflower, cabbage, apple, etc. and made losses of over 300 million rupees to farmers.

4.3 Physiographic Effects on Soil and Agriculture

The physiography of a region is associated with the availability of solar radiation and soil moisture retention capacity, average temperature, precipitation conditions, and ease of crop management that directly affect the soil and cropping system. The climate varies with elevation and influences pedogenic processes (Tsui et al. 2004). The temperature decreases by a gradient of 0.65 °C with an increase of every 100 m elevation, and it is closely related to the increase in wind speed and exposure to solar radiation. The decrease in temperature at a higher elevation is accompanied by larger thermal amplitude (larger variation between diurnal and nocturnal temperature) relative to lower elevations (Tsui et al. 2004).

The directional orientation of the hill slope and the steepness control the micro-climate and water movement. The steep slope areas had poorly developed soils, low fertility, poor water-holding capacity, and high rate of erosion, whereas gentle slope areas had deep soils and good water drainage and aeration in the soil. The interception of solar radiation is closely related to slope angle and direction as the slope directions of Southeast, East, and South exposure are more favorable for exposure to light interception and soil development as compared to the North-facing slope.

The selection of crops and management practices are determined by the physiographic factors along with climatic factors. The slope aspect has a significant effect on the vegetation establishment, plant density, and crop management factors (Stenburg and Shoshny 2001). The high elevation and steep slope areas increase the cost of production as transportation costs and labor cost are high, and farmers add relatively lower agricultural inputs which may cause low agricultural productivity (Li et al. 2014).

4.4 Soil Nutrient

Healthy soil is responsible for the productivity of ecosystems, and healthy soil is that have nutrients abundantly. Although nutrient availability is also a very complex process in an ecosystem, it restricted by several biotic and abiotic factors of the environment, especially in higher altitudes. The concept of nutrient availability can be noticed from two different points of view. From a soil perspective, the flux of nutrients from unavailable pools into pools accessible by plants represents a nutrient supply rate. Ecosystems differ widely in nutrient supply rates because of differences in rates of decomposition, mineral weathering, and other processes. Alternatively, the productivity of individual plants or whole ecosystems can be affected by nutrient limitation, defined as the extent to which productivity is reduced by an inadequate rate of nutrient supply (Wang et al. 2010). In agricultural situations, nutrient supply rate and nutrient limitation are closely linked, although some species have greater flexibility in growth rates and respond better to increasing nutrient supply (Yan et al. 2018).

Biotic factors include all of the living things in an ecosystem, these are plants and animals. Soil fauna and flora are the vital reservoir of diversity and play a necessary role in many soil ecosystem functions; moreover, it is often used to provide soil quality indicators. Soil biotas are thought to harbor a large part of the world's biodiversity and to govern soil ecosystem processes and agricultural productivity in higher altitudes (Jeffery et al. 2010).

4.5 Soil Fauna

Soil is known as the richest source of fauna in the world which contains nematodes, mites, Collembola, Chilopoda, Symphyla, Pauropoda, earthworms, and enchytraeids. Besides these, several other arthropods viz. beetles, diplopods, spiders, and pseudo-scorpion and snails also reside in the soil. Coleman and Wall (2015) have classified the soil fauna on the basis of their body size as megafauna (above 20 mm), macrofauna (2 and 20 mm), mesofauna (200 μm and 2 mm), and microfauna (20 and 200 μm).

4.5.1 Earthworms

Earthworms are known as the engineers of the ecosystem (Brussaard et al. 2012); they have prominent effects on soil structure as a consequence of their burrowing activities and digestion of soil and creation of castings. Earthworms are digested mineral soil or organic matter, mix and enrich it with organic secretions in the gut, through which casts are produced, and then deposit the material as slurry lining their burrows. Mechanism of cast stabilization includes particle organic bonding by polymers secreted by earthworms and microbes, mechanical stabilization of cast by plant fibers and fungal hyphae, and stabilization by drying and wetting cycles and age-hardening effects (Coleman et al. 2012). In earthworm casts, organic matter is mineralized and burrow linings produce zones of nutrient enrichment compared to bulk soil. These zones are known as the drilosphere. Drilosphere is the site of enhanced activity of plant roots and another soil biota (Brussaard et al. 2012). It has also been suggested that earthworm casts constituents' plant growth-promoting substances. Earthworm castings are used to improve the physical properties of soil as soil amendment, which enhance plant growth (Gilbert 2010). Earthworm creates macropores in soil by burrowing it which infiltrates water by functioning as bypass flow pathways within the soils (Calderon-Cortes et al. 2012).

4.5.2 Formicidae (Ants)

Ants are the most significant members of the soil insects and influence soil structure greatly. They live in colonies having different caste system and thus called social in their behavior. Ants have a large impact on their ecosystems. They are major predators of small invertebrates. The abundance of other predators such as spiders and carabid beetles is reduced by the activity of ants (Moore and de Ruiter 2012). Ants are ecosystem engineers, moving large volumes of soil, as much as earthworms do (Garcıa-Palacios et al. 2013). Ants also influence the structure of the soil; this process is important particularly in deserts because earthworm densities are low in deserts (Scharroba et al. 2012).

4.5.3 Termitidae (Termites)

Besides ants and earthworms, termites are the third major group of invertebrates. They have an organized caste system and are considered as social insects (Bignell and Eggleton 2000). The normal food of termite is wood that has come into contact with soil. Maximum termite species construct runways of soil, and some are builders of spectacular mounds. Their enzymes are responsible for degrading the wood in the form of cellulose from the ecosystem at higher altitude (Bignell 2000). Opposite to carbon degradation, prokaryotes are only capable of producing nitrogenase to fix N_2. Certain genera of termite have some bacteria that fix comparatively small amounts of N, but some other, including Mastotermes and Nasutitermes, fix N from 0.7 to 21 g N g^{-1} fresh wt day^{-1} (Breznak 2000). Ngugi and Brune (2012) reported that soil-feeding termites play an important role in terrestrial N cycle; they measured denitrification in the hindguts of two genera of soil-feeding termites. They observed that denitrification significantly ranged from 0.4 to 3.9 nmol h^{-1} (g fresh wt.) N_2O. The result of this study provides direct evidence that soil-feeding termites are more important to the soil ecosystem and also for agricultural productivity in higher altitudes.

4.6 Soil Flora

Similar to soil fauna, soil flora is also important for soil ecosystem and agricultural productivity at higher altitudes. Soil flora mainly leguminous plants, herbaceous plants, and shrubs play an important role in the soil ecosystem at higher altitudes because they release a huge amount of nutrient and energy after decomposition. They are the important components of the nutrient cycle at higher altitudes because they increase the efficiency of the nutrient cycling process and provide a valuable nutrient source for the development of sustainable smallholder farming systems. Leguminous plants used as green manure is a source of organic material with significant benefits for soil and crops, due to the high N_2 fixation capacity, nutrient cycling, and contribution to soil cover (Cobo et al. 2002).

Several types of research have revealed that leguminous plants can supply the crop nutrient demand for N and K, but generally do not play a significant role in sufficient supply of P to meet crop demands (Lupwayi et al. 2007; Mukuralinda et al. 2009). However, it has significant residual effects on long-term P availability after decomposition by releasing organic compounds that reduce the P fixation capacity of the soil (Mafongoya et al. 1998).

Several species of leguminous plants, i.e., *Arachis pintoi, Calopogonium mucunoides, Stylosanthes guianensis, and Stizolobium aterrimus*, have a great capacity to produce high amounts of biomass and accumulate high nutrient concentrations (Matos et al. 2008), after residue decomposition, it becomes available to crops. Residue quality and quantity decide the decomposition and nutrient release in the ecosystem of higher altitudes; however, residue is defined in relation to its chemical and biochemical composition (Matos et al. 2008) because of chemical and biochemical composition of residue influence the activity of decomposer communities (Thonnissen et al. 2000). Nutrient contents in plant materials are correlated with decomposition rates; if there is high nutrient content plant material, then there will be high decomposition and nutrient release, and it also induces microbial growth and activity (Cobo et al. 2002; Thonnissen et al. 2000).

It seems logical that if more than one species can perform the same function, then overall ecosystem functions will be less vulnerable to changes in the populations of a particular species due to environmental stress or pest attack. It is clear that there are trade-offs with more diverse agricultural systems, and that is the kind of diversity matters greatly, but the question is how to design diverse systems that can meet multiple goals in an acceptable way. Inclusion of perennial species in the higher altitudes can provide important benefits to the agro-ecosystems, i.e., less soil erosion and enhanced nutrient flow. The trade-off is that increased shading and microclimate changes may negatively impact the productivity of important annual crop species at higher altitude (Mukuralinda et al. 2009). The effects of climatic conditions can also greatly influence residue decomposition at higher altitude. Environmental factors such as temperature, moisture, and aeration affect the microbial community and activity (Robertson and Morgan 1996) and are therefore related to the decomposition process at higher altitude. Oliveira et al. (2003) observed outstanding effects of wet and dry seasons on *A. pintoi* residue decomposition rate at higher altitude. However, Zaharah and Bah (1999) observed that there are no effects of rainfall on the decomposition and nutrient release rates of gliricidia (*Gliricidia sepium*) at higher altitude.

4.7 Soil Microbes

Soils are the naturally occurring physical covering of the earth's surface and represent the interface of three material states: solids (geological and dead biological materials), liquids (water), and gases (air in soil pores). Whole terrestrial ecosystems and diversity of bacteria, archaea, fungi, insects, annelids, and other invertebrates, as well as plants and algae depend on the different kinds of soils. These soil inhabitants supply food or nutrients for the survival of organisms that exist above- and below-ground. Soils also play key roles in buffering and filtering freshwater ecosystems. Thus, soils are enormously important to human beings and societies. We depend on soils for the basis on which we and our buildings stand and for the production of food, building materials, and other resources; indeed, soils influence nearly all ecosystem services on which we depend. Soil microbes, bacteria, archaea, protozoa, fungi, and mycorrhiza play a vital role in the diversified ecosystem. Soil microbes with immense metabolic diversity lead to the cycling of all major elements (e.g., C, N, P, and other elements), which affects structural and functional soil ecosystems (Dominati et al. 2010).

Biodiversity enhances ecosystem stability and productivity. It is verified for plant communities based on the manipulation of the diversity of functional groups. Microbial community plays a key role in the functioning of the ecosystem which is directly involved in the fertility of environment, soil, and water quality (Singh et al. 2010; Delgado-Baquerizo et al. 2016).

4.7.1 Bacteria

These are microbodies and 100 M to 1 billion bacteria are found in the most fertile soil. They are involved in the decomposition of organic waste and dead plant materials by converting from unattainable to usable forms in most important cycle, i.e., nitrogen cycle. Besides that, they can also directly play a vital role in agricultural productivity by converting an unstable form of nitrogen to stable form of nitrogen, i.e., nitrates. It improves the growth of the crop in the agricultural field (Giller et al. 1997).

4.7.2 Fungi

Fungi are neither plants nor animals. They are hyphae-like structured, and a number of hyphae form mycelium which is 0.8 mm wide and several meters long. They are helpful as well as harmful besides that they have a potential to split down nutrients while other organisms are not able to split such complex compound. Fungi have a symbiotic association with plant roots which is beneficial for their better growth and development by transferring the nutrients, and this association is called a mycorrhizal association. By this association, fungi benefited with the carbohydrate are transported from plants, and again the same food is provided by the plants provide to humans. They performed numerous functions in the soil as decomposer (convert dead organic matter to fungal biomass), mutualists (association of mycorrhizal fungi with plant roots), and parasites (causes reduced production or death when they colonies roots and other organisms) (Rillig and Mummey 2006).

4.7.3 Algae

They are available in the area where moisture and sunlight are present, and in the soil, they range from 100 to 10,000/g. They have the potential to do photosynthesis. The roles of algae in the soil are:

- Maintenance of soil fertility.
- Increase the amount of organic carbon.
- Prevent soil erosion.
- Increase the water retention capacity of the soil.
- Facilitating submerged aeration.
- Improve the weathering of rocks.

4.7.4 Nematodes

Nematodes are not microorganism but responsible for the plant diseases apart from that they are beneficial too. Soil nematodes involve maintaining ample levels of available nitrogen in farming systems relying on organic sources of fertility (Ferris et al. 1998). They feed on plants, algae, bacteria, fungi, and other nematodes. On the basis of the diet, nematodes can be classified into subclasses such as (1) bacterial feeders which consume bacteria, (2) fungal feeders which is feed by cell walls of fungi and consume internal contents, and (3) predatory nematodes eat all kinds of nematodes and protozoa. They are important in mineralization of nutrients and release ammonium while eating bacteria or fungi. They are an indicator of soil quality because of their involvement in soil diversity as well as soil food web (Neher 2001).

4.8 Conclusion

The soil and agricultural ecosystem of the high altitudinal range have a huge biodiversity which is drastically affected by numerous abiotic (temperature precipitation, radiation, etc.) and biotic factors (soil flora and fauna, etc.). It is an exclusive region of a mountainous ecosystem with great environmental variabilities. These variabilities with respect to time in the rate and potential of such factors lead to distinction in environmental and physiography at higher altitude. High altitudinal ecological systems including agricultural system are facing problems due to the climatic inconsistent changes in the pattern of rainfall, warmer cold season and unpredicted storms and frosts. Afforestation and adoption of the intercropping system with fruit tree plantation with local flora will be the alternative source to maintain the health of the ecosystem. However, further studies will lead to the exploration of productive soil ecosystem and advanced agricultural practices for a future scenario.

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Traditional Farming Systems and Agro-biodiversity in Eastern Himalayan Region of India

5

Krishna Giri, Gaurav Mishra, Monica Rawat, Shailesh Pandey, Rajarishi Bhattacharyya, Nabajyoti Bora, and J. P. N. Rai

Abstract

The North Eastern Indian states, namely Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, Sikkim, and hill districts of Assam constitute about 52% area of total Eastern Himalaya. This part of India is a culturally diverse region inhabited by more than 200 tribes in eight states and also recognized as one of the biodiversity hot spots in the world. The entire North Eastern Region (NER) is endowed with rich floral, faunal, and socio-cultural diversity represented by Trans Himalaya, Himalaya and North East India, Bio-geographic realms, an Indo-Burma biodiversity hot spot. Shifting cultivation, also known as *jhum,* is the major farming system in the hilly terrains of NER. *Jhum* cultivation is a cyclic process of slash and burn of community-owned natural forests followed by the cultivation of food crops. After 2–3 years of cultivation in a particular piece of land, the *jhumia* farmers rotate the land and the previously cultivated area is left fallow for regeneration. Wet terrace paddy cultivation in valleys is another traditional farming practice in the NER. These traditional farming practices are unique and tribe-specific which maintains high cultural and farm agro-biodiversity in the region. Alder-based *jhum* and wet terrace paddy cultivation by Angami tribe, Zabo system of Chakhesang tribe, tree-based cultivation of Konyak tribe in Nagaland, paddy-cum-fish farming and *jhum* cultivation by Apatani tribe in Arunachal Pradesh, *jhum* and bun (terrace)

K. Giri (✉) · G. Mishra · R. Bhattacharyya · N. Bora
Rain Forest Research Institute, Jorhat, Assam, India

M. Rawat
ICMR-National Institute of Malaria Research, Dwarka Sector-8, New Delhi, India

S. Pandey
Forest Research Institute, Dehradun, Uttarakhand, India

J. P. N. Rai
G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

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farming by ethnic Khasi tribe of Meghalaya, rice-based farming in Tripura, organic farming, as well as terrace rice cultivation in the river valleys of Sikkim are the predominant traditional farming systems. These indigenous farming systems in the NER represent rich cultural and agro-biodiversity. This chapter attempts to describe unique traditional farming systems, innovations to transform conventional *jhum* cultivation, farm agro-biodiversity and rural livelihood of tribal communities in the NER of India.

Keywords

Traditional *jhum* farming · Terrace paddy cultivation · Agro-biodiversity · North East India · Eastern Himalaya

5.1 Introduction

Shifting cultivation or slash and burn agriculture is the oldest crop cultivation system in North East India (NEI). This system is locally known as *jhum* cultivation and practiced in the hill states *viz.* Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and hill districts of Assam (Deka and Sarmah 2010). Beside North East India, *jhum* cultivation is practiced in hilly tropics of Southeast Asia, the Pacific, Latin America, the Caribbean, and Africa for centuries (Cairns and Garrity 1999; Craswell et al. 1997; Eastmond and Faust 2006; Lawrence and Schlesinger 2001; Ramakrishnan 1992; Stromgaard 1992; Thomaz 2009; Grogan et al. 2012). North East India is a global biodiversity hot spot and culturally diverse region of the world (Giri et al. 2018). The tribal communities inhabiting hilly regions of NEI are dependent on diverse natural resources for their livelihood requirements. Rigorous and undulating mountain terrains in this region coerce the ethnic groups for *jhum* cultivation in natural forests (Fig. 5.1a, b). Due to this mountainous terrain, settled cultivation constitutes a small portion of arable land in lower ridges of hills and river valleys in the form of wet terrace cultivation (Fig. 5.2). In the entire region, diverse and tribe-specific prototypes of *jhum* cultivation blended with unique traditional innovations are prevalent. Diverse cropping with paddy being staple crop is the common practice among all the tribes while in some places, maize and millets are grown as a staple crop in *jhum* land (NEPED and IIRR 1999). Crop diversity in slash and burn *jhuming* system is as high as the diversity of ethnic folks with a documented variation from 15 to 60 crops in a single field (NEPED and IIRR 1999). Besides, paddy, maize, and millets as staple crops, tuber crops, cereals, legume pulses, oilseed crops, and various types of green leafy vegetables are grown in *jhum* fields. The traditional *jhum* cultivation system in Nagaland has been considered as organic farming (Kuotsuo et al. 2014) as cultivation in *jhum* fields is practiced without chemical fertilizer and pesticide inputs.

Traditionally, *jhum* cultivation system is practiced in community-owned natural forests, starting from the selection of land during the winter season where slash and burn operations are to be carried out. The dried biomass is then burnt in the fields

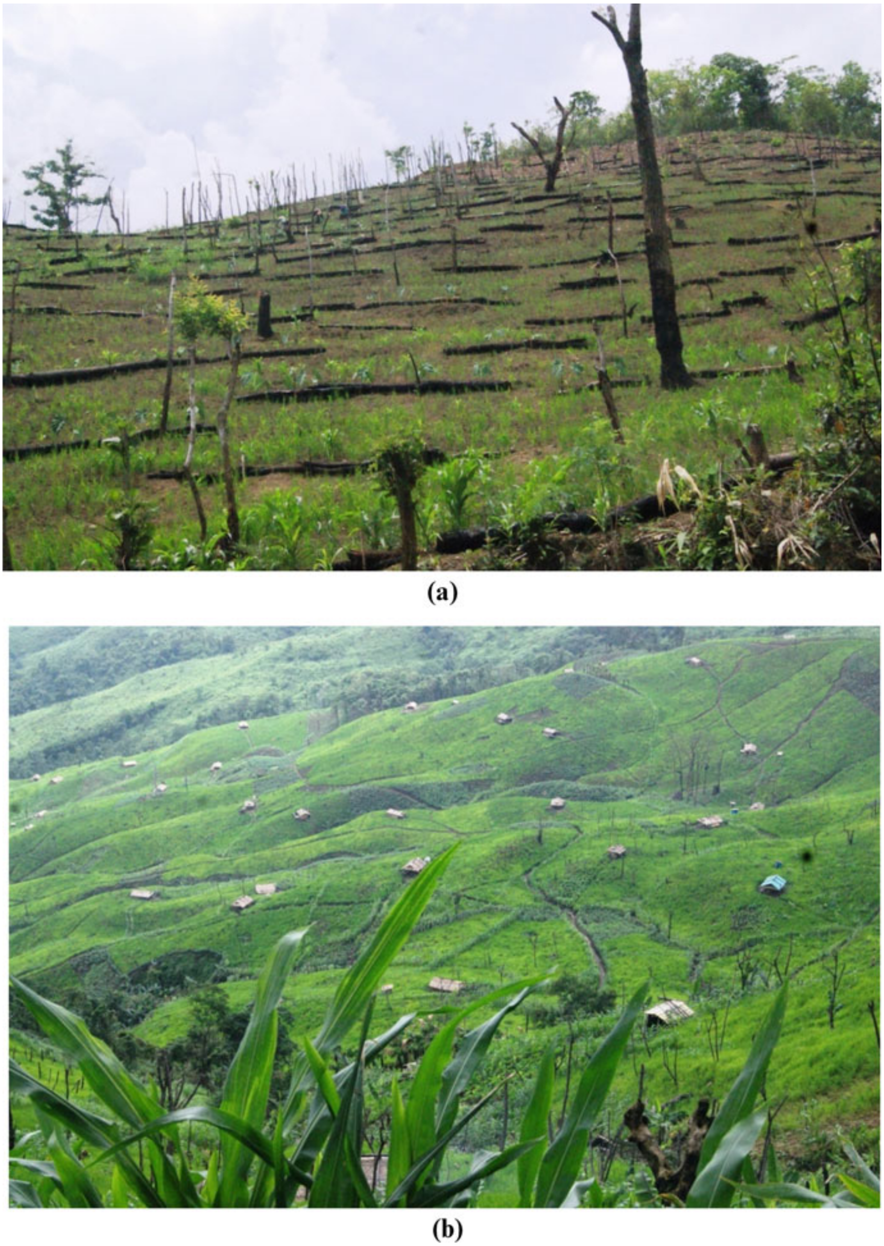

Fig. 5.1 *Jhum* cultivation in Nagaland: (**a**) Wokha (**b**) Mokokchung. (Photographs: Krishna Giri)

Fig. 5.2 *Jhum* cultivation in natural forests and wet terrace cultivation in the valley at Phallong village, Manipur. (Photographs: Krishna Giri)

from February to March. After burning operations, farming communities prepare the land for crop cultivation and dibbling of seeds before the onset of monsoon. Seeds of maize, paddy, pulses, oilseed, and vegetables are dibbled by women while seeds of millets are broadcasted by men workers (NITI Aayog 2018). Crop cultivation in a particular piece of land is carried out for 2–3 years and as the soil fertility declined, the land is abandoned for recovery of vegetation and soil fertility. After 3 years of cropping, the farming communities rotate the land rather than crops. The fallow *jhum* fields are left to regenerate with 15–20 years period which is sufficient to recover vegetation and soil health. During the longer fallow periods of 15–20 years, the land is again recovered through secondary succession and regains the fertility of soils (Fig. 5.3). The secondary climax forests are revisited by *jhumia* communities for crop cultivation and the duration between first cropping to second is known as *jhum* cycle. Increasing population and food grain demand, decreasing land availability due to soil erosion and land degradation, the traditional *jhum* cycle of 15–20 years long has been drastically reduced to 2–5 years. Cultivation of soil exhausting crops and vegetables in a short *jhum* cycle of 2–5 years reduces soil fertility and crop productivity and makes the age-old farming system unproductive and unsustainable (Nath et al. 2015). Therefore, the farming communities have now developed indigenous knowledge-based innovative fallow management practices wherein a variety of soil-enriching legume pulse crops are grown during the fallow period or in mixed cropping system (NITI Aayog 2018). Cultivation of legume pulses as cover crop enrich soil nitrogen through biological nitrogen fixation in association with rhizobium species while the rapid decomposition of leaf litter enrich organic matter and other plant nutrients in the soil (Fig. 5.4). Intensification of

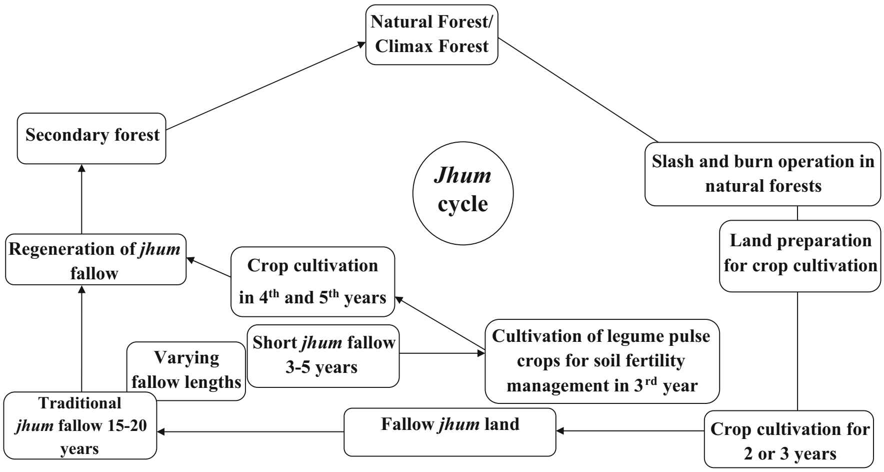

Fig. 5.3 Diagrammatic representation of traditional *jhum* cultivation system

nitrogen-fixing legumes in first-year fallow *jhum,* i.e., third-year after first cropping followed by subsequent cropping in the fourth and fifth year is an innovative management practice in short *jhum* cycle. Management of short *jhum* fallows with traditional and innovative cropping systems are quite prevalent in Nagaland and Meghalaya states.

5.2 Traditional Farming Systems and Agro-biodiversity

Traditional slash and burn agricultural land use system is an age-old cropping system in hilly areas of NEI. Indigenous knowledge and technological intervention-based community-specific farming practices in different states of North East India have gained huge recognition for sustaining livelihood of tribal communities. This chapter attempts to describe the unique farming systems, associated agro-biodiversity and microbiological interventions in *jhum* farming sector of North East India.

5.2.1 Alder-Based *Jhum* Cultivation in Khonoma Village, Nagaland

The Angami community of Khonoma village has developed a unique traditional *jhum* cultivation system by planting *Alnus nepalensis* in their farmland since the origin of this village. *A. nepalensis* also known as Himalayan alder is a non-legume actinorhizal tree that fixes atmospheric nitrogen in association with *Frankia* sp. In this *jhum* system, alder trees grown in the farmland are used to maintain soil fertility by way of leaf litter decomposition, biological nitrogen fixation, and burning of freshly pollarded tree leaves before the cultivation of crops. The land is cropped for 2–3 years and abandoned for 5–7 years. Scientific studies found that the soil fertility

(a)

(b)

(c)

Fig. 5.4 (**a**) Alder-based *jhum* fallow (**b**) Traditional method of land preparation by Angami tribe (**c**) Potato cultivation in pollarded alder *jhum* fields. (Photographs: Krishna Giri) and microbial population is very high in alder-based *jhum* cultivation system which makes it more productive and sustainable than conventional slash and burn *jhum* cultivation. This traditional farming system in Eastern Himalaya has maintained very high agro-biodiversity, soil fertility, and productivity on the one hand and conserved the natural biodiversity on the other. The farming community fulfill their needs of vegetables, fodder, fuelwood, and timber from the alder-based farming system (Fig. 5.4a–c).

However, villagers cultivate paddy in wet terrace fields during Kharif season (Fig. 5.5a, b) followed by beans, garlic, and other vegetables in the winter season. The wet terrace farm of Khonoma village is one of the oldest terrace farming systems in Nagaland. The traditional alder and wet terrace agricultural system of the Angami tribe in Khonoma is highly productive and sustainable to fulfill the food requirements of the villagers. This innovative form of *jhum* and terrace cultivation practice has led to the zero dependency of villagers on surrounding forest resources (Giri et al. 2018). Therefore, the villagers have conserved rich floral and faunal diversity in and around the vicinity of Khonoma village. Due to this unique form of agriculture and lush green forests, the village has been recognized as **"The First Green Village of Asia"** and a popular tourist destination.

5.2.2 Zabo Rice Cultivation System in Kikruma Village, Phek District, Nagaland

Zabo farming system of Chakhesang tribe in Kikruma village, Nagaland, is one of the culturally recognized traditional agriculture practices in NEI. The term Zabo refers to impounding runoff water in the hill slopes which is used for irrigation of paddy fields (Singh et al. 2018). The farming system is an innovation of people developed through indigenous knowledge, wisdom, and skills (Kithan 2014). This farming system in the Eastern Himalayan region of India is an integration of agriculture, forestry, horticulture, fishery, animal husbandry as well as soil and water conservation measures. Water requirement for terrace paddy cultivation is fulfilled through ponds constructed in the hilltops. This traditional farming system is known for rich farm agro-biodiversity where six varieties of paddy are cultivated in *jhum* and terrace Zabo system. Also, maize, Job's tears, beans, oilseeds, vegetables (garlic, chili, onion, brinjal, tomato, potato, cabbage, cucumber, pumpkin, colocasia), and fruits (papaya, banana, plum, peach, lemon, Indian gooseberry, wild apple) are cultivated. Livestock (buffalo, cattle, goat) are reared near the ponds; cattle dung and urine are used for manuring the crops. Fish cultivation is also integrated with water ponds constructed on the farm (Singh et al. 2018). Besides this integrated Zabo farming, Chakhesang tribe practices mixed cropping *jhum* cultivation in hilly terrains. Crop diversity and productivity of Zabo farming is higher than the *jhum* farming system in Kikruma village, which has become possible due to efficient soil, water conservation measure, natural resource management, and utilization practices.

(a)

(b)

Fig. 5.5 (**a**, **b**) Wet terrace paddy cultivation system in Khonoma village, Nagaland (Photographs: Krishna Giri)

5.2.3 Tree-Based Cultivation in Konyak Village, Mon District, Nagaland

Unlike slash and burn, *jhum* cultivation across north eastern states; the Konyak tribe in Mon district of Nagaland maintain trees in the *jhum* fields. In this farming practice, paddy is grown as a major crop along with the management of vast diversity of trees in the *jhum* plots. During vegetation slashing operations in the winter season, trees are retained on contour lines and tender branches are not pruned. Single bole stems are retained to maintain proportionate height and girth while all the unhealthy trees are slashed. Soil fertility of the land is maintained by constructing earthen bunds, cutting the plant leaves, uprooting the weeds followed by decomposition of this plant material. The fresh leaves and uprooted weeds are also used as green manure. Besides paddy, maize, Job's tears, tuber crops, and green leafy vegetables are also grown in the *jhum* fields. They also raise medicinal and aromatic plants along with cereals, pulses, and vegetable crops. Retaining trees in the field is beneficial for soil structure and fertility maintenance. The tree species maintained in *jhum* lands also fulfill the firewood and timber requirements of villagers. Tree-based rice cultivation of the Konyak tribe is among the recognized traditional and sustainable farming system in Eastern Himalaya.

5.2.4 Apatani Fish-Cum-Paddy Cultivation in Ziro Valley, Arunachal Pradesh

Paddy-cum-fish cultivation by the Apatani tribe in Ziro valley, Arunachal Pradesh, is one of the recognized sustainable and integrated agricultural practices, which has been documented as an advanced traditional farming system in NEI. The cultivation system is an example of indigenous knowledge-based technology intervention of wet rice cultivation. The farming system is also known as *Aji* cultivation consists of paddy and fish farming in wet terraces and millets on the bunds of plots (Singh and Gupta 2002). Small pits are prepared in each terrace plots for keeping fish fingerings, and the fields are filled with water during monsoon season. The entire landscape is kept submerged in water, and fishes come out from pits and move around the paddy fields while under less water conditions fishes remain inside the pits for their survival. Traditionally, the paddy fields are classified based on water sources. *Jebi* and *Aane* system indicates the availability of sufficient water supply from natural rain whereas *Ditor* farm is an irrigation source fulfilled cropping system. The fertilization of paddy crop naturally takes place by nutrient washout from the hill slopes. However, manuring of paddy fields provide the nutrition to fishes (Rai 2005). Additionally, recycling crop residues and organic wastes of the villages effectively sustains soil fertility (Jones 1976). The productivity of the Apatani paddy-cum-fish farming system is reported to be 400–500 kg/ha which is 3–4 times of average yield of Arunachal Pradesh (Rai 2005). Studies also revealed that paddy yield under paddy, millet, and fish farming system is higher than the paddy monoculture or paddy and millet in combination with higher input/output ratios in integrated paddy + millet + fish system than two other systems (Kumar and Ramakrishnan 1990).

5.2.5 *Jhum* and Bun (Terrace) Farming in Meghalaya

Traditional *jhum* cultivation in the Khasi hills of Meghalaya is locally known as "Rep Syrti." The farming communities of Khasi hills practice slash and burning *jhum* cultivation in hilly areas for 2–3 years and rotate the land. The agro-biodiversity of Rep Syrti *jhuming* system includes major crops as rice, maize, millets, beans, ginger, chilies, and sesame, along with other locally preferred vegetables (Mohapatra and Chandra 2017). Another prototype of "Rep Syrti" farming system in Khasi hills is *Bun* (terrace) cultivation which is traditional knowledge-based customized farming practice. The term *Bun* in Khasi language is used for the burning of vegetation (Dubey and Sah 2009). The *Bun* farming system in Meghalaya is prevalent in foothills of Himalayan Mountain plantation forests, which involves clearing of the area by removing the grass, shrubs, fallen pine leaves, twigs, branches, and formation of heaps over raised beds. These heaps are locally termed as Bun. The *Buns* are made at regular intervals with 2–7 m length, 1.0–1.25 m width, and 0.20–0.25 m height (Fig. 5.6a, b). The *Buns* are then allowed to dry and are covered with a soil layer and burnt under anaerobic conditions. Once the burning is over, the *Buns* are allowed to cool and crop cultivation is initiated in these fields.

Bun farming system has a wide range of variation and crop diversity, wherein the first crop year ginger is grown in the fields which have very high commercial importance followed by upland rice, maize, and vegetables in the second crop year. Potatoes, beans, ladies finger, tomato, etc. are the most preferred vegetables in *Bun* farming system. Similar to the slash and burn *jhum* cultivation system in other parts of NEI, the Bun fields are also left fallow for rejuvenation of vegetation and soil fertility after cropping for 2–3 years (Majumdar et al. 2002).

5.2.6 Rice-Based Farming System in Tripura

Rice cultivation system in Tripura is centuries-old farming practice which is found in various landforms *viz.* upland rice (in *tillas*), medium upland rice (in *jhum* lands), and lowland rice. Through their wisdom and tradition, tribal communities have developed an integrated farming system, which is popularly known as rice-based farming (RBF) system. Rice, fish, livestock (cow, buffalo, and goat), poultry, fruits, vegetables, and plantation crops are the major components of rice-based integrated farming system in the state (Das et al. 2015).

Due to small landholders having (<1 ha) land, 5–10% of the available land area is developed into water harvesting systems, wherein ponds with 500 m^2 area and 1.25–2 m depth are easily seen in the farmer's field. These water bodies have served as life-saving systems and used for irrigation of land cultivated with fruits and vegetables grown in the peripheries of rice fields. Fish culture in these ponds is one of the activities of the rice-based integrated farming system developed by the farmers with reported productivity of 0.75–1.2 t/ha (Das et al. 2015). In addition to this, farmers also rear cows, goats, and ducks in an around the rice fields, and the

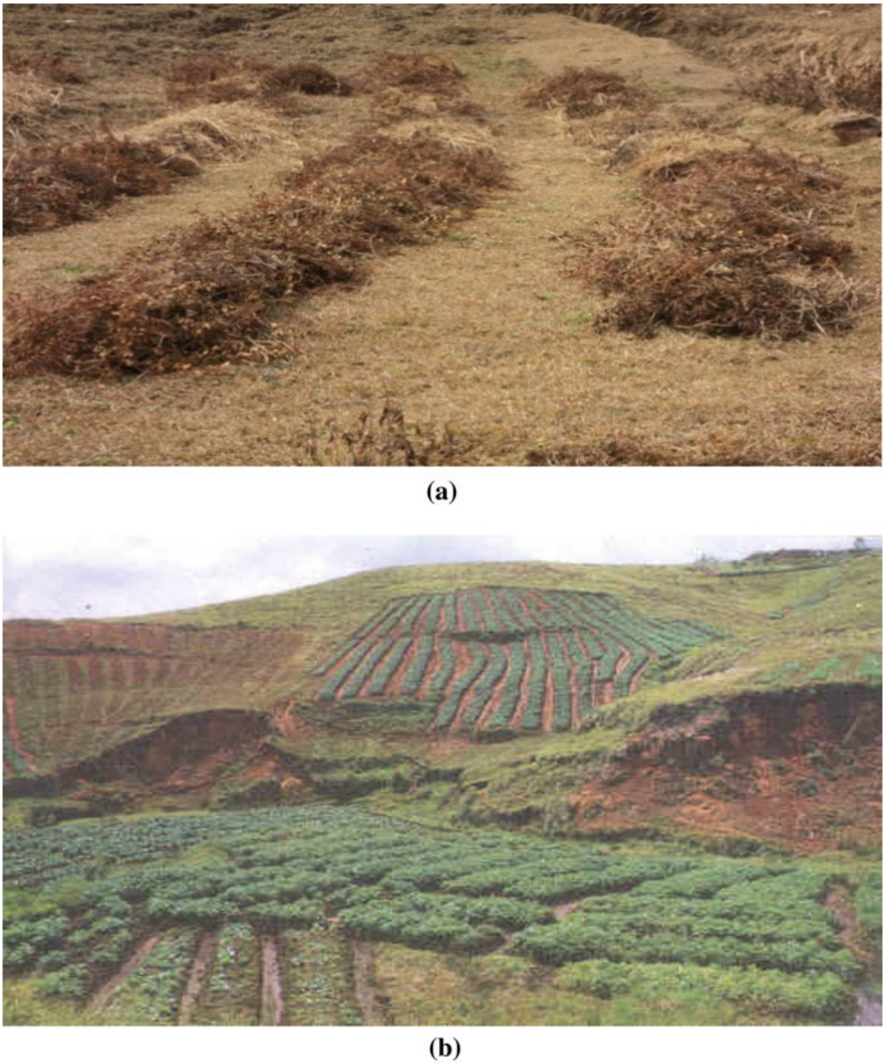

Fig. 5.6 (**a**) Heaps of biomass over raised beds (**b**) Crop cultivation in *Bun* system. (Photographs: Savita Basavaraj)

animal excreta are used as organic manure to fertilize rice fields (Fig. 5.7). Rearing livestock with rice-based farming system strengthen the economic status of these farming communities on the one hand and make the system more productive and sustainable land use on the other. Colocasia, okra, amaranthus, brinjal, and cucurbits are commonly grown vegetables during the rainy season while potato, sweet gourd, banana, leafy vegetables, etc. are grown during the winter season. The fruit trees in the RBF system mainly include banana clumps, guava, coconuts, and few areca nut plants (Fig. 5.8). Although RBF system is time tested, traditionally developed,

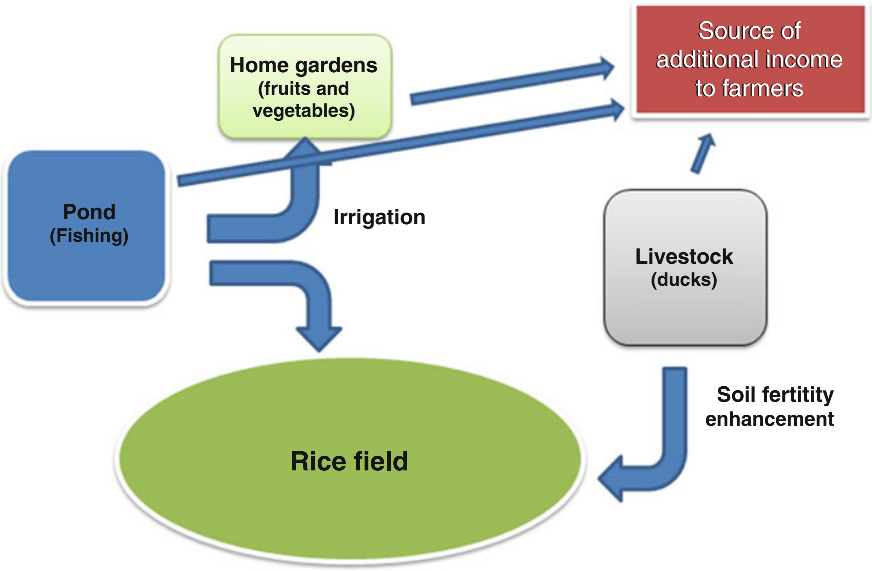

Fig. 5.7 Conceptualized framework of rice-based farming system in Tripura and its multifarious benefits

innovative, productive, and economically viable farming practice in Tripura, however, further enhancement in agricultural technologies and more productive varieties/breeds are imperative to meet the food security of the increasing population in the region (Yadav et al. 2013).

5.2.7 Organic Agriculture and Terrace Paddy Cultivation in Sikkim

Sikkim is the smallest State of NEI with rugged mountainous topography, diverse forests, agroecosystems, traditional farming cultures, and agro-climatic conditions. Similar to the other Eastern Himalayan region, local indigenous communities of Sikkim have developed some innovative strategies to ensure food security and livelihood. The traditional farming system of Sikkim has been represented by four different layers of vegetation, i.e., top layer (multipurpose agroforestry trees), middle layer (fruit species), the lower layer (vegetables), and ground layer (tuber crops) which are totally organic in nature (Sharma et al. 2016). Organic farming is the very old traditional agriculture system in Sikkim. The unavailability of sufficient irrigation sources in the state has led to the adoption of integrated rainfed farming consisting of agriculture, horticulture, and animal husbandry. The rich biodiversity in Sikkim contributes abundant organic matter in the soil and make it more suitable for organic farming. Sikkim has been declared as the first organic state in the country on 19th January, 2016 by the Hon’ble Prime Minister of India and recognized as a

Fig. 5.8 Rice-based integrated farming system **a**, **b** in Tripura. (Photograph: Gaurav Mishra)

harbinger of organic farming, not only in India but around the world (https://currentaffairs.gktoday.in/tags/organic-state).

Maize, paddy, and buckwheat are the major staple crops in the rainfed organic farming system of Sikkim, while urd and rice bean as pulses, soybean, and mustard as oilseed crops. Orange and pears are the major horticultural crops while ginger, cardamom, turmeric, and pepper cultivation as spices. Peas, bean, tomato, and potato are vegetable crops in the state. Cultivation of flowers like cymbidium, rose, gerbera, and anthurium has become a good source of income, and a large number of farmers have adopted floriculture as a commercial venture in the state (State Policy on Organic Farming Government of Sikkim 2004). Terrace rice cultivation in the valleys is a well-recognized traditional farming practice in Sikkim, which is locally known as "thang." Thangs are the flat land riverbanks along with typically terraced slopes in the lower hills contribute to the traditional rice cultivation (Sharma et al. 2009). *Krishna bhog, nuniya, and kataka* are the traditionally grown rice varieties in the terrace farming system of Sikkim which are prominent for their aroma, medicinal importance, and fine grains (Sharma and Liang 2006). Besides rice cultivation, pulses and beans are also grown along the raised bunds of the terrace. However, after harvesting the crops, these terraces are used to grow maize, wheat, buckwheat, oilseeds, and vegetables (Sharma et al. 2016). The terrace system in Sikkim maintains a higher level of agro-biodiversity which helps to sustain the livelihood of people in this region.

5.3 Microbial Interventions in *Jhum* Farming Sector of North East India

Soil harbors a multitude of microorganisms which are beneficial for plant growth and soil health maintenance (Johri et al. 2003; Tilak et al. 2005). Burning of biomass in *jhum* lands negatively affect the soil micro-floral population, decrease soil health and productivity. Though the burning operation is suitable for suppressing obnoxious weeds and pathogenic microorganisms in the soil, at the same time soil beneficial microorganisms also get affected by this operation. Plant growth-promoting bacteria (PGPB) are either free-living or plant symbionts and endophytes that can colonize with plant roots (Mahanty et al. 2017). The current trend in agriculture is focused on reducing the use of pesticides and chemical fertilizers on the one hand and accelerating the search for alternative ways to sustainable agriculture on the other (Smit et al. 2001; Trivedi et al. 2013). The importance of PGPR inoculants has been recognized as a promising alternative to synthetic fertilizers and pesticides in the agriculture sector. Plant growth promotion by rhizobacteria occurs through direct or indirect mechanisms (Persello-Cartieaux et al. 2003). Atmospheric nitrogen fixation, phosphate and potash solubilization, siderophore production for iron capturing and synthesis of growth hormones that enhance plant growth at various stages are the direct effects of PGPR strains (Kloepper et al. 1989; Lynch 1990). However, biocontrol of pathogenic bacteria, fungi, and alleviation of abiotic stress are the indirect actions exerted by PGPR strains. Biocontrol of pathogens happens by the production of antagonistic substances (O'Sullivan and O'Gara 1992) or through the induction of resistance against pathogens (Leeman et al. 1996; Trivedi et al. 2013). Free-living nitrogen-fixing bacteria such as *Azospirillum*, *Acetobacter*, *Azotobacter*, *Rhodococcus*, *Burkholderia*, *Beijerinckia*, *Gluconacetobacter*, *Kosakonia*, phosphate solubilizing and growth regulator producing *Bacillus*, *Arthrobacter*, *Enterobacter*, *Flavobacterium*, *Serratia, Pseudomonas*, symbiotic *Rhizobium*, and *Frankia* are the promising PGPR strains which have been widely studied and found effective in soil fertility and productivity-boosting. Application of these putative PGPR/biofertilizers in *jhum* agroecosystem can be an effective and ecofriendly tool for sustainable land resource management. These organisms upon application will enrich the surface micro-floral population on the one hand and enhanced fertility and crop growth on the other. Chemical fertilizers are exhausted once utilized by the plants, while biofertilizers continuously grow and multiply in the soil. Thus, PGPR strains are the renewable sources of soil fertilization in *jhum* fields.

Impact of *jhum* cultivation on soil microbial diversity was studied in a degraded, moderately degraded and undegraded natural forest in Banderdewa Forest Reserve, Papum Pare district of Arunachal Pradesh, using denaturing gradient gel electrophoresis (DGGE) and polymerase chain reaction (PCR). The DGGE analysis of PCR amplified 16S rDNA fingerprints demonstrated higher bacterial diversity in surface and subsurface soil in undegraded site than the degraded sites. The reduced bacterial diversity in disturbed sites was correlated with the *jhuming* practices in the past. Cluster analysis of the DGGE bands of 16S rDNA fragments revealed significant variation in bacterial genomic structure in the degraded and undegraded site as well as depth where surface soil and undegraded sites were found to be rich in bacterial communities and vice versa (Singh et al. 2011).

Soil microorganisms play key role in soil health and fertility improvement by their multiple plant growth-promoting traits. With this hypothesis Banerjee et al. (2017) carried out a study on plant growth-promoting abilities of microbial strains native to *jhum* cultivation system for promoting the growth of upland paddy. Total 87 (55 bacterial and 32 fungi) were isolated from *jhum* cultivation sites in NEI, and 10 of each bacteria and fungi were screened for plant growth-promoting properties, viz. phosphate solubilization, indole, siderophore, and ammonia production, as well as catalase activity. Based on the preliminary seed germination tests, the isolates were further tested in pots and greenhouse conditions. The effect of tested microbes on plant growth was found to enhance shoot length, root length, and plant height after 4 weeks of showing. The tested bacterial strains SB3, SB5, and SB9 were identified as *Curtobacterium oceanosedimentum, Bacillus methylotrophicus*, and *Bacillus cereus,* respectively. While the fungal strains SF1, SF3, and SF4 were found to be *Penicillium virgatum, Metarhizium pinghaense*, and *Penicillium stratisporum*, respectively. Considering the potential of PGPR in sustaining *jhum* cultivation, Rain Forest Research Institute, Jorhat, Assam has also initiated the application of native PGPR isolates in *jhum* fields of Mokokchung and Kohima districts of Nagaland (Fig. 5.9). As evident from literature, application of plant growth-promoting rhizobacteria (PGPR) as biofertilizers in *jhum* fields of NER is still scanty. Therefore, use of native PGPR for soil fertility and crop productivity

Fig. 5.9 Application of PGPR consortium in *jhum* land in Mokokchung, Nagaland

enhancement of *jhum* cultivation system with a focus on plant–microbe interactions is need of the hour (Banerjee et al. 2017). Application of putative PGPR strains in *jhum* farming sector will be helpful for soil health and crop productivity improvement, restoration of degraded *jhum* fields as well as in achieving agricultural sustainability and social prosperity in NEI.

5.4 Conclusion and Opinion

Indigenous farming systems in North East India have evolved through the co-evolution of local, socio-cultural, and ecological systems. These farming practices exhibit a high level of ecological rational blended with indigenous knowledge of natural resource and agro-biodiversity management. Shifting cultivation in mountainous terrains and wet terrace farming in the valleys is a predominant land use system to fulfill the food grain requirements of rural communities in NEI. The agriculturally diverse *jhum* farming systems of culturally diverse ethnic groups in the past were sustainable, productive, and sufficient to address the food requirements and livelihood sustenance of far rural communities in this region. However, increasing population, continuous land degradation, short *jhum* cycles, and climate change have made these diverse agriculture systems less productive, vulnerable to climate change, unsustainable, and insufficient to meet the food grain requirements of the rural communities in spite of having high agro-biodiversity. Though with the advent of scientific advancement in the agriculture sector, a variety of new models have been developed and implemented in various parts of this region for sustaining the livelihood requirements of these communities. However, the success of new models and practices largely depends on the cultivation of suitable crop varieties and acceptance by farming communities. Implementation of modern agricultural technologies along with traditional knowledge-based natural resource management practices can sustain food security in *jhum* farming system under shortening of *jhum* cycle and changing climate. Development and testing of diverse agroforestry models, tree-based farming, integrated agricultural practices, and application of native plant growth-promoting rhizobacteria/biofertilizers in *jhum* fields have tremendous potential to address the food security of farming communities. Because in many parts of North East India, in spite of having plentiful natural resources, people have not been able to harness them in a sustainable manner. The unique and tribe-specific traditional farming practices described in this chapter are quite successful and productive in their respective areas, but there are rare examples of their replication in other similar agro-climatic conditions of NEI. Therefore, it is opined that combination of scientific interventions and novel technologies along with these indigenous knowledge-based productive agricultural practices can be replicated in suitable agro-climatic zones to transform conventional farming systems into more sustainable, economic, and ecologically resilient farming system.

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Indigenous Agricultural Practices: A Supreme Key to Maintaining Biodiversity

6

Ishwar Prakash Sharma, Chandra Kanta, Tripuresh Dwivedi, and Ranjana Rani

Abstract

Indigenous agricultural practices adopted by locals largely depend on traditional knowledge, common in agricultural system to preserve ecosystem, biodiversity, and useful in maintaining sustainable food and human health. Farmers possess a vast pool of indigenous knowledge in livestock management which reduces external input dependency by utilizing various renewable farm resources as agricultural practices. These practices address the rural development and nature conservation; contribute to maintaining ecosystem of particular area which leads to sustainable use of biodiversity conservation. This knowledge is not systematically recorded; therefore, it is not easily accessible to agricultural researchers; hence, these are valuable practices that provide farmer-to-farmer training or local technology transfer. Different governmental as well as non-governmental policies are designed to maintain biodiversity; hence, indigenous agriculture is one of the most important practices followed for biodiversity conservation. To change the people's mind towards indigenous agriculture through spiritual education is one of the most important factors which could be focused on important ethics; hence,

I. P. Sharma (✉)
Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Patanjali Herbal Research Department, Patanjali Research Institute, Haridwar, Uttarakhand, India

C. Kanta
Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Department of Botany, Doon (PG.) College of Agriculture, Sciences and Technology, Dehradun, Uttarakhand, India

T. Dwivedi · R. Rani
Patanjali Herbal Research Department, Patanjali Research Institute, Haridwar, Uttarakhand, India

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it can preserve both indigenous knowledge and biodiversity for achieving sustainable development.

Keywords
Biodiversity · Indigenous · Practices · Sustainable · Traditional

6.1 Introduction

Indigenous knowledge is a spiritual relationship of natural environment and natural resources utilization which is differs from scientific systems now that need to attach scientifically with scientific knowledges. People with indigenous and traditional knowledge have a valuable association with nature, and they have good understanding about it. They significantly contribute to nature maintenance through use of sustainable natural resources (Beltran 2000). In sustainable agricultural development, this knowledge associates with farmers to natural, physical, and socioeconomic environment from any agro-ecosystem (Bonny and Vijayaragavan 2001). This traditional-based agriculture is followed in every locality in the world. Worldwide approximately 370 million indigenous peoples occupy nearly 22% of the land area; hence, 80% of the world's total biodiversity has been covered (Perroni 2017). People from every locality have their own strategies to select the seeds and seed stocks (Sibanda 1998; Kieft 2001). Nowadays, large number of new varieties have been introduced many of them not fit to indigenous system so that the people from these indigenous systems develop their own system for agriculture in this bases the indigenous varieties conserve to this particular area and fulfill the food need. These indigenous agricultural practices also affect the cost of production. On the other hand, the introduction of these indigenous varieties affects the native biodiversity. It is generally a measure of variation at the genetic, species, and ecosystem level. Biodiversity deals with the close relationship between indigenous flora and fauna including the abiotic factors in particular area (Kimmerer 2002). To fulfilling the human need, biodiversity is very important because it has the conserved genetic pool; so that it has the fundamental values to humans due to availability of our nutritional, cultural, economic, etc. However, nowadays biodiversity is under serious degradation due to rapid and accelerating anthropogenic activities. The indigenous people managed themselves since centuries in different adverse conditions by creation of sustainable livelihood systems, and various forms of knowledge deeply associated with the cultural cohesion allow the communities to maintain sustainable use of natural resources (Sultana et al. 2018). According to Warren (1991), the indigenous knowledge is a knowledge used by local people to make a livelihood in a particular local environment. Indigenous knowledge includes local knowledge, indigenous traditional knowledge, indigenous technical knowledge, traditional environmental knowledge, rural knowledge, traditional ecological knowledge, etc. These values indicate a wide scope of biodiversity values for the people. Indigenous knowledge has a great deal with the agricultural productivity and sustainability. In the indigenous practices, area utilization of synthetic chemicals is zero; hence, agriculture is purely organic as the soil is not polluted, and ultimately the environment is clean with abundant biodiversity.

6.2 Indigenous Knowledge

Indigenous knowledge is traditional or local knowledge which is unique to a community or society or system. It has been developed outside the local system by the researchers, scientists, philosophers, etc. This knowledge generally is going to be extinct which is currently facing endangered conditions due to forgetting or non-exploring situations. According to Louise Grenier, indigenous knowledge is "the unique, traditional, local knowledge existing within and developed around the specific conditions of women and men indigenous to a particular geographic area" (Grenier 1998). This knowledge is closely related to survival and subsistence and helpful in making decisions in food security, health, education, resource management, and other community-based activities. The result of a continuous process of experimentation, innovation, and adaptation is dynamic. It has the capacity to blend with knowledge based on science and technology and should therefore be considered complementary to scientific and technological efforts to solve problems in social and economic development. It is typical to capture and stored in a systematic way because it goes orally from one generation to another; hence, this one is the main disadvantage. Traditional knowledge is characterized in various ways as shown in Fig. 6.1. The main characteristics of this knowledge: generated within communities, location and culture specific, decision-making and survival strategies, not systematically documented, concerns critical issues of human and animal life, dynamic and based on innovation, adaptation, and experimentation, oral and rural in nature.

Fig. 6.1 Characteristics of traditional knowledge

6.3 The Role of Indigenous Knowledge

Recently, the interest in this field has been increased dramatically which is an effective approach to sustainable development. This interest is generally generated within communities for recording and preserving their culture or tradition which are being now the part of institutions in which they are used as invaluable natural resources. Nowadays, the people are excluded from the indigenous systems due to modernization or retrogression which is generally called top-down development. In agricultural sector, the yield is maximizing through introduction of new crops in green revolution, but these crops need to optimum availability of fertilizers and water. Hence, on the point of production the green revolution is a success, but its potential totally depends on valuable resources. Indigenous system is very helpful to promote and maintain biodiversity which can help to promote sustainable future through development of caring values (Sultana et al. 2018). Indigenous communities always live with the environment so that they have the capacity to regenerate the ecosystem and ultimately manage the biodiversity. On the other hand, it can help in education by providing necessary facilities to the local area. The natives from these areas are being associated directly with the environment by knowing about soil, water, climate, biota, etc. This innate knowledge can be easily used in education, and it is very useful in nature conservation which has come from the village. Indigenous system is also useful in food production because in many areas new varieties have been introduced to increase production, but these varieties do not fit into these areas. On the other hand, the indigenous varieties do not require fertilizers, insecticides (because of resistant), and many other agricultural requirements (De Walt 1994; Ty and Cuc 1998).

In forestry development, the indigenous knowledge systems have been used since 30 years to regreen the disturbed hillsides through planting exotic trees because of their resistance to the native environment. So this system is very useful for maintaining ecology of that particular area. One most important role of indigenous knowledge is in medicine use by local people. The knowledge of medicine not only protects people from health threats but also helps in hunger eradication and poverty reduction. In sustainable practices, this knowledge is useful for ecotourism because it has its own vision of development which differs from modern systems (Cunningham 2010). Local people live in most vulnerable ecosystems so that this system is very helpful in knowledge of climate change. In Asia, the climate change threats like typhoons, monsoons, flooding, sea level rising, and salinization of freshwater, etc. can be assessed easily by the indigenous people as they have suffered from consequences of natural phenomena (Conway 2009; Nyong et al. 2007). Similarly, the indigenous people from America and Caribbean are aware of the melting of glaciers, hurricanes, rising of sea level, rainfall patterns, etc. (Kronik and Verner 2010).

6.4 Indigenous Agricultural Practices in India

India is rich in its biodiversity with much diversified agricultural systems. There are some common practices like agroforestry, crop rotations, mixed/inter-cropping, polyculture, and water harvesting performed by the people. In all the states of the country, there is variation in agriculture; the native people have their own agricultural practices like seed processing, seed storing, field preparation, etc. State wise some agricultural practices are mentioned in Table 6.1, and details of some states are as follows.

6.4.1 Meghalaya

Meghalaya is a northeast state located between 25.47–26.10N latitude and 89.45–92.47E longitude. It has rich cultural heritage and luxurious vegetation. Meghalaya also covers the international boundary with Bangladesh. The main tribes from this state are Khasi, Jaintia, and Garo who have their own system of agricultural practices. The Khasi and Jaintia tribe are races of Mongolia while Garo tribe belongs to Tibeto-Burman race. Their cultural practices remain distinctive due to their geography (Jeeva et al. 2006). Mainly shifting and bun cultivation practices are followed by the natives of this state. Shifting cultivation is the most prevalent form of agricultural practices in this state which is one of the most ancient systems of farming started in the Neolithic period around 7000 BC. This system has primitive cultivation techniques with a transition between food gathering and hunting to food production. Generally, this system is predominant in the whole northeast Himalayan region. In this system, it is essential to clear the land, and the agricultural crops are grown for a limited period of one to several years and after that the cultivation moves to a new site. On the other hand, bun or terrace cultivation is a settled cultivation system in hill slopes and valleys since last three decades to provide better production system, conserve soil moisture, prevent land degradation, and soil erosion. In this system, bench terraces are constructed on hill slopes. The space between two buns is leveled using cut and hill method; the vertical interval between the terraces is not usually more than 1 m. This method is helpful preventing soil erosion and retaining maximum rainwater.

Irrigation is one of the most important factors of agricultural production and so various irrigation practices are done by the people of the state. It follows better utilization of all production factors that ultimately increase the crop yield per unit of land. This practice generally provides suitable moisture for crops or lands. It is started at the beginning of rainfall and is helpful to manage cropping pattern in the soil. The most useful practices are bamboo drip irrigation and bench terrace irrigation. Both the practices are predominant throughout the Northeast region of India. Bamboo drip irrigation is an excellent example of evolution of indigenous agriculture systems (Singh 1989). It is widely adopted in Jaintia Hills where hill slopes are steep with low soil depth by using locally available bamboo species. In bench terrace irrigation practice, the hill stream is tapped as it emerges from the forest and finally

Table 6.1 State wise indigenous agricultural practices in India

S. no.	Indian states	Tribes	Indigenous agricultural systems	References
1	Andhra Pradesh	Korra, Killo, Swabi, Ontalu, Kimud, Pangi, Paralek, Mandelek, Bidaka, Somelunger, Surrek, Goolorigune olijukula	Dryland agricultural, Farming system	Padal et al. (2013); Ramprasad (2011)
2	Arunachal Pradesh	Abor, Ahom, Aka, Apatani, Dafla, Tagin, Galo, Khampti, Bugun, Mishmi, Momba, Sherdukpen, Singhpo	Panikheti system, Apatani system, Zabo farming, Alder based farming, Bun farming, Bamboo drip irrigation	Das et al. (2012)
3	Assam	Boro, Dimasa, Chutia, Sonowal, Mech, Tiwa, Garo, Rabha, Sarania, Hajong, Tripuri, Deori, Thengal, Hojai	Shifting cultivation, Double transplanting	Rautaray (2002)
4	Bihar	Gond, Baiga, Pradhan	Smaller plots, Mulching, Making small bunds/rock/ wooden poles, Ploughing	Singh and Sureja (2008)
5	Chhattisgarh	Gond, Abuj maria, Bison horn maria, Muria, Halba, Dhurvaa, Kol, Korba, Kawar, Binjwar	Kharri Method, Lahee Method, Mixed Cropping, Utera Cropping	Singh (2015)
6	Goa	Dhodia, Dubla, Naikda, Siddi, Varli, Kunbi, Gowda,Velip	Kumeri (shifting cultivation), Felling, fixing the dead vegetation, planting or sowing seeds without the plough, weeding	Essays (2018)
7	Gujarat	Bavacha, Bamcha, Rabari, Bhil, Garasia, Dholi, Dungri, Mewasi, Rawal, Tadvi, Bhagalia, Bhilala, Pawra,Valvi, Vasava, Vasave	Intercropping, Agri-Horti systems, Pasture grasses, Silvipastoral system	Dayal et al. (2009)
8	Haryana	Jat, Ahir, Ror, Gujjar, Badgujar, Bachas, Tunwars, Gurs	Tillage, Integrated Weed, Pest and disease Management practices	Sabesh (2006–2007)

(continued)

Table 6.1 (continued)

S. no.	Indian states	Tribes	Indigenous agricultural systems	References
9	Himachal Pradesh	Bhot, Bodh, Gaddi Gujjar, Jad, Lamba, Khampa, Kanaura, Kinnara, Lahaula, Pangwala, Swangla, Beta, Beda, Domba, Gara, Zoba	Mulching, Crop rotation and double cropping, Crop threshing employing animals, Construction of kuhls (water channels), Indigenous drip irrigation, Bamboo drip irrigation, Small ponds, Roof water harvesting, Use of ash, Green manuring	Rawat and Kharwal (2016)
10	Jammu and Kashmir	Gujjar, Bakarwal, Gaddi, Sippi, Balti, Beda, Bot, Boto, Brokpa, Drokpa, Dard, Shin, Changpa, Garra, Mon, Purigpa	Mulching, Roofing, double cropping	Slathia and Paul (2012); Kaloo and Choure (2015)
11	Jharkhand	Munda, Santhal, Oraon, Kharia, Gond, Kol, Kanwar, Savar, Asur, Baiga, Banjara, Bathudi, Bedia, Binjhia, Birhor, Birjia, Chero, Chick, Baraik, Gorait, Ho, Karmali, Kharwar, Khond, Kisan, Kora, Korwa, Lohra, Mahli, Paharia, Parhaiya, Bhumij	Organic manures/ composting, Herbicides, pesticides and other inputs, Earthen bunding, Stone bunding, Spur structure, Brushwood waste weirs, In-situ moisture conservation, Mulching	Dey and Sarkar (2011)
12	Karnataka	Adiyan, Barda, Bamcha, Bhil, Chenchus, Dublas, Gamit, Gond, Hasalaru, Irular, Konda Kapu, Kattunayakan, Kudiya, Kuruman, Malaikudi, Malasar, Malayekandi, Nayaka, Palegar, Beda, Valmiki, Ramoshi, Yerava, Soliga	Kanaja (ground seed storage), Nellu (hanging rope), Seed drilling, Transplanting, Organic farming, multicropping	Shambulingappa, and Mansur (2018); Medegowda and Rao (2013); Shankar (2010)
13	Kerala	Koragar, Maradi, Paniyar, Kurichyar, Kattunaikkar, Mullukkurumar, Adiyar, Kanduvadiyar, Thachanadar, Kanaladi, Irular, Kurumbar,	Slash and Burn Cultivation, Kumeri Cultivation, disease management by using paste of kayakkam (a river bush) and	Velappan (1994); Suresh (2010); Dudhe and Shinde (2002); Luiz (1962)

(continued)

Table 6.1 (continued)

S. no.	Indian states	Tribes	Indigenous agricultural systems	References
		Mudugar, Cholanaikkar, Aranadan, Kadar, Alar, Paniyar, Kadar, Malasar, Malamalasar, Malampadaram, Malappulayan, Urali, Muthuvan, Mannan, Kanikkar, Malandar	avanakenna (castor oil)	
14	Madhya Pradesh	Bharia, Gond, Bhil, Boneya, Damariya, Kawar, Kolam, Majhi, Saharia	Using bone powder, Mixing of Urea and neem, Trash mulching, Use of bukkar, Mixing of seed with neem cake and Ash, Use of dora, Use of indigenous wooden plough	Vinaygam et al. (2006)
15	Maharashtra	Kamadi, Pawar, Warli, Kokna, Dhorkoli	Organic farming, Contour, Bed Planting, Straw Thatching (Mulching), Crop rotation, Precision farming	Bose (2017); Pandit (2001); Shekara et al. (2016)
16	Manipur	Kukis, Nagas	Shifting Cultivation, Bun cultivation, Rice-fish system, Trans-planting of crops	Singh and Bera (2017); Seitinthang (2012)
17	Meghalaya	Khasis, Jaintias, Garos	Shifting cultivation, Terrace or Bun cultivation, Bamboo drip irrigation, Bench terrace irrigation, Alder based farming practice, Aquilaria based farming, Bamboo based farming practice, Bamboo—arecanut-betel based farming, Homestead farming, Khasi pine based farming, Tea based farming	Jeeva et al. (2006)

(continued)

Table 6.1 (continued)

S. no.	Indian states	Tribes	Indigenous agricultural systems	References
18	Mizoram	Dulien, Ralte, Poi, Jahao, Pankhup, Lakher, Paite, Falam, Tangur, Khuangli, Dalang, Sukte, Fanai, Leillul, Mar	Shifting Cultivation, Bun system, Rice-fish system, ZABO system	Dabral (2002)
19	Nagaland	Angami, Chakhesang, Chang, Yimchunger, Konyak	Shifting Cultivation, Terrace cultivation, Alder based cultivation	Kehie et al. (2017)
20	Orissa	Bagata, Baiga, Banjara Banjari, Bathudi, Bhottada, Dhotada, Bhuiya, Bhuyan, Bhumia, Bhumij, Bhunjia, Binjhal, Binjhia, Birhor, Bondo Poraja, Didayi, Gadaba, Gandia, Ghara, Gond, Ho, Holva, Jatapu, Juang, Kandha, Kawar, Kharwar, Khond, Kandha, Kisan, Kol, Lohar	Multi Cropping, Shifting cultivation, Mulching	Singh et al. (2016)
21	Punjab	Sikh, Jats, Khatri, Arora, Saini, Kamboj, Sansi	Shifting, forage-based rotation, soil organic matter utilization, aggregate stability practices, tillage practices	Chaudhry (2011)
22	Rajasthan	Bhils, Minas, Damor, Dhanka, Garasia, Kathodi, Kokna, Koli, Nayaka, Patelia, Seharia	Bidd Cultivation, Khadin Farming System	Sarkar et al. (2015)
23	Sikkim	Bhutia, Lepcha, Limboo	Shifting cultivation, Dhankheti, Sukhabari, Mandarin-Intercrops	Subba (2009); Subba (2008)
24	Tamil Nadu	Adiyan, Aranadan, Eravallan, Irular, Kadar, Kammara, Kanikaran, Kaniyan, Kattunayakn, Kochu velan, Kondakapus, Kondareddis, Koraga, Kota, Kudiya, Kurichchan, Kurumbus, Kurumans, Maha	Crop residue mulching, Summer ploughing followed by leaf litter mulching, Crop rotation and relay cropping, Growing trees on field bunds	Immanuel et al. (2010)

(continued)

Table 6.1 (continued)

S. no.	Indian states	Tribes	Indigenous agricultural systems	References
		malsar, Malai Aryan, Malai Pandaram, Malai vedan		
25	Telangana	Lambada, Koya, Gond, Yerukala, Pradhan	Field bunds, field drains, Perennial grasses on field boundary bunds, Deep ploughing, Furrowing	Rajasekaran (1998)
26	Tripura	Bhutia, Chaimal, Chakma, Garo, Halam, Jamatia, Khashia, Kuki, Lepcha, Lushai, Mog, Munda, Noatia, Orang, Reang, Santal, Tripuri, Uchui	Fish-cum-vegetable culture, Paddy-cum-fish culture, Fish-cum-duckery culture	Saha and Nath (2013)
27	Uttarakhand	Tharus, Jaunsaris, Buxas, Bhotia, Ban Rajis	Spreading of farmyard manure (FYM), Use the mixture of ash and manure, Ash broadcasting, Crop rotation and fallow land, Grazing of farm animals in fallow land	Joshi and Singh (2006); Subrahmanyeswari and Chander (2013)
28	Uttar Pradesh	Bhotia, Buksa, Jaunsari, Raji, Tharu	Scarecrow, Cattle penning, Smoke ripening, Straw mulching, Ash broadcasting, Grain paraheating, Ploughing, Farmyard manuring, Bidahani, Sanda (Double transplanting)	Morya et al. (2016); Gupta (1990)
29	West Bengal	Santal, Oraon, Munda, Bhumij, Kora, Lodha, Mahali, Bhutia, Tamang, Subba, Bedia, Sabar	Indigenous grain storage system, use snail exoskeleton as rodent and repellent	Roy et al. (2018)

channeled to the terraces. In this system, water continuously flows from upper terrace to lower terrace and this makes non-fertile land fertile. The maintaining materials like stones, bags, etc. help to prevent soil erosion.

Tree-based farming practice is one of the most traditional practices followed by the indigenous people of Meghalaya. In this practice, trees are integrated extensively in the crop production practice according to agro-climatic conditions. For this practice the indigenous plants along with cultivated crops are grown together in which the tree species are used for food, fiber, medicine, etc. (Sarma et al. 1997; Jeeva et al. 2006). The field and soil can be improved by this practice. The tree species mainly used in the traditional practices is Alder which is grown together with ginger, maize, potato, sweet potato, and turmeric; Aquilaria grown with areca nut, bamboo, banana, black pepper, and canes; areca nut and coconut cultivated with black pepper, ginger, maize, and turmeric; bamboo species used as bamboo-based farming along with areca nut and betel; Khasi pine grown with ginger, turmeric, paddy, and vegetables.

6.4.2 Uttar Pradesh

Uttar Pradesh is geographically the biggest state in India which has huge agricultural production. The people from the state use a variety of methods to protect crops and improve the yield. Using scarecrow is a very common traditional practice all over the world and a very effective vertebrate pest management. In Uttar Pradesh, the farmers used their old clothes which are filled with grain sacks and straw; painted and a plastic flag attached at the top, during winding the flag and cloth swim in the flow of wind and they fight with vertebrate pests as birds, blue bulls, wild boars, monkeys etc. another practice by the people is cattle penning which is common throughout the world and very successful method for soil nutrient management. In this method, the cattle is kept overnight in the cultivated land and its dung and urine are used as manure. It is usually practiced in summer because at that time no crops are grown. Smoke ripening is another traditional method used by the local people to ripen fruits, and it is useful for removing chemical originated toxic hazards like calcium carbide and ethrel. In this practice, a pit is dug and is covered by straw, then they set fire. This generates smoke inside the pit where unripe fruits are placed, and finally this smoke helps to ripen the fruits. Mulching is another practice used by the people of the state; this is very successful in weed management where mainly plant residues are used as straw for mulching. In many of the places, ash broadcasting is used which is a very effective practice for pest management. For seed storage, preheating is a very effective practice. In this practice, grains heated for a short time kills the insect pests and hardens the seed coat. Farmyard manuring is a very common practice all over the world and a very successful soil nutrient management followed by deny toxic hazards of fertilizers. In this method, decomposed farmyard manure is spread in the field before sowing seeds; this manure rich in humus makes the soil fertile. “Sanda” commonly known as “double transplanting” is another traditional method practiced by the people which is very useful for rainfed rice cultivation. In this practice, 20–25-days-old seedlings are planted in the nearby area and then when the seedlings are 40–45 days old they are transplanted in water-logged fields after monsoon (Morya et al. 2016).

6.4.3 Kashmir

Kashmir is a north state of India. It has good agricultural land and agricultural production. People from the state use their traditional practices to do agriculture. A boundary plantation is one of the most common and oldest practices by the natives in plain paddy fields. In this practice, people plant Salix trees in paddy fields along the sides of canals. Other plants are *Populus deltoides*, *Populus nigra,* and *Aesculus indica*. In the land slopes, agri-silviculture is another beneficial practice by the native people. Hilly areas are completely devoid of vegetation during summer which leads to water scarcity so the people plant forest trees around their farms which provide fuel and fodder. During winter, mustard or mustard-like crops such as *Robinia pseudoacacia*, *Ailanthus altissima*, *Aesculus indica*, *Populus nigra*, *Salix alba*, *Ulmus wallichiana,* and *Juglans regia* are grown along with these trees. In this state, the natives mostly practice agri-silviculture.Mainly, trees like *Cedrus deodara*, *Pinus wallichiana*, *Abies pindrow,* and *Picea smithiana* are grown in hilly areas while in plains *Populus deltoides*, *Populus nigra*, *Salix alba*, and *Robinia pseudoacacia* are grown. Horti-silviculture, is a common practice by the native people; the main purpose of this practice is to make high yield of fruits. Usually, forest trees like *Aesculus indica*, *Ailanthus altissima*, *Populus deltoides*, *Populus nigra*, *Salix alba*, *Robinia pseudoacacia,* and *Ulmus wallichiana* are grown on the boundaries of the fruit trees like *Juglans regia*, *Prunus cerasus*, *Prunus amygdalus*, *Malus pumila*, Peach, Plum, Cherry, Apricot, etc. (Islam et al. 2017).

6.4.4 Jharkhand

People from this state usually practice tillage in the form of 3–4 ploughing. For direct seeded crops, animal dung is powdered and mixed with soil after broadcasting. This practice is much better for mineralization of both macro- and micro-nutrients due to faster activities of soil microorganisms, and it also helps in improving soil water-holding capacity (Dey and Jain 1986). Another practice done with the mixture of dried animal dung and ashes in the field at different places as patches and spreading finally at the time of ploughing. Among the tribal communities in the state, various cropping practices are predominated like legume-based mixed and intercropping, crop rotations, vegetables and potato-based cropping, and paira cropping in rice lowlands. The people from the state develop their own organic manures or composting by using house waste, animal dung, forest litter, animal bedding material, etc. which keeps in a pit where earthworm practices by some farmers. Some farmers use compressed cakes of plant material as manure, e.g., *Madhuca indica*, *Azadirachta indica,* and *Derris indica* cakes, along with farmyard manure. Farmers from the state are not aware of herbicide application so they perform generally hand weeding for weed control; some use kerosene oil and karanj oil to control the weeds. For termite attacks, the people adopt the water-logging method. To conserve water, the people use bamboo drips or small spring water structure in rainy season and this water is utilized by winter crops. Many of the farmers use earthen bunding, an old practice in this area, in which the farmers level their land and prepare earthen bunds in slopes; these bunds are strengthened with hard weeds and bushes. Sometimes, the farmers use stone bunding in which bunds are prepared by using stones. These bunds are found suitable and very effective for soil, water, and nutrients conservation. *In situ* moisture conservation is another old age technique used by the farmers where they select only rice fields because these have sufficient moisture in the soil so the farmers sow linseed or gram for better yield. Since the last 20 years, farmers have been doing mulching by using leaves and paddy straw for ginger production (Dey and Sarkar 2011).

6.4.5 Himachal Pradesh

This is a Himalayan state and its land area is divided into three zones, *viz.* lower hills (350–1500 m) valleys being very fertile for intensive cultivation, mid hills (1500–2500 m) suitable for agro-horticulture, and high hills (>2500 m) which are mostly frozen. Water management is an important agricultural practice used by the people; flooding in the fields is effective against soil-borne pests; alternate flooding and draining is very helpful in controlling insect pests (Panda et al. 1983). Snowfall is a good source of water; ash spread over the snow helps convert snow into water very effectively; this water is collected in small ponds and a canal is built for irrigation (Verma 1998). Throughout the Himalayan ranges, farmers harvest rainwater by build small storage ponds. During rainy season, these ponds are filled with water and is utilized by the people throughout the year, and this controls floods in low hill areas. In hills, among the traditional agricultural practices early ploughing is one of the important practices in which plough the field early morning before evaporation of moisture because the moisture well mixed with the soil particles during ploughing; this moisture is retained by soil for a long time. Natural fog or dew is harvested by the natives to add in the fields. Spring water collection is a process in which the natives collect water in small reservoirs; it is a very common practice in cold deserts and temperate wet Himalayas. Water from these reservoirs is used for agricultural irrigation and domestic purposes. Mulching is the most effective practice used by the people from the state for cultivation and moisture conservation. Surface of the soil is covered with forest tree leaves or grass which conserves soil moisture and also helps to maintain soil temperature. A very thin layer of these mulching materials also helps in enhancing soil fertility. These practices are very cheap and simple to perform so the natives use them to improve the yield and production of crops (Lal and Verma 2008).

6.4.6 Chhattisgarh

Farmers from the state have their own strategies to select the seeds, seed stocks, etc. for agricultural purposes (Sibanda 1998; Kieft 2001). Storing and maintaining local varieties is an important strategy for any local culture mainly the farmers conserve these varieties by mass selection. In a seed technology people from the state remove rogue plants before harvesting to avoid admixture because these rouge plants mature earlier than the main crops, and their culm color is different during weeding and harvesting periods. At the time of sowing seeds, farmers sieve the seeds separate from weed seeds as they are smaller than the main. Tribal farmers generally trust on their own managed seeds on this basis farmers form a huge informal network from which they can exchange and collect the seeds. They examine the quality of seeds by their own observations; if they are satisfied, then they agree to exchange the seeds with others. They have their own indigenous ways in sowing seeds, mainly for rice seeds they use Kharri and Lahee methods. Kharri method is performed in low land areas where water logging is a common problem; to avoid this, farmers broadcast the early to medium maturing seeds in dry land and pulverized soil, the planking is totally avoided in this method. After the onset of first monsoon, the soil receives the first precipitation and seeds get germinated; on this basis, the crop is ready before the logging. In Lahee method, the germinated seeds are broadcasted in well-pulverized moist or puddle soil. This practice is done between mid-June and mid-July where water runoff in the soil is major problem. In cropping system, the native people use mixed cropping in which the farmers normally grow a mixture of legumes, cereals, and oilseeds to fulfill the demand of proteins, fats, and carbohydrates; as a result, the soil fertility also improves. Another cropping practice is utera cropping system; in this, the seeds of next crop are sown before harvesting the current crop. This system is generally adopted in rabi season because it helps farmers to use the available moisture in rainfed areas. In the successful utera cropping, generally seeds of pea, linseed, black gram, and Lathyrus are sown.

The farmers create favorable micro-climate under rainfed farming for diversification of crops and suitable uses of natural resources (Chambers 1990). The Gond tribe from the state usually uses artificial technique of micro-climate whereas the Halba tribe uses the natural. Red gram is grown with finger millet which creates micro-climate. The soil of such climatic condition is more fertile than the normal soil. The farmers usually use the indigenous varieties, and they have their own strategies for identifying the resources, agro-ecosystems, etc. (Lightfoot et al. 2001). So farmers use different micro-farming techniques and practice different specific local varieties; the farmers have classified different micro-farming situation based on soil color, depth, topography, crop, irrigation sources, and other problems. In the case of paddy, the natives have 26 indigenous varieties all are very different from each other phenotypically. Each variety has some specific genetic attributes like drought resistance, water stress resistance, pest and disease resistance, etc. which makes it suitable for a particular micro-climate (Singh 2015). Now the plant breeders of the state are preserving these folk varieties for conservation purpose; hence, on the point of biodiversity these varieties are good sources for future crop and productivity improvement due to variation in their genetic attributes.

6.5 Indigenous Systems in Agriculture

Some common indigenous agricultural practices used by people throughout the world.

6.5.1 Organic Agriculture

Organic agriculture is based on valuable production without any synthetic inputs and using indigenous practices for standardization of ecological processes to increase soil fertility. In the ancient era, our ancestors never used any synthetic chemicals instead they used kitchen ash, poultry manure, etc. The soil loses its fertility after long time by using synthetic fertilizers as a result many factors such as soil erosion, nutrient mining, etc. are following. Organic agriculture provides a broad set of practices to increase productivity in the farms (Boron 2006). Thus, it is a holistic production management system which promotes and enhances the health of agro-ecosystem followed by the increasing biodiversity functions. It must be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment. Organic agriculture plays various vital roles in solving or reducing the problems such as environmental degradation, biodiversity protection, rural development, and nature conservation (Ibeawuchi et al. 2015). In Nigeria, shifting cultivation, mixed cropping or multiple cropping, and crop rotation are practiced for indigenous organic agricultural system.

6.5.2 Multiple Cropping or Mixed Cropping

In this cropping system, two or more crops grow together in a land. The main advantage of this is maintenance of soil conditions, controlling diseases and pests, etc. Thus, the crops together facilitate with the condition of light and shade. This system also permits growing crops in different maturity periods. In indigenous practices, the seeds are sown haphazardly by hand in the field; in mixed cropping, the seeds of all crops are sown simultaneously in the same field. Inter-planting allows cropping systems to reuse their own stored nutrients. In this way, productivity per unit area is higher than the mono-cropping systems (Rankoana 2016).

6.5.3 Crop Rotation

This is a cropping system in which the crops are grown in fallow period. The main feature of this system is a good combination of crops can be grown in a particular sequence in the same area for several years without losing the soil fertility. The main outlook of this system is to improve and maintain soil fertility; prevent the soil from pest, soil-borne diseases, and weeds; control soil erosion, etc. The selection of crops for rotation is always done on the basis of their relationship with each other to ensure complementary or supplementary relationship rather than competitive.

6.5.4 Maintenance of Crops

It is necessary to maintain crop in field subsequently weeding after 4 weeks old crop; the weeds are removed by hand or by using hand hoe without disturbing the crops growth to avoid them competing with the crops for moisture. In many places of the world, weeding is done by mulching on the soil surface. The farmers or local people used to field scaring at the time of crop maturity. In cocoa plantation, tobacco plants are used to prevent insects. In Indonesia, the farmers burn the Jariamun (*Potamogeton malaianusmiq*) plant in rice fields to control pests from the farm (Wahyudy et al. 2012).

6.5.5 Fallowing

In many places, the fields are left fallow for 2–5 years so that the soil regains fertility in this period. During fallowing, domestic animals are driven to graze on the fields and their droppings enhance the soil fertility. Ecological succession is a major process in this area (Buckles et al. 1998).

6.6 Indigenous Agricultural Practices in Relation to Biodiversity Conservation

Biodiversity refers to the variety and variability of life on earth. Biodiversity and sustainable resource use are crucial for ecosystem stability and human survival. However, biodiversity is under assault world over due to rapid and accelerating anthropogenic activities causing persistent decline in species diversity. Biodiversity is typically a measure of variation at the genetic, species, and ecosystem level while indigenous knowledge is the local knowledge which is exclusive to a given culture. Indigenous knowledge is most important to conserve the natural resources which are present in various geographical ranges throughout the world. Indigenous people are very keen on conserving the biodiversity for their survival, which ultimately conserves the whole environment. This knowledge has contributed a lot in biodiversity conservation in the past, but presently this is disappearing (Parajuli and Das 2013). However due to modernization, globalization, environmental threats, etc., this knowledge is restricted to young generations and now gradually it is disappearing. Moreover, exclusionary practices, poverty as the capability deprivation and unequal policies are also responsible for making the indigenous knowledge more vulnerable, eventually affecting the diverse flora and fauna, causing them to be extinct. By the using of natural products such as medicinal plants and different agricultural practices as shifting cultivations, irrigation systems, use of biological pesticides in agriculture, soil fertility management, improve local breeds, etc. all are directly and/or indirectly involve in the disturbance of biodiversity. However, human beings are totally dependent on biological resources for survival, so if these resources are lost or become extinct, then it will ultimately affect human life (Parajuli and Das 2013). Moreover, the people have a great relation with local biodiversity particularly in rural parts of the world. Thus, when indigenous knowledge is lost, we will lose our languages, we will further lose the way of conserving diverse biological resources and the life of younger generations would be difficult if this unique knowledge is not transformed and conserved. Ultimately, the biodiversity of any local area helps people survive so we need to conserve the biodiversity for fulfilling our daily needs.

Indigenous people and their socio-cultural relationships with biological systems have largely been contributing to sustainable conservation of biodiversity, especially in *in situ* conservation (Shrestha et al. 2008). Indigenous people can be referred to any ethnic group who have historically belonged to a particular region or country, and may have different cultural, linguistic, traditional, and other characteristics compared to those of the dominant culture of that region. The indigenous people interact with the available natural resources and maintain them in pristine condition. People who have unique culture within the society have to depend upon the natural resources; hence, they do not only think for themselves but also conserve the natural habitats and individual species for their future generations, and the local people have been using their biodiversity for disease treatment (Subba 2008). Hence, we can strongly say that the indigenous people are always thriving to achieve sustainable development, i.e., indigenous and place-based knowledge always work for sustainable development (Irwin 1995; Semali et al. 2006).

For the sustainable development, various organizations involve in the conservation of biodiversity and traditional knowledge by making various government and non-government policies (Fig. 6.2). The people of United Nations drafted a policy in

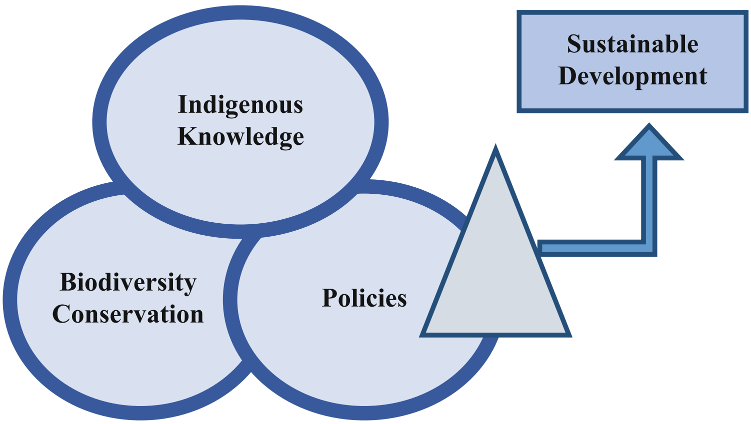

Fig. 6.2 Correlation between indigenous knowledge, biodiversity conservation, and different policies for sustainable development

1993, in which the emphasis was clearly on indigenous issues of human rights in maintaining their distinctive spiritual and material relationship with lands, territories, waters, coastal areas, different kinds of flora and fauna, and other resources which had been occupied traditionally (Sherpa 2005). In 1973, National Parks and Wildlife Conservation Act declared more than 20% of the land area was protected for indigenous people from their original place and made them more marginalized in newer kind of environment. According to this Act, the state government may declare any area to be a sanctuary or a national park if it considers that such an area is of adequate ecological, faunal, floral, geomorphological, natural, or zoological significance for protecting, propagating, or developing wildlife. Another policy is Water Act in 1974; this helps to conserve the local biodiversity. Most important policy for indigenous knowledge protection is Forest Act in 1990 which prevents deforestation and this results in ecological balance.

The concept of sustainable development is embodied in indigenous and traditional livelihood systems (Posey and Dutfield 1997). Furthermore, that knowledge includes the expertise, understanding, and insight of people, applied to continue or improve their livelihood, which can be used in various sectors to achieve the goal of sustainable development in the country. Loss of indigenous languages is also a threat on indigenous knowledge since the traditional knowledge of the communities is passed down orally. Indigenous knowledge is not dispersed all over the space like other types of knowledge as it is confined within few individuals. With the gradual loss of indigenous languages, we will lose at the same time priceless knowledge of other ways of inhabitating nature and of resources for sustainable development. It means culture, language, and biotics are inextricably linked with each other. If we lose the diverse flora and fauna that are nearer to indigenous people, they in turn will perish from this world due to lack of food and other resources. People who have unique culture within the society have to depend upon the natural resources; hence, they do not only think for themselves but also conserve the natural habitats and individual species for their future generations. Socio-cultural practices in a particular area can help protect local biodiversity. This strongly shows that indigenous people are always thriving to achieve sustainable development, i.e., indigenous and place-based knowledge always work for sustainable development (Irwin 1995; Semali et al. 2006).

6.7 Conclusion

The indigenous knowledge and biodiversity correlated with each other so that the effect of one directly observed in other; even other might be disappear. The biodiversity is going to losses due to losses of indigenous knowledge in this scenario the survival of indigenous people is to tuft due to losing of such knowledge, culture, language, etc. This diverse knowledge is going to be extinct from the world due to globalization, poverty as capability deprivation, exclusion of those groups from education and other facilities, recent dangerous environmental threats, etc. Different agricultural practices that are used by the native people directly or indirectly improve the biodiversity. Hence, we need to preserve this unique indigenous knowledge because all the scientific discoveries are explored from the indigenous knowledge; if it is lost, then it will directly affect the livelihood of the great civilization. For the biodiversity conservation, various government and non-government policies are designed. The indigenous agriculture is one of the most important factors to maintain biodiversity through managing soil, gene pools, conserving native varieties, etc. Thus, it is very important to make strong policies in order to conserve both indigenous knowledge and biodiversity. Hence, it is necessary to change people's mind towards spiritual education which focuses on important ethics. In this scenario, we can take help from native or indigenous agricultural systems or technologies in order to increase the efficiency of the indigenous varieties and biodiversity which ultimately preserves the indigenous knowledge and biodiversity for achieving sustainable development.

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Management of Biotic and Abiotic Stress Affecting Agricultural Productivity Using Beneficial Microorganisms Isolated from Higher Altitude Agro-ecosystems: A Remedy for Sustainable Agriculture

7

Yogeshvari K. Jhala, Deepak G. Panpatte, Charles Oluwaseun Adetunji, Rajababu V. Vyas, and Harsha N. Shelat

Abstract

The significant rise in the global population has necessitated a critical search for a sustainable solution that could lead to improvement in agricultural productivity, most especially crop improvement. This will go a long way to feed the ever-increasing population, most especially under unpredictable climate scenario. Therefore, there is a need to optimize the utilization of natural resources that could mitigate against biotic stress affecting agricultural productivity. Atypical example of these is beneficial microorganisms obtained from higher altitude agro-ecosystems. Before a sustainable agricultural production could be achieved, there is a need to maintain an eco-friendly approach that could protect ecosystem functions and biodiversity. Conversely, the utilization of synthetic pesticides has posed a lot of health hazards, an imbalance in the ecosystem, and threats to increase in agricultural production. Hence, one of the key priorities of the current era is to device technologies which offer effective control of pests and diseases, improves plant growth and hazardless to humans, animals and environment. This review reveals some new trends currently used for isolation, screening, characterization, and mass production of beneficial microorganisms isolated from higher altitude agro-ecosystems. On the whole, this review also presents the current scenario on the state-of-the-earth information on registration, strain improvements, mass production, and commercialization of these beneficial

Y. K. Jhala (✉) · D. G. Panpatte · R. V. Vyas · H. N. Shelat
Department of Agricultural Microbiology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India

C. O. Adetunji
Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Edo University Iyamho, Iyamho, Edo State, Nigeria

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microorganism isolated from higher altitude agro-ecosystems. Consequently, this chapter also highlights the needs of development of geneticallyimproved microbial strains from wild type strains isolated from highers altitude eco-system for increasing crop production.

Keywords
Biotic and abiotic stress · Eco-friendly · Biological control · High altitude commercialization

7.1 Introduction

Hill farming faces certain critical problems like remoteness and inaccessibility of resources, incidence of biotic and abiotic stress, peculiarity and instability in the form of moisture stress, poor soil conditions, short growing season, minor land possessions, low productivity, poor post-production management, and poor marketing and networks. Constrains are dominating the opportunities available in hill farming due to underutilization of resources. On the other hand, hills are gifted with rich repository of biological diversity, genetic resources of agricultural crops and microorganisms as well as surplus natural resources. The Green Revolution bypassed the hill farming. To guarantee food and nutritional security, sustainable development of hill agriculture through preservation and thoughtful use of natural resources is really needed. The sensible use of biotechnology in hill farming will not only bring about increased crop productivity but also improves the quality of the produces. Exploiting naturally occurring microbial communities having positive impact on farming system like nutrient cycling, biotic and abiotic stress tolerance, biodegradation of crop residues, and mitigation of greenhouse gas emission will surely help for improving sustainability of hill farming system. Plant growth-promoting rhizobacteria (PGPR) have been well studied for their potential to enhance plant growth and maintain fitness of plants against various biotic and abiotic stresses. PGPR are the natural partners of the plants which can modulate local and systemic responses of plants against biotic and abiotic stresses. PGPR produces secondary compounds which may help plants to fight against pathogens by directly inhibiting growth of the pathogens. Moreover, certain allelochemicals produced by PGPR induce plant immunity against pathogen attack as well as induce tolerance against abiotic stresses. Moreover, versatile role of PGPR are well documented for agricultural system but benefits of PGPR are comparatively less explored for hill farming system. This chapter mainly focuses on the role and mechanisms of action of PGPR strains obtained from high altitude farming system for abiotic and biotic stress tolerance to crop plants grown in high altitude farming system.

7.2 Biological Control of Plant Diseases at High Altitude

Initial step for the development of biological control agents for crop pest and diseases is identification of microorganisms having natural antagonistic ability against plant pathogens (Cook 2008) followed by evaluation for its biocontrol activity under test conditions. It is an important task to get most efficient microbial strains out of natural ecosystem. For isolation of biocontrol agents, two steps must always be kept in mind before selecting the niche for isolation, i.e. targeted crop, pathogen, and its epidemiological stage against which you need to have biocontrol agent. During selection of niche, the restrictions laid down by Convention of Biological Diversity (CBD) (1992) should be taken into consideration. Generally, samples should be collected from pathogen-affected niche, so the chances to get most efficient strains to serve as biocontrol agent will get increased (Validov et al. 2007; Pfender and Wootke 1988). Samples should be collected from various geographical regions to get broad diversity of the microbial biocontrol agents and maximize chances of getting most efficient strain.

The strain which intended to be used as biocontrol strain in high altitude farming should be cold tolerant. Cook and Baker (1983) and Schisler and Slininger (1997) provided various evidence for isolation of antagonists. For example, biocontrol agents obtained from disease suppressive soil may be more efficient for colonization of rhizosphere which provides an added advantage (Larkin et al. 1996), isolating biocontrol microorganisms to safeguard postharvest fruit should be able to colonize fruit surface more rapidly and thoroughly to eliminate the chance of colonization by pathogens or those which compete for the nutrients required for germination of pathogens (Janisiewicz and Korsten 2002). Ghildiyal and Pandey (2008) isolated three species of *Trichoderma,* viz. *T. harzianum*, *T. konengii*, and *T. viride* from forest soil in higher altitudes of Indian Himalayan Region. All the three species were found cold tolerant and with high sporulation at 4 °C within 3 weeks of time. Sporulation induction under low temperature was survival strategy of the strains to thrive into cold environment. All the three strains were found to be antagonist against *Alternaria alternata*, *Cladosporium oxysporum*, *Fusarium oxysporum*, *Pythium afertile*, and a non-sporulating dematiaceous fungi by the production of diffusible and volatile antifungal metabolites.

Dinu et al. (2012) isolated *Beauveria bassiana* (Bals.) *Vuill* from adults of Northern bark beetle *Ips duplicatus* (Sahlberg) showing symptoms of white muscardine from the bark samples of a Norway spruce forest in northeastern Romania (40-year-old trees growing in the hilly area of Botoşani County). The strain of *B. bassiana Vuill* caused 100% mortality of *I. duplicatus* within 4 days after reaching the artificial inoculation. Vashisth et al. (2018) assessed virulence of local EPN isolates belonging to *Heterorhabditis* sp., from Himachal Pradesh, against *Agrotis segetum* (Denis and Schiffermuller). *Heterorhabditis indica* was highly efficient displaying 33.33–93.33% mortality at 40 infective juveniles (IJs)/larva after 96 h of inoculation. *H. bacteriophora* (HRJ) was found superior in soil bioassay against L4 of *A. segetum,* followed by *Heterorhabditis* sp. (HKM) and

Heterorhabditis sp. (HSG) at 10,000 IJs/kg of soil. Mortality rates ranged from 78.33 to 81.67% with indigenous isolates at 7 days after treatment.

Park et al. (2017) purified endophytic fungi from mountain grown ginseng (MCG, Panax ginseng Meyer) and studied for biocontrol efficiency against ginseng pathogens (*Alternaria panax, Botrytis cinerea, Cylindrocarpondestructans, Pythium* sp., and *Rhizoctonia solani*). Out of 129 fungal isolates *T. polysporum* synthesized antimicrobial metabolites to counteract with all pathogens. Ge et al. (2016) obtained a new bacterial strain *B. methylotrophicus* NKG-1 from the rare dormant volcanic soils of Changbai Mountain in China's Jilin Region. *B. methylotrophicus* NKG-1 was capable of suppressing mycelial growth and conidial germination of several plant pathogenic fungi, viz. *Botrytis cinerea*, *Fulvia fulva*, *Fusarium graminearum*, *Rhizoctonia cerealis*, *Bipolaris maydis*, *Valsaceratosperma*, *Fusarium oxysporum*, *Colletotrichum lagenarium*, *Pyricularia oryzae*, *Gloeosporium capsici*, *Alternaria alternate*, *Botryo sphaeriadothidea*, and *Phyllosticta ampelicide* on solid media. Treatment of tomato plants with NKG-1 prior to inoculation of grey mold pathogen *B. cinerea* inhibits growth of pathogen up to 60% in greenhouse experiment. Under field conditions, treatment of tomato seedlings with 100× diluted broth of NKG-1 showed significantly higher growth and yield parameters of tomato. Jain and Pandey (2016) isolated a *Pseudomonas chlororaphis* GBPI 507 (MCC2693) producing antimicrobial compound phenazine-1-carboxylic acid (PCA) from rhizosphere of wheat cultivated in a highland site in Indian Himalayan Region. The isolate exhibited inhibition of phytopathogens in order *A. alternate* > *Phytophthora* sp. > *F. solani* > *F. oxysporum*. Molecular characterization of the isolate further confirmed the production of PCA by GBPI 507 via occurrence of phzCD and phzE genes. Hill farming is facing major problem of the pathogens due to small (less than 0.5 ha) and scattered land holding because of which intensive cultivation with less crop rotation is being carried out in hilly region. This results in high population of pathogens and increase in disease and pest incidence. Negi et al. (2005) isolated four strains of *Pseudomonas fluorescence,* viz. Pf-102, Pf-103, Pf-110, and Pf-173 from rhizosphere of vegetable crops grown in Garhwal Himalayas. In liquid culture assay, isolates showed 60–100% inhibition of mycelia growth of *F. solani f.* sp. *Pisi*. Further studies shown that Pf-102 and Pf-103 inhibited growth of *F. solani f.* sp. *pisi* by fungistasis, whereas Pf-110 and Pf-173 were lytic in their action.

For the development of commercially viable product, it is essential to screen out best strain from the large bulk of the isolates obtained (Blum 2007) as a large number of isolates show antagonism under model system but only few can satisfy commercial needs. Screening of the biocontrol agents by considering commercial viability of the product will help to come out with economically best biocontrol agent. The screening process not only comprises the choosing best isolates out of bioefficacy studies but on the basis of growth kinetics of the microbes in feasible laboratory media (Schisler and Slininger 1997). Lone et al. (2017) isolated fifty-six *B. thuringiensis* strains from ten diverse areas of northwestern Himalayas. Further identification of isolates was accomplished based on the presence of bipyramidal, spherical, flat, and irregular crystal shapes; SDS-PAGE analysis of spore-crystal mixtures shows the presence of 130, 70, and 100 kDa protein bands, PCR analysis with primers for eight *cry* and *cyt* gene families and 13 *cry* gene subfamilies. The results revealed that most of the isolates revealed the occurrence of crystal protein and demonstrated various blend of insecticidal genes, among which c*ry*1 was most abundant (57.1%). After screening of the isolates under in vitro testing conditions *B. thuringiensis* isolate JK12 showed greater fatality against *H. armigera.* Similarly, Validov et al. (2007) screened out microbial antagonists by unique methodology in which the soil samples were freeze dried and spray dried before potential bacterial antagonists against *Fusarium oxysporum* f.sp. radicis-lycopersici were isolated from such samples. Benefit obtained out of the method includes the antagonist obtained out of the research were resistant to industrial drying process and thereby appropriate for viable mass production. Similarly, for isolation of the antagonists against apple scab pathogen *Venturia inaequalis*, Köhl (2010) picked isolates which grow best with maximum spore production using cereal-based solid media, cannot grow at human body temperature, tolerant to low temperature, and humidity making them commercially viable. Screening of antagonists should be based on large number of criteria such as natural characteristics required for better field performance, toxicological profiles, mass production through fermentation as well as features of permissible property rights and marketing (Blum 2007). Berg et al. (2001) selected bacteria obtained from rhizosphere by three different selection methods. They analysed in vitro antagonism against *Verticillium dahlia* and other phytopathogenic fungi, synthesis of fungal cell wall degrading enzymes, and plant growth-promoting effects on strawberry seedlings. Isolates obtained from triple screening method did well under greenhouse conditions as compared to marketed biocontrol products. Even if majority of studies related to isolation and screening of biocontrol agents emphasize on in vitro inhibition of test pathogen on agar medium, many of the researchers showed no relationship among in vitro inhibition test and field efficiency of biocontrol agents. Burr et al. (1996) reported no connection among the capability of bacteria and yeasts for in vitro inhibition of *Venturia inaequalis* and its ability to control apple scab. Likewise, Milus and Rothrock (1996) described that bacteria displaying highest levels of inhibition under in vitro conditions were not operational under field for controlling Pythium root rot of wheat. Characterization of biocontrol strains is significant stage in the direction of commercialization and registration of the native strain as biocontrol agent to be marketed. Preliminary identification of the isolates can be done up to species level by DNA sequence analysis. In case if identification of the strain up to species level is not possible, then the strains are screened for safety and toxicity of isolated strain in medical and microbiological databases such as the German Collection of Microorganisms and Cell Cultures (http://www.dsmz.de) and regulations in European Commission, 2000 (Brimner and Boland 2003). Moreover, intellectual property rights of isolated strain in connection with targeted pathogen and ecosystem should be studied through data mining.

7.2.1 Biocompatibility with Other Soil Activity

A very important feature of sustainable agriculture is to achieve a balanced agro-ecological practices that do not affect the ecosystem, ensure plant health without any adverse effect after application of agricultural pesticides which includes beneficial microorganisms isolated from the higher altitude which has been utilized for the management of biotic and abiotic stress. A sustainable agricultural practices should embrace soil biodiversity, adequate recycling of nutrients, nutrient balancing between soil microorganisms and organism matter, and ecologically stable environment (Hendrix et al. 1990). Also, soil microorganism has been highlighted as a crucial component necessary for the maintenance of soil biomass, and it consists of actinomycetes, fungus, bacteria, collembolans, nematodes, diplopoda, arthropods, and earthworms (Davies 1973). Several microorganisms play a crucial part in bio-geocycling of nutrients in an environment, most especially for the maintenance of organic agriculture. It has been observed that bacteria and fungi dominate soil decomposing activities which involve carbon, energy, nitrogen, and other nutrient fluxes, but it has been observed that some invertebrates participate in the N flux (Swift and Anderson 1993). They similarly play a crucial role in regulation of soil ecosystem processes where they perform crucial roles such as transforming atmospheric nitrogen into organic forms, suppressing soil-borne pathogens by antagonism, hormones, allelochemicals, vitamins, vital chelators, and decomposing litter and nutrient cycling. Therefore, soil microorganism isolated from higher altitudes are important for the maintenance of a sustainable agro-ecosystem but depends largely on choice of management practices which support soil biological activities such as inhibition of soil nematodes and insects, biocontrol of weeds, synthesis of plant growth hormones, plant growth promotion: alterations in seed germination, floral growth, root and shoot biomass, biodegradation of synthetic pesticides or industrial contaminants, improved nutrient use efficiency, enhanced drought tolerance of plants (Paoletti et al. 1994).

Wang et al. (2011) evaluated the influence of plant–soil–enzyme communications on plant structure and diversity available in four different alpine meadow communities. The activities of the soil enzymes secreted by soil microorganisms were evaluated by the amount of enzymes available at different layers from meadow type and upper soil layers. Also, it was noticed that there was a relationship between the aboveground biomass of functional groups and coverage per functional group in four alpine meadows. Furthermore, the level of soil enzyme activity and soil microbial biomass were greatly influenced by an enormous level of soil nutrients inputs as a result of droppings from plant biomass. It was also observed that the level of soil enzymes showed a relationship between plant primary makings to change in vegetation and soil physiochemical features. Their study shows that some factors like community productivity, original soil conditions, and composition of plants are necessary in the maintenance of the activity, microbial biomass, and towards the regulation of plant community.

Ashaduzzaman et al. (2011) studied the influence of salinity-sodicity on the level of soil microbial enzymes and bacterial population from the soil obtained from the

Bay of Yellow Sea, Incheon, South Korea. It was observed that the soil sample closer to coastline exhibited more standards of saline-sodic soil, and soil obtained from sites 1.5–2 km away from coastline were not significantly pretentious by interruption and spray when all the following parameters were considered: seawater electrical conductivity, exchangeable sodium percentage, pH and sodium adsorption ratio. Moreover, it was observed that the halotolerant bacteria exhibited similar trends as observed for the physicochemical properties when compared to the intolerant bacteria, and enzymatic activities had contradictory trends. Also, significantly positive relationships were discovered among electrical conductivity, exchangeable sodium percentage and pH with sodium adsorption ratio and exchangeable sodium percentage. On the other hand, electrical conductivity, exchangeable sodium percentage, sodium adsorption ratio showed a significant negative correlation with enzyme activities and bacterial populations. Study exhibited that, there is an important association between salinity-sodicity and sampling distance from coastline which constitute the major factor that induce stress that affect microbial and biochemical characteristics.

Verma and Suman (2018) wrote a comprehensive review on the effect of various environmental factors on growth and yield of wheat crops planted in different six mega environmental agro-ecological zones in India on the basis of climatic conditions. Some of the environmental factors considered were salinity, temperatures, pH, drought and soil types, and the type of soil microorganisms. Some of the families isolated from the wheat microbiome include *Proteobacteria, Bacteroidetes, Gemmatimonadetes, Actinobacteria, and Firmicutes.* The various agro-ecological zones have been reported to contain several groups of microorganisms which constitute a unique ecosystem and great sources of important biomolecules, genes that could help in the prevention of pests and diseases, most especially under hearse environment. They include xerophiles, acidophiles, thermophiles, alkaliphiles, psychrophiles, and halophiles. It was observed that these microbiomes play a significant role in the maintenance of soil health, plant growth, fertility, and mitigation of abiotic stress majorly by the production of phytohormones (auxin, cytokinin, and gibberellins), solubilization of potassium, zinc, phosphorus, or indirectly via the production of ammonia, iron-chelating compounds, hydrolytic enzymes, hydrogen cyanide, and other bioactive molecules that could suppress the growth of other soil pathogens.

7.2.2 Recent Trends in the Strain Improvements

It has become necessary to isolate the gene responsible for the biological control activity in a particular biological control agent most especially from microorganisms before it could be utilized effectively for the management of biotic and abiotic stress affecting agricultural productivity. The recent trends in the application of molecular biology has enhanced the application of numerous techniques that could be used to enhance their biological control of biotic and abiotic stress. This is normally through recombination techniques and genetic modification. Examples include Agrobacterium-mediated transformation, transposon mutagenesis, protoplast fusion, and other transformation techniques are applied in the genetic improvement of beneficial microorganism that could perform the role biological control agents. Moreover, whenever an undesirable trait is observed in the biological control agent, transposon mutagenesis could be used to suppress or delete unwanted traits (Zeilinger et al. 2005).

Protoplast fusion has identified as techniques that permit the introduction of beneficial traits from one microorganism to another distinct promising strain. They have been employed to improve biological control attributes of biocontrol agents. This has been applied most especially to improve the biocontrol prospective of *T. harzianum* and enhance the level of specific proteins. Protoplast fusion of two biocontrol strains of *T. harzianum* leads to generation of offspring strain with more enhanced biological control efficacy. Afterward, rapt relocation of fungal genetic sequences encoding for factors essential in biocontrol will be conceivable once such sequences are accessible. This permits relocation of complex characters without prior knowledge of gene regulating the biological control traits and genetic recombination among an organism which exhibits sexual recombination. The application has been used specifically for various *trichoderma* species for intergeneric, inter-strain and interspecific crosses (Linda and Charles 2002). Some of the challenges of protoplast fusion include the extemporaneous manifestation of somatic mutations which might affect the influence of oligonucleotide-mediated techniques, a problem in the selection and low rates of the gene modification. Despite the highlighted challenges, the commercial application of these methodologies should be estimated in a small time (Li et al. 2007).

The application of genetic modification techniques has been utilized for improvement of biocontrol effectiveness of biocontrol agents (Morrissey et al. 2002). Resca et al. (2001) studied synergetic effect of *P. fluorescens* F113Rif (pCUP9) and *P. fluorescens* F113Rif (pCU8.3) on sugar beet in microcosm experiments. Moreover, *P. aeruginosa* strain PNA1 was isolated from rhizospheric soil of chickpea plants in India. The mutant strain FM13 lacking in phenazine synthesis was found using transposon mutagenesis from wild strain PNA1 (Schnider et al. 1995). Also, the influence of trpC mutation enhances the inhibitory effect of *Pythium* spp. and damping-off of *P. vulgaris* in lettuces. The biological efficacy might be linked to the influence of trpC mutation that enhances the influence of Anthranilate which is an intermediate of tryptophan biosynthetic pathway which might have blocked the growth of *Pythium* responsible for the damping effect in lettuce plant.

Several researchers have also validated the application of physical mutagens like UV-irradiation and chemical mutagens for the enhancement of the activities performed by the wild strains in order to develop a new biotype with enhanced biological control effectiveness (Wafa 2002). Galvão and Bettiol (2014) utilized ultraviolet-B (UV-B) radiation to enhance the biological potential of *Lecanicillium* isolates and on its capability to prevent the rate of sporulation of rust lesions. The result obtained showed that strain CCMA-1143 was the most tolerant among all the screened *Lecanicillium* isolates and possess LD50 = 1.63 kJ/m^2 of UV-B which led to inactivation and prevent the rate of spore germination of the coffee leaf rust lesions after spraying the biological control agents. Their study shows that UV-B could enhance the biological control effectiveness of the biological control agent and their eventual utilization as a biopesticidal agent.

Wafa (2002) utilized UV mutation to enhance some biological control traits of *P. fluorescens* against *R. solani* and *F. solani, F. oxysporum* f. sp. *lycopersici.* Some of the active compounds responsible for the biological control activity produced were pyrrolnitrin, antibiotics, siderophore pigment production, phloroglucinol, and phenazine. Increased in the rate of antibiotic and fluorescence was observed on King's medium from the mutant stains when compared to wild type. Adetunji et al. (2018) formulated granular pasta formulation containing strains of *Lasiodiplodia pseudotheobromae and Pseudomonas aeruginosa*, and their effect was tested on some weeds. It was observed that the bioherbicidal formulation containing BH4 formulated from the mutant strains of *Pseudomonas aeruginosa* C1501 and *Lasiodiplodiapseudotheobromae* C1136 exhibited an enhanced bioherbicidal effectiveness when compared to the formulation containing the wild strains.

Mukherjee et al. (2003) established that cloning of mitogen-activated protein kinase-encoding gene, tvk1 obtained from *T. virens* improves the biological control potential, conidiation, and the rate of mycoparasitic exhibited by the tvk1 null mutants. The mutant strains showed more expression of mycoparasitism-related genes when confirming the rate of its antimicrobial effectiveness against the *R. solani* which was the plant pathogen used during the experiment. Moreover, the null mutants exhibited enhanced protein secretion than the wild type which was evaluated as the level of lytic enzymes released into the submerged fermentation (Zaldivar et al. 2001). Further, biological control assay carried out showed that the null mutants were more effective as compared to synthetic fungicides and wild type. Efficiency of null mutants could also be related to increases in the rate of sporulation in the liquid culture, whereas the wild type didn't show any traits of sporulation which shows that the Tvk1 acts as negative modulator in sporulation and host sensing *T. virens*.

For effective commercialization of these strains, some beneficial microorganism with unique qualities like stability, easy formulation, and colonization could be enhanced using biotechnological techniques by generating a transgenic strain that possessed multiple modes of action. Huang et al. (2004) transformed gene responsible for 1-aminocyclopropane-1-carboxylic acid deaminase that triggers the plant development by separating abrupt precursor of plant ethylene into *P. fluorescens* from strain CHAO. This led to a drastic improvement in plant growth and enhanced the biological properties of the biological control agent used during their study (Wang et al. 2000).

Most of the reported microorganism that has been established to perform biological control effectiveness showed it in three different forms which include competition, antibiotics, and exploitation. Most of them have the capability to protect plant roots against pathogen responsible for several plant diseases. Several generals have been reported with biological control traits under various in vivo and in vitro trials. Examples of these genera include *Agrobacterium, Arthrobacter, Azotobacter, Bacillus*, *Enterobacter, Pseudomonas, Burkholderia, Rhizobium* and *Bradyrhizobium, Serratia,* and *Stenotrophomonas.* The production of antibiotics has been highlighted as one of the most crucial mechanisms utilized by the beneficial and agricultural important microorganism for their plant growth-promoting bacteria against phytopathogens. Soil *pseudomonads* produce 2,4-diacetylphloroglucinol, pyoluteorin, tropolone, amphisin, oomycin A, cyclic lipopeptides, tensin, and phenazine while soil *Bacillus*, *Streptomyces*, and *Stenotrophomonas* sp. produce xanthobaccin, kanosamine, oligomycin A, and zwittermicin A. Other prominent metabolites were lipopeptides, and polyketide has been reported to be synthesized by *B. amyloliquefaciens* which has been displayed to be involved in the biocontrol activity of soil-borne pathogens and prevention of their proliferation on crops. Furthermore, another typical example of antibiotics produced by beneficial microorganisms is hydrogen cyanide which has been reported to possess antimicrobial effectiveness against black root rot of tobacco caused by *Thielaviopsis basicola* (Lanteigne et al. 2012). In the same way, some *Pseudomonas* also demonstrate biological control effectiveness against the bacterial canker of tomato using HCN and 2,4-diacetylphloroglucinol. Some enzymes have also been described to be involved in plant growth promotion most especially to relieve biotic and abiotic stress from plants. Examples of these enzymes include proteases, β-glucanase, phosphatases, chitinases, dehydrogenase, and lipases. The modes of action utilized by these microorganisms are to demonstrate hyperparasitism by breaking the cell walls of the pathogen they wanted to attack using cell wall hydrolases (Suman et al. 2015). Some of the pathogenic fungi include *Sclerotium rolfsii, Rhizoctonia solani, Phytophthora* sp.*, Botrytis cinerea,* and *Pythium ultimum* (Arora 2013).

7.2.3 Commercialization of Biological Control Agents

Characteristic features of commercial biopesticides include economical production, long-term storage strength, satisfactory field persistence, ease of handling, and consistency in efficiency of controlling targeted pest/s. Biocontrol research mostly emphasize on the development of bioinoculants that can effectively compete with chemical pesticides, but now, commercial success of biopesticide formulations depends on the fact to develop biocontrol agent to control the pests which could not be controlled by any chemical pesticide or which stands in state where chemical use is banned. World over organic farming is fastest rising area of agriculture offering higher prices of produces. Organic farming do not allow use of chemical pesticides which increases chances of effective commercialization of biopesticides (Behle et al. 1999). Once usefulness of the biopesticide production has been established it will lead to switch to other sectors. The Environmental Protection Agency (EPA) has set up a Biopesticide Pollution and Prevention Division (BPPD) for quicker registration of biopesticides. The average duration for registration of biopesticides is 12 months, and cost of registration is very low as compared to chemical pesticide registration. In spite of regulatory encouragements, comparatively limited biocontrol agents reached to market. The reasons for limited commercialization of biocontrol agent includes lack of acquaintance with production and formulation development technology of biocontrol agents. Many of the scientist groups are working on the development of cost-effective and easy technology for biocontrol agent manufacturing to come out from such technological hurdles. Many of the countries like the USA prefer batch liquid cultivation of microbial products, and scientists are also looking for devising technologies to maximize biocontrol product yield with adequate quality. Newer formulation approaches have also been deliberated to maximize stability of developed product and performance upon delivery to field.

Majority of biocontrol products comprising of microorganisms serves as biocontrol products in majority of countries. So, government regulations for biocontrol agents are more or less similar to chemical plant defence products. Thorough toxicological analysis is essential to assure that there are no human or animal associated with usage of this product. Moreover, studies are necessary to guarantee that no environmental hazards will be there after usage of such products. Meanwhile industries also have to consider technological cost for production and formulation of biocontrol agent, genetic steadiness of antagonist, market size of developed biocontrol product, and prospects of patent safety for application (Whitesides et al. 1994).

Though much evidences and literature are available signifying the potential of biocontrol agents as eco-friendly biopesticides and biofertilizers, their commercialization is still considered a bottleneck in the development of sustainable agriculture worldwide. The high expenses of the registration procedure for a single biocontrol agent is a challenge for the commercialization of biocontrol agents, signifying the reason for few registered biocontrol agent products in the global biopesticide market. There are several commercially available biopesticides to farmers. As per an estimate, 175 registered biopesticide active ingredients and 700 products are existing worldwide. In India, only 12 biopesticides have been registered out of which, 5 were bacteria, three fungal, two viruses, and two plant products. Among numerous bioproducts, *B. thuringiensis* (Bt), *T. viride*, *Metarhizium, B. bassiana,* Nuclear Polyhedrosis Virus (NPV), and neem are popularly used in plant protection. According to the EPPO standards, PM 6/1 and PM 6/2, there are certain recognized lists of biological control agents classified as indigenous, introduced, and established biocontrol agents. However, this list does not include the list of microorganisms being used as BCAs. In India under the insecticide act of 1968, there are a total of 31 gazetted microbes registered as BCAs comprising of 23 fungi and bacteria, 6 entomopathogenic fungi, and 2 baculoviruses.

7.3 Abiotic Stress

Changing climate is the major limiting factor for decrease in crop productivity as it leads to abiotic stress (Padgham 2009; Grayson 2013). Major abiotic stress includes drought, low or high temperature, salinity and acidic conditions, light intensity, submergence, anaerobiosis, and nutrient starvation (Wang et al. 2003; Chaves and

Oliveira 2004; Agarwal and Grover 2006; Nakashima and Yamaguchi-Shinozaki 2006; Hirel et al. 2007; Bailey-Serres and Voesenek 2008). According to an estimate, drought has affected 64% of the global land area, 13% of land area suffer from flood (anoxia), 6% by salinity, 9% by mineral deficiency, 15% land area covered by acidic soils, and 57% is affected by cold (Mittler 2006; Cramer et al. 2011). Plants often manage harsh conditions of environment with their inherent metabolic capability (Simontacchi et al. 2015) and reprogramming of metabolism according to changing conditions (Swarbrick et al. 2006; Shao et al. 2008; Bolton 2009; Massad et al. 2012). Many times plant gets assisted by microbiome present in their surrounding niche for reduction of adverse effect of climate change (Turner et al. 2013). Inherent metabolic and genetic abilities of microorganisms enable them to fight with adverse environmental conditions (Sessitsch et al. 2012; Singh et al. 2014). Colonization of plants and their surroundings by microorganisms induce different local and systemic resistance responses which can improve plant's metabolic potential to battle against abiotic stresses (Nguyen et al. 2016).

From biochemical, physiological, and molecular studies of plant–microbial interactions, it is ascertained that microbial colonization have great impact on plant response towards stress (Farrar et al. 2014). Microorganisms develop adaptive features to combat against several biotic and abiotic stresses as it is being continuously exposed to either biotic stress like pathogens, foreign metabolites, or abiotic stresses like temperature, pH, moisture content, nutrient availability, etc.

7.3.1 Impact of Abiotic Stress on Plants

Plants get adversely affected by variation in environmental conditions posing abiotic stress for growth and development of plants. Extreme environmental conditions can be defined as high or low level of temperature, salinity, water level, moisture content, nutrients keeping optimum level of abiotic factors as baseline. The first obvious reaction of plants towards abiotic stress appears at cellular level followed by physiological symptoms which are visible. Physiological response of plants towards water stress includes decreased photosynthesis, reduction in leaf size, root growth, seed numbers, seed size and seed viability, delayed flowering and fruiting which ultimately restricts plant growth and productivity (Osakabe et al. 2014; Xu et al. 2016). Overexposure to light causes photooxidation which increases concentration of greatly reactive oxygen intermediates to change biomolecules and enzymes that upon continuous exposure to excessive light results into loss of plant productivity (Li et al. 2009). Freezing (cold) and high temperature (Koini et al. 2009; Pareek et al. 2010), acidity, salinity, and alkalinity of soils (Bromham et al. 2013; Bui 2013), pollutant contamination rigorously influence plant growth and productivity. Upon stimulation received form stress, plant exhibits instant response by activating stress-induced signalling cascade (Chinnusamy et al. 2004; Andreasson and Ellis 2010) which results in biosynthesis of phytohormone-like jasmonic acid, ethylene, abscisic acid, and salicylic acid (Spoel and Dong 2008; Qin et al. 2011; Todaka et al. 2012), build-up of phenolic acids and flavonoids (Singh et al. 2011; Tiwari et al. 2011), amplification of numerous antioxidants and osmolytes as well as activation of transcription factors (TFs) to offer safety to plants against stress (Koussevitzky et al. 2008; Atkinson et al. 2013; Prasch and Sonnewald 2013).

7.3.2 Plant–Microbe Interactions for Alleviating Abiotic Stress

Association of plants with microorganisms plays a central role in existence of both partners in stress conditions. Microorganisms mediated Induced Systemic Tolerance (IST) can enhance survival of plants under abiotic stress. Effects of abiotic stresses can be reduced by accumulation of osmoprotectants, production of superoxide radical scavenging mechanisms, omission or compartmentation of ions by efficient transporter and symporter systems, synthesis of precise enzymes involved in regulation of plant hormones are certain mechanisms that plants have developed for adaptation to abiotic stresses (Des Marais and Juenger 2010; Parida and Das 2005; Santner et al. 2009; Shao et al. 2009). Microorganisms are having indigenous metabolic and genetic potential which can help to reduce adverse effect of abiotic stresses in plants (Gopalakrishnan et al. 2015). Efficiency of rhizospheric microbes belonging to genera *Pseudomonas* (Grichko and Glick 2001; Ali et al. 2009; Sorty et al. 2016), *Azotobacter* (Sahoo et al. 2014a, b), *Azospirillum* (Creus et al. 2004; Omar et al. 2009), *Rhizobium* (Alami et al. 2000; Remans et al. 2008; Sorty et al. 2016), *Pantoea* (Amellal et al. 1998; Egamberdiyeva and Höflich 2003; Sorty et al. 2016), *Bacillus* (Ashraf et al. 2004; Marulanda et al. 2007; Tiwari et al. 2011; Vardharajula et al. 2011; Sorty et al. 2016), *Enterobacter* (Grichko and Glick 2001; Nadeem et al. 2007; Sorty et al. 2016), *Bradyrhizobium* (Fugyeuredi et al. 1999; Swaine et al. 2007; Panlada et al. 2013), *Methylobacterium* (Madhaiyan et al. 2007; Meena et al. 2012), *Burkholderia* (Barka et al. 2006; Oliveira et al. 2009), *Trichoderma* (Ahmad et al. 2015), and cyanobacteria (Singh et al. 2011) for plant growth promotion and fighting with several abiotic stresses has already been established. Isolation, screening, and application of stress-tolerant microorganisms especially from hilly regions could be sustainable alternative from ensuring high crop productivity in stress susceptible hilly regions. Application of *T. harzianum* improved oil content of NaCl affected Indian mustard (*Brassica juncea*). It also enhanced uptake of vital nutrients, greater build-up of antioxidants and osmolytes as well as reduced NaCl uptake (Ahmad et al. 2015). Chang et al. (2014) reported that application of *Pseudomonas* sp. and *Acinetobacter* sp. to barley and oats can improve the production of IAA and ACC deaminase in saline soil. Similarly, mitigation of salt stress in tomato by *Streptomyces* sp. strain PGPA39 (Palaniyandi et al. 2014), drought stress alleviation in maize (Naveed et al. 2014b), and wheat (Naveed et al. 2014a) as well as salt stress mitigation in Arabidopsis (Pinedo et al. 2015) by *Burkholderia phytofirmans* strain PsJN was reported earlier. The potential of microorganisms colonizing plants can induce local or systemic tolerance response against stress for its survival while assisting plants to preserve their growth and development by fixation, mobilization, or production of nutrients, hormones, and organic phytostimulant compounds. Such multidimensional activities of microbial communities make them sturdy, sustainable, and dynamic choices for abiotic stress alleviation approaches for crops.

7.4 Cross-Protection Against Abiotic and Biotic Stress

Plants express non-specific generalized response against abiotic stress which leads to cross-protection of plant against biotic stress too. For instance, increased production of quaternary amines such as glycine betaine, not only improves plant's tolerance to water scarcity, but also provides defence against frost and salinity stress. PGPR inoculation response can be better under adverse environmental situations such as flooding (Grichko and Glick 2001), drought (Mayak et al. 2004; Yuwono et al. 2005; Belimov et al. 2009), metal toxicity (Belimov et al. 2005), or nutrient shortage (Egamberdiyeva 2007). However, the survival of inoculated strain under the challenging environment is the prime factor to determine efficiency for plant protection (Strigul and Kravchenko 2006). Inoculated microbes perform better under stress condition as indigenous microbial community growth gets limited due to death of microbes by stresses and that is why inoculated strains will get enough nutrients to survive in the absence of competition by indigenous microflora (Ramos Solana et al. 2006). Colonization of plants by beneficial PGPR can induce signalling pathway within the plants providing protection to the plants against stress (Choudhary and Johri 2008; Walters and Fountaine 2009). Such resistance pathway known as induced systemic resistance (ISR) found to be operative against both biotic and abiotic stress as plant's immune system contains two branches: one countering to pathogen virulence factors and other identifying and reacting to elicitor molecules of non-pathogenic bacteria (Jones and Dangl 2006). Chakraborty et al. (2006) and Barriuso et al. (2008) showed that *Bacillus* sp. can induce ISR that enhance plant's tolerance against abiotic stress. This phenomenon of priming is not totally decoded yet at molecular level; it is assumed to be connected with gathering of inactive signalling proteins that become activated and transduced, upon further encounter of plant with same stresses (Conrath et al. 2006). Furthermore, study of gene expression pattern of *Arabidopsis thaliana* primed with *Paenibacillus polymyxa* upon exposure to drought or pathogenic bacterium *Erwinia carotovora* confirms that genes involved in plant reaction to biotic and abiotic stresses may be co-regulated (Timmusk and Wagner 1999). In similar manner, constitutive expression of gene *Osmyb4* encoding a transcription factor involved in cold tolerance of rice gives rise to increased tolerance of transgenic *A. thaliana* to both abiotic (salt, UV, ozone, drought) and biotic (viruses, bacteria, fungi) stresses (Vannini et al. 2006). In the same way, the promoter system encoding osmotin protein that accumulates and protects plants against salt stress is also responding to ethylene and ABA; viral and fungal infections; wounding as well as to the abiotic stresses salinity, drought, and UV radiation (Liu et al. 1995). Though, osmotin proteins leading to salt tolerance gets accumulated only when salt stress and fungal infection are there which indicates that regulatory control for cross-protection are somewhat difficult and happen not only at the level of gene expression, but also during translational and post-translational changes (La Rosa et al. 1992). In the same line, Xiong and Yang (2003) reported that disease resistance and abiotic stress tolerance in rice are inversely modulated by an ABA-inducible mitogen-activated protein kinase (MAPK). This MAPK is activated by pathogen, wounding, drought, salt, cold, etc. and enhances tolerance to drought, salinity, and cold stress upon over-expression. Suppression of MAPK genes significantly improved resistance against *Magnaporthe grisea* and *Burkholderia glumae* pathogens, whereas tolerance to drought, salinity, and low temperature was significantly reduced.

7.5 Conclusion

Environmental stress like biotic and abiotic are the foremost regulating factors for crop productivity. Drought, salinity, flooding, high and low temperature, air pollutions, etc. are major abiotic stresses whereas phytopathogens including insect, nematodes, microorganisms, and weeds comprise of biotic stresses. To achieve sustainable food production from limited land area especially in the hilly region farmers having small land holding, scientists needs to find out low cost strategy to manage biotic and abiotic stresses. Microorganisms prevailing in soil are having extraordinary ability to withstand environmental stresses and when they are associated with plants they enable plants to fight against stresses. A large number of mechanisms have been proposed for microbial management of biotic and abiotic stresses in agricultural system. Hilly region soils are rich in microbial population especially those who can better tolerate abiotic stresses. Exploring the potential of high altitude microbial life will provide the most efficient means to mitigate future environmental challenges.

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Microbial Diversity in North Western Himalayan Agroecosystems: Functions and Applications

8

Vandana Jaggi, N. T. Brindhaa, and Manvika Sahgal

Abstract

North Western Himalayan region is home to varied ecosystems ranging from completely snow-covered mountain slopes, alpine meadows, dense forests, and agricultural terraces across altitudinal gradients. The pristine habitats of Himalayan region have been the hotspot for the exploration of microbial diversity. Both classical and molecular approaches have been utilized in biodiversity studies. Lately metagenomic analysis based on next-generation sequencing has become common to estimate the abundance and diversity of both bacteria and fungi. Further, 16S rDNA sequencing-based metagenome analysis help in determining the taxonomic composition of resident microbial community. Microbial communities of mountain ecosystems are unique and diverse. The microbial community structure in this region is governed by various factors including temperature and altitudinal gradients, climate change, soil characteristics, and plant species diversity. Forests and agriculture are primary livelihood source in Himalayas, so investigation of microbes in these niches becomes important. Acidobacteria, Actinobacteria, and Proteobacteria are dominant phyla in high-altitude cold Himalayan desert soil while Firmicutes and Bacteroidetes at lower altitude. In contrast, Firmicutes followed by Proteobacteria dominated hot springs, while Acidobacteria is followed by Actinobacteria alpine meadows. Considering that microbes are inherent component of mountain agroecosystems that are faced with constraints such as remoteness and inaccessibility, marginality, and fragility in terms of moisture stress and the poor soil conditions and a short growing season. Therefore, a comprehensive approach with emphasis on microbial component is required as a long-term strategy for agriculture sustainability in the region.

V. Jaggi · N. T. Brindhaa · M. Sahgal (✉)
Department of Microbiology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

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North Western Himalayan region · Metagenome · Microbial diversity · Pristine habitats · Agroecosystem

8.1 Introduction

The Himalaya is the world's youngest mountain range. It extends over 3500 km in Afghanistan, Pakistan, China, India, Nepal, Bhutan, Bangladesh, and Myanmar covering an area of about 43 lakh km^{2}and separates Indo Gangetic plains from Tibetan Plateau. The Himalayan mountains are origin for three main river systems: the Ganges, the Brahmaputra, and the Indus. The Himalayan mountain range within - Indian Territory is collectively known as Indian Himalaya Region (IHR). It represents highly fragile and vulnerable mountain ecosystems and covers an area of about 5 lakh km^2 (~16.2% country's total geographical area) between 26°20′ and 35°40′ N latitudes and 74°50′ and 95°40′ E longitudes. IHR is spread over 12 states of the country (Fig. 8.1). The IHR is broadly divided into North-West, North-East, and Trans Himalaya (Rodgers and Panwar 1988). There is a great variation in three geographical features (i.e., latitude, longitude, and altitude) which causes immense diversity of climate and habitat within the region (gbpihed.gov.in). The region is rich in biodiversity. It provides forest cover and rivers. Forests are crucial for biodiversity conservation while rivers are the major resource for water. The region is known for its landscape and commodities (https://www.slideshare.net/basil777/the-indian-himalayan-region).

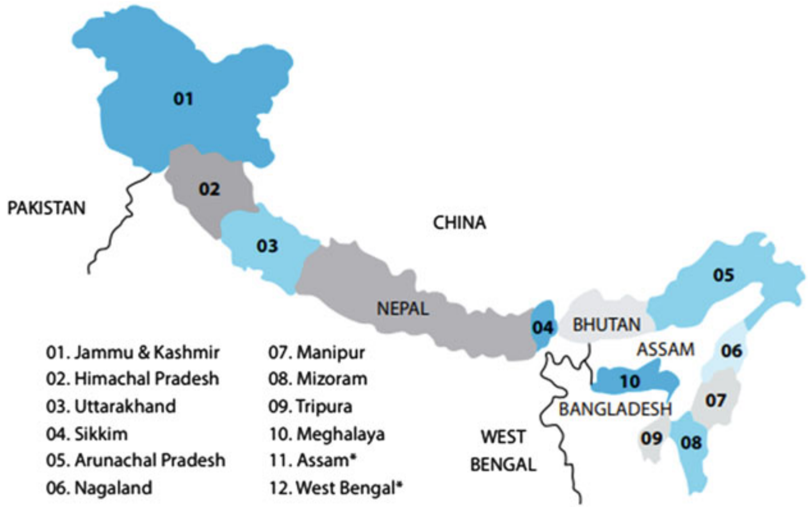

Fig. 8.1 The Indian Himalayan Region. (Source: *National mission on Himalayan studies*)

Table 8.1 Physiographic parameters of North Western Himalayan region

State	Area (km^2)	Latitude	Longitude	Proportion of state geographic area to IHR area (%)	Climate	Rainfall (mm)
Jammu and Kashmir	222,236	32° 17′ to 37° 5′ N	72° 40′ to 80° 30′ E	41.65	High altitude temperate (humid to cold arid)	<1200
Himachal Pradesh	55,673	30° 23′ to 33° 13′ N	75°43′ to 79 °4′ E	10.43	Hill temperate (per humid to sub-humid)	1200–1800
Uttarakhand	53,483	28° 43′ to 31° 27′ N	77° 34′ to 81°02′ E	10.02	Hill temperate (per humid to sub-humid)	1200–1800

Source: Modified from Sidhu and Surya (2014)

North Western Himalayan (NWH) region is located between 28° 43′ N and 37° 05′ N latitude, 72° 02′ E and 81° 02′ E longitude with an altitudinal gradient from 300 to 6000 m abmsl. It is spread over the states of Jammu and Kashmir (32.3865° N, 75.5173° E), Himachal Pradesh (31.1048° N, 77.1734° E), and Uttarakhand (77°34′ 27″ to 81°02′ 22″ E longitude and 28°53′ 24″ to 31°27′ 50″ N latitude), covering 10% (approximately 33.12 M ha) of the total geographical area of India (Table 8.1). The altitudinal expanse causes variation in climate from cold arid zone to temperate, sub-tropical temperate, transitional, and low-altitude sub-tropical. The climate variation in this region promotes specific pattern of vegetation that include cold deserts, coniferous forests, subtropical forests, alpine meadows, grasslands, and agricultural lands (Padma 2014a, b; Tewari et al. 2017). Majority of NWH region is covered by forests, grazing land, or alpine meadows. Agricultural land constitutes less than 10% of the net area. The distribution of agricultural land in different NWH states is highest in J&K (856 thousand ha) followed by UK (758 thousand ha), and HP (604 thousand ha) (Singh et al. 1998; Sharma et al. 2008; Chandel and Malhotra 2006).

8.2 Ecosystems in North Western Himalayas: Structure and Diversity

Latitudinal and elevation gradients in NWH region cause climate and soil-type variations (Sawhney et al. 2007). The region of North Western Himalaya passing through Jammu and Kashmir up to Ladakh is composed of various climatic zones with different soil texture and varying altitude peaks. Its characteristic features include glaciers, lush green meadows, valleys, and varying elevation zones harboring a plethora of microbes. Similarly Himanchal Pradesh has wide geoclimatic variations and is divided into four agroclimatic zones, viz. Shivalik hills, mid hills, high hills, and cold dry zone corresponding to subtropical, subhumid, temperate wet, and temperate dry climates, respectively. It has several specialized ecological niche, e.g., thermal springs, cold deserts, glacial lakes, and salt mines. Large area of the state is covered by hardwood and softwood forests. It is host to several natural and artificial lakes. Several rivers originate from this region (Řeháková et al. 2011). The soils are shallow to deep, well to excessively drained, sandy, loamy-skeletal, coarse-loamy, fine-loamy, and calcareous/non-calcareous with low exchange capacity and high heavy metal toxicity. The soils are slightly acidic to neutral on high reaches and neutral to slightly alkaline on lower hills. The organic carbon content of the soils is high to very high. The mean annual rainfall ranges from 600 to 1300 mm, and the mean annual temperature varies between 12 and 14 °C (Sen et al. 1997). The abrupt rise of the mountains from less than 500 m to over 8000 m, complex topography, geology coupled with intensive biotic influence, has given rise a variety of ecosystems in North Western Himalaya. It includes high altitude cold deserts, hot springs, forests, alpine meadows or grasslands, wet lands and agroecosytem with alpine, and temperate and subtropical vegetation (Padma 2014a, b; Pandey 2012).

8.2.1 The Cold Deserts

The temperate zone of NW Himalayan ranges are home to cold habitats at higher altitudes. These are largely snow covered with subfreezing temperatures between −20 °C and +10 °C. These cold deserts are surrounded by land masses where the average temperature is relatively higher. There are dramatic seasonal shift governed by altitudinal gradient. At lower altitude in winters, there is snow cover and subfreezing temperature, whereas summer has intense sunshine. In NWH region, the cold deserts are present in Ladakh (J&K), Spiti region and part of Kinnaur district (HP). Ladakh with the elevation of 2900–5900 m abmsl is the coldest region on earth (average temperature −30 to −70 °C) (Shaheen et al. 2012). The region has very short growing seasons due to extreme climatic conditions and nonfertile soil. Except for a few patches of alpine meadows, Ladakh and Spiti are almost devoid of vegetation cover. Few stunted cedars and willows are found on moist strips. The region is characterized by cold day temperature, high wind velocity, foggy atmosphere, and short day length and consists of glacier-fed streams. These are least habited regions (https://whc.unesco.org).

8.2.2 Hot Springs

There are about 340 hot spring sites all over India. Half of them are in the Himalayan region, mainly in the northwestern Himalaya. NWH region has high thermal gradient (>100 °C/km) and heat flow (Shankar 1988; Chandrasekharam 2000). There are more than 20 hot spring sites in Jammu and Kashmir State (Craig et al. 2013), 50 in Uttarakhand, and 10 in Himanchal Pradesh. In Himanchal Pradesh, majority of hot springs are located in the Beas, Parvati, and Satluj valleys. Manikaran lies in the Parvati valley, Vashishtin in Beas valley, and Tattapani in Satluj valley. In Uttarakhand region of Himalayan mountains, moderate to high temperature springs have been reported along the banks of the major rivers—Yamuna Tons, Bhagirathi, Mandakini, Alaknanda, Dhauli Ganga, and Kali (GSI 1991). Some of the well-known hot springs are located in Soldhar, Suryakund, Tapovan (Gangotri, Yamunotri), Gangnani, Badrinath, Garam pani near Nainital, Kapkot, and Gaurikund, located at the valley of Birhi Ganga and Kosi. Several hot springs also occur between Pala and Gangnani, north of Uttarkashi (Alam et al. 2004; Chandrasekharam 2005; Anthwal et al. 2010).

8.2.3 The Forest Ecosystems

The region has various forest types (Champion and Seth 1968; Singh and Singh 1991). There are tropical deciduous forests in Terai and Bhabhar, subtropical, temperate, and Alpine forest at higher elevation (1500–2600 m abmsl). Temperate forests are dominated by Chir pine (*Pinus roxburghii*) and oaks (*Quercus*), blue pine (*Pinus wallichiana*), silver fir (*Abies pindrow*), chilgoza pine (*Pinus gerardiana*), and spruce (*Picea smithiana*) at 2500 m abmsl. In Jammu and Kashmir region, coniferous forests occur between 2000 and 2800 m abmsl, *Pinus wallichiana* (around 2800 m), *Cedrus deodara* (1800–2800 m), *Abies pindrow* (2500 m), and deodar (1800–2600 m). At an elevation above 2800 m abmsl, there are broad leaved mixed forests comprising *Betula utilis* trees mixed with *Abies pindrow* (Dar and Sundarapandian 2016).

Himanchal Pradesh region of NWH comprises moist and dry temperate mixed forest, subtropical forest, tropical moist deciduous, subtropical dry evergreen, and tropical dry deciduous forest. They comprise deodar (*Cedrus deodara*) as dominant species, whereas blue pine (*Pinus wallichiana*), chir pine (*Pinus roxburghii*), fir (*Abies pindrow*), and spruce (*Picea smithiana*) are other species. Generally, deodar and ban oak (*Quercus leucotrichophora*) are found between 1700 and 2500 m abmsl and moru oak (*Quercus himalayana*) and kharsu oak (*Quercus semecarpifolia*) at or above 2500 m abmsl.

The Uttarakhand part of NWH consists of coniferous forest, deciduous forest, and subtropical forest. The coniferous forest consists of deodar (*Cedrus deodara*) as dominant species along with chir pine (*Pinus roxburghii*), fir (*Abies pindrow*), blue pine (*Pinus wallichiana*), and spruce (*Picea smithiana*).

8.2.4 Alpine Meadows

Alpine meadows or grasslands are natural herbaceous formations with scattered trees and shrubs, located on mountain slopes. They cover 171,646 km^2 which represents 25% of the Himalayan region. They are locally known as “Marg” in

Kashmir, "Kanda" or "Thach" in Himachal Pradesh, and "Bugyal" in Uttarakhand. The meadows are usually located above the tree line which is usually marked by birch–rhododendron (*Betula utilis–Rhododendron campanulatum*), fir (*Abies pindrow*), or brown oak (*Quercus semecarpifolia*). The meadow vegetation typically comprises tussock forming grasses, sedges, and herbaceous plants. The common grass species are *Andropogon munroii, Chrysopogon gryllus*, *Danthonia jacquemontii, Dactylis glomerata*, *Koeleria cristata*, and *Themeda triandra*. The grasslands are dominated by a large number of herbaceous plants such as *Anemone rivularis, Anaphalis cuneifolia*, *Origanum vulgare, Ranunculus hirtellus, Rumex nepalensis, Senecio chrysanthemoides*, and *Taraxacum officinale* (Lal et al. 1991).

8.2.5 The Wetlands

The wetland ecosystem constitutes river courses, margins of shallow lakes, and man-made water bodies. NWH region is drained by several rivers such as Chenab, Ganges, Indus, Jhelum, Ravi, Sutlej, Sharada, and Yamuna. The Himalayan region also has several lakes which can be classified into four groups: (1) structural lakes, formed by folds or faults due to the movements in earth's crust (e.g., Nainital lake in Uttaranchal); (2) glacial lakes which are formed in and around glaciers; (3) remnant lakes which were originally structural but represent the remnants of vast lakes (e.g., Dal lake in Kashmir, Tso Kar, Pangong, Tso Moriri, and Tso in Ladakh); and (4) natural dammed lakes, i.e., temporary water bodies formed along the river courses due to the deposition of rocks or debris, e.g., Gohna Tal in Garhwal, Uttaranchal. The wetlands are highly productive and dynamic ecosystem.

8.2.6 Agroecosystems

The mountain ecosystems play a crucial role by providing livelihood to about 10% of the world's population. In terms of area coverage, agriculture is the third most important land use in Himalayan region, whereas agriculture is the most populated land use practice. About 14% of the IHR is under agriculture. Mountain agriculture is mainly rainfed millets, cereals, and pulses along with vegetables and fruits being traditional crops grown in major areas.

About 68% of total 53,485 km^2 area of Uttarakhand is covered by forest and 14% under agriculture (Sati 2005). The state has two divisions: Kumaun and Garhwal. The highly productive agricultural area is known as the Tarai, and it comes under Kumaun division having altitude between 900 and 1500 m (Bisht et al. 2006). Typically there are two crop seasons—the kharif season from July to November, characterized by warm weather and plentiful monsoon rain, and the rabi season from December to April, much cooler in the first 2 months and dry (Koranne 1996). At higher altitudes (>2200 m), there is only one crop season a year, viz. kharif (July–October) as the region remains snow covered for almost 6 months. The

Uttarakhand Himalaya is a hotspot of agro-biodiversity which varies with altitudinal gradient. In all, 86 agronomic and 11 horticultural crops are cultivated (Mehta et al. 2010). The traditional farming systems are "Barahnaja" and "agroforestry" (Singh and Tulachan 2002; Sati et al. 2009). Barahnaja is the mixed farming system where varieties of crops are planted on the same terrace during kharif or monsoon season. Crop community includes cereals/millets, pulses, oilseeds, vegetables, spices, and fiber plants. The vines climb on to sturdier crops. It gives the look of natural forests as plants grow up to different levels. This is the sustainable system with more overall productivity and sustained soil fertility. In agroforestry system, there is a tree zone in between rainfed crop terraces. Among various agroforestry models, agri-horti-silviculture is very commonly practice in Uttarakhand. The line planting of trees on terrace risers is common. The species used for line planting is *Grewia optiva* (common name Bhimal) which is a multipurpose tree providing fodder, fuel, and fiber. Besides, *Boehmeria rugulosa* (daar), *Celtis australis* (nettle tree or hackberry), *Toona ciliata* (mountain cedar), *Mangifera indica* (mango), and *Ficus roxburghii* are also planted. Various other species such as *Vitex negundo* (nirgundi), *Erythrina suberosa* (Dhaul dhak), and *Lannea coromandelica* (gurjon tree) are planted as hedges around homestead. In J&K, 70% population gets livelihood directly or indirectly through agriculture. J&K is divided into three agro-climatic zones, namely Jammu (subtropical zone), Kashmir (temperate zone), and Ladakh (cold arid zone) having their distinct geo-climatic conditions which largely determines the cropping pattern and crop productivity (Khan et al. 2018). Paddy is the dominant crop of Kashmir zone, followed by wheat, maize, oilseeds, pulses, fruits, vegetables, and fodder. In Jammu region, wheat is the main crop followed by maize, paddy, pulses, oilseeds, fodder, fruits and vegetables, and other crops while in Ladakh zone, barley is the chief cereal crop followed by wheat and pulses (Andrabi 2018). The rice, maize, and wheat together account for about 84.00% of the total area under food crops and constitute 97.9% of total food grain production. However, in Himanchal Pradesh, maize and paddy crops are major crops grown during kharif season, whereas wheat and barley crops in rabi season. Potato, vegetables, and ginger are the main commercial crops of the state. The area under potato, vegetable, and ginger (Green) cultivation is 15, 75, and 2.90 thousand ha, respectively (Anonymous 2019).

8.3 Constraints of Hill Agroecosystem

The Himalayan region is diverse in its topography, climate, and natural resources. In spite of the diversified varieties of crops cultivated in NWH states, the region is faced with low crop productivity. The major reason is the low average size of holding. More than 94% holdings are less than 2 ha. Moreover, the area is vulnerable to natural disasters and climate change. In addition, anthropogenic activities, nutrient imbalance, invasive weeds, and diseases are major constraints to hill agroecosystem. The above-listed factors lead to shift in the microbial community (Bhati and Zingel 1997). The bacterial diversity and abundance are influenced by geological and ecological factors such as vegetation type, soil temperature, and nutrient status (Bryant et al. 2008; Young and Crawford 2004). Soils represent the most diverse and important ecosystem on earth. Aboveground biodiversity of agroecosystems influences soil biota and their functions which in turn affects crop growth and soil sustainability. Therefore, microbial soil communities are the most sensitive and rapid indicators of changing land-use pattern (García-Orenes et al. 2009).

8.3.1 Climate Change

Mountains are facing enormous pressure of climate change (Nogués-Bravo et al. 2007). Dimri and Dash (2012) observed the increase in T_{max} between 1.1 and 2.5 °C in NWHs. With rising temperatures, areas covered by permafrost and glaciers are decreasing. Consequently there is a rise in sea level (Beniston 2003). Changes in the hydrological cycle may significantly change precipitation patterns of Himalayas leading to changes in river runoff and ultimately affecting hydrology and nutrient cycles. Climate change increases the risk of extinction of species (Hannah et al. 2007; Sekercioglu et al. 2008). Moreover, microorganism perfectly adapted to phycrophilic temperatures are greatly influenced by the melting of surface layers. Global warming is changing the basal temperature of the ice, going from cold to polythermal, causing the growth of mesophilic, thus leading to change in the diversity and composition of microbial populations (Garcia-Lopez and Cid 2017).

8.3.2 Natural Disasters

Natural disasters viz. floods, landslides, earthquakes, and soil erosion have increased due to anthropogenic activities (www.fao.org). A recent example is natural disaster at Kedarnath in June 2013 in which a cloud burst; flooding and landslides caused immense loss of human lives and natural resources. Landslide events have increased. Moreover, the rate of soil erosion is very high and may be caused by water and wind. Erosion leads to loss of fertile topsoil, nutrients, and vegetation, change in soil properties, and decreased soil fertility (Chen et al. 2015) as a result microbial community structure and dynamics get affected. Multiple ecosystem functions and services driven by micro- and macro-organisms including decomposition, plant productivity, nutrient cycling, and regulation of greenhouse emissions are negatively impacted (Orgiazzi et al. 2018). By modifying soil communities, soil erosion also influences these services (Rekhi et al. 2000; Wakene et al. 2007). Soil microorganisms are sensitive to sudden environmental changes and play critical part in regulating ecological processes such as litter decomposition, soil nutrient turnover, as well as the plant growth performance (Liu et al. 2010; Wardle et al. 2004).

8.3.3 Nutrient Imbalance and Soil Organic Carbon Losses

Nutrient imbalance and soil organic carbon loss are the major issues. It is driven by removal or *in situ* burning of crop residues, no or least addition of organic manures, and intensive cultivation. In addition, excessive and/or imbalanced nutrient inputs may pose risk of nutrient imbalance. Soil organic matter content seems to be the main factor responsible for the different microbial community structure in soils. The abandonment of agriculture has led to increments in microbial biomass, richness, and shifts in the microbial community structure (Zornoza et al. 2009).

8.3.4 Anthropogenic Activities

Anthropogenic activities such as deforestation, grazing, lopping/cutting of trees for fodder and fuel wood, and construction of dams, tunnels, and hydroelectric projects affect the ecosystem diversity. These activities significantly alter the soil characteristics, including physical, chemical, and biological properties. Agricultural management influences soil microorganisms and soil microbial processes. Soil microbes play an important role in forest ecosystems through decomposition of organic matter and carbon and nutrient cycling. Soil microbial communities are very sensitive to forest land-use changes. The tree species and composition strongly affect the structure and function of microbial communities in forest soils (He et al. 2012). Thus, conversion of forest type could induce significant shifts in soil microbial community. In the Himalaya, the major cause of habitat degradation and loss is uncontrolled levels of grazing by livestock (Kala and Rawat 1999). Overgrazing alters the function, structure, and organization of the ecosystem by reducing richness and diversity of species, increasing the proportion of unpalatable weed species, accelerating soil erosion, and depleting the nutrient pool (Petit et al. 1995). The exorbitant grazing harms the ground flora and impedes regeneration of dominant tree species in the area (Malik et al. 2016), excessive use of pesticides can drastically modify the function and structure of soil microbial communities, thereby altering the normal functioning of terrestrial ecosystems, which in turn has important implications for soil quality (Pampulha and Oliveira 2006).

8.3.5 Biological Constraints

The major biological constraints are weed, pests, and diseases. On the one hand, these affect plant growth and yield and on the other soil biotic component including microorganisms. Soil biota contributes substantially to the resistance and resilience of agroecosystems to abiotic disturbance and stress (Brussaard et al. 2007). Change in soil microbial community structure and composition can result in rapid multiplication of pathogenic microorganisms. This in turn causes imbalance in the soil microbial structure resulting in poor soil and crop health, yield, and quality (Xing et al. 2010). The predominant weeds in NWH are *Brachiaria ramosa,* *Cannabis sativa, Cyperus rotundus, Fumaria parviflora, Galinsoga parviflora, Oxalis latifolia, Phalaris minor*, and *Polygonum plebeium*. Insect pests viz. leaf miners, beetles, thrips aphids, and scarabaeids decrease the global crop production by 35%. In all, 116 species of scarabaeids have been reported from NWH (Mehta et al. 2010). The increasing incidence of soil-borne and foliar diseases, especially angular leaf spot (causal organism *Phaeoisariopsis griseola* Sacc.) and *Rhizoctonia* root rot (causal organism *Rhizoctonia solani* Kühn), is becoming a major constraint for the profitable cultivation of bean both in hilly regions as it leads to 13.2% crop loss. The good soil quality is positively correlated to higher abundance of beneficial microbes which in turn result in better plant growth, lower disease incidence, higher nutrient contents, and soil enzyme activities. For example, Wang et al. (2017) studied microbial diversity of healthy vs wilted plant through Illumina sequencing of 16S rRNA gene amplicons and observed that soil microbial composition and diversity between healthy and bacterial wilt-infected soils were distinct. The microbial community varies at different plant growth stages. Healthy soils exhibit higher microbial diversity than the infected soils. The plant beneficial microbes including *Acremonium*, *Agromyces*, *Bacillus*, *Bradyrhizobium*, *Chaetomium, Lysobacter*, *Mesorhizobium*, *Micromonospora*, *Microvirga*, and *Pseudonocardia* are abundant in the healthy soils rather than infected soils. Moreover enzyme activities invertase, catalase, and urease as well as soil pH, available phosphorous and potassium content, are all significantly increased in the healthy soils.

8.3.6 Indigenous Farming Practices in North Western Himalayas

Microbial community structure is correlated to land-use pattern. Besides, disturbances caused by forest management can alter soil characteristics and subsequently influence the diversity and structure of the microbial community (Hartmann et al. 2015). The predominant cropping system and management practices play an important role in changing the soil physicochemical properties and microbiome composition and diversity. For example, shifting cultivation leads to tremendous loss of soil nutrients and natural vegetation. Cultivation of -non-cereal crops such as alfalfa ((*Medicago sativa*) Linn.) and grass clover (*Trifolium repens* Linn.) are known for soil organic matter conservation. In rubber plantations, abundance of *Acidobacteria* in soils is largely unchanged while copy number of *Chloroflexi* is significantly increased (Kerfahi et al. 2016). There is a considerable difference in proportion of abundant bacterial groups in native and regenerated per humid montane forest soils (Lin et al. 2011). Crop rotation can also modify soil mass and fertility factors and thus influence microbial community structure (Benítez-Páez and Sanz 2017). Traditional farming systems wherein varieties of crops are cultivated on same land are reservoirs of microbial diversity. With the introduction of high-yielding variety (HYV) of food and fruit crops, the farming systems have diverged from mixed crop cultivation to mono-crop cultivation, leading to the loss of agrobiodiversity. Therefore, conservation of the indigenous farming systems is needed for overall environmental, economic, and social development (Singh et al. 1998).

8.4 Microbial Diversity in Cold Deserts

8.4.1 Bacteria

Himalayan mountain region spreading across 3500 km from west to east is one of the richest bioresource of unique microflora particularly bacteria, fungi, and actinomycetes. The glaciers and permafrost regions are reservoir of psychrophilic/pscychrotolerant bacteria and actinomycetes. Ladakh and Zanskar are dry cold deserts where winter snow is the main source of water. The soils are highly saline and a huge resource of halophilic and radiation-resistant bacteria and fungi. The imperfectly weathered rock fragment in this area is inhabited by lithotrophic microorganism. The psychrophilic microorganisms are abundant in these regions and are the source of cold active proteases, lipases, amylases, and alkaline phosphatases.

The bacterial diversity studies of Himalayan cold desert through real-time PCR revealed that bacteria and diazotrophs were in less abundance at high-altitude Gangotri soil ecosystem than moderate-altitude Kandakhal soil of NWH. Metagenomic study of the region revealed that high-altitude soil was dominated by *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* while low-altitude soil by *Bacteroidetes* and *Firmicute*s. The major conclusion is that there is a selective proliferation of gram-negative bacteria at higher altitude Gangotri region (Kumar et al. 2019).

Srivastava et al. (2014) analyzed culturable rhizobacteria growing at varying altitude in Garhwal Himalaya. In all, 44 rhizobacteria belong to 11 different genera from Kedarnath wildlife sanctuary, 8 rhizobacteria from Srinagar valley, and 16 rhizobacteria at Pauri. Among rhizospheric bacteria, *Bacillus* sp. is the most dominant genera followed by *Pseudomonas* sp. In addition, other identified genera are *Azotobacter* sp., *Alcaligenes* sp., *Enterobacter* sp., *Klebsiella* sp., *Flavobacterium* sp., *Micrococcus* sp., *Staphylococcus* sp., *Serratia* sp., *Staphylococcus*, and *Xanthomonas* sp. In the soil samples from western Himalayan region, 45% of culture bacterial strains belong to Proteobacteria, with 39% belonging to γ-Proteobacteria. Firmicutes is the second most abundant phyla (32%) followed by Actinobacteria (16%) and Bacteroides (6%) (Table 8.2). Of the total 18 genera observed, most of the strains belong to genus *Bacillus* (30%) followed by *Pseudomonas* (24%) and *Actinobacteria* (12%). Cultured bacteria from this region are represented by *Actinobacter, Aeromonas, Alishwanella, Brevundimonas, Exiguobacterium*, *Flavobacterium, Hydrogenophaga, Iodobacter, Microbacterium, Pontibacter, Rheinheimera, Rhodococcus, Streptomyces, Vogesella, and Yersinia*. Isolates belonging to *Pseudomonas, Bacillus*, and *Arthrobacter* show maximum diversity. In all four, phylotypes are considered new species as they share <97% sequence identity with the previously described bacteria. Most of the *Arthrobacter* species have been frequently reported from cold environments. Commonly distributed *Arthrobacter* species in cold environments include *A. agilis, A. sulfureus, A. oxydans, Pseudomonas syringae, P. frederiksbergensis* and *P. spinosa* (Meyer et al. 2004; Shivaji et al. 2005). Several species like *Pseudomonas fluorescens, Acinetobacter johnsonii, Flavobacterium columnare*, and *Bacillus cereus* are abundant

Table 8.2 Dominant bacterial phylum and their representative species in North Western Himalayan region

Phylum	Class/order	Bacterial species	References
Actinobacteria, 17.78%	Actinomycetales	*Arthrobacter antarcticus, A. aurescens, A. chlorophenolicus, A. koreensis, A. methylotrophus, A. nicotianae, A. nicotinovorans, A. nitroguajacolicus, A. polychromogenes, A. psychrochitiniphilus, A. psychrolactophilus, A. sulfonivorans, A. sulfurous*, and *A. ureafaciens*	
Proteobacteria, 42.57%	Pseudomonadales	*Pseudomonas aeruginosa, P. antarctica, P. azotoformans, P. baetica, P. cedrina, P. corrugate, P. costantinii, P. deceptionensis, P. extremaustralis, P. extremorientalis, P. fluorescens, P. fragi, P. frederiksbergensis, P. geniculata, P. gessardii, P. graminis, P. jessani, P. kilonensis, P. koreensis, P. lurida, P. mediterranea, P. moraviensis, P. orientalis, P. pavonaceae, P. peli, P. plecoglossicida, P. poae, P. psychrophila, P. putida, P. reactans, P. rhodesiae, P. simiae, P. stutzeri, P. syringae, P. teessidea, P. tolaasii, P. trivialis, P. vancouverensis*, and *P. xanthomarina*	Pradhan and Lee (2010)
Firmicutes, 32.94%	Bacillales	*Bacillus acidicola, B. altitudinis, B. amyloliquefaciens, B. aryabhattai, B. baekryungensis, B. cereus, B. cibi, B. circulans, B. firmus, B. flexus, B. licheniformis, B. marisflavi, B. megaterium, B. mojavensis, B. muralis, B. psychrosaccharolyticus, B. pumilus, B. simplex, B. sonorensis, B. subtilis, B. thuringiensis*, and *B. weihenstephanensis. Exiguobacterium mexicanum, E. acetylicum, E. antarcticum, E. artemiae, E. aurantiacum, E. homiense, E. indicum, E. marinum, E. sibiricum*, and *E. coli; Paenibacillus amylolyticus, P. lautus, P. pabuli, P. terrae, P. tylopili and P. xylanexedens; Planococcus antarcticus, P. donghaensis*, and *P. kocurii*	

(continued)

Table 8.2 (continued)

Phylum	Class/order	Bacterial species	References
Cyanobacteria, 1.17%	Oscillatoriales—65%	*Leptolyngbya foveolarum, Leptolyngbya*	Řeháková et al. (2011)
	Nostocales—20%	*Benthonica, Planktothrix agardhii, Plank*	
	Chroococcales—15%	*Clathrata, Geitlerinema acutissimum, Limnothrix redekii, Nostoc linckia, N. punctiforme, Nodularia sphaerocarpa, Gloeocapsopsis pleurocapsoides, N. spongiaeforme*, and *Cyanobium parvum*	

in Himalaya but are rarely reported from psychrophilic environment elsewhere. Several isolates have shown phylogenetic affinity with isolates from glaciers in China, permafrost environments in Siberia and Bolivia, and very few with isolates from Antarctica or Arctic Circle. The phylogenetic affinity of bacterial phylotypes with those of nonpolar and polar habitats imply towards the same selective mechanism in all these regions (Gangwar and Kaur 2009).

Rhizosphere of high-altitude plant *Zanthoxylum armatum* DC growing at three different locations in Garhwal Himalaya has been explored. Cultured rhizobacterial strains belong to eleven different genera at Kedarnath wildlife sanctuary, eight at Srinagar valley, and six at Pauri. Among the identified genera, *Bacillus* is the most dominant followed by *Pseudomonas* at all three locations. Other genera identified in rhizosphere are *Flavobacterium, Xanthomonas, Klebsiella* sp., *Staphylococcus, Azotobacter, Serratia, Enterobacter, Alcaligenes,* and *Micrococcus* (Srivastava et al. 2014). Bacterial diversity in cold desert area under potato cultivation at Mana Village (Chamoli district, Uttarakhand) has been analyzed. The soil in these regions remains influenced by snow for 6 months (October to April) before and after crop season. Although the soil is nutrient poor, microbial population is in the range of 10^7 colony-forming units per gram. Microbes with plant growth-promoting properties are dominant and consisted of *Bacillus, Pseudomonas, Penicillium, Actinomycetes,* and Yeast.

8.4.2 Cyanobacteria

The cyanobacterial and microalgal diversity at altitudinal gradient of 3700–5970 m abmsl in Ladakh Mountains has been explored. Abundance of cyanobacteria component is high with 70.9–98.6%, whereas microalgae account for 29% of total biovolume. The cyanobacteria from Nostocales dominate at higher altitude sites (subnival zone) while Oscillatoriales at lower altitude (alpine meadows). Nostocales are represented by *Nostoc* and *Scytonema*. At high altitude, Oscillatoriales are minor component and among them *Phomidium* dominates. *Leptolyngbya* spp. is abundant and *Microcoleus vaginatus* is nearly absent. The Cyanobacterial order Chroococcales is present in higher volumes where Nostocales are dominant. Together the phototrophic communities are composed of 20 morphotypes, 14 for cyanobacteria, 10 for green algae, and four for diatoms. Lahaul-Spiti, the largest district of Himachal Pradesh, is divided into Lahaul and Spiti Valley. It is a part of the Indian cold desert in the northwestern Himalayas and covers an area of 13,883 km^2 with the elevation of 2400 and 6400 mabmsl. Climatic conditions are dry temperate, and the area remains covered with snow for more than 6 months. In this region, high-altitude mountain lakes are characterized by low temperature, low buffering capacity, and low nutrient level (Psenner et al. 2002; Catalan et al. 2006). The biodiversity of these lakes is scanty, with prokaryotic cyanobacteria as the major component. Singh et al. (2016) studied the diversity of cyanobacteria in four high-altitude lakes, namely Sissu Lake, Chandra Tal, Suraj Tal, and Deepak Tal of Himachal Pradesh, India through morphology and 16S rRNA gene sequence analysis and identified 20 cyanobacterial species belonging to 11 genera. These are *Cyanobium parvum, Geitlerinema acutissimum, Gloeocapsopsis pleurocapsoides, Leptolyngbya antarctica, L. frigida, L. benthonica, L. foveolarum, L. lurida, L. valderiana, Limnothrix redekii, Nostoc linckia, N. punctiforme, Nodularia sphaerocarpa, N. spongiaeforme, Planktothrix agardhii Plank clathrata, Pseudanabaena frigida, Synechocystis pevalekii, Phormidium autumnale*, and *P. chalybeum* (Fig. 8.2).

8.5 Bacterial Diversity in Sub-Glacial Lakes

Sub-glacial lakes have been explored for microorganism diversity. Sahay et al. (2012) reported the presence of highly diverse bacterial population belonging to Proteobacteria (α, β, γ), Firmicutes, and Actinobacteria in sub-glacial Pangong Lake in Kashmir region of Indian Himalayan. Culturable bacteria of this lake show distinct morphology and pigmented colonies. Population density of cultured bacteria is more at subsurface than at surface and sediment. This is in contrast to freshwater and brackish water lakes where highest bacterial density is in sediment. Several unique culturable bacterial species such as *Alishewanella* sp., *Brevundimonas* sp., *Ochrobactrum* sp., *Sphingomonas* sp., and *Tsukamurella* sp. have been reported in Pangong Lake. Unculturable bacteria are abundant in sediment samples of Pangong Lake. The distribution and occurrence of bacterial communities in different zone of sub-glacial lake may be determined by factors such as nutrient availability, photochemical properties of water, oxic–anoxic interface, and the presence of other communities. Culturable bacteria from subglacial lakes exhibit the production of cold adaptive enzymes such as protease, amylase, xylanase, and cellulose.

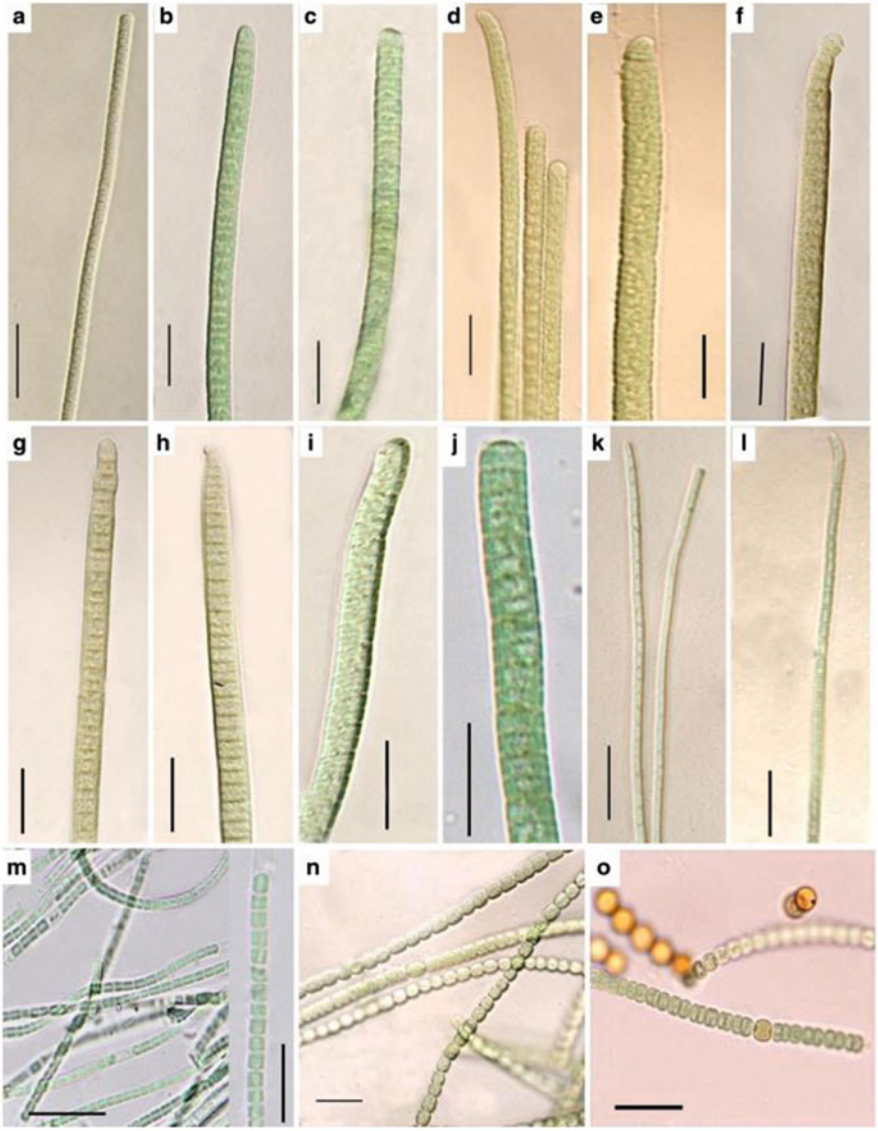

Fig. 8.2 Cyanobacterial species isolated from high altitude lakes of Lahaul-Spiti (Source: Singh et al. 2014). (**a**) *Leptolyngbya benthonica*, (**b**, **c**) *Phormidium autumnale*, (**d**, **e**) *P. chalybeum*, (**f**–**h**) *Planktothrix agardhii*, (**i**, **j**) *Plank clathrata*, (**k**, **l**) *Geitlerinema acutissimum*, (**m**) *Pseudanabaena frigida,* and (**n**, **o**) *Nodularia sphaerocarpa*

8.6 Hot Spring Diversity

Himalayas have unique hot springs at high elevation across NW part of Indian Himalayas. A few have been explored for their microbial diversity. These are Chumathang, Khirganga, Kasol, Manikaran, Tattapani, and Vashisht in Himanchal Pradesh (Kumar et al. 2004; Verma and Shikot 2014; Priya et al. 2016; Yadav et al. 2015) and Soldhar and Ringigad in Uttarakhand (Kumar et al. 2004).

Manikaran thermal spring located in Beas and Parvati valley geothermal system is the hottest in the country with temperature of 89–95 °C and pH 7.8–8.2 and is abundant in cultured thermophile (60–90 °C) and thermotolerant (37–60 °C) bacteria. Majority of isolates are gram positive with highest number of Firmicutes (86%) followed by Proteobacteria (10%) and Bacteroides (4%). Fourteen different genera namely *Anoxybacillus, Bacillus, Brevibacillus, Brevundimonas, Burkholderia, Geobacillus, Paenibacillus, Planococcus, Pseudomonas, Rhodobacter, Thermoactinomycetes, Thermobacillus, Thermonema,* and *Thiobacillus* are identified in Manikaran Lake (Sahay et al. 2017). The results provide evidence that *Bacillus*- or *Bacillus*-derived genera are ubiquitous and predominant under hot spring environment. All these bacteria produce one or more thermoadaptive enzymes such as protease, amylase, cellulase, and xylanase. At Soldhar and Riggad hot spring *Bacillus* sp. has been reported (Kumar et al. 2004, 2005; Trivedi et al. 2006; Sharma et al. 2009; Pandey et al. 2014).

In addition, thermophilic Cyanobacterial sp. *Spirulina meghniana, Chlorogloeopsis, Lyngbya hieronymusii, Pseudanabaena galeata* have also been reported from these two hot springs (Bhardwaj et al. 2010, 2011). *Spirulina meghniana* and *Chlorogloeopsis* are new record for hot springs in Uttarakhand. Recent study of Soldhar and Tapovan hot springs documents *Pseudomonas* spp. Analysis of bacterial population at Tapovan kund (moderate temperature hot spring) reveals a high percent of amylolytic bacteria at Tapovan and proteolytic at Soldhar (Ranawat and Rawat 2017).

8.7 Microbial Diversity in Forest Ecosystem

8.7.1 Bacterial Diversity

Coniferous forests are dominant in NWH. Cedrus, Pinus, and Taxus are the major coniferous tree species in these forests. Soil microbes are the essential components of natural forests. They provide essential nutrients to their host plants (Nazir et al. 2009) and are responsible for proper ecosystem functioning (Hackl et al. 2004). Microorganisms are responsible for geochemical cycling of elements. They maintain soil chemical composition and release limiting nutrients, so that they are available for the proper growth of plants (Heneghan et al. 1998; Nazir et al. 2009). Phosphorus is one of the essential plant nutrients which plays critical role in N-fixation and resistance to diseases. In forest soils, phosphorous is present as both inorganic P (from weathering of parent rocks) and organic P (from decayed organisms). It is made available to plants upon solubilization or mineralization through microbes. The bacterial diversity in Himalayan region soils has been investigated through both culture-dependent and culture-independent approaches. Of various culture-independent approaches, metagenomic approach is widely preferred and has reported the presence of bacterial phyla such as Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria in the mountainous soil (Gangwar and Kaur 2009; Pradhan and Lee 2010; Shivaji et al. 2005; Srinivas et al. 2011; Joshi 2018). Majority of cultured phosphate-solubilizing bacteria (PSB) belong to Gammaproteobacteria (Gupta et al. 2015). NWH forest ecosystems are rich in inorganic P solubilizing bacteria. The population of inorganic P-solubilizing bacteria in *Dalbergia sissoo* natural forests at Tanakpur, Lachhiwala, and Pantnagar was found to be 1.2×10^4, 1.55×10^4, and 1.06×10^4 cfu per gram, respectively (Joshi 2018). The same study reported that OTUs (based on illumina sequencing) belonging to genera *Flavobacterium, Bradyrhizobium, Candidatus, Solibacter, Phenylobacterium, Flavisolibacter,* and *Rhodoplanes* were relatively more abundant at Tanakpur and Lachhiwala, whereas *Williamsia, Blastocatella, Methylobacterium,* and *Brevibacterium* were at Pantnagar forest. The bacterial isolates from Himalaya depicted better inorganic P-solubilizing efficiency than those from non-Himalaya (Gyaneshwar et al. 2002). The phosphate-solubilizing bacteria (PSB) from Shisham rhizosphere were identified as belonging to genera *Pseudomonas, Streptomyces, Klebsiella, Staphylococcus, Pantoea, Kitasatospora*, and *Micrococcus* (Joshi et al. 2019). Several previous studies reported that the most important P-solubilizing bacterial strains from forests belong to the genera *Bacillus, Enterobacter, Pseudomonas,* and *Rhizobium* while fungi belong to *Aspergillus* and *Penicillium* (Behera et al. 2014; Nazir et al. 2017). Therefore, bacterial and fungal strains isolated from these regions have the potential to be developed as biofertilizers to increase the P availability in soils (Khan et al. 2009).

8.7.2 Mushroom Diversity in Forest Ecosystem

Mushrooms are fungi belonging to family Basidiomycetes. They grow in diversified niches of forest ecosystem. They predominantly occur during rainy and spring season. Globally, Agaricales is the most represented mushroom (Lakhanpal 2014). It is represented by 15 families, 59 genera, and 300 species (Table 8.3). Tricholomataceae is one of the largest families of Agaricales. World over it is represented by 98 genera. Of the total 41 genera present in India, 25 have been reported from the Himalayas and 15 exclusively from NW Himalayan region. Second widely recorded family from NW Himalayan region is Boletaceae. It consists of 7 genera and 57 species.

In all, 23 edible mushrooms are found in the Northwestern Himalaya (Semwal et al. 2014). Twenty-one of these are Basidiomycetes and two Ascomycetes. The common edible species are *Agaricus, Amanita, Astraeus, Hericium, Macrolepiota, Morchella, Monotropa, Pleurotus,* and *Termitomyces,* whereas species of *Auricularia, Cantharellus, Lactarius, Ramaria, Russula*, and *Sparassis* are less common (Fig. 8.3). *Cordyceps sinensis* and several species of *Morchella* are reported in high-altitude region of North Western Himalayas during spring season.

Table 8.3 Major Agaricomycetes species reported in North Western Himalayan region

Order	Family Class—Agaricomycetes	Genera	Representative species
	Boletaceae	*Austroboletus*	
Agaricales		*Boletus*	*Boletus edulis and B. hoarkii*
		Gyproporus	
		Leccinum	
		Strobilomyces	
		Suillus *Tylopilus*	
	Amanitaceae	*Amanita*	*Amanita caesaria* and *A. vaginata* (edible)
	Russulaceae	*Lactarius*	*Lactarius delicious* and *L. sanguifluus*
		Russula	*Russula brevipes*
	Cantharellaceae	*Cantharellus*	*Cantharellus cibrarius* and *C. minor* (edible)
		Craterellus	*Craterellus cornucopioides*
	Agaricaceae	*Agaricus*	*Agaricus augustus., A. arvensis, A. campestris, A. placomyces,* and *A. arvensis*
		Cystoderma	*C. amianthinum*
		Lepiota	*L. acutesquamosa, L. clypeolaria*, and *L. cristata*
		Macrolepiota	*M. procera*
		Leucoagaricus	*L. rubrotinctus*
		Leucocoprinus	*L. cepaestipes*
	Hygrophoraceae	*Hygrophorus*	*H. ebureneus, H. pudorinus*, and *H. pustulatus*
		Hygrocybe	*H. psittacina, H. conica*, and *H. calopus*
		Camarophyllus	*C. pratensis*
	Pluteaceae	*Pluteus*	*Pluteus cervinus, P. dryinus, P. ostreatus, P. membranaceus*, and *P. fossulatus*
		Volvariella	*V. volvacea, V. bombycina and V. pussila*
	Physalacriaceae	*Armillaria*	*A. mellea, A. obscura*
		Oudmansiella	*Oudemansiella radicata*
		Flammulina	*Flammulina velutipes*

Source: Lakhanpal (2014)

8.8 Microbial Diversity in Alpine Meadows

The grasslands are found above the treeline at an altitude between 3000 and 5200 m. The Chippla kedar alpine meadows in Pithoragarh district and Bedini Bugyal of Chamoli district of Uttarakhand have been explored for their microbial diversity. In these soils, bacterial and fungal biomass is estimated to be 1–2 and 2–5 tons per ha, respectively (Standing and Kilham 2007). Nacke et al. (2011) by pyrosequencing technique revealed that the dominant bacterial

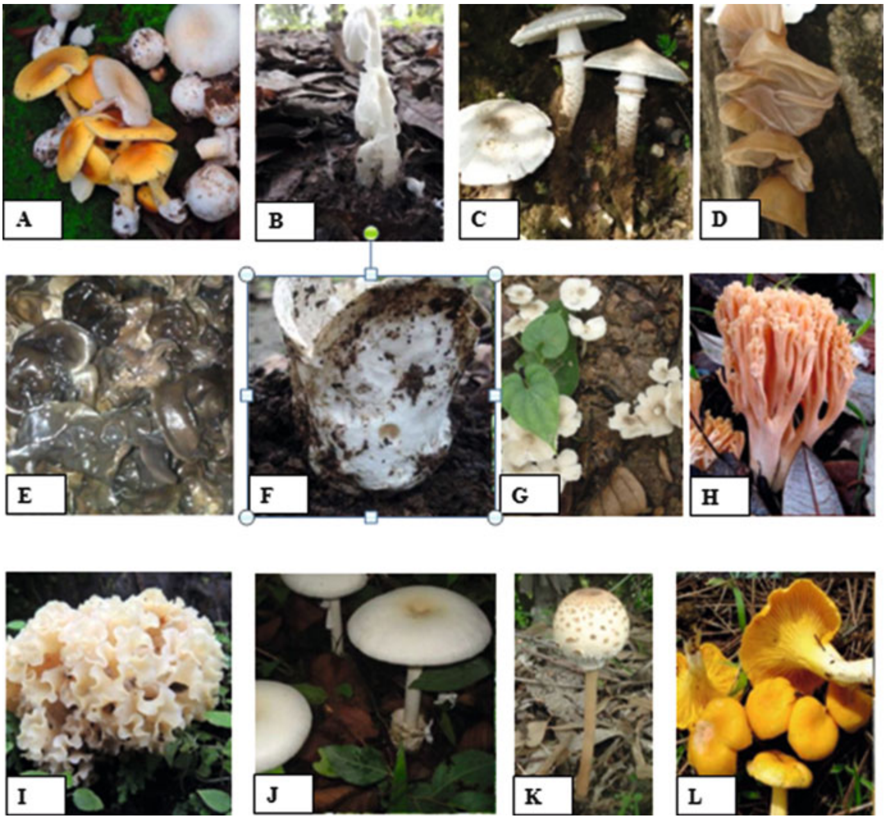

Fig. 8.3 Diversity of mushroom in North Western Himalayan region. (**a**) *Amanita hemibapha*, (**b**) *Monotropa uniflora*, (**c**) *Termitomyces heimii*, (**d**) *Auricularia auricula-judae*, (**e**) *Auricularia polytricha*, (**f**) Large cup like volva of *Amanita chepangian*, (**g**) *T. microcarpus*, (**h**) *Ramaria botrytis*, (**i**) *Sparassis crispa*, (**j**) *Amanita chepangian*, (**k**) *Macrolepiota procera*, (**l**) *Cantharellus cibarius*. (Source: Semwal et al. 2014)

phylum in grasslands ecosystem are Acidobacteria (18.7%) followed by Actinobacteria (16.1%), than Alphaproteobacteria (11.4%), Betaproteobacteria (5.9%), Bacteroidetes, and Verrucomicrobia. Metagenomic analysis has confirmed the presence of bacterial phyla Proteobacteria, Acidobacteria, Verrucomicrobia, Actinobacteria, and Bacteroidetes at 25 grassland sites (Leff et al. 2015). The grassland soils of Pithoragarh (latitude 29°35′N and longitude 80°15′N, 1615 m abmsl) and forest soil of Almora (latitude 29°37′N and longitude 79°40′E, 1250 m abmsl) were reported to be rich in culturable Pseudomonas isolates. Functionally these 22 Pseudomonad isolates were characterized as having biocontrol potential against phytopathogenic fungi such as *Alternaria brassicae, Colletotrichum falcatum, Fusarium oxysporum f.* sp. *lycopersici, Gloeocercospora sorghi, Phytium ultimum*, and *Rhizoctonia solani f.* sp. *Sasakii* (Rajwar 2014).

8.9 Plant Growth-Promoting Bacteria in Himalayan Agroecosystems

High-altitude regions of NWH have extreme temperature conditions. The microbes inhabiting these places are well adapted to low temperature conditions. They are rich in cold adaptive enzymes. Thus, microbial abundance and diversity in such areas are of particular interest. Although, microbial species richness and diversity of the region are very high, very little has been explored (Pandey et al. 2006). Microbes of such habitats are difficult to culture and have slow growth rate (Greenland and Losleben 2001). The NWH region is a hot spot of biodiversity, and several new cold-tolerant bacterial species have been isolated from this region (Shivaji et al. 2005). Among the extremophiles, Psychrophiles are the most abundant in terms of biomass, diversity, and distribution in cold climate. Psychrophilic microorganisms have the unique ability to survive and grow at subzero temperatures (Satyanarayana et al. 2005). A change in the environmental conditions away from the optimum is a major factor limiting the productivity and geographical distribution of many species, including important agricultural crops in the hilly regions of NWH. Psychrophiles, psychrotrophs, and cold-adapted microorganisms are present virtually in all cold areas, and many of them also possess plant growth-promoting and biocontrol traits (Premon et al. 1996; Pandey et al. 2006). Microbial extracellular enzymes such as amylase, cellulose, chitinase, β-galactosidase, β-glucosidase, lipase, protease, pectinase, and xylanase with optimal activity at low temperature help in the adaptation of life in cold habitats (Yadav et al. 2016). Selvakumar et al. (2009) isolated *E. acetylicum* strain 1P from rhizospheric soil of an apple orchard located at an altitude of 2200 m abmsl in Almora district (29°36′N and 79°40′E) of NWH. The volatile compounds produced by *E. acetylicum* strain 1P were found to be a plant growth promoter as well as the potent inhibitor of phytopathogenic fungi including *F. oxysporum, R. solani*, *S. rolfsii,* and *Pythium*. Such highly adapted microbial strains have potential for use as biofertilzers in hilly areas. The selection of native functional plant growth-promoting microorganisms is a mandatory step for reducing the use of chemical fertilizers. Psychrotrophic bacteria such as *Pseudomonas corrugata, Pantoea dispersa,* and *Serratia marcescens* isolated from different location in the NWH possess potential plant growth promotion activity (Pandey and Palni 1998a, b; Trivedi et al. 2005a, b; Selvakumar et al. 2011). Similarly, *Pseudomonas* sp. strain PGERs 17 (MTCC 9000) isolated from garlic root sample grown in sub-alpine site in the North Western Himalayas (Pithoragarh, 1900 m abmsl) is another important isolate that could be developed as a suitable inoculants for winter season crops grown in the alpine and sub-alpine regions (Mishra et al. 2008). Plant growth-promoting bacteria (PGPB) found in NWH region includes genera, *Arthrobacter, Bacillus, Brevundimonas, Burkholderia, Citricoccus, Exiguobacterium, Flavobacterium, Janthinobacterium, Kocuria, Lysinibacillus, Methylobacterium, Microbacterium, Paenibacillus, Pseudomonas, Providencia, and Serratia*. Microorganisms belonging to genera *Arthrobacter, Janthinobacterium, Paenibacillus glacialis, Pseudomonas,* *Planococcus, Psychrobacter, Sphingobacterium*, and *Sporosarcina* produce certain secondary metabolites and enzymes which directly or indirectly influence the growth crop (Pandey and Palni 1998a, b; Pandey et al. 2001).

8.10 Conclusion

The Himalayan mountains are the source of remarkable ecosystems such as cold deserts, hot springs, glacier lakes, forest ecosystem, alpine meadows, and agroecosystem based on altitudinal and climatic variations. However, these diverse ecosystems harbor plethora of microorganisms including bacteria and fungi. The cold desert areas represent unique climatic conditions with negligible summer monsoon rains. Our analysis of the bacterial communities from cold deserts at different high-elevation areas showed that communities were dominated by similar phyla, particularly Proteobacteria, which were reported as dominant also in previous studies on snow from Arctic and Antarctica. However, each area hosted significantly different bacterial communities. Each of the unique ecosystem in Himalayas harbors distinct microbial flora. The alpine meadows harbor antagonistic proteobacteria especially Pseudomonads. Natural forests in mid hills and Tarai region have abundance of Acidobacteria, a considerably primitive phyla. Moreover, forest soils are rich in phosphate-solubilizing bacteria. *Bacillus*- or *Bacillus*-derived genera are ubiquitous and predominant under hot spring environment. The microorganisms residing in extreme environment are good source of enzymes to be used in agriculture and industry. The Himalayan region is home to *Morchella* and *Cordyceps* which are not reported in plains and plateau region of the country. These edible mushrooms have high medicinal value and are rich in antioxidants. Agriculture is dominantly practiced in the valleys and on terraces of lower and middle hills. These are famous for tropical and temperate food production. The common crops grown are millet, maize, and rice. Among fruits, apple is the dominant fruit, whereas peach, plum, apricot, pears, and walnut are also widely grown. Mountainous vegetation has manifold functions. It helps in regulating floods and flow in streams and combating the climate change and greenhouse effects. Presently soil erosion is the major concern for sustainable agriculture, forests, maintaining eco-friendly environment and livelihood for Himalayan people. Moreover, natural disasters and anthropogenic activities are causing a huge loss of biodiversity including microorganisms in the Himalayan region. The extinction of natural diversity possesses the challenge to explore, identify, and conserve the biological diversity including microorganism diversity. There is a need to investigate pristine habitats for microbial diversity. Such efforts can retard the impact of climate change and provide sustainable livelihood to the local populace. Therefore, an implication of appropriate strategy and action plan is needed for the conservation and management of the microorganism diversity.

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Biodiversity of Microbial Community: Association with Sustainable Hill Agroecosystems

9

Harshita Negi, Anju Rani, Samiksha Joshi, and Pradeep Kumar Sharma

Abstract

The hill environments cover about 27% of the total land surface of earth, and the Himalaya lies in North East India, comprised of 65% mountainous area. Agriculture is the primary source of income in hills; however, it has several intrinsic limitations, i.e. inaccessibility, underutilization of natural resources, climatic impact, and environmental limitations. There are various factors affecting the productivity of the hill agroecosystems including soil conditions, change in precipitation, temperature, and climate change in recent years. In addition, nowadays the traditional agriculture practices have been influenced by modernization which have ignored the key resource base of traditional crops in hills and resulted in considerable loss of microbial diversity. Microorganism in soil plays a crucial role in preserving the soil health, its productivity, and allover ecosystem functioning. The diverse population and metabolism of bacteria, fungi, and archaea enhance the availability of essential minerals and trace elements through their complex redox reactions and colonization into the soil. Considering the constrains and limitation of hill agriculture, it is essential to investigate about the microbial functional signatures, viz. energetics and regulatory circuits and structural genes regulating different metabolic pathways, which can directly

H. Negi
ONGC Energy Centre, SCOPE Minar, Delhi, India

A. Rani (✉)
Department of Life Sciences, Graphic Era (Deemed to be University), Dehradun, Uttarakhand, India

S. Joshi
Agriculture Microbiology, Crop Production Division, ICAR, VPKAS, Almora, Uttarakhand, India

P. K. Sharma
Department of Environmental Sciences, Graphic Era (Deemed to be University), Dehradun, Uttarakhand, India

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address to the potential of microbial functions that are related to agroecosystem processes. The advancement in the molecular techniques, viz. high-throughput metagenomics enabling the soil scientists to investigate the microbial communities in detail to harness their potential for productivity of hill agroecosystems.

Keywords
Hill agroecosystem · Microbial diversity · Nutrient cycling · Soil biota · Mineralization

9.1 Introduction

Biodiversity of microorganisms refers a vast array of the smallest forms of life, i.e. bacteria, archaea, and microscopic eukaryotes, that are ubiquitous in nature and estimated to contribute about 50% of the living protoplasm on earth. Microorganisms in fact considered as the first form of life on earth which were originated about 3–4 billion years ago (Pandey 2015). Microorganisms are the smallest but powerful enough, capable of harnessing a vast range of energy sources even in habitats of extreme heat, cold, salt, acidity, pressure, and darkness. Depending on the habitat studied, about 0.1–10% of microbial species are known so far (Panizzon et al. 2015).

Further, hill agroecosystem of Indian Himalayan Region (IHR) and Andean Highlands is restricted by a common set of environmental limitations, i.e. difficult terrain, little access to irrigation, low temperature climate, low fertile soils, threat of pests and pathogens, severe top soil erosion, etc. Moreover, small and marginal farm-holders have limited resources, for example, mechanized tillage, inadequate infrastructure, crop varieties, quality fertilizers, and modern irrigation facility (Yarzábal 2015). The agroecosystem mainly depends upon the diverse microbes that fertilize the soil by decomposition and recycling the organic matter, fixing nitrogen, enhancing the availability of essential minerals and trace elements, destroy soil pollutants, through their complex redox reactions. The various metabolic processes of microorganisms imparts the fundamental role in the sustainable functioning of agroecosystems through accomplishing its productivity (Bonkowski and Roy 2005). Many microorganisms also act as biocontrol agents of pests and diseases in agricultural crops and various plants, thus providing the fundamental underpinning of agroecosystem (Andreoti et al. 2009).

Furthermore, in high altitudes of agroecosystems, the psychrotolerant microorganisms colonize the soils and play important roles for promoting the plant growth; thus collectively called as plant growth-promoting microorganisms (PGPM). The cold-adapted PGPM impart the mobilization of soil nutrients and execute the inhibition of the growth of plant pathogens, ultimately resulting in increased productivity of hill agriculture (Pandey and Yarzábal 2018). Literature review revealed that recent research studies focused on the importance of the PGPM for sustainable agriculture in the hill agroecosystems. For instance, psychrotolerant fungi (*Aspergillus, Cladosporium, Penicillium,* and *Trichoderma*) with narrow or wide range of temperature tolerance reported in Indian Himalayan Region (Dhakar and Pandey 2015); psychrophilic diazotroph *Pseudomonas migulae* (Suyal et al. 2014), and psychrotroph *Pseudomonas palleroniana* reported in western Himalayan Region (Soni et al. 2015). The bacterial groups *Acidobacteria, Gemmatimonadetes, Chloroflexi, Planctomycetes, Actinobacteria, Bacteroidetes, Nitrospira, Firmicutes, Cyanobacteria, Chlorobi,* and *Verrucomicrobia* reported in North Western Himalayas (Yadav et al. 2015, 2016). Furthermore, even though there is continued use of agricultural practices in the limited area of agroecosystems, soil remains fertile and nutrient rich owing to plant growth-promoting and biocontrol properties of some microorganisms for a range of diseases and pathogens of agriculture (Andreoti et al. 2009). In addition, the recent research studies indicate that the microbial diversity of agroecosystem is considered as an indicator for the quality and productivity of its soil, as illustrated by metagenomics study (Orellana et al. 2018).

9.2 Soil Health for Sustainable Agroecosystem

Agroecosystem is an interaction between natural ecosystem (communities of plants and/or animals interacting with their physical and chemical environments) and human society which has been exploited by human to produce food and other commodities for consumption and other processing (Zhu et al. 2012). The health of any agroecosystem can be defined by seven parameters which include constancy, flexibility, diversity, complexity, efficiency, and equality.

With increasing degradation of ecosystem across the globe, it is advisable to pay more attention to the ecosystem health. The ecosystem and agroecosystem have similarities with each of them having its own specialty. The only difference between agroecosystems and ecosystems is its human interference in the former one.

Agroecosystem health research has the following features:

- Assessment of agroecosystem health
- Relationship between soil quality
- Water quality
- Co-relationship between agroecosystem health and the human health
- Role of Integrated Soil Management (ISM) and Integrated Pest Management (IPM) to agroecosystem health
- Effect of biological indicator on ecosystem
- Assessment of transgenic crops on agroecosystem
- Role and effects of agricultural practices

9.2.1 Soil Biota

Soil quality is determined by its biological, chemical, and physical properties (Dominy and Haynes 2002; Chen et al. 2003). Although soil organic matter (SOM) is the key factor for assessment of soil quality, however microbial activity is a chief indicator of soil quality. Microbial activity is the chief indicator in predicting changes in soil properties (Ruark and Zarnoch 1992). Microbial activity is a sign of microbiological processes of soil microorganisms (bacteria, actinomycetes, and fungi) present in different magnitude depending on the soil system.

Soil harbours numerous microorganisms and other living forms. The magnitude, diversity, and activities of these organisms differ from soil to soil, which in turn is governed by SOM content, soil texture, pH, temperature, moisture content, aeration, etc. (Subler and Kirsch 1998; Spaccini et al. 2002). Among all soil microorganisms, bacteria are the most abundant organisms followed by actinomycetes, with least count of fungi and algae (Chen et al. 2003). Dominance of any organism, in particular, microenvironment depicts adaptation of this form in existing environmental conditions. The uppermost layer of soil shows an abundance of microorganism due to availability of organic food in the form of dead and decayed plant litter (Ruark and Zarnoch 1992; Spaccini et al. 2002). Soil microorganism performs numerous activities which include mineralization of organic nutrients, processing of plant litter into soil organic matter, which in turn help in the uptake of inorganic nutrients by plants and improved soil quality (good aeration and soil aggregation) by reducing soil bulk density, respectively (Franzluebbers et al. 1999; Dominy and Haynes 2002; Spaccini et al. 2002).

Natural ecosystem possesses nutrients in balance in the soil. Cropping of soil results in loss of these nutrients like carbon and nitrogen. Replenishment of these nutrients (lost by plant uptake) is required to maintain productivity of soil. Cropping is dependent on soil microorganisms to form and decompose SOM (soil organic matter) as an incessant nutrient supply (Subler and Kirsch 1998). Some agricultural practices such as application of sewage sludge, manure, or compost positively influence soil microbiological activity (Benckiser and Simarmata 1994). Thus assessment of SOM (by quantification of C & N fixation) will be an indicator of soil microbial activity which in turn indicates soil quality (Yamakura and Sahunalup 1990).

- Each organism in soil plays a crucial role in preserving the soil health, its productivity, and allover ecosystem functioning.
- The activities of certain organisms such as worms and termites enhance porosity of soil by their activities thus affect soil structure.
- Soil biota plays a key role in decomposition process and nutrient cycling.
- Certain soil organisms act as a biocontrol agents and thus protect crops from pest attack.

- Many microbes (*Rhizobium* and *Mycorrhiza*) shows symbiotic association with plants and help in uptake of water and mineral nutrients, therefore help in growth of plant biomass.
- Various soil organisms also help in remediation of pollutants and thus prevent their accumulation in soil.

9.2.2 Nutrient Cycling Associated to Microbial Community

The mountain ecosystem play a vital role as 10% of world's population depends upon mountain resources such as water, agricultural products, and minerals (United Nations 2001). In hill ecosystem, low soil temperature, elevated moisture, and less rate of evapotranspiration in most of the growing season lead to slow litter decomposition and low turnover of soil organic matter. Therefore due to persistent cold and wet soil conditions, microbially mediated activities such as breakdown and nutrient mineralization become limited and generate an ecosystem "bottleneck" (Chapin 1980). The reduced rate of nutrient supply to plant roots results in slow plant growth low rate of nutrient cycling between plants and soils.

Soils attribute in sustainable agroecosystems being a habitat of remarkable biodiversity leads to more productivity of crops. Soil microbial diversity (bacteria, fungi, archaea, and protists) is sustained in soil by nutrients released by the plants (as root exudates) and decomposition of organic waste (Paungfoo-Lonhienne et al. 2012).

There is urgent need to manage sustainable agroecosystems for efficient nutrients recycling thus, limiting dependence on additional nutrients and energy resources. Soil biota convert organic forms of N, P, and S into soluble NH^{4+}, NO^{3-}, $H_2PO_4^{-}$, HPO_4^{2-}, and SO_4^{2-}, the ionic forms absorbed by root cells. Absorption of ionic form of nutrients results in high yield of productive crops (Whalen 2014).

Therefore, it is essential to know the role of soil biota (microbes & macrofauna) in decomposition and mineralization processes occurs in soil (Fig. 9.1). The biological conversion of organically bound nutrients to soluble ions is a two-step process that proceeds as follows (Whalen et al. 2013).

(a) Decomposition: Breakdown of organic matter into smaller particle size which in turn results in more surface area. More surface area facilitates microbial attack and hydrolysis of smaller particles into monomeric units which gets adsorbed on microbial surface for further action.
(b) Mineralization: The intracellular enzymes of microbes help in breakdown of adsorbed monomeric units for assimilation and energy release. Once the energy need of microbial cells is fulfilled, excess ions can be released into the soil upon cell lysis and make ions accessible for plant uptake.

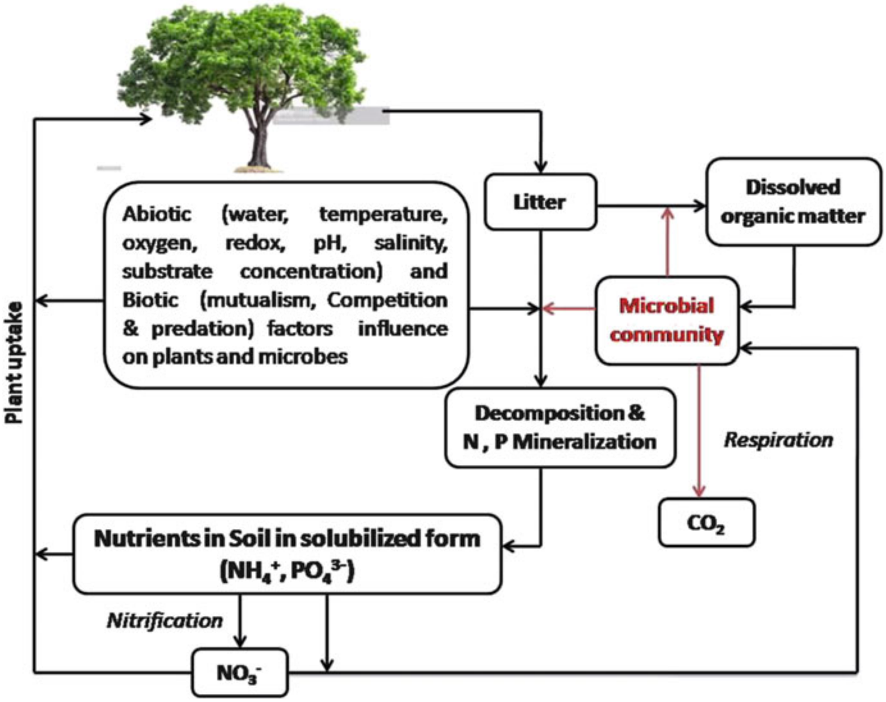

Fig. 9.1 Conceptual diagram of factors affecting decomposition and mineralization of organically bound nutrients and nutrient uptake by plant

9.2.3 Soil Health Management

Agricultural land management greatly alters soil characteristics in terms of physical, chemical, and biological properties (Jangid et al. 2008). This is particularly important in case of unsuitable land management and climatic variation/limitation (prevailing drought conditions, scarcity of rainfall) which leads to soil erosion, loss of fertility, and decrease in soil biodiversity (Caravaca et al. 2002). Soils put through tilling can be more prone to loss of soil microbiota due to desiccation, mechanical destruction, soil compaction, and less availability of nutrient resources (García-Orenes et al. 2013). In view of the above, soil health management is necessary to enhance soil microorganisms and microbial processes by altering quality and quantity of plant residues in soil system. Although some studies have reported the use of organic fertilizers (manure and sewage sludge) to enhance the activities of soil microbial communities (Enwall et al. 2007; Ruppel et al. 2007) however, frequent application of manures may introduce faecal microbial flora into soil which in turn can change endogenous microbial flora.

To manage soil health, it is mandatory to develop and implement novel sustainable agricultural practices. Although many practices have been employed to reduce erosion rate and for enhancing soil quality and aggregability (Garcia-Orenes et al. 2009, 2013), it is important to study their impact on microbial diversity and structure. Alteration in the soil microbial community can be studied using PLFA, t-RFLP, RFLP, DGGE/TGGE, and RISA/ARISA molecular techniques (Muyzer and Smalla 1999). Application of these techniques can give information that can be used to measure how environmental factors/agricultural practices contribute to changes in microbial community structure.

9.3 Microbial Biodiversity of Mountains/Hills

The hill environments cover about 27% of the total land surface of earth and are habitat of a great diversity of microbial species (Convention on Biological Diversity 2019). The hill ecosystems tend to host species richness and endemism (species being unique to a defined geographic location) as compared to the lowlands.

Exploring the extent of microbial biodiversity of the entire IHR, ranging from the Kashmir to Ladakh (lies at northeast) and to Zanskar (lies in east), reported as the hotspots of exclusive microbial biodiversity, owing to the different geographical entities. In Ladakh region, dry high plains and saline soils are a huge resource of halophilic microorganisms; however, the major high altitude areas of Kashmir Himalaya are a great source of psychrophilic microorganisms with potential source of cold active enzymes. The greater part of the Himalayas that lies below the snow line harbour mostly mesophilic bacteria, actinomycetes, potential endophytes, and symbiotic fungi (Hassan 2015).

However, Himalaya that lies in North East India comprises 65% mountainous area. In these mountain ecosystems, the endemism levels are comparatively higher, particularly at medium elevations in the tropics and warmer temperate latitudes. However, the hills at the lower altitudes support the exceptional biodiversity owing to ample range of ecosystems into a relatively small area. Therefore, Himalaya is one of the "hotspots" of biodiversity due to great variations in the climatic conditions, topography, and geography resulting in a wide variety of habitats ultimately responsible for the colonization of microorganisms (Joshi et al. 2017).

9.3.1 Bacterial Community

Based on the microscopic identification for cell shape, metabolism, Gram's staining, and genetic identification, over 11,000 species of bacteria have been identified. Bacteria is most dominating group of microorganism inhabiting the agroecosystems. Diverse group of bacteria studied from hill agroecosystems, reported as mesophilic, thermophilic, and psychrophilic in nature, which also act as plant growth promotory at certain temperature.

The literature review illustrating that the diversity and abundance of bacterial communities are seen to be influenced by altitudinal gradients (Young and Crawford 2004). The higher bacterial diversity at medium elevations on the Mount Lushan, located in the Central China, has been reported by Meng et al. (2013); however, higher bacterial diversity has been reported at higher altitudes in comparison to medium altitudes on Mt. Halla in South Korea (Singh et al. 2014). However, Zhang et al. (2013) do not support the above given fact. Fierer et al. (2013) illustrated that there was no significant differences in soil bacterial diversity along altitudinal gradients in Southwestern highlands of Saudi Arabia and Appalachian Mountains of Eastern Peru, as revealed by the pyrosequencing results. Furthermore, the factors responsible for these variations are still unknown (Shen et al. 2013). Using qPCR and Illumina sequencing, Siles and Margesin (2016) reported higher bacterial abundance at higher altitudes in Alpine forests. Therefore, literature review illustrates that along with high altitude there is no specific geographical pattern of soil bacterial diversity.

For instance, mesophilic bacterial species reported from North Western Himalayas in India, belonging to *Acidomonas, Enterobacter, Acetobacter, Campylobacter, Acidophilum, Acinetobacter, Actinomyces, Aerococcus, Listeria, Alcaligenes, Azotobacter, Rhizobium, Bacillus, Carnobacterium, Cellulomonas, Burkholderia, Corynebacterium, Flavobacterium, Kurthia, Mesophilobacter, Micrococcus, Renibacterium, Streptococcus, Enterococcus, Lactobacillus,* and *Kocuria* (Thakur et al. 2015). Thermophilic bacterial species, viz. *Bacillus licheniformis*, *B. sonorensis*, *B. tequilensis, Paenibacillus ehimensis*, and *Staphylococcus epidermidis* reported from Soldhar and Ringigad hot springs under the Indian Himalayan Region located in Uttarakhand state of India (Pandey et al. 2014). Psychrophilic Gram-negative bacteria *Serratia marcescens,* reported from 29°36′N and79°40′E, altitude 1600 m masl (metres above sea level) for plant growth-promoting activity at 4 and 15 °C temperature (Selvakumar et al. 2008). Bacterial species *Azotobacter chroococcum* and *Azospirillum brasilenses* reported at subtropical (1200 m altitude) for an increase in plant growth (Trivedi et al. 2012). Bacteria of *Pseudomonas* species reported from cold deserts of Lahaul and Spiti for increasing the growth of sea buckthorn (Gulati et al. 2008, 2009, 2010).

Seasonal variations found to affect the bacteria diversity in the agroecosystem. For instance, in the Colorado Mountains, the division Acidobacterium was found to be most abundant in spring season while in winters, the community of Actinobacteria and members of the Cytophaga, Flexibacter, and Bacteroides division were dominated. However, some species like α-proteobacteria were seen to resist temperature fluctuations (Lipson and Schmidt 2004). Diversity of fluorescent *Pseudomonas* reported from the rhizospheric ecosystems of cold hilly states of India, i.e. Himachal Pradesh and Uttarakhand (Negi et al. 2005; Shanmugam et al. 2008).

The plant growth promotory activity were found to be induced by different genera of bacteria in the agroecosystems (Table 9.1), either through fixation of atmospheric nitrogen, production of plant growth regulators (hormones), solubilization of minerals, siderophores production, bio-stimulation, and biocontrol activity or by a combination of any of these mechanisms.

Table 9.1 Bacteria species contributing for plant growth in hill agroecosystems

Bacteria	Location	Crop	Reference
Pseudomonas sp.	Western Indian Himalaya	Bean *(Phaseolus vulgaris)*	Suyal et al. (2015)
Providencia rustigianii, Pseudomonas cedrina, Pseudomonas deceptionensis, Pseudomonas fragi, Pseudomonas moraviensis, Psychrobacter frigidicola, Sanguibacter suarezii and *Yersinia ruckeri*	North-western Himalayas	Wheat (*Triticum* sp.)	Yadav et al. (2015)
Bacillus sp.,*Pseudomonas* sp.	Indian Himalaya Region	Jhum cultivation	Pandey et al. (2011)
Pseudomonas sp.	Indian Himalaya Region	*Dianthus caryophyllus* L.; *Camellia sinensis* L.; *Hippophae rhamnoides* L.; *Vigna mungo* L.	Gulati et al. (2010)
Serratia marcescens	29°36′N and 79°40′E, altitude 1600 m	Summer squash (*Cucurbita pepo*)	Selvakumar et al. (2008)
Azotobacter chroococcum, Azospirillum brasilense	Sikkim Himalaya	Maize (*Zea mays*)	Pandey et al. (1998)
Bacillus subtilis, Bacillus megaterium and *Pseudomonas corrugata*	Indian Himalayan region	Tea	Trivedi et al. (2005, 2012)

9.3.2 Archaeal Community

Hilly areas of higher altitudes have been recently identified as environments rich in archaeal diversity. Ecological distribution and metabolic diversity of archaeal community is found to be limited compare to the bacterial diversity. Siles and Margesin (2016) reported archaeal community to be dominated by *Thaumarchaeota* at Alpine forests over an altitudinal gradient of 545–2000 m using qPCR and Illumina sequencing. Several studies suggest that Archaea contribute a key role in ammonia oxidation in the alpine as well as freshwater lakes (Vila-Costa et al. 2016). Auguet and co-workers (2012) also observed that the water–air interface in the high mountain lakes were suited for the development of *Thaumarchaeota*, a phyla closely related to ammonia oxidizers. Thus, freshwater lakes of high mountains are found as natural laboratories of great interest, which can significantly improve the current knowledge of archaeal ecology and their biology (Auguet and Casamayor 2013).

9.3.3 Eukaryotic Community

Microeukaryotes are crucial components of hill agroecosystem and contribute highest genetic diversity within the Eukarya domain. Among microeukaryotes the microalgae were found as important components of soil environments, specifically in semiarid and arid zones. They have various important functions such as improving soil structure, prevention of soil erosion (Kubečková et al. 2003), and enhancing the establishment of plant seedling (Belnap and Eldridge 2001) etc.

The single cell eukaryote, i.e. fungi is the second dominating group of microorganism inhabiting hill ecosystems after bacteria. Furthermore, fungi are the most diverse and largest group of living organisms with species richness thus contribute higher biomass in the soil as compared to bacteria. Among all, *Penicillium* is reported as the most abundant and diverse genus present in the rhizosphere at higher altitudes of Himalaya located in Sikkim and Uttarakhand, India. Furthermore, commonly occurring species of *Penicillium* in Himalayan ecosystems were *P. janthinellum, P. raistrickii, P. chrysogenum, P. pinophilum, P. oxalicum, P. aurantiogriseum, P. javanicum,* and *P. purpurogenum.* Other genera reported to contribute to the fungal flora of Uttarakhand Himalayan region were *Aspergillus, Myrothecium, Epicoccum, Cladosporium, Fusarium*, *Paecilomyces, Gangronella,* and *Trichoderma* (Rai and Kumar 2015).

From the Eastern Himalayan range, major diversity of fungi reported from phyla Zygomycota and Ascomycota. Further, seven dominant orders corresponding to these phyla are Pleosporales, Hypocreales, Eurotiales, Calosphaeriales, Mortierellales, Capnodiales, and Mucorales. *Eurotiomycetes* reported as the dominant species from four altitude ranges (1–500, 500–1000, 1000–1500, and 1500–2000 m) followed by *Sordariomycetes* (Devi et al. 2012). The symbiotic association of plants roots with fungi is the main promoting factor for the plant growth in hill agroecosystems. Arbuscular mycorrhizal fungi reported mainly from North East Himalaya support the Jhum crops (Talukdar and Thakuria 2015).

9.3.4 Metabolic and Genetic Diversity

Microbial community is the richest stockpile in molecular and metabolic diversity, providing basis for all ecological mechanisms, i.e. food chain, biogeochemical cycles, and maintenance of dynamic relationship with higher organisms (Hunter-Cevera 1998). Further, the metabolic diversity of microorganisms is significantly important for the proper functioning of the agroecosystem to maintain ecological processes, i.e. nutrient cycling, soil aggregation, organic matter decomposition, and controlling pathogens within the ecosystem. Several studies have been preferably focused on the functional traits rather to just taxa (Fierer et al. 2013). This will help to provide a better understanding of biogeochemical cycles, other ecosystem functions, and microbial responses for change in environmental factors (Reiss et al. 2009). Nowadays, it is also important to investigate about the microbial functional signatures, viz. energetics and regulatory circuits and structural genes regulating different metabolic pathways, which can directly address to the potential of microbial functions that are related to agroecosystem processes.

Advancement in the molecular techniques, viz. high-throughput metagenomics has enabled scientists to investigate these microbial communities in detail (Lu et al. 2012). Despite of advancements in molecular approaches, most of the studies were focused on the taxonomy of microbial community (Dick and Tebo 2010), and profiling of microbial distribution at the phylogenetic level (Wang et al. 2012), however, no study has been reported on the functional gene level of microbial community.

The genome of the whole microbiota collectively found on earth, termed as metagenome which contains all genetic content including the uncultivable and culturable subset as well (Handelsman et al. 1998). Sequencing of microbial DNA isolated from agroecosystems (metagenomic studies) revealed the existence of a tremendous variety of yet uncultured microorganisms, indicating that the true microbial diversity in nature is much higher than currently identified. The genetic information illustrated from the metagenomic studies provides the structure, evolution, and origin of genetic material, which could be applicable for the benefit of environmental stability. Therefore, literature review indicates that the microbial diversity is directly related to stability of the agroecosystem (Yamanaka et al. 2003).

9.4 Hill Agroecosystem

Diverse agroecosystem of the Himalaya is a consequence of biological, social, economic, and environmental factors existing there. Agrobiodiversity covers a vast array of biological resources linked with agriculture (Palni et al. 2001). Among the inhabitants of Himalayan region, most of the communities sustain through farming. Out of total Himalayan region, 15.8% are slightly rainfed and irrigated farmland, whereas 69% is occupied by rangelands, pastures, wastelands, and rest 15.2% includes rocky mountains and mountains covered by snow permanently (Pratap 2011). Therefore, the primary source of income of the inhabitants is through agriculture sector (45% of the total regional income).

9.4.1 Traditional Hill Agroecosystem

In the hills, traditional agroecosystems have been replaced by modern agroecosystems to increase yield and economy, which has resulted in a considerable loss of biodiversity. Present agricultural practices have ignored the key resource base of traditional crops by focusing more on new technologies using external resources. Preference of technologies over traditional agricultural practices has resulted in loss of biological diversity along with danger to the natural ecosystem. Maintaining flexibility in both human and ecological systems to optimum level is the most feasible way to adapt to climatic variation (Palni et al. 2001).

9.5 Factors Affecting Hill Agroecosystem

As per climate change report, the temperature and precipitation will rise 1.4% and 3%, respectively, by 2020 in India. This will lead to floods, droughts, cyclones, and forest fires (IPCC 2001). The hilly areas usually are rigorously affected by large-scale deforestation, soil erosion (micronutrients loss), scarcity of water, acidic soil, high intensity rainfall, snow and frost, and lack of infrastructure in the hostile topography. Therefore, adaptation is vital as climate change can cause undesirable impacts on the agriculture sector and adaptation can reduce the vulnerabilities.

A number of studies have proven that the microbial distribution in soil is dependent on soil properties, environmental factors such as climate and topography (Griffiths et al. 2011; Terrat et al. 2017). The regulators of soil biota distribution can vary in different ecosystems. A study carried out by Dequiedt et al. (2009), in France, concluded that the soil properties and land cover influence the spatial distribution of bacterial communities more than topography and climate change. A study carried out by Chen et al (2015) suggests that the biographies variation of the microbial population of soil is more linked with precipitation and soil factors (Fig. 9.2).

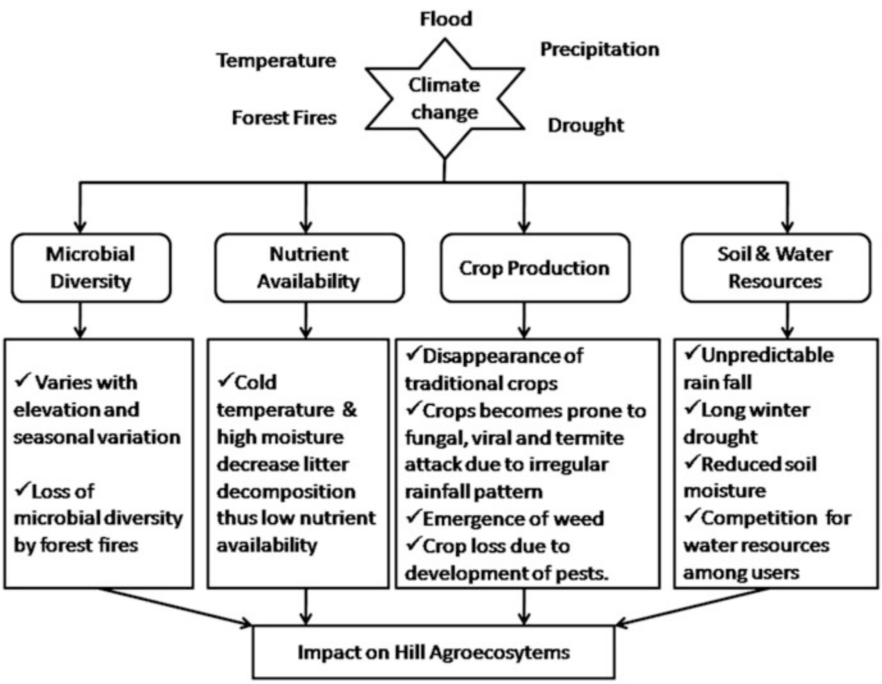

Fig. 9.2 Effect of climate change on the hill agroecosystems

9.5.1 Temperature

Temperature is a major factor affecting hill agroecosystems as it is observed to decline with elevation. Elevational gradients of seven different temperate regions were studied and reported with constant responses of ecosystem properties to elevation (Mayor et al. 2017). Reports showed that with decreasing elevation availability of soil nitrogen for plant growth increased which means that warming may enhance nitrogen availability in plants. However, availability of phosphorus was not found to be controlled by elevation. Ratio of nitrogen to phosphorus availability was found to be constant across different elevation gradients, whereas varied greatly across low elevation regions. This indicates that the balance is mainly governed by temperature which varies according to elevation. Furthermore, it was found that availability of nutrients to plants at different elevations were also linked to soil organic matter, quality of organic matter, and microbial composition (Mayor et al. 2017).

9.5.2 Climate Change

Hill agroecosystems is considered as one of the unique systems to study climate changes and its associated impacts. The reason behind mainly includes the rapid changes that occur with elevation over relatively short horizontal distances, the hydrology and vegetation (Whiteman 2000). Climate change projections indicated a significant variation in global warming among different regions; particularly, at high latitudes and elevations (Gobiet et al. 2014). Recently, IPCC highlighted high sensitivity of hills with respect to climate change in its latest report. Furthermore, this may cause loss in the habitat and biodiversity, deterioration of freshwater quality, landscape modifications, etc. (Huss et al. 2017). The response may vary with geographical region, rate of climate change, and ecological domain (Beniston 2003).

9.5.3 Farming Technique

Over the past several decades, hill agroecosystems were well known for following traditional farming practices, where people worked on the major land portion and ploughed with bullocks(Pandey 1982). Traditional farming system has become complex which includes livestock husbandry, forestry, and arable cropping where changes within any one of the components will significantly affect the others (Mahat 1985). High level of climatic changes and difficult topography makes this farming system extremely labour intensive. Majority of farmers in hills have small and marginal arable land holdings where they practice integrated farming. Integrated crop-livestock farming system is integral part of hill agricultural production systems. This system is an economically and environmentally sound diversified production system in hills. Two major types of land pattern observed among hill agroecosystems are rainfed *bari/pakho* and irrigated *khetland*. The rainfed *bari/pakho* is mainly followed on sloping terraces and unirrigated valley bottoms whereas *khetland* is usually distributed along the river banks and which is bunded for flooding under different irrigation systems. Khetland is found to be more productive, with good cropping potential as compared to bariland. However, land with rainfed pattern far exceeds irrigated one, which makes bariland more valuable in the regions where it is available. Availability of small-scale irrigation schemes can lead to possible conversion of bariland into khetland for farmers at lower altitudes. Cropping practices commonly followed are maize-based on the non-irrigated bariland and rice-based on irrigated khetland.

9.6 Conclusion

Agriculture in hilly areas is not accessible to modern technologies due to topographical variation and socioeconomic constraints. Moreover, hill agriculture also has intrinsic limitations such as inaccessibility, underutilization of natural resources, and climate impact. The declination of natural resources at significant rates due to existing environmental conditions demands restoration of hill agroecosystems employing sustainable technologies. Although farmers of hilly regions are using traditional knowledge/technologies to get accustomed to climatic variations at temporal and spatial scale, yet intervention of scientific and technical approaches is necessary.

Hill ecosystem is an abundant reservoir of microbial diversity comprising bacteria, fungi, archaea, and actinomycetes population. The distribution of microbes depends upon various factors, for example, soil properties, rainfall pattern, topography, and agricultural practices. Among various factors available, nutrients in soil influence microbial diversity most. The microbial population which are well adapted to extreme conditions prevailing in hill agroecosystems help in maintaining soil health and plant growth by facilitating nutrient uptake via decomposition and mineralization processes.

9.7 Future Prospects and Recommendation

Hill agroecosystems are significantly important as contributed to the significantly higher microbial diversity with wide range of adaptability. The diverse microbial community imparts various roles in the productive and sustainable farming at the hill agroecosystems. However, owing to intrinsic limitations, farmers in hilly areas should adopt an integrated farming system, which involves simultaneous management of land, water, vegetation, farm animals, and human resources. The use of integrated farming system in hilly areas can prevent soil erosion and can increase cropping potential of soil. In view of sustainable management of hill agroecosystem, there is an urgent need to implement appropriate multidisciplinary approaches. Although various studies have been done to study microbial diversity of hilly areas, yet specific investigation is required to know the effect of physical, chemical, and biological obstacles to the conversion of organically bound nutrients into an inorganic form. Apart from this, it is also necessary to know the effect of biotic and abiotic factors on microbial activities. These studies will assist farmers to manage their agricultural practices accordingly and to know the amount of chemical fertilizers to be added in case of insufficient nutrient supply through microbial activity.

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Soil Microbiota: A Key Bioagent for Revitalization of Soil Health in Hilly Regions

10

Amir Khan, Viabhav Kumar Upadhayay, Manisha Panwar, and Ajay Veer Singh

Abstract

Soils of hill regions encompass diverse organic and inorganic substances, where nutrient cycling of these matters and associated factors maintain soil fertility. However, due to rigorous anthropogenic activities and constant exposure of cold temperature the biological behavior of hilly soil get transformed which makes soil more acidic with low nutrient availability. These are the key factors for diminution of plant growth performance and productivity in the hilly regions. In the present era, various strategies such as agricultural interventions, use of chemical fertilizers, etc. are being practiced to improve the soil health. Such strategies are labor demanding, expensive, and affect the ecosystem negatively. Such practices in long term can change the physical, biological, and chemical properties of soil, and may also degrade the quality of soil by lowering down its fertility. On the contrary, microorganism based practices have the potential to restore soil health quite efficiently without disturbing the soil ecosystem. Soil-dwelling microorganisms can promote organic matter management, nutrient cycling, soil aggregation, and soil fertility by means of various mechanisms for sustainable agriculture. These mechanisms include nitrogen fixation, mobilization of phosphorus, potassium, zinc, and iron by the frequent secretion of assorted organic acids and low molecular weight metal chelators, i.e., siderophore. Moreover, soil microorganisms improve soil stability by producing organo-polysaccharides, which act as a gluing agent for soil aggregation. Therefore, microbial inoculation in the soil is considered a striking strategy for maintaining soil health without deteriorating soil physicochemical properties. This manuscript focuses on the splendid role of soil microorganisms in nutrient cycling and their implication as a

A. Khan · V. K. Upadhayay · M. Panwar · A. V. Singh (✉)
Department of Microbiology, College of Basic Sciences and Humanities, GBPUA&T, Pantnagar, Uttarakhand, India

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sustainable management strategy for reinstating the fertility of hill regions soil for less reliance on chemical or artificial fertilizers.

Keywords
Psychrophiles · PGPR · Soil health · High hills · Integrated nutrient management

10.1 Introduction

Since prehistoric times, agriculture is the main center for supply of food and employment for major population residing in the hilly regions. The geographical regions of Jammu and Kashmir are mountainous, where agriculture contributes to about 65% of the state revenue. Hilly region encompasses extensive altitudinal variation that attributed to the variability in the ecosystem including variation in altitude, temperature, soil texture and composition, nutrient accessibility, and microflora composition. Generally, the rotational farming is the major practice at hilly regions, but regions with very high altitude where the extremely cold environment prevails provoke employment of monocultural farming which can only be doable in summer. Maize, rice, pulses, millets, wheat, fruit crops, and oilseeds are the major food crops grown in the higher region of hills. Besides, farmers also cultivate some underutilized crops like buckwheat, grain amaranth, black cumin, and saffron. Sustainability and high yield agricultural cropping system chiefly depend upon the native climatic conditions, soil fertility, soil texture, and available nutrients. However, soils of higher altitude are typically characterized as acidic in nature and possess low nitrogen and phosphorus accessibility considered as the principal cause for elevation in disease incidence and reduction in crop growth and productivity. Shrestha et al. (2004) stated that every year about 32 t/ha of topsoil is degrading and leads to a reduction in maize productivity. World's population primarily depends on crop-based nutrients diet, but crops with low nutrients value cause malnutrition and health-related risk (Forouhi and Unwin 2019). Therefore, several plant breeding techniques and anthropogenic activities such as intensive use of pesticides, herbicides, chemical fertilizers, and growth hormones are practiced to reinforce the soil health and crop production in all over the world. The main drawback of nutrient fertilizers application in the soil is their large proportion rapidly convert into fixed form in soil and hence become unavailable for plants (Upadhayay et al. 2018). Moreover, chemical-based fertilizers also show negative impacts on soil health as the rampant application of such fertilizers leads to the descent in soil fertility and native soil microbial ecology.

A number of efforts are taken by the governments of many countries to address the problem and for getting an effective and long-lasting solution to augment the nutrient availability, fertility, stability in soil, crop yield, and ecosystem sustainability. Therefore, the application of potential microflora such as bacteria, fungi, and actinomycetes can endorse plant growth by the revitalization of unavailable nutrients of the soil (Yadav et al. 2016). Such microflora restore nutrients availability and fertility in soil by means of various types of mechanisms such as atmospheric nitrogen fixation, solubilization of phosphorous, potassium, silicon, and zinc by acid production, chelation of metals (iron, zinc, magnesium, etc.) by secreting ionophores such as siderophores (Parveen et al. 2018). Soil microorganisms also secrete various extracellular enzymes essentially required for degradation of polymers and also for the transformation of inorganic nutrients by oxidation or reduction. Moreover, soil associated microbiota secrete exopolysaccharides (EPS) and some gluing agents that promote the aggregation of soil. Production of phytohormone and various metabolites by the soil-dwelling microorganisms assists in plant growth and also reported to confer resistance to the plant from the pathogen attack and also improves the soil health (Bargaz et al. 2018). Indigenous microbial strains of hill region are well adaptive for the native climatic and soil conditions as they can acclimatize easily and therefore they can participate in nutrient management for efficient plant growth promotion. Hence, the application of indigenous microflora is advantageous for nutrient recovery and sustainable agriculture.

10.2 Importance of Soil Nutrients

The top few inches of earth's crust composed of innumerable organic and inorganic elements providing a favorable platform not only for plants growth but also suitable for flourishing growth of microbes. Among the animal-plant-soil continuum, still the soil is the most neglected one. Moreover, various anthropogenic activities are also resulting in degeneration of soil quality in hill regions. Soil nutrients directly regulate the hormonal status of plants, which in turn regulates the physiological properties of plants. Moreover, the standing nutrient condition of the soil dictates the distribution of vegetation in geological regions of hills. Besides, it also affects physicochemical assets of soil, viz. friability, water holding capacity, bulk density, etc. Plants require nutrients for their metabolic activities and growth. Deficiency of any nutrient triggers diseases development and negatively affects the plant growth. Similarly, soil microbiota also require nutrients (micro and macronutrients) for their growth. There are 18 elements which are reported essential for plant growth, classified broadly into macronutrients (C, H, N, O, S, P, K, Ca, and Mg) and micronutrients (Fe, B, Zn, Cu, Mn, Mo, Cl, Co, and Ni) according to their required quantity of nutrient. Each element has its specific function and their physiological role has been well characterized (Table 10.1). To start with, carbon is the major element of plant skeleton and essentially required for their metabolism. Availability of nitrogen is a crucial factor for plant metabolic activities and essential part of the protein, DNA, hormone, and chlorophyll. Phosphorous is important for energy generation, while potassium, boron, and silicon regulate the disease resistance (Singh et al. 2011; El-Ramady et al. 2014). Micronutrients are accountable to maintain the osmotic pressure and relative continuum inside the plant cell. In addition to nutrition, the higher concentration of micronutrients than their threshold level also affects plant growth through toxicity leading to disease development.

10.3 Factors Affecting Nutrient Status of Soil

The dynamics of soils in hilly terrain is very complex in many ways. A huge hill region is endlessly experiencing degradation of soil, accountable for diminution of soil inherent capacity to support the metabolic activities for plant growth due to the deficiency in the nutrient reservoir of soil. The nutrient status of soil holds the most prominent role in this regard. Therefore, it is an important issue for serious debate and research. There are several factors such as temperature, altitudinal gradient and precipitation, agricultural practices, and microbiological processes, which influence the carbon pool, nutrients cycling, fertility, growth pattern, subtle moisture adherence, seedbed hold, and several other prominent effects in hilly agriculture. Numerous studies are done by various researchers so far but there is still uncertainty on the master factor regulating nutrient management and plant distribution. Diverse abiotic and biotic environmental features are responsible for determining the nutrient composition of the soil and can be categorized into broad areas (Zu et al. 2018).

10.3.1 Temperature and Altitude Gradient

Increase in the elevation temperature decreases accordingly. Therefore, a variation in climatic conditions can be seen at different altitude range that directly affects the soil properties. Continuous exposure of low temperature declines the biological transformation rate of soil nutrients, which results in reduced accessibility for nutrients. However, altitude gradient is also blessed with variation in landscape, i.e., broad or steeper landscape. Thick and nutrients rich soil horizons are generally found in broad hill landscape due to the high filtration rate occurring through the rapid flow rate of water, whereas steeper landscapes form thin and nutrient deficient soil horizons due to the low flow rate and higher leaching.

10.3.2 Agricultural Practices

The soil nutrients pool is critically based on cropping system in the agricultural regions. Repeated and monoculturing cropping pattern reduces the magnitude of some nutrients in the soil, making imbalance in the nutrient reservoir of soils. Whereas, an unscientific practice, i.e., jhum or shifting cultivation also drastically degrades land and reduces its quality. It involves cutting of forest, bushes on steeply slope, and then cultivating on such land for 2 or 3 years and after that, they burn the land and depart from there for the selection of a new site to repeat the same process. It results in the reduction of forest cover that leads to soil erosion and reduces water holding capacity. Moreover, it also reduces carbon (C), nitrogen (N), and other nutrient content of soil (Sati and Rinawma 2014). Sharma (1998) estimated that in Northeastern Himalayan region experiencing loss of 88,346 tons topsoil, 10,669 tons of N, 0.372 tons of P, and 6051 tons of K is due to the shifting cultivation.

10.3.3 Soil Microbiota

Besides the above factors, soil microbiota (bacteria, fungi, actinomycetes, etc.) also regulate the soil properties and considered as the functional backbone of the ecosystem, resides in soil and performs essential functions for the management of soil health and ecosystem sustainability. But their composition varies through different climatic conditions. A healthier soil retains its C:N ratio to 10:1. But due to low-temperature the decline in availability of nitrogen leads to high C:N ratio in soil, which slows down the microbial decomposition rate (He et al. 2016). Generally, only cold-adaptive and cold tolerant microorganisms can prevail in hill region due to the low-temperature persistence (Kumar et al. 2019). They acquire their nutrition from the soil and lead to the transformation of various insoluble macro and micronutrients, along with the balance organic matter of soil by their unique cold-active enzymes and make soil fertile to support the development of the plants.

10.4 Properties of Soil at Higher Altitude

Properties of soil are an important aspect because it demonstrates the class and health of soil. Healthy soil is a fundamental component for crop production. Temperature, moisture, and precipitation rate are variable at an altitudinal gradient of mountains. Generally, soil nutrients, texture, and horizon depth are governed by these factors. Generally, hills with a broad and gentle lands experience high vertical water movement, thereby forming a thick layer of soil horizon, whereas steeper hill forms thin soil horizon due to reduced water movement. Majority of Himalayan regions, hill regions of Africa, Bangladesh, and Ethiopia are characterized as a stressful climate with low temperature and soil with acidic in nature, and hence restricted the microbial conversion of N and P, leading to dearth of nitrogen and phosphorous content. Moreover, the soils of such regions also possess the toxicity of manganese and aluminum that is the major cause of poor soil fertility and reduced production. The soils of cold desert areas such as Leh-Ladakh hills are suffering through high salinity stress and are sandy and clay type, coarse-textured, poor in water retaining capacity and mineral nutrients, and have low biological activity. Diverse altitudinal ranges of Himalaya are characterized by unique climatic conditions that govern the different rainfall level, snowfall, organic matter decomposition, soil texture, mineralogy, and fertility. Himalayan soil is generally sandy loam and clay type and acidic in nature (pH below 5.5) and about 65% of soil of Northeastern region experiencing high acidity along with deficiency of nitrogen and phosphorous (Suyal et al. 2017). Similarly, soils of African hills are also suffering by acidity with a pH of less than 5.2 (45–50% soil) and about 65–80% of soil is deficient for phosphorous and 60% soil for nitrogen along with low fertility. Among all factors, biological entities residing in the soil play a very crucial role in determining the soil quality. Nutrient cycling, aggregate stability, size, water preserving capacity, and soil organic matter instigate by microbial actions. Microbial composition in the soil also depends on the temperature, pH, and soil constituents.

Low-temperature environment is an excellent factor for the growth and multiplication of psychrophilic microorganism. They possess the cold-active enzymes which carry out the biological conversion in soil and are also important for industrial purposes. Van Horn et al. (2013) investigated the impact of some abiotic features such as organic matter, pH, sulfates in controlling the biodiversity of microbes in Antarctica soils and found that members of *Actinobacteria* and *Acidobacteria* phyla were majorly prevailing under low elevation site rich in organic carbon, whereas at a higher elevation with high humidity *Firmicutes* and *Proteobacteria* were dominant. Similarly, Himalayan soils hold the substantial diversity of psychrophiles and psychrotolerant microorganisms which are able to manage soil health and crop production.

10.5 Why Require Nutrient Management?

Nutrient management can be defined as the practices that maintain the nutrient balance in the soil and keep remains it healthy in terms of nutrient content, moisture content, and soil organic matter for the support of the production of economic goods. Good management practices ensure the availability of soil nutrients and enhance plant productivity. But in hilly region, soil is drastically degrading due to its steeply sloping landscape, soil erosion by heavy rainfall, continuous exposure of cold temperature as well as it is becoming acidified because at high altitude cation removal becomes high via water runoff, and leaching process makes hill soil gradually acidified, leading to the accumulation of increased soluble Al^{3+} concentrations in clay particles. Increased soluble Al^{3+} concentrations boost its uptake by the plant that disturbs the plant nutrient balance and also reduces regular plant nutrient uptake along with the occurrence of major diseases (Awasthi et al. 2017). Moreover, various anthropogenic activities are also causing a nutrient imbalance in soil and also leading to degradation and erosion of soil at a higher rate. Therefore, in present scenario of the world, management of soil nutrient in hill region is a primary issue of research.

It is expected that the world's population will achieve up to 9.8 billion by 2050; hence, crop production should be enhanced for the feed of such population in a very sustainable manner. Therefore, the soil nutrient management is an issue of great concern because it directly or indirectly depends upon the crop production, human health, soil constancy, and environmental sustainability. Besides, soils of Northeastern Himalayan region are nitrogen and phosphorous deficient, because nitrogen and phosphorous are converted into their unavailable forms, which are inaccessible for the plants. Moreover, soil genesis in hilly area generally depends upon water runoff, deposition, and erosion, which gives different soil properties at the region of hills. Such processes are different due to its topographical variation of hills. Some hills have broad landscape with a gentle slope; it allows boosted water infiltration through the quick flow of water vertically via soil particles causes high deposition of nutrients and such landscape generates thick and well-developed soil horizons. Whereas some other hills have steeply sloping landscapes that do not permit that much vertical water flow through soil particles, hence less deposition is there which leads to the development of a thin and exhausted soil horizon. It also triggers the high soil erosion rate which is the main cause of land degradation and nutrient imbalance due to the removal of the top soil layer, rich in nutrient and soil organic matter. It negatively affects the environment and introduces instability in agricultural productivity. In addition, continuous cultivation practices, without or uneven amendments of nutrients in soil, lead to dearth of nutrients reserves and organic matter in the soil. It also triggers acceleration in degradation and erosion of soil, and hence ultimately reduces crop productivity and causes a discrepancy in environmental sustainability. According to the Karki et al. (2004) hill region of Nepal was facing the deficiency of zinc, molybdenum, and boron at several ecological belts; such zinc was deficient in Terai region; therefore, farmers applied zinc phosphate fertilizer without appropriate advice or proper guidance which resulted in the soil nutrient imbalance and reduced soil fertility. Therefore, identification and development of proficient and multifaceted conservation practices for soil nutrient management, crop productivity enhancement, sufficient and nutritious food diets, and conservation of remaining natural resources are the key future challenges. The current situation not only requires the nutrient management or enhanced crop productivity but it requires more, that is all together with sustainability in the environment.

10.6 Strategies of Nutrient Management in Soil

The biological, physical, and chemical properties of hilly soil are imbalanced due to various environmental factors (biotic and abiotic) which reduces its inherent capacity to support the crop production that may lead to shrivel the food quantity for the consumption of continuously increasing world's population. Therefore, for current scenario, practicing of promising strategies for nutrient management, soil conservation, and improved crop production strategies is required to get the more outputs than inputs. In the current era, several techniques are in practice that include agronomical approaches, use of chemical fertilizers, microbial-based approaches, and integrated nutrient management system for nutrient management and soil conservation.

10.6.1 Agronomical Approaches

From the ancient time, natural nutrient cycling has occurred from the soil to the plant and then the animal after that is come back to the soil by the degradation and decomposition of plants and animals. Repeated and intensive cultivation of the same crop declines the content of specific nutrients and increases other nutrients content in the soil which is not utilized by such crop, hence makes imbalance in nutrient composition of hilly soil. Therefore, researchers are making efforts for developing alternate and co-cultivation cropping plans for the reestablishment of soil nutrients. Cropping pattern includes inter-plantation of legumes with cereals which improve the soil fertility by efficient and balanced utilization of nutrients, which reduce production risk and return better to the farmers. Research of 7-year in Northwestern China has been reported around 4–11% high organic matter and nitrogen deposition in the soil during intercropping of faba beans (*Vicia faba* L.), wheat, and corn in comparison to monoculturing of those species in rotations, and triggered the increase in organic matter and nitrogen sequestration rate (approximately 184 and 45 kg ha^{-1} $year^{-1}$ for, respectively) (Cong et al. 2015). Moreover, plant breeding techniques are practicing for the development of novel germplasm that is able to grow in acidic soil and has resistance for cold environmental conditions. Tripathi (2001) selected the novel germplasms of maize, upland rice, wheat, and soybean, which were able to grow under acidic conditions. Similarly, Indian Agriculture Research Institute (IARI) released various varieties of wheat (var. HS 507), rice (var. Pusa Sungandha 5), and lettuce (var. Great lakes) for efficient cultivation in hilly region. In the modern era, researchers are focusing on "conservation agriculture (CA)" which encompasses an efficient farming system that prospers numerous events such as reducing the soil disturbance, maintains permanent soil cover and diversification of plant species. Moreover, "conservation agriculture (CA)" also develops a farming system which increases agricultural practices that preserve natural resource along with high production and also protecting the environment from certain negative aspects such as soil erosion and land degradation. Furthermore, such approaches usually improve the availability of water and its infiltration into the soil, which ultimately encourages the process of carbon sequestration through reduced SOC decomposition.

10.6.2 Use of Chemical Fertilizer

Now it is well-known that the soil of hilly region showed different soil properties as per their topography and observed the deficiency in macro (N and P) and micronutrients (Zn, S, and Fe) and acidic nature of hilly soil. Moreover, repeated monoculture cultivation practices and co-cultivation practices without appropriate nutrients addition in the soil lead to the depiction of the nutrient reservoir of soil. Because plant requires nutrients supplied by soil and it takes a very long time to decompose and return nutrients to the soil. It leads to the reduced fertility of soil declining the productivity of crops. Therefore, there is a need to add only an appropriate quantity of nutrients regularly in the soil to maintain its health. From the several past decades, the exploitation of organic and inorganic synthetic fertilizers is practicing for the replenishment of nutrient reservoir of soil. Synthetic fertilizers are produced through complex industrial processes or mined by earth crust, generally obtained from the nonliving sources. Most synthetic fertilizers are alerted by blending, purification, or mixing for easy handling. Nutrient management through chemical fertilizers application (both organic and inorganic) generally focuses on nitrogen and phosphorus management in soil. Application of different types of chemical fertilizer offers rapid availability of various nutrients for plants. Therefore, its early days were glorious when the application of synthetic fertilizers showed a massive positive change in crop productivity rate. But the long-term intensive, inappropriate, and unplanned utilization of synthetic fertilizers coincided with numerous problems such as a large proportion of chemical fertilizer convert into insoluble form makes soil infertile and become unavailable for the plants. They are toxic and pose environmental and water pollution, and high cost is another disadvantage associated with it.

Different soil type requires different concentration and types of fertilizer such as sandy soil requires high and multiple doses due to high leaching. Corn and green leafy vegetables require a high dose of nitrogen then root crops. Therefore, optimization of the dose of fertilizer application according to the requirement is a great challenge since its keen target is the efficient utilization of fertilizer to augment crop yield and soil nutrient revitalization with environmental safety. For example, in a 9 years phase from 1996 to 2005, application of N and P fertilizers increased about 51% for enhanced crop production but the crop improvement was only 10% in China (Zhang et al. 2012). Besides, appropriate, optimized, and required concentration of chemical fertilizers can replenish the soil nutrient reserve. Under cold temperature, the soil becomes very cold that hinders the fertilizers utilization from the soil. To overcome this problem, liquid chemical fertilizers can be applied by foliar spraying to absorb the nutrients by the plants. Incomplete fertilizers (i.e., fertilizer possesses one or two nutrient separately instead of a combination of many nutrients together) can be used separately as per the soil requirement for the replenishment of soil. A study was conducted in the southern hill region of China from 1998 to 2009 and observed that appropriate fertilizer application was able to sustain the soil pH at 5.89, and extensively improved soil organic matter and total nitrogen (i.e. 22% and 17.8%, respectively) in comparison with control (Dong et al. 2012). Hence the application of suitable fertilizers concentration under optimized conditions is an excellent strategy to augment soil fertility, nutrients pool, and yield of plants.

10.6.3 Role of Microorganisms in Reinstatement of Soil Nutrient

Microbial diversity in the soil system is a functional unit that performs numerous activities essentially required for maintaining the fertility of soil. Bacteria, actinomycetes, fungi, and algae are the main components of soil microbial diversity; however, the proper exploration of soil inhabiting microorganisms presented significant roles in sustainable agricultural development, and also in large-scale industrial and commercial applications. The growing concern of soil health, crop production, environmental sustainability, and socioeconomic impacts of climatic variation and chemical dependent conventional agricultural practices developed seek for the identification of alternative tools or techniques in order to develop agriculture practices in more sustainable manner. It is a well-known fact that soil microbiota (bacteria and fungi) play a central role in functioning of the ecosystem. Soil microbiological, microbial eco-physiological, and biogeochemical findings strongly reveal that the soil microorganisms perform biological transformations in soil that ultimately establish the carbon, phosphorous, and nitrogen cycling rates in the ecosystem (Nadeem et al. 2018). Although the composition of soil microbiota affects by various ecological factors but indigenous microbiota, i.e., well adaptive for the native climatic conditions, positively and negatively regulate the processes such as soil genesis, nutrient cycling, plant interactions, and decomposition of dead material in the soil and hence governs the crop production (Siles et al. 2016). In addition, soil microbiota secret gluing agents such as exopolysaccharide that triggers the aggregation of soil, also improves macroporosity to the subsoil surface that stimulates water transmission and declines runoff.

Microbes are the living biological entity, therefore, its application is a sustainable and eco-friendly approach for the improvement of soil fertility, nutrient balance, and crop production. Therefore, eco-friendly approach of present era, i.e., microbes based biofertilizers hold great promises as the key player for sustainable agro-ecosystems. Biofertilizers are the single or group of microorganisms having potential bioactive metabolites to carry out necessary conversion to encourage the complex plant-microbe-soil interactions (Stamenkovic et al. 2018). A group of microorganisms (bacteria and fungi) that display the plant growth promoting (PGP) traits are termed as plant growth promoting microorganisms (PGPMs) and are utilized for the development of biofertilizers (Singh et al. 2013). Biofertilizers are generally classified into four categories such as (1) nitrogen-fixing bacteria (2) phosphate solubilizing bacteria, (3) composting microbes, and (4) biopesticides (Pathak and Kumar 2016). PGPMs govern soil nutrient management and crop production by various mechanisms such as biological nitrogen fixation, solubilization of various nutrients such as phosphorous, zinc, silicon, and chelates metals by siderophores secretion and enhance their availability in the soil. Furthermore, they also produce secondary metabolites extracellularly such as indole acetic acid (IAA), gibberellic acids (GAs), antibiotics and suppress the pathogen attack by Induced systemic resistance (ISR). As discussed above that the soil of various hill regions is acidic in nature, less fertile, and deficient in available macronutrients (C, N, and P) and micronutrients (zinc, iron, and molybdenum) and crops are also suffering from some degree of pathogen attack (Singh et al. 2017). Moreover, the temperature of hill regions generally remains low and inversely decreases with the increase in distance from the ground, hence the PGPMs should be cold-adaptive or cold tolerant for the survival and efficient functioning. Therefore, it is essential to explore indigenous or region-specific plant growth promoting microorganisms to mitigate the problems in a sustainable manner (Hamdan 2018). Previously, various hill regions have been explored by numerous researchers to find the potential PGPMs in order to develop a potent biofertilizer for nutrient management and yield enhancement but unfortunately, the microbiota of a very less number of hill regions has been documented till now. Some of the cold-adaptive plant growths promoting microorganisms are recorded in Table 10.2.

10.6.3.1 Microbial Mechanism for Nutrient Management

Life of most living organisms is sustained by soil. Numerous groups of microorganisms reside in the soil and take part in the biogeochemical cycles to maintain soil fertility and sustainability (Singh and Shah 2013). They carry out multifaceted processes to boost the organic matter content and stimulate nutrients availability in the soil. Exact functional mechanisms of most of the processes are not fully revealed until now. Some of the mechanisms are summarized below in forthcoming sections.

10.6.3.1.1 Nitrogen Fixation

Soil is the hub of various nutrients essential for living organisms. Nitrogen (N) is the most vital nutrient necessitating for standard plants growth and metabolism. But often its availability for plants befalls in risk due to the loss through leaching, i.e., a common phenomenon in steeper sloppy landscape and excess denitrification. While, diatomic form (N_2) of nitrogen is abundantly, i.e., 78%, present in the atmosphere, but due to its diatomic structure atmospheric nitrogen is inert. Various physical, chemical, and biological techniques are practicing for the formation of plant-utilizable nitrogen forms from the atmospheric free nitrogen. However, physical and chemical methods require huge energy and these are not economically feasible. Diazotrophic microorganisms reside in the soil mainly includes bacteria and archaea possess unique metabolism to transform atmospheric free nitrogen into ammonia by a unique set of enzymes called as nitrogenase enzyme complex, a process called as biological nitrogen fixation (BNF) (Pérez-Montaño et al. 2014). Nitrogenase enzyme complex comprised of two units, i.e., dinitrogenase reductase and dinitrogenase. Dinitrogenase reductase encompasses high reducing power, therefore donates an electron to the latter enzyme, which actually converts atmospheric nitrogen into ammonia. Later, through nitrification, this ammonia gets converted into nitrite and nitrate by Nitrosomonas and Nitrobacter, respectively. Of note, globally around two-thirds of available ammonia produced by BNF, whereas rest conversion is governing by physical method and Haber–Bosch process**.** On the basis of habitat, diazotrophs are classified into two groups which include free-living diazotrophs (*Herbaspirillum* and *Azospirillum*), reside in bulk or rhizospheric soil whereas, the bacteria fall in the second group, forms symbiotic alliance with leguminous and non-leguminous plants includes *Rhizobia*, *Frankia*, and *Bradyrhizobium*. These symbiotic nitrogen-fixing bacteria establish an association with host plant and form a highly specialized structure called as nodule inside the host, where they colonize and carry out nitrogen fixation. In order to produce one molecule of ammonia, 16 ATP gets consumed.

$$N_2 + 8\,H^+ + 8e - +16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

Symbiosis and nodule formation is the specific host-microbe interaction. Although it is not only controlled by the host or microbe but also by variety of factors which play a very crucial role and which include soil type, nutrients, temperature, climatic conditions. The maximum rate of biological nitrogen fixation by diazotrophs is generally achieved at mild temperature. Jacot et al. (2000) conducted an experiment for the evaluation of nitrogen fixation at different altitudes and observed that up to certain altitude limit (approx. 2100 amsl), the biological nitrogen fixation is well adaptive for the native climatic conditions. Meta-analysis study revealed that the formation of nodule, their numbers, and activity rate get limited by the scarcity of P, S, and K (Divito and Sadras 2014). Generally, the soil of high altitudes faces phosphorous limitation that also reduces the activity of nitrogenase enzyme complex. Fungi generally do not contribute for fixation of the atmospheric nitrogen but colonization of vesicular-arbuscular mycorrhiza facilitates the high availability of P, S, and K and other necessary nutrients to the nitrogen-fixing microorganisms and stimulates the nitrogen fixation rate. Moreover, vesicular-arbuscular mycorrhiza also provides shelter for free-living diazotrophs. Microbes also participate in the reversion of fixed nitrogen into diatomic atmospheric nitrogen through denitrification to maintain nitrogen cycle occurring in the ecosystem. It is governed by heterotrophic bacteria such as *Paracoccus denitrificance*, *Thiobacillus denitrificance* via anaerobic respiration process, they use nitrate as terminal electron acceptor for the utilization of organic matter and converts nitrate into atmospheric nitrogen as the final product of metabolism.

10.6.3.1.2 Soil Organic Matter Management

Soil organic matter majorly comprised of dead organic material, living plants parts, and soil micro and macro biota (microorganisms and soil animals). Soil organic matter imprints great impact on properties of the soil, governs ecosystem functioning. A small alteration in the amount of organic matter shifts the CO_2 concentration in the soil. It gives structure to the soil and makes it fertile and nutrient rich. Total soil organic carbon content is strictly controlled by two intricate processes, i.e., net primary production and its decomposition rate. Plants, algae, and cyanobacteria are autotrophs, and transfer atmospheric carbon dioxide (CO_2) into the soil as photosynthetic organic material. Most of the soil microorganisms are heterotrophic and they depend upon the primary producers or intermediates for carbon, nutrients, and energy needs. They utilize plant, animal, or microbial carbon for the generation of their biomass and some part of fixed carbon returns in the ecosystem and remaining remains in the soil by a process called decomposition. The decomposition process is majorly driven by the soil bacteria and fungi, whereas only 10–15% of carbon decomposition contributed by other entities. Saprophytic bacteria (*Acetobacter, Bacillus stearothermophilus*) and fungi (*Rhizopus*, *Mucor*, *Aspergillus*, *Penicillium*) converts plant, animal organic material into their microbial biomass (cell material), organic acids, and CO_2. Saprophytic organisms utilize simple components of plants and animal such as sugars, amino acids, lipids. Therefore, initial decomposition process occurred very rapidly but it occurs at a slow rate when saprophytic organisms decompose insoluble complex compounds such as chitin, cellulose, lignin, and hemicelluloses. Variety of enzymes released by microorganisms are necessary for the decomposition of such compounds such as cellulase for cellulose decomposition and chitinase for chitin, lignin peroxidase for lignin degradation and hence different microbial communities (autotrophs and heterotrophs) reside in the soil leads to the carbon balance in the ecosystem.

10.6.3.1.3 Phosphate Mobilization

Phosphorus is required for both plant and animal for energy generation and metabolisms; therefore, it is considered as the second crucial macronutrient after nitrogen. Neutrally a huge reservoir of organic and inorganic phosphorous (i.e., inorganic (apatite), organic (inositol phosphate or phytate, phosphomonoesters, and phosphotriesters) is mixed in the soil. Besides, the soil of hill regions illustrated a very minute amount of available phosphorous for the plants, because major portion of phosphorus has converted into their salt or insoluble form. Of note, the reason is the highly reactive nature of inorganic phosphorous, it reacts easily with metal such as Fe, Al, and Ca and approximately 75–90% of P get converted into insoluble salt forms making it inaccessible for the plants. Although plants can only metabolize two forms of phosphorous, i.e., monobasic ($H_2PO_4^-$) and dibasic (HPO_4^{2-}) ions, hence called as soluble forms of phosphorous. Therefore, to cope up with such problem farmers are applying chemical phosphatic fertilizer in fields, but here also a small quantity is utilizing by the plants and bulk of P is becoming insoluble and creating soil infertility and environmental pollution (Singh et al. 2010; Singh et al. 2018). Therefore, exploration of psychrophilic phosphate solubilizing bacteria, able to solubilize and mineralize insoluble form of phosphorous under low-temperature environment, can be an effective and sustainable approach. Mohammadi (2012) stated that phosphate solubilizing microbes based biofertilizers are the most promising strategy to solubilize insoluble phosphorous. Bacterial genera like *Pseudomonas, Bacillus, Massilia, Paenibacillus* are the common genera having psychrophilic phosphate solubilizing bacteria (Dutta and Thakur (2017); Zheng et al. (2017); Rajwar et al. (2018). Microbes generally mobilize soluble phosphorous through direct and indirect mechanisms. Under indirect mechanisms, microbes utilize organic matter for their growth and secrete low molecular weight anions such as citric acid, gluconic acid, oxalic acid, succinic acid, acetic acid for the solubilization of phosphorous by exchanging phosphorous anion with metals on absorption site of soil by a process called ion exchange. Moreover, these hydroxyl and carboxyl anions sequester the cations and thereby enhance phosphorous solubilization in the soil. Psychrophilic phosphate solubilizing bacteria (PSB) also secretes various phosphate solubilizing enzymes like phytases and phosphatases to cleave the C-P bond of insoluble organic phosphorous. Whereas indirect mechanisms exert releasing of numerous proton (H^+) during NH_4^+ assimilation that declines the pH and solubilizes phosphorous. Secondly, heterotrophic bacteria residing in the soil secretes CO_2 during respiration, which reacts with soil water and leads to the formation of carbonic acid, which declines the pH and resulted as solubilize phosphorous (Fig. 10.1). Besides, soil microbes assimilate the phosphate for their energy generation; hence they can govern the phosphorous management in the soil.

10.6.4 Integrated Nutrient Management (INM)

In the past centuries, population and their food demands were low; therefore, farmers relied solely on compost, farmyard manure, litter, and crop residues to maintain the

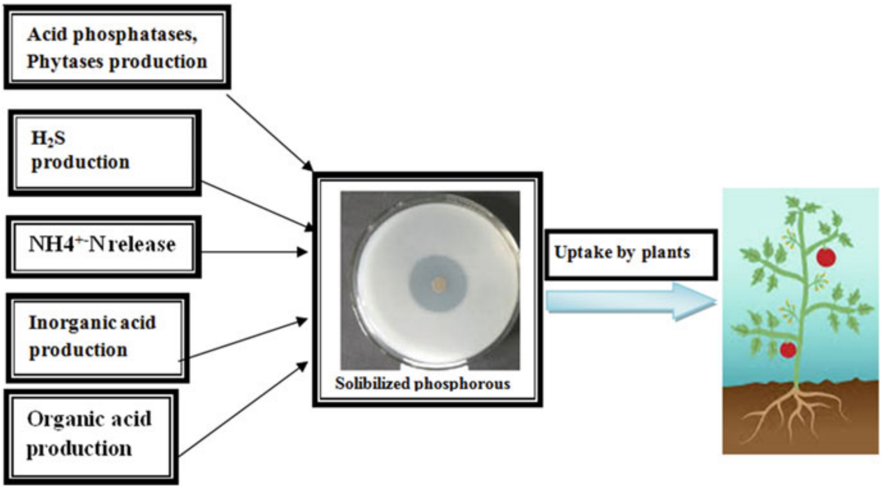

Fig. 10.1 Mechanisms adopted by microbes for phosphorous solubilization (Khan et al. 2019)

soil composition and better crop production. However, in recent decades the population density became very high, consequently, the demand for food supply has been also extremely raised. For the fulfillment of such demand, farmers intensively applying chemical fertilizers for doubling the production, which is causing serious environmental threat. Therefore, the ambition of present day research is the maintenance of sustainability in the ecosystem by efficient use and conservation of natural resources to assure the future human needs and shelter, and along with the improved quality of soil and environment. To achieve such goals a combined application of traditional and modern-day techniques has to be suggested. INM is a kind of united approach, refers to the use of limited and optimized quantity of chemically synthesized fertilizers together with the crop residues, microorganism based biofertilizers, farmyard manure (FYM), compost, and other living nutrient rich degradable materials in equilibrium for efficient use of fertilizer to augment the soil fitness and crop production in a sustainable manner (Shah and Wu 2019).

The study of Smaling et al. (1992) enlightened the need of INM for agricultural sustenance, mostly for those areas that are suffering from reduced soil fertility. INM comprises both traditional approaches such as application of natural living entities along with modern techniques, i.e., judicious and optimized quantity of fertilizer that makes it economically feasible and environment-friendly cropping systems (Fig. 10.2). According to Wu and MA (2015), the main aim of INM is the optimization of chemical fertilizer input along with natural fertilizer according to the nutrient demand of soil and execution of fertilizer application at appropriate timing for improved soil fertility and efficient output. Recently Warjri et al. (2019) conducted field trial on rice in the hill region of Meghalaya to investigate the impact of collective application of chemical fertilizers along with farmyard manure on the nutrient accumulation in soil and observed that the integrated application

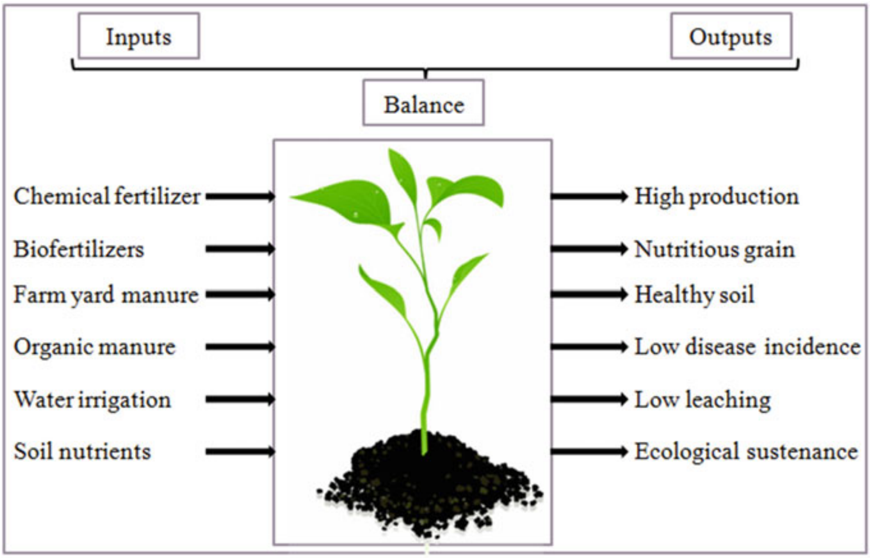

Fig. 10.2 Integrated nutrient management system for sustainable plant growth

significantly improved the soil organic matter, NH_4^+ exchange, soluble NO_3 phosphorous, and potassium availability. Similarly, Kumar et al. (2012) conducted an experiment in Northeast hill region of Meghalaya in acidic soils and reported the liming in combination with integrated nutrient management practice augmented soil nutrients concentration as well as showed three folds increase in maize productivity. Hence, inputs and outputs of all macro and micronutrients, soil organic matter get managed through integrated nutrient management (INM). Moreover, it improves soil water retaining ability and reduces the nutrient losses occur through leaching, immobilization, runoff, and volatilization. INM is economically, ecologically, and socially feasible practice that is practiced by farmers very easily to augment the productivity along with maintaining soil health. Therefore, it is becoming the most popular and acceptable by farmers.

10.7 Conclusion

Soils of hill regions are generally illustrated as degraded, acidic in nature along with the deficiency of nitrogen and phosphorous. In addition, altitudinal variation causes decline in the temperature with increase in elevation accordingly. Therefore, multifarious approaches in "soil nutrients management" are necessitated for improving the soil health to maintain sustainable agriculture in hilly regions, where soil experiences degradation due to environmental factors. Application of chemical fertilizers within the limit assisted in nutrient management but rampant exploitation of such agricultural practices is supposed to be dreadful which disturbs soil nutrient, fertility, and ecosystem sustainability. Therefore, an alternative of chemical fertilizers in form of soil beneficial microorganisms is an escalating approach to improve the soil fertility via various microbiological transformations of nutrients in soil along with the minimum cost and less laborious, and does not show the negative impact on soil characteristics. Soil microorganisms are the “soil engineers” which directly or indirectly participate in nutrient management, and as a “living catalyst” expresses a positive impact on soil health. The roles of soil microbiota in nutrient cycling such as mobilization of nutrients (N, P, K, Zn, Fe, etc.) and organic matter disintegration are admirable where the microbial activities sustain the nutrients level in the soil. Improvement in soil stability through commencing the process of soil aggregation is another important aspect of soil microorganisms. However, indigenous microorganisms adapt existing environmental conditions, i.e., psychrophiles in the hill region of soils, and as key inhabitants of soil, exhibit functional traits regarding nutrient management and also help in plant growth promotion. Soil microorganisms possess various unique characters that are most important in the revitalization of soil in sustainable agriculture with avoiding huge reliance on the application of chemical fertilizers.

10.8 Future Prospects

Application of microorganisms in soil nutrient management and soil vitalization are wider, but existing challenges are still to be addressed in future research. The further research is required for employing microorganisms as “natural fertilizer” for providing a natural way of maintaining the soil fertility/vitality with extra efficiency and circumventing the approaches advocated for using chemical fertilizers. The intense study based on molecular approaches is also required to generate high throughput information about soil microbiome, and to draw metabolic functions of microorganisms in nutrient cycling. The modern research is also directing the future for promotion of using microbial based fertilizers at large scale for solving the purpose of soil revitalization and soil nutrient management.

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Characteristics of Microbial Community and Enzyme Activities in Higher Altitude Regions

11

Vikas Sharma, Digvijay Dahiya, and D. Vasanth

Abstract

The high altitude regions around the world consist of an interesting group of landscapes with diverse features and microbial diversity. These regions differ individually and possess their own characteristic features such as lakes, glaciers, desserts, volcanoes, and forests. The microbial diversity found in these remote areas is uniquely adapted to the challenges of high altitude such as cold temperature, lack of water and biomass, seasonal variation in climatic conditions, and solar radiations. The unfortunate lack of studies on the microbial communities at high altitudes has made it necessary for the researchers to explore unique microbes surviving in the extreme conditions of higher altitude along with the enzymes they produce to survive. The novel characteristics of the enzymes obtained from these regions are expected to be industrially important which demands the need of their in depth understanding. The mountainous locations are among the highly prone areas to be affected by the global warming which ultimately leads to changes in the structure of microbial community and even extinction of microbial species. In this chapter, we discuss various higher altitude sites, their climatic conditions and the factors affecting the microbial community structure. We also present the seasonal variation in the enzyme activities and their correlations with various factors such as C/N ratio, amount of biomass, fungal/bacterial number ratio, and change in altitude. In addition, the predominant microbial species found at various high altitude niche regions were discussed.

Keywords

Microbial communities · Enzyme activities · Microbial diversity · Mountains · Psychrophiles

V. Sharma · D. Dahiya · D. Vasanth (✉)
Department of Biotechnology, National Institute of Technology Raipur, Raipur, Chhattisgarh, India
e-mail: dvasanth.bt@nitrr.ac.in

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11.1 Introduction

The studies on microbial diversity in higher altitude regions are of much interest nowadays. As there is a growing effort to document all existing life, it is essential to reach all corners of the globe to find rare species that may have a chance of going extinct due to global warming. High altitude regions are among those extreme geological location that has been ignored in this effort of microbial search and discovery (Connon et al. 2007). High altitude locations are characterized with cold temperature, rapid changes in temperature, rise in total amount of radiation in a year, and decrease in vegetation. The amount of precipitation received may vary from one geographic location to another which in turn impacts the vegetation level and biomass. The correlation between the amount of plant biomass and the number of microorganisms is pretty straightforward, so it stands the reason that the high altitude regions with lesser plants will have lower amount of microorganisms (Jha et al. 1992; Xu et al. 2015).

Mostly pyschrophilic microbes are present in higher altitude regions and their enzymes show activity at low temperature and site-specific stress conditions (Dumorné et al. 2017). Spore forming variants are usually found to be more successful at higher altitude regions due to their ability to survive at extreme temperature, pH, and pressure (Mandic-Mulec and Prosser 2011) and are also important nitrogen recyclers and biodegradation agents. Some species of *Pseudomonas* and *Bacillus* have been reported for their role as biocontrol agents and plant growth enhancers in colder regions (Pandey et al. 1999). Bacterial species such as *Carnobacterium, Rhodococcus,* and *Serratia* isolated from higher altitude regions have found their applications in bioremediation due to their biodegradation characteristics (Kaira et al. 2015; Leisner et al. 2007), whereas strains that are pathogenic in nature are important for clinical relevance (Wenzler et al. 2015; Brooke 2008). Enzymes produced by the cold adapted microbes isolated from alpine soils have found their role in industries due to their numerous advantages, thus are important targets for the biotechnology industry (Dumorné et al. 2017; Barria et al. 2013; Barroca et al. 2017). High demand of novel psychrophiles and enzymes that shows optimal activity at lower temperature attracts the scientific community to explore extreme locations of high elevation around the world.

Majority of the work has been done on the altitudinal gradients and existence of flora and fauna across low altitude landscapes (Kessler et al. 2011; Mccain 2005; Rahbek 2005). But the most abundant and diverse class of organisms of earth are still not well studied (Torsvik and Øvreås 2002). The current literature lacks the studies that considered elevation and climate at the same time (Siles et al. 2016). Microbial community present in the soil circulates the carbon, nitrogen, sulfur, phosphorus, and numerous metals, thus plays an important role in the foundation of earth's biosphere. These microbes are also crucial for the decomposition of litter in an efficient manner (Schimel and Bennett 2004; Balser and Firestone 2005; Carney and Matson 2005).

The processes involved in the ecosystem are strongly affected by the microbial diversity of a community because these microbes play an important role in the respective processes. It is well known that fungal and Gram-positive bacterial strains are more susceptible in utilizing complex compounds when compared with the Gram-negative bacterial strains (Waldrop and Firestone 2006). The functioning of an ecosystem is influenced by the variations in community structure and microbial diversity which ultimately affected the overall turnover of soil organic matter (Stroud et al. 2007; Baumann et al. 2013; Wang et al. 2013). Diversity of microbes in the soil plays a significant part in the interactions and functioning (Hines et al. 2006; Stroud et al. 2007), changes in climate (Singh et al. 1989), and biogeography of an ecosystem (O'Malley 2007; Fierer et al. 2009) which demands the thorough study of their spatial patterns and driving factors. Hence, there is a need for improvement in the thorough understanding of microbial biogeography and the environmental factors associated with them.

A fewer studies are reported on the altitudinal patterns of soil bacterial and fungal communities which mainly projected the variation in the structures of microbial communities with the increase in elevation (Fierer et al. 2011). The factors including soil pH, carbon/nitrogen ratio (C/N), and climatic conditions showed positive correlation with the diversity and composition of the microbial community (Fierer et al. 2011; Lucas-Borja et al. 2012; Shen et al. 2013). The seasonal variation leads to change in the temperature and water level on high altitudes which ultimately affects the microbial biomass and the production of enzymes (López-Mondéjar et al. 2015). The climatic conditions also affected the plant biomass which can be seen during the leaf falling in the autumn season (Koranda et al. 2013). Not all mountain ecosystems are similar though. There is huge amount of variations at different sites of the same altitude such as the presence of lakes, forests, completely dry volcanic soil, etc. Some mountains with active or inactive volcanoes are found to have similar chemosynthetic modes of obtaining energy as are found in deep sea vents (Connelly et al. 2012). The study of such sites may even shed some light on the theoretical functioning of microbes on planets that may harbor life due to the possible presence of water, like Mars (McEwen et al. 2011). There is an urgent need to study the mountainous microbial communities as the threat of global warming looms over their existence. The global warming leads to receding glaciers which exposed the underneath soils to different environmental conditions, thereby altering the microbial community structures (Allison et al. 2010).

A study of the colonization of deglaciated soils at high altitudes revealed that the *cyanobacteria* were the ones to colonize during the initial period which leads to increase in soil stability and then arrival of moss, lichen, and vascular plants takes place (Schmidt et al. 2008). The microbes present in the soil play a major role in carbon flux between soil and atmosphere (Falkowski et al. 2008). The 2007 report of IPCC mentioned that the concentration of CO_2 was 380 ppm in the year 2005, which was 80 ppm more than the average of 650,000 years before it. The microbial biomass remains stable or decreases with the increase in global warming but there is no case of increase (Vanhala et al. 2011; Feng and Simpson 2009). The fungal numbers were also found to decrease with increase in global warming (Vanhala et al. 2011). The usual mode of studying high altitude microbes has been by typical culture techniques in which only the microbes that grow in nutrient rich media grow with high growth rates, which unfortunately excludes a large number of microorganisms that live in these areas (Kaeberlein et al. 2002). This is why the recent attempts at isolating microorganisms make use of the genetic sequences and other advanced techniques. The major techniques being used today for high altitude microbial studies are 16S rRNA, MALDI-TOF, diffusion chamber based isolation, and the use of metagenomic tools (Pandey et al. 2019) (Table 11.1). This chapter presents the detailed analysis on the effect of change in altitude and season on the microbial community structure and enzyme activities. We also discussed various sites around the world that were actively studied for their mountainous microbiota and their specific biogeographical features.

11.2 Study Sites and Their Climatic Conditions

Several higher altitude locations were explored for their microbial diversity and microbial activities around the world. The higher altitude regions studied are mainly from India, China, Nepal, Italy, Mexico, borders of Argentina and Chile, Africa, and Spain (Fig. 11.1).

11.2.1 Higher Altitude Locations Studied in India

A study was conducted in Meghalaya state of India at two different sites Byrnihat (100 m) and Shillong (1500 m). These locations are at a distance of 90 km away from each other and are situated between latitude 26″N and longitude 92″45′E at Byrnihat and latitude 25″34′N and longitude 92″47′E at Shillong (Table 11.2). The climatic condition in Byrnihat and Shillong is monsoonic type which varies from tropical to subtropical. December and January are among the coldest months of these places, whereas May to September time period remains moist and warm. The average maximum and minimum temperatures measured were 28 and 17.5 °C at Byrnihat, while at Shillong they were 20 and 10.9 °C, respectively. The average rainfall recorded was 367 mm at Shillong and 212 mm at Byrnihat. Gneisses, granite, and schists were the major type of soil present in these areas which were originated from the hard rock, whereas the texture of the soil is mainly reddish brown sandy loam formed through laterization (Kshattriya et al. 1992). A fungal strain was isolated for the production of laccase enzyme from the soil of cold desert, Mana (30°46′24.8″N; 79°29′33.4″E; located around 3238 m above mean sea level), and nearly 4 km from Badrinath, in Chamoli district of Garhwal Himalaya, India (Dhakar and Pandey 2016). Kumar et al. investigated the microbial diversity and physiochemical properties of the soil from the Himalayan cold desert of Gangotri. The higher altitude soil ecosystem was finalized for the collection of soil samples of Gangotri located at the 30.9947°N and 78.9398°E (Kumar et al. 2019). Whereas Pandey et al. (2019) studied the bioprospectation of cold adapted microbes of Pindari Glacier region located at 33°5′–30°10′N to 79°48′–79°52′E and cold desert at 30°46′24.8″N; 79°29′33.4″. In this study, they covered a wide range of altitudes between 1800

Table 11.1 Microbial diversity at higher altitudes

Study location	Altitude (in m.a.s.l.)	Major microbial community	Type of gene study	References
Iztaccihuatl Volcanic Complex, Mexico	4950 (Monte de Venus lake), 5065 (La Panza glacial) and 5200 (El Pecho glacial)	Bacterial diversity *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Acidobacteria*, *Cyanobacteria*, *Armatimonadetes*, *Candidatus Saccharibacteria*, *Parcubacteria*, and *Firmicutes*	16S rRNA gene sequencing (phylum level)	Calvillo-Medina et al. (2019)
Indian Himalayan Region (IHR)	1800–3610 (Pindari glacier region and cold desert)	*Bacillus*, *Pseudomonas* species and genera *Alcaligenes*, *Carnobacterium*, *Lysinibacillus*, *Microbacterium*, *Paenarthrobacter*, *Rhodococcus*, *Serratia,* and *Stenotrophomonas*	16S rRNA gene sequencing	Pandey et al. (2019)
Gangotari, Indian cold desert	3415	*Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Cytophaga*, *Flavobacterium, and Bacteroides* (CFB). Psychrophillic diazotrophs such as *Arthrobacter humicola*, *Brevibacillus invocatus*, *Pseudomonas mandelii,* and *Pseudomonas helmanticensis*	Real-time quantification and DGGE analysis of 16S rDNA and *nif*H genes	Kumar et al. (2019)
Luoshijiang Wetland, in the Yunnan–Kweichow Plateau, Yunnan province	2056	*Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Gemmatimonadetes*, and *Verrucomicrobia*	16S rRNA gene clone library analysis	Zhang et al. (2014)

(continued)

Table 11.1 (continued)

Study location	Altitude (in m.a.s.l.)	Major microbial community	Type of gene study	References
Volcanoes in the Atacama region of Argentina and Chile	5500–6330 (Volcano Socompa and volcano Llullaillaco)	Bacteria: *Actinobacteria*, *Chloroflexi*, *Proteobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Acidobacteria*, *Plantomycetes*, *TM7*, *Firmicutes* Eukaryotes: *Fungi*, *Cercozoa* Archaea: *Thaumarchaeota*	Amplicons based bi-directional sanger sequencing	Lynch et al. (2012)

and 3610 m above sea level including the alpine, subalpine, and temperate zones of the Indian Himalayan region. The location is known for heavy rainfall and snowfalls. The mean temperature measured in these regions lies near about 5.5 °C in the month of January and 21.5 °C in August, whereas pH of the soil lies in the range of 4.5–6.5 at the sampling site.

11.2.2 Higher Altitude Locations Studied in China

The variation in structure and functioning of microbial diversity of Changbai Mountain in the Northeast China (Jilin Province) located at the 126°55′-129°00′E; 41°23′-42°36′N was investigated. This mountain is among the most promising locations for the study of highly conserved ecosystems on earth to explore novel microbial communities that spread along the border of North Korea and China. In the northeast region of China, Changbai mountain is the highest mountain which possesses a well-defined vertical distribution of forests. The mean annual temperature (MAT) increases from the highest part (2691) of the mountain to the lowest part of the mountain (540), whereas mean annual precipitation (MAP) decreases from 1340 to 750 mm (He et al. 2005). The changes in climatic conditions and topography of the mountain resulted in four vertical zones of vegetation and are categorized as: (a) mixed coniferous and broad-leaved forest (MCB) which is located below 1100 m; (b) dark-coniferous spruce-fir forest (DCF) which is located between 1100 and 1700 m; (c) Ermans birch forest (EB) which is located between 1700–2000 m, and (d) alpine tundra (AT) which is located above 2000 m. The type of soil found in MCB region is Albi-Boric Argosols and in DCF, EB, and AT region is cambisols (Shen et al. 2013), all of which are derived from volcanic ash (Xu et al. 2015). Tan et al. studied the effect of removal of snow from the microbial biomass in a Tibetan alpine forest. The site of study is located in a 120-year-old natural alpine forest of China which is in the Bipenggou Nature Reserve of Lixian County of Sichuan, China.

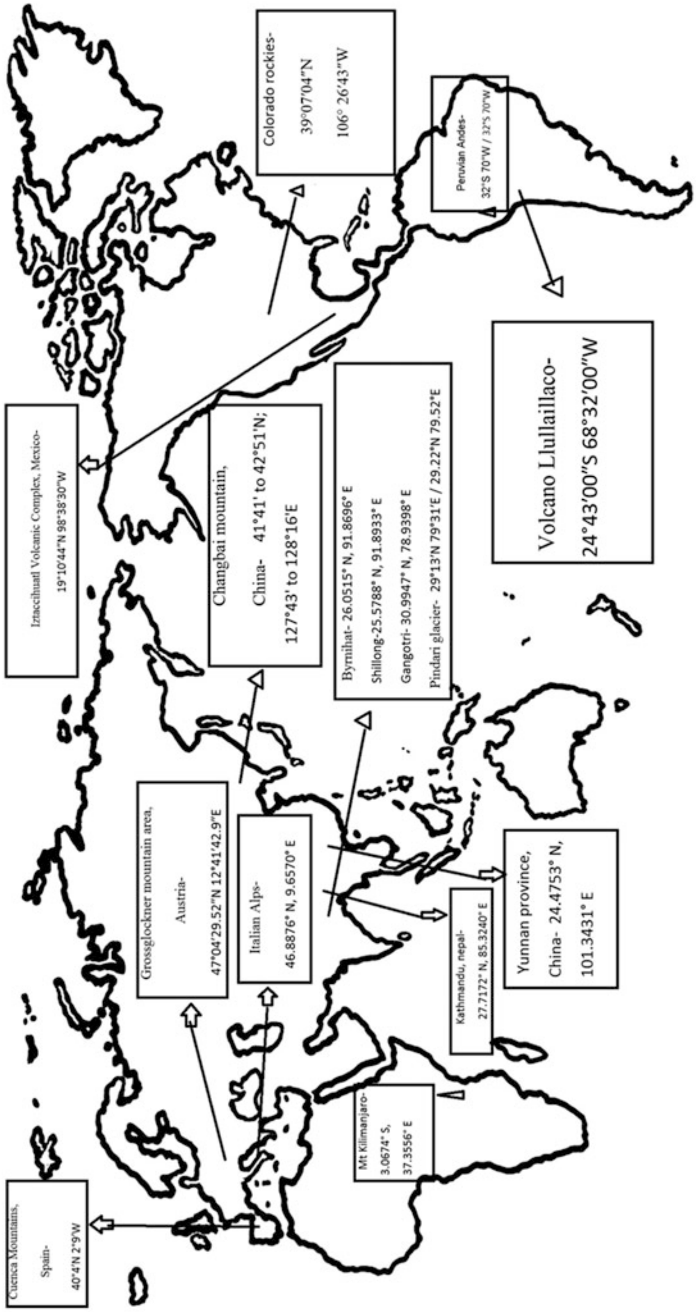

Fig. 11.1 Higher altitude locations studied around the world

Table 11.2 Summary of various higher altitude study locations

S. No.	Study locations (a.s.l.)	Enzyme studied	Effect of increase in altitude	Correlation with fungal/ bacterial number	Reference
1.	Byrnihat, India (100 m MSL)-Lower altitude and Shillong, India (1500 m MSL)-Higher altitude	Cellulase	Decrease in activity	+ve	Kshattriya et al. (1992)
		Amylase	Decrease in activity	+ve	
		Invertase	Increase in activity	No correlation	
2.	Byrnihat, India (100 m MSL)-Lower altitude and Shillong, India (1500 m MSL)-Higher altitude	Dehydrogenase	Variable	+ve	Jha et al. (1992)
		Urease	Decrease in activity	+ve	
		Phosphatase	Variable	+ve at lower altitude only	
3.	Changbai mountain, China 1. Mixed coniferous and broad leaved forest (1100 m) 2. Dark coniferous spruce fir forest (1100–1700 m) 3. Ermans birch forest (1700–2000 m) 4. Alpine tundra (>2000 m)	β-Glucosidase	Decrease in activity	+ve	Xu et al. (2015)
		N-acetylglucosaminidase	Decrease in activity	+ve	
		Acid phosphatase	Decrease in activity	+ve	
		Leucine aminopeptidase	Decrease in activity	+ve	
4.	Italian Alps 1. Submontane (545–570 m) 2. Montane (1175–1200 m) 3. Subalpine (1724–1737 m) 4. Alpine (1965–2000 m)	Phosphomonoesterase	Increase in activity	+ve	Siles et al. (2016)
		Arylsulfatase	Increase in activity	+ve	
		Protease	Increase in activity	+ve	
		β-Glucosidase	Increase in activity	+ve	

(continued)

Table 11.2 (continued)

S. No.	Study locations (a.s.l.)	Enzyme studied	Effect of increase in altitude	Correlation with fungal/ bacterial number	Reference
		Xylosidase	Increase in activity	+ve	
		Cellobiohydrolase	Increase in activity	+ve	
5.	Lower Jinsha river, China elevation range (680–1840 m)	Polyphenol oxidase	Increase in activity	NA	Xiao et al. (2019)
		Saccharase	Increase in activity	NA	
		Cellulase	Increase in activity	NA	
		Catalase	Increase in activity	NA	
		Urease	Increase in activity	NA	
		Alkaline phosphatase	Increase in activity	NA	
6.	Grossglockner mountain area, Austria (1500–2530 m)	Dehydrogenase	Decrease in activity	+ve	Margesin et al. (2009)
7.	Cuenca Mountains, Spain 1. Arcas forest (960 m) 2. Majadas forest (1350 m) 3. Tragacete forest (1670 m)	Dehydrogenase	Increase in activity	+ve	Lucas-Borja et al. (2012)
		Alkaline phosphatase	Increase in activity	+ve	
		Urease	Increase in activity	+ve	
		β-Glucosidase	No effect	+ve	

This site is located at 31°15′28.10″N, 102°53′29.34″E and 3580 m a.s.l. in the Eastern Tibetan Plateau (Fig. 11.2). The annual mean precipitation was recorded as 850 mm, while annual mean temperature is 3.0 °C with maximum of 23.1 °C in the month of July and minimum of −18.0 °C in the month of January, respectively. Soil

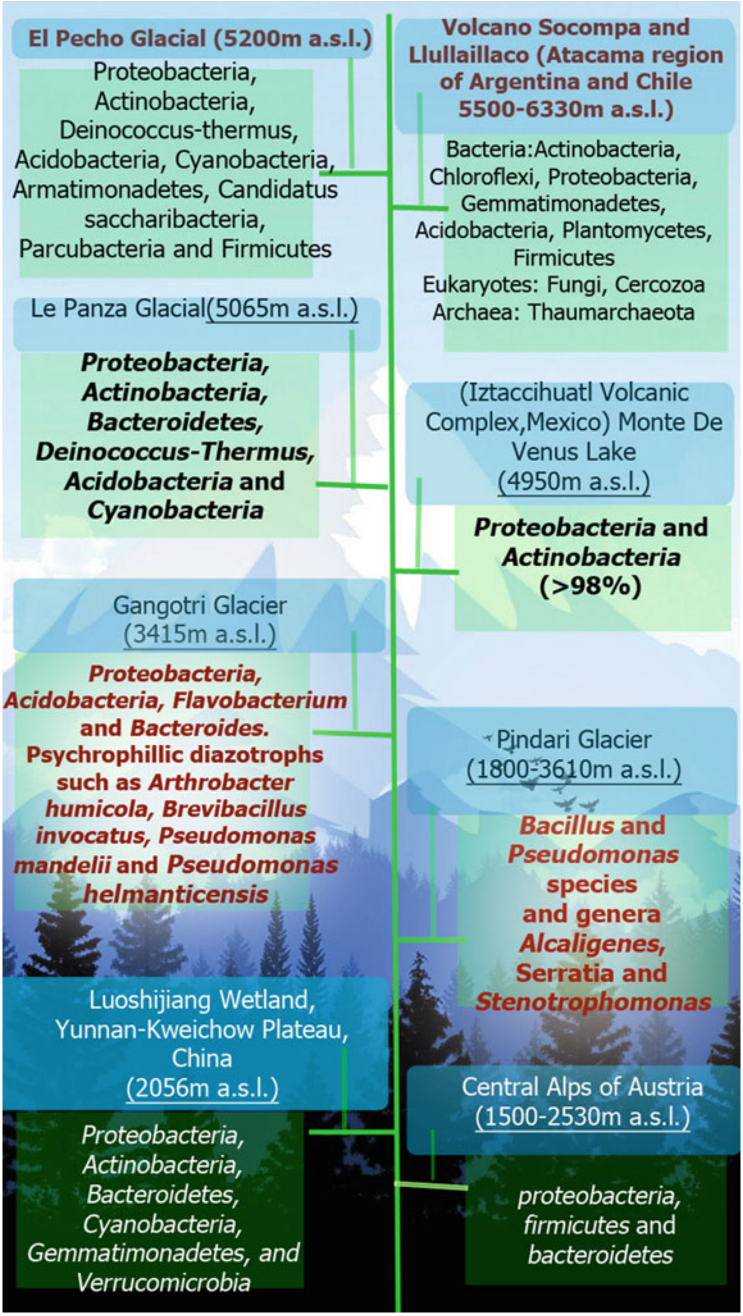

Fig. 11.2 Study locations with their altitude and predominant microbial communities

temperature shifted to below 0 °C and during the whole cold snow season remains frozen from late November to mid-April (Wu et al. 2010). The sample soil is categorized as Cambic Umbrisols with a 15 cm deep layer of organic matter (Tan et al. 2014). The enzyme activities and nutrient composition of soil collected from dry-hot valley region of southwestern China was studied along an altitudinal gradient which lies between 680 and 1840 m a.s.l. The study site lies in the lower region of the Jinsha River in eastern Ningnan County of Sichuan province which is located at 26°54′-27°09′N, 102°54′-103°02′E. The major soil type present in this area is classified as an Ustic Ferrisols under the Chinese taxonomic system. The climatic conditions and vegetation vary in this region along the elevational gradient. Elevations >1500 m refer to a subtropical climate having mean annual precipitation of 700–850 mm, a mean annual evapotranspiration of about 1600 mm, and a mean annual temperature of 14.2 °C. Elevations <1500 m belong to the typical dry-hot valley region and possess a mean annual precipitation of 600–700 mm, a mean annual evapotranspiration of about 3500 mm, and a mean annual temperature of 21.8 °C (Xiao et al. 2019). Zhang et al. (2014) reported the Bacterioplankton communities from four different sites of the Luoshijiang Wetland. The Luoshijiang Wetland, a typical freshwater wetland in the Yunnan-Kweichow Plateau, is situated in Dali City of Yunnan Province. The wetland is surrounded by farmlands and occupies an area of 1 km^2 with an elevation of approximately 2056 m. This wetland is adjacent to the Erhai Lake, which is also the second largest high altitude lake in Yunnan Province. The annual precipitation and the annual mean air temperature at this site were 1000–1200 mm and 15.7 °C, respectively. The study sites include A as the no vegetation zone located at 25°57′25″N–100°06′06″E, B as the reed-planted zone at 25°57′12″N–100°05′59″E, C as the densely water-lily-planted zone at 25°57′4″N–100°06′00″E, and D as the sparsely water-lily planted zone at 25°56′55″N–100°05′59″E. Tang et al. (2012) investigated the diversity in bacterial communities in the Zoige Wetland soil of the Qinghai-Tibetan Plateau of China.

11.2.3 Higher Altitude Locations Studied in Other Parts of the World

Siles et al. investigated the four types of forests in the South Tyrol region of Italian Alps. The alpine region consists of four diverse vegetation zones that dominate in the reported area including submontane, montane, subalpine, and alpine. The zone submontane is located 8 km south of Bozen/Bolzano at an elevation of 545–570 m a.s.l. and is above the Small Lake Montiggl. The mean annual temperature was recorded to be 11 °C and the annual precipitation was found to be approximately 900 mm at this zone with the mild continental climatic conditions consisting of submediterranean influences (Bonavita et al. 1998; Margesin et al. 2014).The montane zone is situated 300 m north of the village center of Klobenstein on a small hill at an elevation of 1175–1200 m a.s.l. The mean annual air temperature recorded as 7.4 °C with an annual precipitation of 950 mm, whereas the climate is montane-continental. The soil type was classified as dystric cambisol in case of submontane and montane sites. The subalpine site is located below the Rittner Horn and is around 7 km north of Bozen/Bolzano at an elevation of about 1724–1737 m a.s.l. The soil contained a thick layer of raw humus and its type was categorized as Haplic Podzol. The mean annual air temperature measured as 4.0 °C with an annual precipitation of 1000 mm with the subalpine-continental climatic conditions (Bonavita et al. 1998; Margesin et al. 2014). The alpine site is located at a height of 1965–2000 m a.s.l. at the tree line below the mountain Schwarzseespitze. The mean annual temperature of this site is 2.4 °C and an annual precipitation is of 1050 mm with alpine-continental climatic conditions. The subalpine and alpine soil type is Haplic Podzol. The pedogenic substratum consists of rhyolite in all of the four zones (Siles et al. 2016). In another study, the soil samples were collected from different places of Kathmandu Valley (Kirtipur, Balkhu, Dhobighat, Gwarko, and Putalisadak) and the Rautahat District of Nepal to produce the secretory enzyme laccase. The altitude of these sites ranges from 1600 to 2303 m above sea level (Yadav et al. 2019). The microbial diversity was investigated in the mineral soils of the Atacama region. Soil samples were collected in mid-February during the austral summer at elevations ranging from 5500 to 6330 m a.s.l. on Volcán Socompa and Volcán Llullaillaco, located at the border of Argentina and Chile. This work was a part of global survey of biological diversity at high altitudes in the Andes, Rockies, and Himalayan mountain range (Lynch et al. 2012).The seasonal and vertical distribution of picoplankton were reported in the Alchichica lake which is situated at 19°24′N, 97°24′W; 2340 m a.s.l. The lake belongs to a group of six maar lakes, thus considered as a crater lake, located on the eastern region of the Trans-Mexican Volcanic belt of the central Mexican Plateau. It covers surface area of 2.3 km^2. It possesses maximum depth of about 62 m, while its mean depth is around 41 m; thus, the lake is one of the deepest lakes of Mexico. The Alchichica lake is oligotrophic with a salinity of 8.5 g/L and alkaline pH between 8.6 and 9.4. The Chl a concentration of the lake was recorded as <5 μg/L (Ramírez-Olvera et al. 2009) and high concentration of sodium–magnesium and chloride–bicarbonate ions was present. The climatic conditions of the location are semi-arid with annual precipitation of <500 mm and a mean temperature of 12.9 °C. The lake is warm-monomictic type, with mixing during the cold dry season and thermal stratification from April to December (Pajares et al. 2017). Margesin et al. studied the microbial community structure and their activities in the soil of alpine and subalpine regions in the Grossglockner mountain area of Austrian central Alps. Soil samples were taken along the southern and northern slopes at subalpine area with an elevation of 1500–1900 m, under packed vegetation, up to the forest line and alpine area with an elevation of 2300–2530 m, under scattered vegetation, above the forest line. All the soil samples were predominantly gneiss (silicate) type (Margesin et al. 2009). The soil samples of Mount Kilimanjaro of Africa were studied for the decomposition of soil organic matter with the increase in altitude via enzymatic catalysis. The temperature sensitivity of the process was also investigated. The site is located adjacent to the Machame route at 3°4′33″S 37°21′12″E. The altitude gradient lies in between the colline zone to middle subalpine zone within a range of 950–3020 m a.s.l. (Blagodatskaya et al. 2016). Lucas-Borja et al. presented the characteristics of content present in the Mediterranean humid soil with the diversity of microbes present in the location. The study was conducted in the Cuenca Mountains of east-central Spain. During the experimentation, six forest areas and two tree diversity levels including monospecific and mixed pine forest were considered at three stages of altitudes. At the lower altitude of up to 960 m a.s.l. a monospecific Spanish black pine forest stand and a mixed forest stand were studied. At medium altitudes of up to 1350 m, a monospecific Spanish black pine forest stand and a mixed forest stand named Scots pine and Spanish black pine were investigated, whereas at the altitude of above 1670 m, a monospecific Spanish black pine forest stand and a mixed forest stand named Scots pine and Spanish black pine were studied (Lucas-Borja et al. 2012).

11.3 Microbial Diversity of High Altitude Regions

The mountainous regions of the world are home to numerous and interesting microbial populations with their own unique adaptations for survival. Various higher altitude locations around the globe showed novel characteristics and diverse range in the microbial communities. In this section, we take a look at the different studies that sought to catalogue the variety of microorganisms in various mountains around the world. The Central Alps of Austria have availability of both Alpine and subalpine regions making a suitable location for high altitude microbial study. A change in microbial community structure is to be expected due to the change in altitude with the total number of microorganisms decreasing in general (Uchida et al. 2000; Lipson 2007), but the proteobacteria showed an increase along with psychrophilic Gram-negative bacteria and fungi (Margesin et al., 2009) (Fig. 11.3). The method of investigation also had a bearing on the strains obtained, like proteobacteria and β-proteobacteria which showed up majorly on the culture-independent methods, while γ-proteobacteria and Firmicutes were shown to be present in high number in the culture dependent studies with bacteroidetes present in lower numbers (Margesin and Miteva 2011). The Gram-positive bacteria were outnumbered by Gram negative as the altitude increased, which may be due to their tolerance to freeze-thaw cycle and changes in pH. The Himalaya and the Arctic region have been shown to have psychrotolerant fungal species like *Aspergillus, Cladosporium, Penicillium,* and *Trichoderma* (Dhakar and Pandey 2016). Dhakar et al. isolated a novel strain of psychrotolerant fungus identified as *Cladosporium Tenuissimum* with the ability to produce laccase (Dhakar and Pandey 2016). Similarly, Yadav et al. isolated several *Actinomycetes* and *Streptomycetes* strains from the Kathmandu Valley and observed the production of laccase from a strain identified as *Pestalotiopsis* spp (Yadav et al. 2019). Himalayas contain both vegetated areas and arid regions. But there are some mountain ranges that remain permanently without water and vegetation, which make them interesting to study for the microorganisms that survive there. One such study involved the higher regions of Atacama region spanning Argentina and Chile. The study took place at approximately 6000 m a.s.l. and in the extremes of daily high and low temperatures as well as intense solar radiation (Lynch et al. 2012). In these mountains, at elevations of 5000 m a.s.l. and above, a Mars like atmosphere prevails

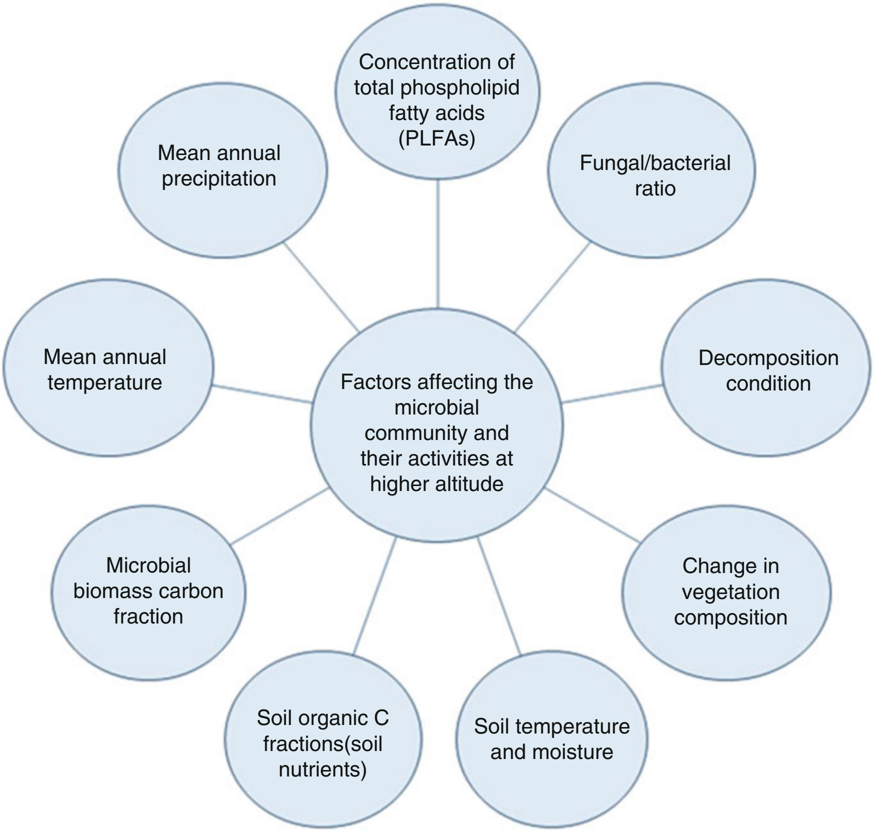

Fig. 11.3 Factors affecting the microbial community at higher altitude regions

devoid of any plant or animal life. There has been a lot of interest in the Atacama region but it has received little attention from a biology point of view (Connon et al. 2007). The 16S and 18S targeted microbial study revealed that at lower levels *Pseudonocardia* dominates, while the dominance shifts to *Ktedonobacter* with increase in altitude. Eukaryotic microorganisms were found in only 7% of the total samples, while the Xerotolerant *Cryptococcus albidus* clade members were present majorly. There was a minor number of archae as well from the phylum Thaumarchaeota, which can utilize trace quantities of ammonia and oxidize it for energy. Autofluorescence at 680 nm was used to discover low levels of algae and cyanobacteria containing chlorophyll in the higher reaches of Himalayan soils.

Similar method used in Atacama indicated the absence of any chlorophyll containing microorganisms which made it necessary to look for other sources of energy the existing microbes may use from the volcanic areas of the peaks. Another factor affecting the survival of microorganisms is the cooling rate which drastically decreases their number at 1.5 °C/h, while at slower cooling rates they were largely unaffected. The cooling rates recorded on Volcán Llullaillaco, the major site of this study, has linear cooling rates faster than 1.5 °C/h in the range of 1.83 °C/h. The only other place having similar cooling rates is the Peruvian Andes and measured during the austral winter in barren, peri-glacial soils and is found to be faster than in some studies on Himalayas, especially Tibet. The sequence data revealed that the major Actinobacteria is very similar to *Pseudonocardia asaccharolytica* which is known to be capable of oxidizing dimethyl sulfide (DMS). The other dominant lineage is from a branch of *Ktedonobacter racemifer* which is a facultative "carboxydovore." The upper atmosphere contains microorganisms that can be dispersed globally by precipitation, which may be the source of these microbes in the Atacama mountains (Lynch et al. 2012).

There are similar mountain ranges to the one in above study that are approximate in environmental and geological conditions like the subnival zones of Colorado Rockies and Central Andes. Although these soils appear barren, and the deep ice pack slows down any soil evolution, unexpected amount of nitrogen and carbon cycling have been observed by some studies. The process of mineralization by microorganisms has been documented by the researchers, who observed that the fungi was majorly involved in organic material mineralization during the melting of snow, while in the summertime, bacteria dominated the process. Although the Andes sites in Peru were higher and drier than the sites in Colorado (82 μg C/g), the Andes (140 μg C/g) was found to be hosting much more life forms as indicated by the amount of microbial biomass. But there was correlation between water content and biomass as well (King et al. 2008)

The features that can be found on some of the mountains around the world are water bodies such as lakes, which can be formed by inactive volcanoes or other kinds of depressions. Lakes at high altitudes may differ in the type of microbial life they have from other areas of the same mountain. It is thus necessary to study and contrast microbial life in high altitude lakes from mountains or other low level lakes. One such study was done by Pajares et al. (2017) that examined seasonal and altitude variations in picoplankton and their ability for ammonium oxidation and denitrification through effects on their gene distribution in the Lake Alchichica (2340 m a.s.l.) of Central Mexican Plateau. Another such crater lake has been studied in the Iztaccihuatl Volcanic complex in Mexico (Calvillo-Medina et al. 2019). It is a Pleistocene stratovolcano complex, with an active volcano, three glaciers, and two crater lakes. The study done by 16S rRNA gene amplicon method revealed the presence of several extremophiles well adjusted to this kind of climatic conditions. Three sites were chosen for sample isolation, namely Monte de Venus crater lake (4950 m a.s.l.), glaciers La Panza (5065 m a.s.l.) and El Pecho (5200 m a.s.l.). In all three samples taken together the *Proteobacteria* pervaded with a comparative percentage of 31–93% distantly followed by *Actinobacteria* with 6–15% of the total and *Bacteroidetes* at 1–17%. Other smaller groups present were *Deinococcus-Thermus, Armatimonadetes, Candidatus Saccharibacteria, Parcubacteria, Cyanobacteria, Firmicutes,* and *Acidobacteria.*

Another study in similar vein was done by Zhang et al. (2014) which was concerned with high altitude wetlands and its microbial communities. The site of study was Luoshijiang wetland situated in the Yunnan-Kweichow Plateau, China. The 16S rRNA study revealed the presence of *Actinobacteria, Bacteroidetes, Gemmatimonadetes, Proteobacteria, Cyanobacteria,* and *Verrucomicrobia* with only *Bacteroidetes, Proteobacteria,* and *Verrucomicrobia* common to all the testing sites. The study of correlation between the various microbes and the chemical properties of water revealed that positive correlations exist between pairs of *Actinobacteria* and *Gemmatimonadetes* with nitrile nitrogen and *Alphaproteobacteria* with dissolved phosphorus. Also present were members of genus *Luteolibacter* and *Haloferula*, but only in a few samples and in lower percentages. Of special interest among these is *Verrucomicrobia*, which is universally distributed and capable of using several carbon compounds (Arnds et al. 2010). Although the dominant nature of *Verrucomicrobia* in freshwater lakes has been studied, but its correlation with the dissolved phosphorus and nitrogen has been ignored (Arnds et al. 2010; Kolmonen etal. 2011). Another predominant freshwater microorganism is *Proteobacteria* which is supported by the findings of Sommaruga and Casamayor (2009) showing the dominant presence of *beta-proteobacteria* in lakes around the Mount Everest as well as the largest Tibetan lake called Lake Namco (Liu et al. 2013). *Alpha* and *gamma proteobacteria* take precedence over *beta-proteobacteria* in more saline lakes situated at high altitudes as shown with the study of 16 high altitude lakes on the Tibetan Plateau (Zhang et al. 2014).

While the Tibetan Plateau is consistently very dry place, the western part of Himalayas is very diverse. A study by Kumar et al. (2019) in the Gangotri Glacier, which is also the second largest glacier in Himalayas after Siachen, has revealed several novel microbial communities. The study was spurred by the fact that the Gangotri Glacier is receding very fast and the soil left uncovered may change its microbial mix and rapid documentation is required before that happens. Two sites were selected for comparison purposes, one at Kandakhal (1532 m a.s.l.) and Gangotri (3415 m a.s.l.) using the metagenomic analysis, several bacterial lineages were revealed among which the ten major phyla in order of decreasing percentages were *Proteobacteria* (38.49%), *Acidobacteria* (17.88%), *Actinobacteria* (14.48%), *Bacteroidetes* (7.89%), *Gemmatimonadetes* (7.87%), *Chloroflexi* (5.94%), *Nitrospirae* (1.08%), *Cyanobacteria* (0.93%), *Firmicutes* (0.9%), *TM7* (0.84%), and *Elusimicrobia* (0.55%). A number of unidentified OTUs were also present suggesting the exciting possibility of finding novel strains. In terms of abundance, the bacterial classes in decreasing order were *Alphaproteobacteria* (16.88%), *Beta-proteobacteria* (9.44%), *Acidobacteria*-6 (7.86%), and *Actinobacteria* (7.33%). There were both culturable bacteria on Gangotri site as well as a major number which are unculturable by normal methods. The authors of the study used a specially devised diffusion chamber to facilitate growth of some of the unculturable strains. The resulting cultures were found to be of *Acidovorax facilis*, *Arthrobacter pascens, Arthrobacter equi, Dyadobacter endophyticus, Paenarthrobacter nirogunajacolicus, Pseudomonas baetica, Paenarthrobacter siccitolerans, Pseudomonas mandelii, Pantoea gaviniae,* and *Pseudomonas frederiksbergensis*. In addition, there were some psychrophilic strains that were easily cultured using low temperatures, such as *Arthrobacter humicola, Pseudomonas helmanticensis*, *Brevibacillus invocatus,* and *Pseudomonas mandelii*.

Another study in the Himalayas by Pandey et al. sheds more light on the types of microorganisms that survive in harsh high altitude climates. The researchers here did not stick to a single site but studied various altitudes and regions such as temperate, subalpine, alpine, and cold desert areas of Pindari Glacier with altitudes ranging from 1800 to 3610 m a.s.l. The study made use of both 16S rDNA sequencing and MALDI-TOF analysis to study the diversity of microbes. A comparison of the MALDI-TOF, 16S rRNA, and biochemical methods confirmed that the use of MALDI-TOF for bacterial isolate identification is efficient and fast. The use of this technique revealed the major bacterial genera as *Bacillus, Rhodococcus, Pseudomonas, Stenotrophomonas, Serratia, Lysinibacillus, Paenarthrobacter, Alcaligenes, Carnobacterium,* and *Microbacterium* with *Bacillus* being the largest group, largely due to their spore forming ability making it easier for them to survive under harsh conditions of pH, temperature. and pressure. Another dominating genus was *Pseudomonas* which has already been known to survive easily in colder environments. The rest of the represented species are known majorly for bioremediation (Pandey et al. 2019).

All the above studies have shown us how much variations there can be at different altitudes and differing mountain ranges of the same altitude. Yet, there are some common groups that are consistently found at higher altitudes such as *Proteobacteria*. Other factors that modify the microbial biodiversity at these high altitudes are moisture content, nutrients available as well as the presence of special features like volcanoes.

11.4 Characteristics of Microbial Enzymes Produced at Higher Altitude Sites

The enzymatic activity of several microbial communities at higher altitudes has been studied in detail by the researchers around the globe. The major focus of their study lies on the variation of activities in different seasons throughout the year. It is suggested from the literature that the type of vegetation and litter affects the production of enzymes. The climate conditions, respiratory activity, and chemical properties of soil directly influence the quantity of enzymes (Sinsabaugh and Linkins 1989; Kshattriya et al. 1992). The activities of enzymes such as cellulase, amylase, and invertase isolated from the extracts of leaf litters of four different forests of different ages of lower and higher altitudes located in Northeast India were studied. It was observed that the enzyme activities were lower in case of litters at higher altitude than the litters of lower altitude, whereas cellulase and amylase showed the variation in their activities with the change in season at both altitudes. Cellulase and amylase activities were found to be increased during the decomposition of litter, whereas invertase activity increased only at the beginning stages of litter decomposition. The numbers of fungi and bacteria significantly correlated with the amylase and cellulase activities, whereas the activity of invertase remained unaffected with the microbial population. Angiosperm litters showed higher enzyme activities than the coniferous litters in the higher altitude regions (Kshattriya et al. 1992). The activities of dehydrogenase, urease, and phosphatase enzymes were determined from the microbial population of four different forest areas of lower and higher altitude regions. The forest stands considered in the study did not have the same regeneration stage and it was noticed that the activity of dehydrogenase enzyme positively correlated with fungal numbers in the four locations. The correlation was significantly positive regarding microbial number and urease activity at the lower altitudes of less degraded forests. But at higher altitudes, only fungal population numbers showed positive relation with urease activity. However, no significant correlation was observed between phosphatase activity and microbial population in case of higher altitude regions, but it was significantly positively correlated to number of fungi and organic C in soil, in the case of lower altitude (Jha et al. 1992). Xu et al. reported the higher activity of *N*-acetylglucosaminidase, β-glucosidase, leucine aminopeptidase, and acid phosphatase at mixed coniferous with broad-leaved forest than the other studied sites of high altitude regions. The soil nutrients and soil organic matter decomposition displayed positive correlation with the enzyme activities (Xu et al. 2015). A psychrotolerant fungus *Cladosporium tenuissimum* isolated from the Indian Himalayan region specifically a cold desert has been reported to be producing extracellular laccase enzyme. The enzyme showed optimum activity at temperature and pH of 14 °C and 5.5, respectively (Dhakar and Pandey 2016). Siles et al. (2016) reported a positive correlation of microbial activity with the increase in altitude. The increase in microbial activity ranges from 1.7 times (sulfatase) to 4.8 times (β-glucosidase). During the study, it was observed that enzymes showed highest activity at alpine site except xylanase, whereas Schinner (1982) demonstrated higher xylanase activity and SOM content in alpine soils. PLFA-related analysis displayed a location specific effect of enzyme production. The production of enzymes such as acidic phosphatase, arylsulfatase, protease, and cellobiohydrolase was increased in spring than in autumn, while β-glucosidase and xylanase remain unaffected with the change in season (Siles et al. 2016). The production of laccase enzyme was investigated from a fungal strain *Pestalotiopsis* spp. CDBT-F-G1 which possesses an approximate molecular mass of 43 kDa with pH and temperature optima of 6 and 60 °C, respectively (Yadav et al. 2019). Pastor et al. (2019) studied the role of various factors that control the enzymes during the processing of organic matter present in the streams of high arctic region. It was observed that phenol oxidase and phosphatase activities were lower in alluvial regions than the solifluction regions. Major drivers controlling the enzyme activities at high arctic are dissolved organic carbon and availability of nitrogen (Pastor et al. 2019).

Xiao et al. 2019 studied the enzymatic activities of cellulase (CEL), polyphenol oxidase (PPO), saccharase (SAC), urease (URE), catalase (CAT), alkaline phosphatase (ALP) in the increasing order of altitude of a dry valley in Southwest China. They reported a direct positive correlation of absolute enzyme activities with altitude but the specific activities were not that straightforward, which first decreased and then increased, except for phosphatase. The enzyme activities increased with an increase in nutrients such as nitrogen, which had an increasing trend with altitude rise and this abundant nitrogen concentration may be responsible for overcoming the excretion and other metabolic limitations of microbes (Sinsabaugh et al. 2002; Xu et al. 2015). But this does not apply to enzymes that degrade biomass and form elemental cycles, which may not be affected by changes in elevation (Nottingham et al. 2015; Chang et al. 2016). They found that the changes in soil SOC does not have to be reflected in the changes in enzyme activity per unit SOC and its different from the activity calculated based on soil mass. The specific activities of immobilized extracellular enzymes were more informative than the absolute activities regarding ecology and enzymatic efficiency (Raiesi and Beheshti 2014; Zhang et al. 2015). For this study site in the dry-hot valley region of China, the increase in altitude correlated more to specific enzymatic activity than absolute enzymatic activity. King et al. (2008) reported the higher phosphatase and peptidase activities in the soil of Peru and higher activities of phosphatase and β-glucosidase in the soil of Colorado. β-Glucosidase isolated from Colorado had positive correlation to C from microbial biomass, while phosphatase showed correlation with dissolved organic C and with microbial biomass C but none of them had significant correlation to water content in the soil. The phosphatase and peptidase enzyme activities in Peru had no correlation with either soil carbon content or the amount of water in soil. The dehydrogenase activity of soil collected from the Grossglockner mountain area (Austrian central Alps) shown to be decreased with the increase in altitude. But higher activity of dehydrogenase was observed in alpine soils than the subalpine soils (Margesin et al. 2009).

11.5 Factors Affecting the Microbial Community and Their Activities at High Altitude

The climatic conditions, chemical properties, and respiratory activity of soil directly influence the quantity of enzymes (Sinsabaugh and Linkins 1989; Kshattriya et al. 1992). Various studies suggested that microbial communities of soil at higher altitude depend on wide range of factors. It is reported from the literature that majority of the enzymes including dehydrogenase (Margesin et al. 2009) showed decrease in their activity along the altitude gradients but in a report on the Mediterranean soil showed lower enzyme activities at lower altitudes. The studies suggested that the factors related to altitude including soil moisture and temperature play major role in levels of enzyme activities (Lucas-Borja et al. 2012). It was observed that the increase in pH values increases the levels of total phospholipid fatty acids (PLFAs) in the soils of beech/beech oak forest located in the Northern Germany (Bååth and Anderson 2003). The high altitude regions generally possess low temperature conditions; therefore, fungal strains are more prominent in these areas which ultimately increased the fungal/bacterial ratio (Margesin et al. 2009), whereas Djukic et al. (2010) reported that variation in the composition of vegetation and decomposition condition affected the structure of microbial community in the higher altitude regions of Austrian limestone alps. The change in climatic conditions with the season tends to change the soil enzyme activities of that particular site. It was observed from the previous studies that increase in elevation leads to harshness in the environmental conditions such as cold climate, change in vegetation, and improper nutrient conditions, which ultimately affected the microbial activities, thus climate shifts also alter enzyme activities (Xu et al. 2015).

In addition, fraction of organic carbon in the soil and microbial biomass carbon was effectively related to the soil enzyme activities (Wang et al. 2013). However, there is still unclear image of the major influencing factors of soil enzyme activities.

Xu et al. (2015) reported that change in altitude tends to change the structural and functional patterns with the microbial community of the soil. Thus, they hypothesized the following three patterns in the microbiological characteristics of the soil:

1. Increase in altitude leads to decrease in soil microbial biomass.
2. Enzyme activities are primarily controlled by the nutrients present in the soil, whereas changes in the microbial composition of the soil are mainly regulated by the C/N ratios and pH of the soil.
3. There should be a positive correlation between the microbial biomass, SOM decomposition, and enzyme activity.

Forests located in the Italian alpines were investigated to observe the effect of change in season from the spring to autumn in high altitude regions on the microbial properties of the soil. Therefore, they studied several factors including soil temperature, microbial activities, community level physiological profiles (CLPP), microbial abundance and community structure, PLFA profiles, and physiochemical properties in each season. The concentration of nutrients in the soil and soil organic matter (SOM) was found to be increased, whereas enzyme production was highly dependent on the site and season. The correlation between site factors and incubation temperature for soil microbial activities confirms the variation in microbial communities. Site-specific effects were noticed for CLPP. It was observed that site-specific, altitudinal and seasonal effects influence the microbial community structure and its functioning. Correlation study suggested that altitude was the major factor for the determination of variation in the biotic and abiotic characteristics of the sites, while seasonal variation does not show any major effect (Siles et al. 2016). Singh et al. presented a report on diversity of microbes at various extreme locations. According to their study, the extremity of the environment increases with the altitude gradient which ultimately resulted in the lower levels of microbial biomass and enzyme activity. It was also hypothesized that the composition of microbial community varies with the change in environmental conditions (Margesin and Miteva 2011). An increase in culturable psychrophilic heterotrophic Gram-negative bacteria and fungi was observed with the increase in altitude in the Austrian Central Alps soils and the dominance of proteobacteria was found among them. The microbial activity of dehydrogenases also decreases with increasing altitude (Margesin et al. 2009). A decrease in microbial species was noticed with the elevational gradients in the cold soils of Himalayan mountains and the abundance of proteobacteria and β-proteobacteria was also reported in a culture-independent study, whereas the culture dependent study displayed the dominance of γ-proteobacteria, Firmicutes, and low number of bacteroidetes (Margesin and Miteva 2011; Singh et al. 2019).

The enzyme activities were lower in case of litters at higher altitude than the litters of lower altitude, whereas cellulase and amylase showed the variation in their activities with the change in season at both altitudes. Cellulase and amylase activities were found to be increased during the decomposition of litter, whereas invertase activity increased only at the beginning of litter decomposition. The number of fungi and bacteria significantly correlates with the activity of cellulase and amylase; however, invertase activity remains unaffected with the microbial population (Kshattriya et al. 1992).

The forest stands considered under study were at different stages of regeneration and it was found that microbial population numbers were lower in highly degraded forests than in less degraded ones. The factors including fungal and bacterial population numbers, enzyme activities, organic C, and soil moisture were considered for the measurement of their correlation coefficient. It was noticed in all the forest stands that fungal population numbers develop a positive correlation with dehydrogenase activity. A notable level of positive correlation was observed between microbial population numbers and urease activity at the lower altitudes of less degraded forests. But at higher altitudes, only fungal population numbers showed positive relation with urease activity. However, no significant correlation was observed between phosphatase activity and the abovementioned factors in case of higher altitude regions, but in lower altitude regions, phosphatase enzyme possesses positive correlation with fungal population numbers and the concentration of soil organic C (Jha et al. 1992). Xu et al. reported a variation in PLFAs profiles with the change in altitude. The ratios of fungal/bacterial (F/B) number and the Gram-positive/Gram-negative (G+/G−) bacteria hiked with the increase in altitude. Various factors including the soil moisture, silt and clay fraction, mean annual temperature (MAT), mean annual precipitation (MAP), phosphorus concentration, and nitrate nitrogen correlate with the changes in the composition of microbial community of the soil. The nutrients present in the soil were significantly correlated with the variation in soil enzyme activities. The rate of decomposition of soil organic matter (SOM) showed positive correlation with the microbial enzyme activities and PLAs profiles (Xu et al. 2015).

11.6 Concluding Remarks and Future Perspectives

The mountainous regions around the world have always been a source of life owning to their role in regulating the water cycle. The large number of glaciers that adorn the upper levels of the highest mountains are the source of constant supply of freshwater to the inhabitants living in all the areas lying below. The microbial communities on these locations are adapted to extreme conditions like cold temperature and different levels of moisture content. But the threat of global warming is the first to reach and affect these areas, eroding glaciers and exposing underlying soils to new environments. These processes cause the loss of existing species of microorganisms and they may be replaced by a different set of microbes. Therefore it is of utmost importance to study the existing microbial community in these areas with an alarming approach but sadly that has not been the case as the impact on microbial life has largely been ignored. There is a distinct possibility of finding new species and their associated enzymes that may be useful to us industrially. Although the remote dry mountains have been studied geologically, the biological studies have had a very slow start. The study of barren high altitude zones like Atacama, Andes, or Colorado Rockies has revealed that the microorganisms surviving in such areas are carbon limited due to the absence of any plant life and dependent on the presence of ice packs whose melting may endanger the particular ecosystem. Although the old system of microbe culturing had limitations in growing various strains that existed in high altitudes, thus recent developments in metagenomics and techniques like 16S rRNA and MALDI-TOF have increased the chances that we can document most of the life forms that exist on the mountains. Soil organic matter (SOM) decomposition is yet another issue that is still very unclear in terms of how it occurs and requires a lot of further research, although an increase in SOM content with altitude and proportionally the number of fungi/bacteria have been observed. Study of microorganisms in these remote and untouched environments will also help us in hypothesizing the kind of atmosphere and life that existed on prehistoric earth.

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Psychrotolerant Microbes: Characterization, Conservation, Strain Improvements, Mass Production, and Commercialization

12

Pankaj K. Mishra, Samiksha Joshi, Saurabh Gangola, Priyanka Khati, J. K. Bisht, and A. Pattanayak

Abstract

Exploring cold habitats offers untapped sites for screening and harnessing potential/novel psychrotrophic microbes bestowed with the characteristic to grow near 0 °C and optima lying in mesophilic range. These microbes are of great commercial importance and find multiple uses in different areas such as industries, pharmaceuticals, and agriculture as they are potential producers of enzymes, peptides, biodetergents, antibiotics and acquire multiple plant growth-promoting traits. Utility of such cold-active microbial strains is of immense need for high altitude agroecosystems due to the unique climatic conditions. Hence, it is crucial to identify, characterize, and conserve these beneficial microbes that maintain their functional properties under cold temperature conditions. This chapter is likely to provide some more insights into the recent developments associated with improvement and large-scale production of psychrotolerant microbes as well as scaling up for commercial production.

Keywords

PGPR · Psychrotolerant · Characterization · Strain improvement · Conservation · Commercialization

12.1 Introduction

Temperature plays an important role in colonization of microbial communities under natural environments. Cold temperature induced stress gains prime importance in global ecology due to pervasiveness and superiority of cold-tolerant microbes which are considered as most efficient colonizers on earth (Russell 1990). Psychrotolerant

P. K. Mishra (✉) · S. Joshi · S. Gangola · P. Khati · J. K. Bisht · A. Pattanayak
ICAR—Vivekananda Institute of Hill Agriculture, Almora, Uttarakhand, India

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microbes can grow near 0 °C with growth optima above 30 °C, therefore also known as cold-tolerant mesophiles (Morita 1975). Psychrotolerant microbes are highly widespread as compared to psychrophiles because of broad temperature range. These microbes show highly extended lag periods when grown on growth medium under in vitro conditions. Various habitats such as Antarctic lakes, snow, glaciers, ice cap cores and cloud droplets have been found to be colonized by psychrotrophs (Yadav et al. 2015). Complete genome sequences of numerous novel and potential cold-tolerant microbes are available (Singh et al. 2016). Most of the novel species belong to different phylum of domain Bacteria (Actinobacteria, Bacteroidetes, Cyanobacteria, Proteobacteria, Spirochaetes, Firmicutes, Chlamydiae, Mucoromycota, Chloroflexi, Nitrospirae, Gemmatimonadetes, Planctomycetes, and Verrucomicrobia), Archaea (Euryarchaeota and Thaumarchaeota), and fungi (Mucoromycota, Basidiomycota, and Ascomycota) (Zachariah et al. 2017). Moreover, several fungal communities have also been investigated in colder regions, with respect to their role in ecosystem services such as nutrient immobilization, mineralization, and biodegradation (Wang et al. 2015).

Recently, psychrotolerant microbes have attained the focus due to enormous biotechnological applications in different agricultural, medicinal, and industrial sectors. They are able to produce variety of enzymes, antibiotics, peptides, antifreezing compounds and also exhibit multiple plant growth-promoting attributes (such as siderophore, indole acetic acid, ammonia, and hydrogen cyanide production; solubilization of zinc, potassium, and phosphorus; biocontrol activity against phytopathogens) (Yadav et al. 2017). Shukla and co-workers (2016) have reported biodegradation of agricultural waste by developing consortium of cold-tolerant *Paecilomyces* sp., *Eupenicillium* sp., and *Bacillus* sp. Extreme environmental conditions are quite common on earth and the microbial diversity associated with cold areas has attracted the special interest of the scientific community due to its wide range applications. Despite this fact, very less attention has been given towards bacterial population growing at low temperatures which may be due to their slow growth rate and difficulties associated with handling of these bacteria. Hence there is a need to explore such potential microbes. This chapter presents the psychrotolerant microbes applicability and their different perspectives for future endeavour.

12.2 Plant Growth-Promoting (PGP) Traits of Psychrotrophs

Cold-tolerant (psychrotrophs) microbes are crucial in sustaining the production and productivity of any agroecosystem as they retain their activity at suboptimal range of temperature. These microbes can enhance plant growth either directly, e.g., by N_2 fixation, phosphate solubilization, producing siderophores and various phytohormones or indirectly via inducing resistance against plant pathogens.

One of the most important macronutrient majorly affecting plant growth is nitrogen. Microbes with nitrogen fixation potential are reported as efficient alternatives to N fertilizers for promoting plant growth. Rhizobia associated with soybean root nodules in cold conditions of North America were found more efficient in nitrogen fixation and nodulation as compared to the plants of Southern region with warmer climate (Zhang et al. 2003). Verma and co-workers (2016) introduced several genera such as *Azotobacter, Arthrobacter*, *Azoarcus*, *Bacillus*, *Serratia, Gluconacetobacter*, *Pseudomonas*, *Klebsiella*, *Enterobacter,* and *Azospirillum* that are capable to fix N_2 under low-temperature condition.

PGP cold-tolerant bacteria can solubilize and mineralize the complex form of nutrients to their simpler form as very less proportion of essential macronutrients is available in soil. Das et al. (2003) reported solubilization of phosphorus in cold conditions by *Pseudomonas* sp. for the first time at 10 and 25 °C. Cold-tolerant species such as *P. putida* (Pandey et al. 2006), *P. fragi* (Selvakumar et al. 2009a), and *P. lurida* (Selvakumar et al. 2011) were isolated from different cold temperature regions, and showed significant increase in different plant growth parameters. Some species of *Aspergillus, Paecilomyces*, and *Penicillium* were also able to solubilize phosphorus through the production of organic acids and phosphatase enzymes (Dhakar and Pandey 2016).

Microbes produce siderophores which help in iron assimilation. Selvakumar and co-workers (2011) found the presence of siderophore production ability in *Pseudomonas lurida* M2RH3 isolated from low-temperature conditions. In another study a mutant of cold-tolerant *Pseudomonas fluorescens* was introduced by Katiyar and Goel (2004) which showed increased rhizosphere colonization along with 17 fold higher siderophore production. Inoculation of this strain in *Vigna radiata* plants has shown positive effects on its growth.

Indole-3-acetic acid (IAA) is an essential phytohormone and has positive effect on growth of plants (Patten and Glick 2002). A psychrotolerant *Pseudomonas jesenii* was found to produce IAA and promote plant growth when associated with native *Eleusine coracana*, *Cajanus cajan*, *Vigna radiate*, *Cicer arietinum*, and *Vigna mungo* (Kumar et al. 2014). Several IAA positive cold-tolerant bacterial strains were isolated from cold regions of Northwestern Himalayas and Leh, Ladakh, India (Yadav et al. 2015). Mishra et al. (2009) have documented IAA production by psychrotolerants *Pseudomonas* sp. strain NARs9 and PGERs17. Seed bacterization with these strains showed enhanced plant growth parameters of wheat seedlings.

ACC deaminase-producing bacteria helps to regulate ethylene levels ensuring that it does not impair the plant growth (Glick 1995). Verma et al. (2015) reported 25 cold-tolerant bacterial strains as ACC deaminase producers. *Pseudomonas putida* UW4, a psychrotolerant bacteria was found to enhance growth of *Brassica napus* at low temperature by producing ACC deaminase (Cheng et al. 2007).

Several species of *Trichoderma* were reported to inhibit different phytopathogens at low temperatures (McBeath 1995). In addition, a novel cold-tolerant bacteria *Exiguobacterium acetylicum* was isolated from North–Western Indian Himalayas showing antagonism against various phytopathogens like *Fusarium* sp., *Rhizoctonia* sp., *Sclerotium* sp., and *Pythium* sp. under in vitro conditions. Considering the multiple traits of psychrotrophs, a lot more efforts are required to decipher the potential bacterial strains capable of promoting plant growth under cold environmental conditions (Selvakumar et al. 2009b).

12.3 Characterization

Cold-tolerant microbes can be characterized through culture-dependent and culture-independent approaches. Very small proportion of microbial diversity can be assessed by cultivable methods described below due to the limitation of laboratory methods. Culture-independent methods can easily determine 100- to 1000-fold greater number of microbial communities in comparison to conventional/traditional methods.

12.3.1 Cultivable/Culturable Methods

Isolation of cold-tolerant microbes can be done through enrichment or serial dilution methods followed by spread or pour plating on specific medium. Analysing large number of samples with more diversity is very time-consuming using this approach. Several non-selective and selective culture media are used for cultivation of microbes. Various genera or species dominant or present in small numbers can be detected and recovered using these medium. Yadav and co-workers (2018) mentioned different specific growth mediums for archaea (standard growth media, chemically defined medium, and halophilic medium), bacteria (Kings' B agar, Ammonium mineral salt, Antarctic bacterial medium, Jensen N_2-free agar, T3 agar, R2 agar, Tryptic soya agar, and Yeast mannitol agar), and fungus (Czapek Dox Agar, Rose Bengal agar, Sabouraud dextrose agar, and Potato dextrose agar). Isolation can also be done by giving variable abiotic stress conditions. Furthermore, these microbes can be checked for different functional traits qualitatively like nitrogen fixation (Boddey et al. 1995), phytohormones production (Bric et al. 1991), zinc solubilization (Fasim et al. 2002), ammonia production (Cappuccino and Sherman 1992), siderophore production (Schwyn and Neilands 1987), HCN production (Bakker and Schippers 1987), and phosphate solubilization (Pikovskaya 1948).

12.3.2 Non-cultivable/Unculturable Methods

12.3.2.1 Fluorescence Microscopy

Fluorescence microscopy is one of the most extensively used methods in microbial ecology with several advantages. It is rapid, easy to use, and makes visualization possible for spatial distribution of cells in sample. Viable cells can be easily differentiated from dead cells using suitable combinations of fluorescent stains. Epi-fluorescence microscopy and confocal scanning laser microscopy are mainly used for analysing the samples. Evaluation of results is either done manually or computer-aided. Image analysis provides detailed information which includes object recognition and measurement, image acquisition, processing, segmentation, and data output. Also, it allows rapid quantification of several other parameters (Kepner and Pratt 1994).

12.3.2.2 Molecular Techniques

Two major techniques applied for identification of bacteria at molecular level are PCR and hybridization. Hybridization method was first introduced and widely applied for microbial detection and their identification. Evolution of PCR techniques has led to the present scenario, where hybridization is mainly used in combination with PCR. Advancement leads to introduction of microarray technique which allows detection of large number of sequences simultaneously (Hacia et al. 1998).

Community analysis techniques allow examination of microbial communities from multiple samples simultaneously and compare genetic diversity from different habitats. Several techniques used for such kind of analysis include temperature-gradient gel electrophoresis (TGGE), denaturing-gradient gel electrophoresis (DGGE), and single-stranded conformational polymorphism (SSCP) (Turner et al. 2002). Several other techniques such as amplified ribosomal DNA restriction analysis (ARDRA), random amplified polymorphic DNA (RAPD), ribotyping, pulsed-field gel electrophoresis (PFGE), repetitive element sequence-based PCR (rep-PCR), and amplified fragment length polymorphism (AFLP) can be performed for genetic fingerprinting of bacterial isolates (Maukonen et al. 2003).

The major challenge faced by extremophiles is to obtain enough biomass during cultivation. This may occur due to harsh conditions that extremophiles need to grow (Ferrer et al. 2007). Vester and co-workers (2015) recently reviewed few techniques to deal with this problem and make the cultivation easier of such microbes. Metagenomics a culture-independent approach is an alternative for such population which involves DNA extraction of an environmental sample followed by the construction of metagenome libraries (Temperton and Giovannoni 2012).

12.3.3 Techniques Used to Culture the "Unculturables"

Cultivation of certain bacteria is made possible only when co-cultivated with helper strains due to their dependency on valuable bacterial interactions (Nichols et al. 2008). Helper strains release different factors into the environment which act as growth stimulator for uncultivable bacteria even in the dearth of helper strain. Nichols and co-workers (2008) reported growth stimulation of *Psychrobacter sp.* when grown in media conditioned with cell-free extracts or spent culture supernatants derived from helper strains. Signalling molecules are also found to be responsible for growth promotion. Studies revealed that the growth-promoting factor was identified as 5-amino-acid peptide and responsible for growth stimulation of *Psychrobacter* sp.

Another approach by Kaeberlein et al. (2002) simulates the natural conditions in vitro by constructing a diffusion chamber with a membrane which acts as channel for transfer of substances from the natural environment and helps in growth of bacteria. Reports are available on similar diffusion chambers used to culture "uncultivable" bacteria from freshwater (Bollmann et al. 2007) and marine environments (Nichols et al. 2008). Studies conducted with such chambers reported a greater diversity of recovered isolates than on conventional agar plate method.

12.4 Conservation

Worldwide distribution of approximately 758 microbial culture collections across 76 countries has been recorded by World Data Centre for Microorganisms (WDCM). Microbial resource centres facilitate long term preservation of microbes or microbiomes with high quality standards. It serves researchers or industries with several purposes as they get supplied with authentic microbial strains as reference material from these centres. In addition, it plays a key role in maintaining the balance of ecosystem which further secures biological resources on this planet for future purposes. Most of the bio-resource centres follow functional guidelines of World Federation for Culture Collections (WFCC) and best practices of the Organisation for Economic Development and Cooperation (OECD) (DSTI/STP/BIO (2007)9/REV1). Conservation of bio-resources and their use at global level follows the regulations under the Nagoya Protocol of CBD with special reference to "Biological Diversity (BD) Act 2002 and Rules 2004" of India (Sharma et al. 2018).

Cold-tolerant microbes are important constituents of ecosystems on earth with great biotechnological and agricultural importance. Different factors such as climate change, global warming, and anthropogenic activities may result in diversity loss. Therefore, their preservation is most important. Recent advancement in molecular techniques has found novel strains which are needed to be preserved to make them available for exploitation as well as for other research purposes. Microbial conservation approaches mainly involve "in situ", "ex situ", and "*in-factory*" form of conservation.

12.4.1 In Situ Conservation

"In situ" (on site) conservation is the most suitable way of conserving viable populations in their ecosystem and natural habitats. It provides long-term preservation of microbes under their natural habitat and is essential for places where no previous investigations have been made. Moreover, it involves description, management, and monitoring of biodiversity within the ecosystem where it is found. This approach can target either selected species or whole community (Heywood and Dulloo 2005). Convention on Biological Diversity (CBD), a treaty declared in June 1992 at the Rio de Janeiro Earth Summit, recognized the importance of conservation of biodiversity, however, subsequently GW Griffith advocated Global Strategies for Microbial Conservation (GSMC) was also introduced (Griffith 2012). Conservation of cold-tolerant microbes mainly those which are on the edge of extinction is needed on priority basis.

12.4.2 Ex Situ Conservation

"Ex situ" (off site) conservation preserves and maintains different microbial species in artificial media and are taxonomically well described. Microorganisms are being conserved in laboratories using specialized methods for their present and future applications. The microbes of specific nature can be protected and utilized in sustainable way in these repositories. In this method, the microbial activity is minimized by imposing different conditions. Different preservation and maintenance approaches available are sub-culturing, agar beads method, mineral oils, gelatin discs, cryopreservation, spray drying, freeze drying, desiccation, and silica gel storage. Out of these, freeze drying and cryopreservation are considered as most efficient and widely used methods for long-term preservation of microorganisms (Sharma et al. 2019a, b). Culture collections play a crucial role in long term preservation of cold-tolerant microbes. Many microbial resource centres are available for conservation in different countries of the world, following the guidelines of Organisation for Economic Development and Cooperation (OECD).

At an international level, International Union of Biological Sciences (IUBS) and World Federation for Culture Collections (WFCC) are mainly responsible for the development of culture collections (WFCC 2014). In all about 31 culture collections are registered with WDCM in India among which very few serves the scientific society. Among all of them, Microbial Culture Collection (MCC), Pune is declared as one of the biggest culture collection by WFCC because of high number of strain holding. It was established in 2007 by Dept. of Biotechnology, Govt. of India, New Delhi (Sharma and Shouche 2014) (Table 12.1). Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh recognized by World Intellectual Property Organization (WIPO), Geneva,

Table 12.1 Largest culture collections with holdings worldwide

Rank	Culture collections	Country	Holdings	Website links
1	Microbial Culture Collection (MCC)	India	164,652	http://www.nccs.res.in/mcc/index.html
2	NITE Biological Resource Centre (NBRC)	Japan	127,694	https://www.nite.go.jp/en/nbrc/
3	Northern Regional Research Laboratory (NRRL)	USA	96,200	https://nrrl.ncaur.usda.gov/
4	American Type Culture Collection (ATCC)	USA	75,079	https://www.atcc.org/
5	Culture Collection University of Gothenburg (CCUG)	Sweden	40,500	https://www.ccug.se/
6	Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)	Germany	33,784	https://www.dsmz.de/
7	Japan Collection of Microorganisms (JCM)	Japan	24,784	https://jcm.brc.riken.jp/en/
8	Korean Collection for Type Cultures (KCTC)	Korea	23,175	https://kctc.kribb.re.kr/En/Kctc.aspx
9	China Centre for Type Culture Collection (CCTCC)	China	22,073	https://www.wipo.int/budapest/en/idadb/details.jsp?id=5827

Source: www.wfcc.info

Switzerland collectively holds above 9000 cultures (mtccindia.res.in). Also, National Agriculturally Important Microbial Culture Collection (NAIMCC), ICAR-NBAIM, Mau, Uttar Pradesh is a Designated Repository (DR) recognized by National Biodiversity Authority (NBA), Government of India, for conservation of agriculturally important microbial wealth of the country (Sharma et al. 2019a, b). Furthermore, microbial culture collection for conserving and bioprospecting cold-adapted fungi of Indian Himalayan Region (IHR) has been established in the microbiology lab of G. B. Pant National Institute of Himalayan Environment & Sustainable Development (GBPNIHESD) (Pandey et al. 2019).

12.4.3 In-Factory Conservation

"*In-factory*" approach is an intermediate type of conservation and mainly followed by agricultural or industrial sectors. This method conserves through keeping them in normal conditions of use by two different ways. One is dynamic strategy where no restrictions are imposed on the use of strains, except mixing it with any culture of different origin. However, other method, i.e., static conservation follows strict restrictions to maintain the culture and avoid any type of changes (Sharma 2016).

12.5 Strain Improvements

Genetic improvement of microbes is of immense importance in biotechnology and agriculture field. To fulfil the demands, strain improvement using different approaches has large contribution during the last decades.

12.5.1 Gene Cloning and Mutagenesis

Quantitative as well as qualitative enhancement of applications needs strain improvement as it helps to attain desirable changes at gene level (Fig. 12.1) which controls the productivity of organisms genetically. Kulakova and co-workers (1999) reported five times more active production of serine alkaline protease (SapSh) after cloning the gene encoding protein from *Shewanella,* a psychrotolerant strain Ac10 to *Escherichia coli.* Similar kind of study was performed with *Pseudoalteromonas* sp. present in seawater of Antarctica (Guoying et al. 2011). Another approach is site-directed mutagenesis. Tindbaek et al. (2004) constructed Savinase-S39 hybrid from subtilisin enzyme using site-directed mutagenesis. This hybrid exhibited improved low-temperature activity and wide substrate range. Saranraj et al. (2013) reported that mutants of cold-tolerant *Pseudomonas fluorescens* strains GRSI and PRS9 showed increased solubilization activity under laboratory and natural conditions. In addition, random mutagenesis is found to be superior from a commercial point of view (Twardowski and Małyska 2015) and can be achieved by different chemical or

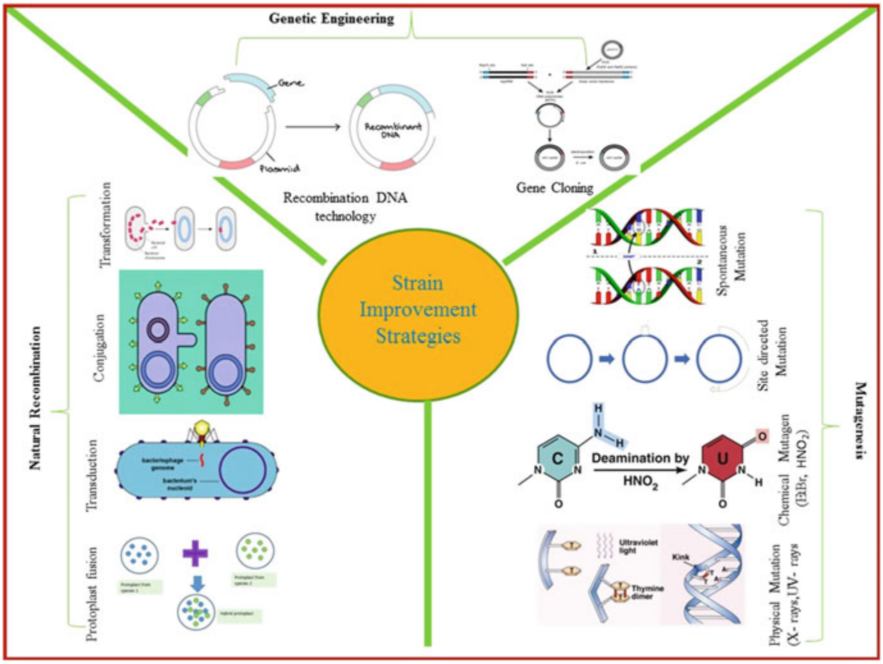

Fig. 12.1 Strain improvement strategies

physical mutagens. Consequently, SOS system helps to repair induced DNA damages through making DNA alterations (Bridges 2001).

12.5.2 Atmospheric and Room-Temperature Plasma (ARTP)

ARTP approach developed for strain improvement has gained more attention and also made commercially available nowadays. This system does not produce harmful radiations, toxins, or any other kind of waste deleterious for the environment and thus requires minimal training. Thus, it has been proved as the most reliable and effective technique leading to generation of large number of random mutations. Ottenheim et al. (2018) reported its successful application in improving strain properties of diverse bacterial and fungal species for increased production of different valuable products. Dong et al. (2017) isolated and identified a cold-adapted strain as *Pseudomonas* sp. producing esterase from the Glacier of Tianshan, Republic of China. Moreover, they generated mutants of the same strain using this technique and screened them for enhanced esterase activity.

12.6 Mass Production

Fermentation is a process designed for mass production and to achieve maximum product yield with minimum manufacturing costs (Hellmuth and Van den brink 2013). These two targets can be fulfilled by adjusting optimum conditions for strain and product. Various factors affecting strain physiology and fermentation conditions to which microbes are adapted need to be considered. Some of the important fermentation aspects include pH, temperature, medium composition, aeration, and mixing. Mostly batch type fermentations are preferred over others, but more productivity is found to be achieved by fed-batch method. Strain related information is necessary for fed-batch operations, to avoid limitations. Another essential aspect is efficient mixing in the bioreactor as it leads to optimal distribution of nutrients and oxygen in the entire medium (Zlokarnik 2000). Continuous fermentations are rarely used in industries due to increased risk of contamination.

12.6.1 For Enzyme Production

Cold-active enzymes are important and widely used with large number of applications in different sectors, but their production cost limits their use (Kademi et al. 2005). Therefore, it is of major interest to increase their production by optimizing culture conditions during fermentation processes. Use of cheap substrates may result in enhanced productivity and decreased cost of production (Dominguez et al. 2003).

(a) **Production and media development:** Microbial enzyme production can be done by submerged culture (Ito et al. 2001) and solid-state fermentation methods (Chisti 1999). Solid-state fermentation (SSF) uses low-cost substrates as nutrient sources for microbial enzyme production, which mainly includes by-products obtained from agricultural industries. This may add value to the final product with reduced cost (Menoncin et al. 2008). Several studies optimized pH, temperature, carbon, nitrogen, and dissolved oxygen concentration for different microbes (Elibol and Ozer 2000). However, this may require large space, complex media, machinery, high energy demand, more capital, and good control systems (Satyanarayana 1994). Immobilized cell culture has also been useful in few cases (Hemachander et al. 2001). It increases the rate of reaction and also facilitates in downstream processing (Gunasekaran and Das 2005).

(b) **Purification:** It helps to increase the purity level of enzyme with enhanced biocatalyst activity (Balaji and Ebenezer 2008). The methods mainly used for purification include gel filtration, extraction, precipitation, crystallization, ion exchange chromatography, and affinity chromatography. Techniques used for purification should be fast, high yielding, low cost, and liable to large-scale operations.

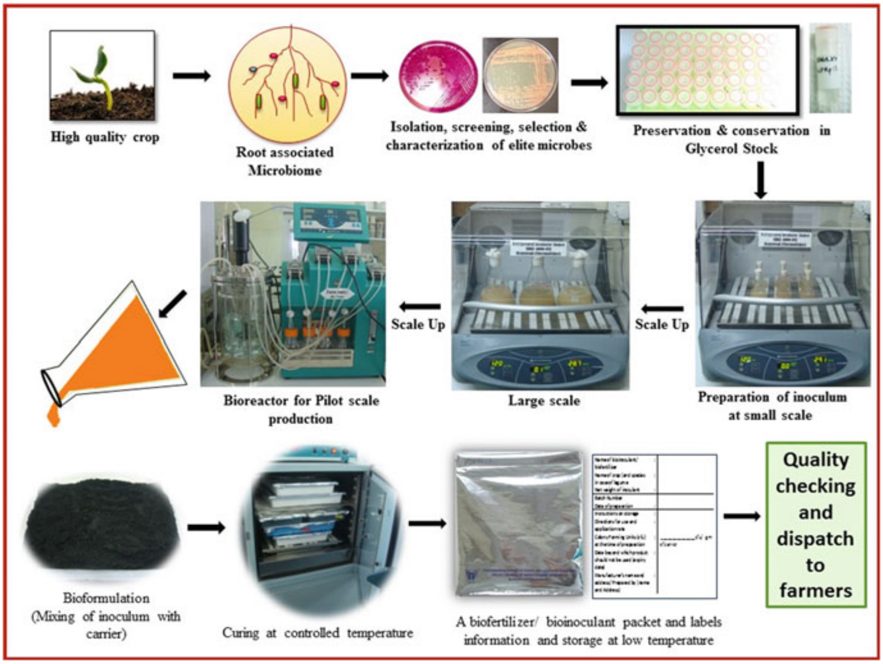

Fig. 12.2 A scheme showing large-scale production preparation of bioformulation/bioinoculant

(c) **Shear tolerance:** This step involves application of emulsifiers and strong mechanical agitation to improve interfacial area in the bioreactors. Various studies demonstrated shear-associated inactivation of many enzymes at liquid–liquid or gas–liquid interfaces (Chisti 1999).

(d) **Three-dimensional structure:** It is important to investigate and understand three-dimensional structures of enzymes and association between their structure and function (Saxena et al. 2003). Furthermore, factors responsible for enantiospecificity and region specificity are also essential to make enzymes suitable for specific applications. Sharma et al. (2001) studied 3D structure of 12 lipases belonging to different microbial origin.

12.6.2 For Bioinoculant Production

Mass production of bioformulation/bioinoculants (Fig. 12.2) requires specific environmental and nutritional conditions for the growth of microorganism. This can be obtained through solid, semi-solid, or liquid fermentation methods.

In liquid fermentation a low-cost medium is required with appropriate nutrient balance (Nakkeeran et al. 2004). However, variety of organic substrates can be used for the solid-state fermentation. Media consisting of inert carriers with food bases can be used for mass production of biocontrol agents. Solid substrates such as wheat bran, sawdust, straws, sorghum grains, farmyard manure, moistened bagasse, paddy chaff, decomposed coir pith, and substrates rich in cellulose are selected for inoculums production. Incorporation of some antagonistic bacteria can be done to the organic manures to manage plant parasitic nematodes. This step is further followed by incubation at 35 °C for 5–10 days coupled with frequent mixing under sterile conditions along with water to maintain moist conditions, which aid in the proliferation of the bacteria (Siddiqui and Mahmood 1996). The enriched organic manure with biocidal value could be used as bioformulation which further helps in pest control and also augments plant growth.

12.7 Commercialization

Psychrotolerant microbes have commercial importance because of their wide advantages in different areas. Cold adaptation involves numerous modifications in structure, molecular architecture, biochemistry, and physiology of these microorganisms (Ramana et al. 2000). Presently, they have been recognized for various economical as well as ecological advantages over mesophilic and thermophilic microbes (Soror et al. 2007). Cold-active enzymes are discovered with novel properties like high catalytic efficiency at low temperatures which helps to save time and energy loss (Margesin et al. 2005). Different cold-tolerant microbes showed synthesis of these enzymes (Zeng et al. 2003). Vazquez et al. (2008) reported production of extracellular protease enzyme from eight Antarctic cold-tolerant *Pseudomonas* sp. strains. Several studies proved psychrotolerant yeasts as good candidates for production of different cold-active enzymes like pectinase, lipase, protease, amylase, cellulase, and esterase (Carrasco et al. 2016). Several fungi, belonging to the species of *Penicillium, Cladosporium, Phialophora,* and *Aspergillus* isolated from Indian Himalayas, possessed characteristics of producing extracellular laccase enzyme (Dhakar and Pandey 2016). In addition, several studies have also revealed the role of cold-tolerant microbes (PGPR) in making bioformulations through which they can improve nutrient availability to the plants (Verma et al. 2017). Nowadays, exploitation and commercialization of cold-tolerant microbes in biotechnological and agricultural sector is of great interest.

12.7.1 In Food Industry

Psychrotolerants and their enzymes have great utility in this area. They are not only beneficial due to their high specific activity but also for their easy inactivation when required. Inactivation of these enzymes can be done by relatively low heat. Milk coagulating enzymes obtained from cold-tolerant microbes have the advantage of controlled casein coagulation for maintaining the quality of whey. Few products of microbial rennet are commercially available in developed countries with different brand names such as Marzyme® (DuPont, USA), Rennilase 50TL, and Moelilase. Cold-active neutral protease obtained from *Bacillus subtilis* is also being marketed under the commercial name eutrase. The enzyme enhances the flavour intensity by reducing the time for ripening. Furthermore, they facilitate various purposes like beer treatment, in bakeries, meat tenderization, etc. (Margesin and Schinner 1994).

12.7.2 In Bioremediation

Psychrotolerant microorganisms have potential to degrade various compounds in different environmental habitats. They can be used for removal of several pollutants such as dodecane, hexadecane, naphthalene, and toluene at low temperatures. Few degradative genes like ndo B and todC I responsible for catabolism of naphthalene and toluene, respectively, have also been detected in psychrotrophs using the molecular approaches like hybridization and PCR (Banerjee et al. 2016). Moreover, a psychrotrophic species of *Rhodococcus* is found to degrade diesel and small chain-alkanes at low temperature. Cold-adapted proteases are also found with good potential for wastewater treatment and bioremediation in protein contaminated cold environments. Cold-adapted fungi, *Penicillium pinophilum* obtained from Pindari Glacier showed simultaneous production of lipase and ligninolytic enzymes under variable situations which proved beneficial for biodegradation at low temperature (Dhakar and Pandey 2016). Various other species are also investigated for their degradation potential of several waste products (Refai et al. 2015).

12.7.3 In Fine Chemical Synthesis

Polyhydroxyalkanoate (PHA) compounds, which belong to polyesters group and can serve as intracellular carbon and energy reserves, have been found to be produced by few cold-adapted microbes. These compounds are of great industrial interest due to their elastomeric and thermoplastic properties and as sources for fine chemical synthesis. Other enzymes like cold-adapted esterase and lipases have been found to exhibit a greater stereospecificity during fine chemical synthesis (Methé et al. 2005).

12.7.4 In Detergent Industry

Biodetergents are preferred over synthetic detergents due to their better cleaning properties and low energy input as they work at lower temperatures (Kuddus and Ramteke 2011). Recombinant enzymes with improved stability and high catalytic efficiency can be used, at low temperatures (Narinx et al. 1997). Serine protease (CP70) cold-active enzyme was reported to be synthesized by *Flavobacterium balustinum* with its optimum temperature lowered by 20 °C on comparing with conventional detergent protease like Savinase. *Stenotrophomonas maltophilia* obtained from Gangotri Glacier soil produced alkaline protease with enhanced stability and activity at pH 10 and 20 °C temperature. Detergents containing this enzyme showed tremendous results with high efficiency for removal of proteinaceous stains at low temperature (Kuddus and Ramteke 2009, 2011). Certain other enzymes added to detergents such as lipases, glycosidases, and subtilisin to hydrolyse macromolecular stains are poorly active at the temperature of tap water, thus can be substituted with psychrotolerant enzymes (Feller and Gerday 2003).

12.7.5 In Textile Industry

Psychrotolerant microbes are very good source of enzymes for the textile industries. Different enzymes have shown great utility in these industries for getting better methods of production and finishing of fabrics. This includes amylases for desizing of clothes, laccases for textile bleaching, cellulases for denim finishing (Araujo et al. 2008). Proteases have shown their unique importance in providing different finishes to wool and silk fibres (Najafi et al. 2005). Furthermore, it may help in degumming process of silk without causing any damage.

12.7.6 In Peptide Synthesis

Kumar and Bhalla (2005) have found successful application of microbial proteases in biologically active peptide synthesis. Besides several advantages associated with enzymatic synthesis of peptide, there are also some disadvantages. Presently, several studies are going on to find the solutions (Kumar and Bhalla 2005). More knowledge about the properties and biological functions of enzymes may lead to advancement of techniques for large-scale production of biologically active peptides.

12.7.7 As Plant Growth-Promoting (PGP) Bioinoculants in Agriculture

Commercialization of cold-tolerant microbes for plant growth promotion includes different stages: isolation of elite strains, screening, pot tests and field evaluation/efficacy, mass production and formulation development, fermentation methods, formulation viability, toxicology, industrial linkages, and quality control (Doraisamy et al. 2001). Biofertilizers are good replacements of synthetic fertilizers to get better soil fertility as well as a novel tool to increase the productivity with sustainable benefits to the agriculture. These microbes are referred as PGP microbes as they colonize the roots and stimulate the growth of plants. Cold-tolerant bacterium characterized as *Pantoea dispersa* strain 1A (Selvakumar et al. 2008a) were reported to been endowed with multifarious PGP traits such as siderophore production, P solubilization, HCN production, and IAA production. In another study Gulati and co-workers (2009) recorded major improvement in the growth parameters and yield of maize, pea, barley, and chickpea under field conditions after inoculating with *Acinetobacter* sp. strain BIHB 723 exhibiting multiple plant growth-promoting activities. Several studies on application of psychrotrophic bacteria in agriculture have been reported from India, mainly along the Himalayan region (Mishra et al. 2008, 2009, 2010; Vyas et al. 2010; Selvakumar et al. 2008a, b, 2011; Gulati et al. 2009). Low temperature is a major factor affecting growth and productivity of many economically important crops such as rice, wheat, and soybean (Solanke and Sharma 2008). Cold-tolerant microbes can be helpful to plants by alleviating the harmful effects.

12.8 Conclusion

Cold-tolerant microorganisms are extensively distributed in the agroecosystems. The potential of microbes to grow over wide range of temperature makes them attractive for multiple applications in industrial, agricultural, and allied sectors. Microorganisms endowed with multifarious plant growth-promoting traits could be used as bioinoculants. However, their full potential for any commercial use as biofertilizers to improve hill agriculture productivity is to be harnessed in coming years. High-throughput analysis techniques allow improved understanding of microbial diversity, colonization ability, and identification of potential microbes that retain different functional traits under low-temperature conditions in the field. Cold-active enzymes are also drawing major attention in biotechnology and require extensive efforts to overcome several bottlenecks such as high enzyme cost, low stability, and the low biodiversity of psychrotrophic microbes explored so far. Therefore, efforts have to be made to attain higher production of these beneficial compounds through alteration by genetic engineering. Also global strategies should come for conservation of this hidden microbial treasure in their habitats. In future, the field of application of these organisms will be enlarged more and will open up many newer eras in agriculture and industries because of their unique characteristics and easy accessibility.

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Understanding Cold-Adapted Plant Growth-Promoting Microorganisms from High-Altitude Ecosystems

13

Himani Singh, Nupur Sinha, and Prachi Bhargava

Abstract

Psychrophiles are found almost in all the ecosystems at low temperatures. They are of great importance as they act as models to study the mechanics for survival at low temperature and can be used to extract several enzymes and secondary metabolites which are useful in various industries say healthcare, food, detergent, tannery, etc. This chapter focuses on the basic modifications of psychrophiles at cellular, molecular and functional levels, their applications in different spheres of life and how these strategies can be mimicked in human lives.

Keywords

Psychrophiles · PGPR · High altitude · Cold-adapted enzymes

13.1 Ecological Diversity of Cold-Adapted Microorganisms

Low temperature suits best to psychrophiles for their growth and reproduction. The cold-adapted microorganisms are present at higher altitudes and in deep blue seas where the temperature is below 15 °C. Though these ecosystems are too harsh for survival, still diverse microbial communities survive facing all the challenges with the help of adaptations at various levels. The challenges include availability of

H. Singh
Institute of Biosciences and Technology, Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India

N. Sinha
Amity Institute of Biotechnology, AUUP, Noida, Uttar Pradesh, India

P. Bhargava (✉)
Institute of Agricultural Sciences and Technology, Shri Ramswaroop Memorial University, Barabanki, Uttar Pradesh, India
e-mail: prachi.bio@srmu.ac.in

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nutrients, IR radiations, excessive UV radiations, change in pH and very high osmotic pressure (De Maayer et al. 2014). Psychrophiles may be autotrophic, chemotrophic or heterotrophic based on their mode of nutrition. They play an important role in nutrient turn over and production of biomass in low-temperature environments and have significant role in various industries. Psychrophilic microbes find their importance in food preservation and degradation of organic matter in low temperatures where artificial cold environmental conditions are generated in vitro. Ironically most of the food spoiling bacteria are adapted to man-made cryo-environments. Pse*udomonas*, *Psychrobacter*, *Staphylococcus* and *Photobacterium* have often been isolated from psychrotolerant bacteria in artificial cold environments. Some of the factors that govern the existence of the genera in artificial cold environments have been summarized below:

- Possibility of shift of ambient environment to cold environment
- Possibility of invasion by their own basic cellular and molecular components
- Ability to propagate rapidly
- Presence or absence of oxygen at low temperatures

These genera are considered to be genetically diverse and have mechanisms for adapting to cold environments.

In addition to the above attributes, marine psychrophiles have cell membranes made up of lipids that do not harden in cold environment. Moreover, the presence of catalase atoms in psychrophiles help them to adapt to the natural conditions (higher concentration of hydrogen peroxide at low temperatures) under which these microbes endure. Three types of psychrotolerant H_2O_2-safe microscopic organisms have been disengaged from channel reservoirs of a fish egg preparing plant that utilizes H_2O_2 as a fading operator. Certain varieties of obscure bacterial species exist with specific varieties of natural adjustment systems (e.g., enzymatic efficiency of catalase and its cellular localization) contingent upon the natural H_2O_2 focus and delicacy of cells. Therefore, it is really hard to surmise general rules that may clarify the limit with respect to numerous psychrophiles to adjust their genomic and metabolic highlights to their local cold natural surroundings. The physiological studies of individual strain of proteins and genes show high level of psychrophilic adaptation (Rodrigues and Tiedje 2008; Casanueva et al. 2010). Various omics technologies have been utilized to ponder different capacities in microorganisms developed under various cold temperatures (Allen et al. 2009; Fondi et al. 2016). These adaptations work in a synergistic way at both genomic and metabolic levels to help the microorganism lead a smooth life in cold environment (Math et al. 2012). One of the example is the adenylate cyclase present in the cell membrane which gets activated at low temperature, aiding in smooth functioning of metabolic pathways. Various such cold adaptations will be discussed in detail in this chapter.

13.2 Effects of Low Temperature on Microbes

Low temperature can affect the microorganism in various ways. Reduction in growth rate and number of cell, variation in cell composition and nutritional requirements are some direct effects while other indirect effects include solute solubility, cell density, nutrient distribution and osmotic adjustment of the membrane. Microbes sense the decrease in environmental temperature with the help of their cellular responses like stiffness in their membrane, which is a very important membrane-associated sensor. Cold signal transduction pathway in microorganism is a two-component system. The signal with the help of sensors reaches the response regulator which in turn upregulate the genes involved in membrane fluidity in cold-adapted microorganism. The lipid bilayer maintains the cell permeability and transportation of essential solutes in liquid–crystalline phase. When temperature decreases, the functional phase of lipid transits into gel form due to which membrane fluidity is lost. Gene for fatty acid desaturases includes membrane lipid and protein phosphorylation and dephosphorylation, which induce phosphorylation of cytosolic protein (Jagtap and Ray 1999). Composition of fatty acid varies according to external temperature. At low temperature, there is more unsaturation owing to saturases, more methyl branching, alteration in fatty chain length, increase in the ratio of ante-iso to iso branching and change in the ratio of sterol and phospholipid contents. In 2008, Coa-Hoang et al. stated that cold shock induced membrane injury which triggered high rate of cell inactivation in microbes like *Escherichia coli* and *Bacillus subtilis*. Other adapting features like secretion of cold-shock proteins (Czapski and Trun 2014), molecular RNA chaperones, osmotic solutes (cryoprotectants) (Kawahara et al. 2008), enzymatic denaturation, incorrect protein tertiary and quaternary structures and intracellular ice formation play a pivotal role in the existence of microbes in cold environments.

13.2.1 Cell Membrane-Associated Changes

Microorganisms exhibit significant tolerance to chilling by reducing the damage in their membranes. Downshift in temperature reduces membrane fluidity and induce permeability in response to increased phase transition of membrane phospholipids (Cao-Hoang et al. 2010). Cells growing at 37 °C have more saturated fatty acid content (laurate) while at low temperature the content of laurate decreases and is substituted by unsaturated fatty acid (palmitoleate), which increases membrane fluidity and decreases membrane phase separation. Enzyme fatty acid desaturase causes unsaturation of fatty acids in *Bacillus subtilis* in preexisting membrane phospholipids (Aguilar et al. 2001). The gene of enzyme desaturase is regulated by a sensor called DesK kinase which activates the transcriptional activators DesR at cold temperature (Albanesi et al. 2004). Figure 13.1 illustrates the mechanism of unsaturation of already present fatty acids and maintenance of membrane fluidity by desaturase enzyme.

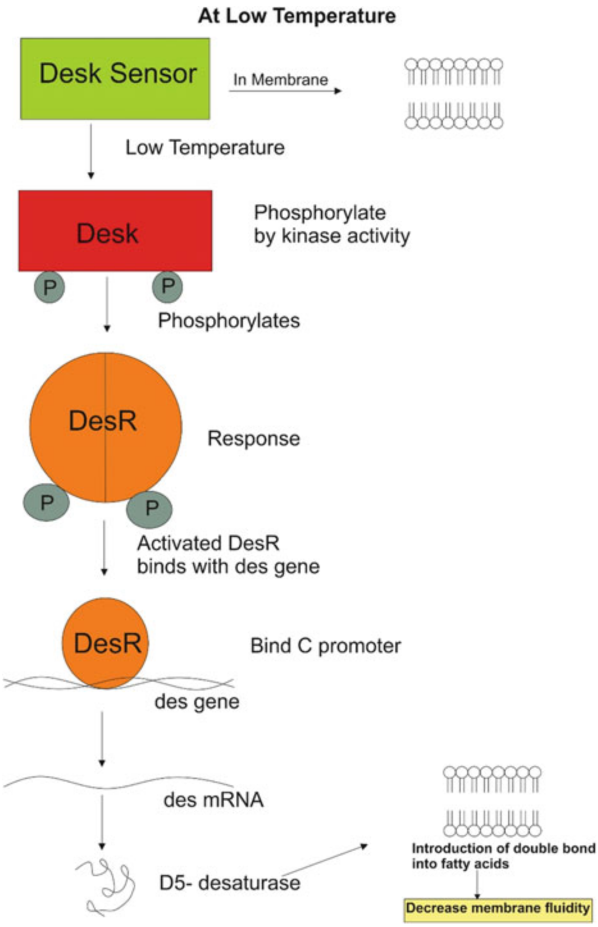

Fig. 13.1 Mechanism of unsaturation of already present fatty acids by desaturase enzyme at low temperature

13.2.2 Role of Cryoprotectants

Cryoprotectants (CRPs) are small molecules or chemical chaperones that provide defence mechanism against cold stress (Kawahara et al. 2008). These compounds include sugars like monosaccharides (glucose, fructose), disaccharides (sucrose, trehalose, etc.), polyamines, polyols (alcohol sugars such as glycerol and sorbitol) and amino acids (glycine, alanine, and proline). CRPs may be secreted outside the cell or may be located at intracellular level. Secreted CRPs can lower the freezing of water (Bouvet and Ben 2003) while their intracellular counterparts increase the total internal solute concentrations so as to regulate the osmotic pressure and maintain the osmolarity prior to freezing. CRPs have been reported in various bacteria like *Lactobacillus*, *Pseudomonas* and *Pantoea*. During cold shock, glycine betaine controls the aggregation of cellular proteins and regulates the fluidity of the membrane (Chattopadhyay 2002). Almost similar conditions have been reported in food-borne pathogen *L. monocytogenes*, where glycine betaine maintains high osmolarity at chilling stress (Angelidis and Smith 2003). Another cryoprotectant trehalose accumulates on both sides of the cell membrane and conserves intracellular water to stabilize cell membrane against freezing (Sano et al. 1999). Exopolysaccharides (EPS) synthesized by psychrophiles in cold environment have polyhydroxyls which prevent ice nucleation of water, enzyme denaturation and lysis of cell (Feng et al. 2014). ESPs store water and minerals and assist in cell aggregation, cell coating and formation of biofilm (microbial cells adhere to each other within an indigenous matrix of extracellular polymer) and maintain the viability of cells (Qin et al. 2007). Fungi *Mortierella elongata*, has some characteristics which favour their growth at low temperatures. These features include increased the amounts of intracellular trehalose, stearidonic acid and absence of ergosterol lipid when subjected to cold stress (Weinstein et al. 2000). Ergosterol is the main sterol in fungi which makes lipid membranes more rigid and decrease their membrane permeability; hence, deficiency of ergosterol causes the membrane more liable to cold-induced damage. Therefore, *M. elongata* increases the production of trehalose as an adaptation method in low temperature. Trehalose is the most effective cryoprotectants in thermotolerance in the fungi *Neurospora crassa* and *Cunninghamella japonica* (Neves et al. 1991; Tereshina et al. 1991). Dong and Chen found that at 4 °C cultured cell extracts of *Methanolobus psychrophilus* R15, there is upregulation of a new type of adenosine derivative which acts as osmotic solute in cold condition. Another cryoprotectant Cor26 is accumulated in *Pseudomonas fluorescens* KUIN-1 bacteria and aspartate in *Methanococcoides burtoni* in response to cold temperature. Aspartate is known to increase the affinity of GTP binding to elongation factor 2 while the action of Cor26 is unknown.

13.3 Cold Acclimation Proteins and Cold-Shock Proteins

Psychrophiles release a group of ~20 proteins during steady-state growth at cold temperature referred as cold-acclimation proteins (CAPs). The level of these proteins increases constitutively at low temperatures which help microorganism to adapt in cold climate (Phadtare 2004). They regulate protein synthesis and are essential for viability in cold condition. RNA chaperone CspA are usually cold-shock proteins reported in mesophiles and function as Caps in cold-adapted bacteria. The function of CAPs is not yet explored much; however, it has been revealed that these proteins regulate cell cycle and cell growth at lower temperature. *Pantoea ananas* KUIN-3 release a cold acclimation protein, Hsc 25, which has the potential of refolding the cold-denatured enzymes (Kawahara et al. 2008).

When environmental temperature comes down suddenly, psychrophilic bacteria show cold-shock response and release cold-shock proteins (Csps). These are (65–75 aa in length) nucleic acid-binding proteins (Czapski and Trun 2014). Cold-shock proteins neutralize various detrimental effects of fall in temperature and hence facilitate the cells to adjust with a transient overexpression that affect a number of molecular and cellular processes (Phadtare 2004). At cryo-temperature, RNA structures stabilize and become non-dynamic that induce premature transcription and translation termination. However, protein folding is disorganized, and ribosome function is hindered. Csps function as RNA chaperones helping in the sliding of ribosomes on target mRNA. This activity can be inhibited due to secondary structures of RNA at cold stress. Due to chaperone activity of Csps, single-stranded state of RNA is maintained (Barria et al. 2013). All Csps are ancient proteins which have some key conserved structure which includes five antiparallel strands that makes a ß-barrel. Csps that comprise a single nucleic acid-binding domain are known as cold-shock domain (CSD). CSD consists of two RNA binding motifs referred as ribonucleoprotein 1 and 2 (Lee et al. 2013). These binding motifs open tightly packed nucleic acid molecules which are inaccessible for translation (Chaikam and Karlson 2010).

Proteins that are constitutively synthesized in cell are called housekeeping proteins. Cold shock does not lower the production of these proteins, whereas the expression of Csps is enhanced with aggravated cold shock (Ermolenko and Makhatadze 2002). These CSPs reduce the expression of housekeeping gene and maintain the folding of important proteins. Hence cells adapt for temperature downshift at slower rate. Chaikam and Karlson (2010) reported that Csps are actively associated with the maintenance of chromosome folding. CspA was the first reported cold-shock protein in *Escherichia coli* (Goldstein et al. 1990). Previously it was reported that *E. coli* CspAs consist of nine homologous proteins (CspA to CspI). CspA consists of 13% of total cell proteins at cold condition while at 37–40 °C it is declined to lower levels (Lee et al. 2013). During cold or before freezing, Csp proteins are overexpressed in *Lactobacillus* strains which increase the survival rate of the cells. Human pathogen *Listeria monocytogenes* becomes less virulent under refrigerated condition due to the removal of *cspA*, *cspB*, and *cspD* genes which regulate the synthesis of the virulence factor listeriolysin O (Schärer et al. 2013). CspA from psychrophilic *Psychromonas arctica* was overexpressed in *E. coli* which increases the rate of cell survival and cold resistance in hosts by tenfold after repetitive freezing and thawing in polar environments (Jung et al. 2010). In Antarctic bacterium *Psychrobacter* sp. G, three CSP genes *Csp1137*, *Csp2039* and *Csp2531* have been identified with their regulatory sequence (Song et al. 2012). Csp genes of *Yersinia enterocolitica* 8081 and *Yersinia pseudotuberculosis* IP32953 share the maximum homology with csp genes of *E. coli* K-12 W3110 (Kanehisa et al. 2016). Enteropathogenic *Yersinia* psychrotrophs (spread by eatables and cause enteric illness yersiniosis) bear a locus having CspA duplication gene (cspA1 and A2) (Neuhaus et al. 1999).

13.4 Ice Nucleators and Antifreeze Proteins

Ice nucleators are the proteins that act as an ice crystal surface at low temperature (0 °C). They induce freezing and control the energy required for ice formation by ice crystal surface arrangement on water. Some bacteria have the potential of ice formation at low temperature. These bacteria are reported as "ice plus" bacteria. They have ice nucleation-active protein (Ina protein) located on the outer bacterial wall, which act as potent nucleating centre for ice crystals. *Erwinia herbicola* produces highly potent ice nucleators which show optimum activity at subfreezing temperature (Kozloff et al. 1983). Hirano et al. (1985) found that ice-nucleating bacteria live on the surface of leaves and induce frost damage when the temperature goes down. *Pantoea* (Lindow 1983), *Xanthomonas* (Kim et al. 1987) and *Pseudomonas* (Obata et al. 1987) are some examples of cryotolerant ice-nucleating bacteria.

Antifreeze proteins (AFPs) are ice-binding proteins that inhibit ice crystal formation and growth in any bacterium (Gilbert et al. 2005). They control the ice from melting by binding irreversibly to its surface. AFPs induce high thermal hysteresis activity and inhibit ice recrystallization even at milli-molar concentrations. Fungal AFP from a mold *Typhula ishikariensis* also possesses ice-binding properties (Cheng et al. 2016). Re-crystallization of ice is robustly reduced due to binding of antifreeze proteins with multiple ice planes. Ice-nucleating and antifreeze activities of AFP have also been identified in Arctic rhizobacterium *Pseudomonas putida* GR12-2, which is an extracellular glycolipo protein (Muryoi et al. 2004). There are three domains in ice-nucleating proteins, namely N, R and C. N domain facilitate the binding of ice-nucleating proteins (INP) to lipids and carbohydrates, and ice formation and ice nucleation activity are related to R-domain and C-terminal domain (Kawahara et al. 2008). Yamashita et al. (2002) reported that *Moraxella* was the first reported bacteria in Antarctic region that synthesizes an AFP for its survival in extreme cryo-environment. Ca^{2+}-dependent AFPs have been reported from *Marinomonas primoryensis* bacteria which is dominantly found in Antarctic lake (Gilbert et al. 2005). Psychrophilic phytopathogenic fungi have extracellular AFPs which check the freezing of hyphae (Robinson 2001) and make sure the accessibility of substrate by checking the rate of freezing of nutrients. Hoshino et al. (2009) reported that various genera belonging to Basidiomycetes, Oomycetes and Ascomycetes release AFPs which control freezing of extracellular environment and check the growth of mycelia at very low temperature.

13.5 Cold-Adapted Enzyme

In low temperature, the rate of chemical reactions is very slow because there is inadequate kinetic energy to conquer enzyme activation barriers (in ground state (substrate) and activated state). Psychrophiles release enzymes that show high specific activities at lower temperatures called as cold-adapted enzymes. These include cellulases, lipases, proteases, amylases, xylanases, pectinases, keratinases, esterases, catalases, peroxidases and phytases are perform important role under very harsh climatic as they represent low activation energy and high catalytic activity (Kuddus et al. 2011). Reaction rate (*kcat*) of cold-adapted enzymes is highly independent of temperature.

Cold-adapted enzymes increase structural flexibility to cope with freezing at low temperature (Collins et al. 2008). These adaptations involve molecular dynamic simulations of distinct stabilizing interactions either in the enzyme or at the active site of enzyme (implicated in catalysis). Some relevant factors include electrostatic interactions like reduced no of ion pairs, hydrogen bonds and hydrophobic interaction, reduced proline and arginine residues in loops, location of glycine residues, decreased cofactor binding, increased interaction with the solvent and reduced inter-subunit interactions (Siddiqui and Cavicchioli 2006). Amylases are very common enzyme found in microorganisms, plants and animals. The α-amylase reported in *Pseudoalteromonas haloplanktis* (AHA), also the most studied cold-adapted enzyme is monomeric, has multiple domains and shows Ca^{2+}- and Cl^{-}-dependent properties (Siddiqui and Cavicchioli 2006). D'Amico et al. (2003) reported that activation energy is reduced in psychrophilic α-amylase (35 kJ mol^{-1}) as compared to thermophilic α-amylase (70 kJ mol^{-1}), so k_{cat} of psychrophilic α-amylase is increased 21-folds at low temperature. Binding of substrates require low energy, so binding affinity of cold-adapted enzymes is lesser, and substrate binding is highly accessible. Several cold-adapted enzymes comprise a more labile and localized flexibility (flexible catalytic site) than other protein structure (Siddiqui et al. 2005). In cold-adapted enzymes, buried amino acids are smaller and show lesser hydrophobicity than their mesophilic and thermophilic counterparts. Hydrolysis and transesterification of fatty acid esters are catalysed by another class comprising of hydrolytic enzymes called esterases and lipases. Esterases differ from lipases on mode of their kinetics and specificity of substrate (Chahiniana and Sarda 2009). EstSL3 esterase, a novel cold-adapted enzyme from *Alkalibacterium* sp. SL3, shows close similarity to lipases extracted from *Alkalibacterium* and *Enterococcus* (Wang et al. 2016). Many cold-adapted enzymes have broad cavities to contain H_2O molecules and/or ligands (Giordano et al. 2015). Cold active pectinases abundantly used in the food-processing industry isolated from *Cryptococcus* have pectinolytic activity (35–36 U/mL at 9 °C) and synthesizes pectinase by glucose as carbon substrate (Birgisson et al. 2003). Some psychrophilic yeasts reported in Japan have pectinolytic activity only at 5 °C and are not capable to survive at high temperature (Tomoyuki et al. 2002). *Aureobasidium pullulans* strain produces pectinase enzyme at cryo-temperature which shows higher pectinase activity of 0.7–0.8 U/mL at 12 °C (Merín et al. 2011). Fungal strains *Aspergillus awamori* isolated from Himalayan region not only has maximum pectinase activity but also produce high amount of psychrophilic xylanases and cellulases (Anuradha et al. 2010)

13.6 RNA Degradosomes in Psychrophiles

Psychrophilic microbes have a multiprotein complex called degradosome which is engaged with the debasement of delivery moiety RNA and the handling of ribosomal RNA which is directed by non-coding RNA. The degradosome consists of enzymes like RNA helicase B, polynucleotide phosphorylase and RNase E (Carpousis 2002; Feng et al. 2001; Cho 2017). The amount of RNA in any cell varies with time for instance, in *Escherichia coli*, the time period of messenger RNA is approximately in the range of 2–25 min, whereas it may live more in other microscopic organisms. RNA is degraded even in resting cells, and the resulting nucleotides are reused for crisp rounds of nucleic acid synthesis. The amount of RNA formed by degradosomes is significant as related to quality guideline and quality control.

All life forms have numerous enzymatic tools for debasing RNA, for example, ribonucleases, helicases, 3′-end nucleotidyltransferases, 5′-end topping and decapping catalysts and RNA-restricting proteins which utilize RNA as substrate. RNA degradosome of *Escherichia coli* comprises four essential parts: (1) hydrolytic endo-ribonuclease RNase E, (2) phosphorolytic exoribonuclease PNPase, (3) adenosine triphosphate (ATP)-subordinate RNA helicase (Rh1B), and (4) glycolytic compound enolase. The degradosome of Antarctic bacterium *Pseudomonas syringae* contains ribonuclease E and RNA helicase. Polynucleotide phosphorylase is present in *Escherichia coli*, and this enzyme controls the quality of ribosomal ribonucleic acid. But the composition of degradosome of the Antarctic counterpart possess another exoribonuclease, ribonuclease R. In *Escherichia coli*, it is well known that ribonuclease R can degrade RNA molecules, and in this process, it does not require (ATP) but helicase requires ATP due to which energy is conserved by cells at low temperatures as well (Purusharth et al. 2005; Hardwick et al. 2010; Carpousis et al. 2009). The metabolic enzyme aconitase is found in *C. crescentus* and phosphofructokinase is found in *B. subtilis* degradosomes though enolase enzyme is present in both the psychrophiles. The bacteria regularly adjusts the gene expression to survive in the harsh environments. Ribonucleases (RNases) control the expression of regulatory proteins and protein-coding RNA by degradation and maturation. Exoribonucleolytic capacities are available in polynucleotide phosphorylase (PNPase) and RNase R in the human pathogen *Streptococcus pyogenes*. An exoribonuclease, PNPase is the focal 3′-to-5′ exoRNase partaking in RNA damage (Lécrivain et al. 2018; Chandran and Luisi 2006; Chandran et al. 2007). A considerable number of the Csp family proteins are in charge of RNA adjustment and debasement. Moreover, mRNA is stable in cold conditions due to the presence of chaperon functions of Csps. CspA also destabilizes the secondary structure and maintains its structure in a single-stranded state, which is necessary for its degradation. CspE acts in the opposite manner, and it stops RNA degradation. CspE binds to poly-A tails, which interfere with their degradation by PNPase, and it stops RNA cleavage by RNase E (Feng et al. 2001; Khemici et al. 2008; Prud'homme-Généreux et al. 2004). A DEAD-box helicase called DeaD in *E. coli* is added into the degradosome which can degrade RNA, under cold conditions.

13.7 Plant Growth Promotion by Agricultural Microbes in Cold Climate

The psychrophilic microorganisms help in the growth of plants under adverse conditions, their promotion and adaptations under harsh environments such as extremes of temperatures, high salt conditions, extremes of pH and drought stresses and are termed as plant-associated extremophilic microorganisms. They possess diverse plant development advancing characteristics, and hence, these productive and potential organisms might be used as biofertilizers to enhance the productivity and maintain the well-being of soil giving a push to the highly talked sustainable agriculture (Verma et al. 2016).

Certain strains of rhizospheric microorganisms, known as plant growth-promoting bacteria (PGPB), invigorate plant development and wellness. Various microorganisms promoting the yields are significant for keeping up the supportability of harvest generation in horticulture. Microorganisms related with harvests can be rhizospheric, phyllospheric, and endophytic based on their location. The rhizosphere contains roots and is affected by the addition of substrates that influence microbial action. A number of microorganisms are found to be associated with the plant rhizosphere which helps in the growth and development of the plant belonging to genera *Azospirillum, Bacillus*, *Pseudomonas*, *Rhizobium,* etc. (Verma et al. 2014). The epiphytic microorganisms are most versatile in nature as they endure high temperature (40–55 °C) and UV radiation. The phyllospheric microorganisms include *Agrobacterium, Pseudomonas,* etc. which can survive in harsh conditions such as extremes of temperature (Nutaratat et al. 2014).

The endophytic living beings are those microorganisms that colonizes in various aerial and subaerial parts of the plant, viz. root, stem or seeds without expediting any ruinous effect on the host plant. These microorganisms have been extracted from plants including wheat (Verma et al. 2013), soybean, pea, common bean, chickpea, pearl millet and rice (Suman et al. 2016). Various examples of endophytic microbial species are *Achromobacter, Azoarcus*, etc. (Verma et al. 2014). Microscopic organisms isolated from harsh temperature conditions are adjusted to live under stressful temperature conditions. Many optimizations have been used to isolate psychrotolerant and psychrophilic microbes from soil. The growth of cold-tolerant Antarctic bacterium can be increased by supplementing the minimal media supplements like amino acids which improved the growth rate of psychrophilic bacteria when the temperature was lowered from 11 °C to almost freezing point of water, i.e. 5 °C.

Indole-3-acetic acid (IAA) is a vital phytohormone secreted by PGPR which enhances overall plant development (Selvakumar et al. 2008). This IAA-secreting capacity of psychrophilic microorganisms acts as a marker tool for their identification while looking at the physiological or environmental conditions. Auxin production in microscopic organisms is controlled by the proline amino acid-dependent pentose phosphate pathway (Sahay et al. 2017). *Pantoea dispersa* and *Serratia marcescens* show their maximum IAA-creating capacity at 4 and 15 °C, respectively. Seed treatment with these bacterial strains significantly improved plant biomass and supplement take-up of wheat seedling developed at cold temperatures. Introduction of seeds with these mentioned strains upgraded the seed germination, root growth and shoot lengths of wheat plantlets developed at low temperatures (Sahu and Ray 2008).

Another bacterial framework that influences plant advancement is the nearness of compound 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This catalyst enhances the overall development and improvement of plants. Bacterial strains that have ACC deaminase can diminish the ethylene combination even in virus infections, thus curbing the negative impact on plants. Plants having ACC deaminase may adjust to this troublesome situation by cutting down ethylene level similar to normal stresses. Few psychro-tolerant bacteria producing ACC deaminase promote plant development even at low temperature that too under high osmotic pressure.

13.7.1 Nitrogen Fixation

Nitrogen fixation is a very essential process in the soil which is performed by many bacterial species that have the capacity to absorb atmospheric nitrogen and convert it into nitrogenous substances that furnish important nutrients for plants. These microbes synthesize the nitrogenase enzymes that form ammonia from nitrogen N_2. These processes require biological energy in the form of adenosine triphosphate (ATP). The nitrogen-fixing microbes may be free-living or symbiotic. The basic source of energy for some of the nitrogen-fixing microbes living freely is sunlight while others depend on organic matter present in soil. Soil microorganism *Azotobacter* is an aerobic heterotroph, and *Clostridium species* are active in conditions that do not have oxygen. Both the groups of microorganisms (free-living and symbiotic) can fix only minimal amounts of nitrogen, but still they are important for the survival of various plants in the environment.

Symbiotic microbes thrive on plant roots, forming root nodules. *Rhizobium* is an important member of this group which lives symbiotically with various members of leguminaseae family (peas, clover, beans, peanuts, soybeans). *Frankia* is an actinomycete associate with several plant families including species of temperate region trees, for example, *Alnus* and *Myrica*, the arid-region *Acacia*, and the tropical-region *Casuarina* and *Ceanothus*. Crop rotation helps to introduce good amounts of N_2 into the soil for efficient crop production.

Cyanobacteria have a pioneering role in fixing atmospheric nitrogen. They are very active in media which is very shallow such as flooded rice fields and marshy areas. An aquatic microbe, *Anabaena,* lives in a shallow medium in association with *Azola* which is a water fern, and its symbiosis can produce a high quantity of nitrogen per hectare annually, which is sufficient for rice production.

Nitrogen cycle involves various processes such as fixation, ammonification, nitrification and denitrification. Each step involves specialized microbes, and the consequences depend on the physiological state of the soil. The nitrogen cycle in the soil is also affected by the atmospheric processes. Partial removal of nitrogen in the soil releases N_2O, which is a very harmful and strong greenhouse gas responsible for global warming. Carbon dioxide and methane are other greenhouse gases that come out from the soil in special circumstances. N_2 fixation in the deep Arctic or Atlantic Ocean is the most important source of nitrogen where nitrogen is limited in system. N_2 fixation that occurs in ice-free summer waters contributes up to 30% of the N_2 fixation in the Arctic Ocean. Nitrogen fixation in freshwater is relatively common at high altitudes, but still nitrogen fixation in oceans is considered to be common. In Arctic region, when ice melts due to increase in temperature, the net production of nitrogen is increased in Arctic Ocean due to marine nitrogen fixers (Arrigo et al. 2012). A sufficient amount of nitrogen fixers is required to increase the productivity of nitrogen via Arctic nitrogen cycle which affects the primary producers that form the foundation of the food chain (Popova et al. 2012). This data helps us to conclude that N_2 fixation can also occur at minimum temperatures (Moisander et al. 2010) and at very high altitudes (Sohm et al. 2011; Díez et al. 2012) where it was believed that nitrogen fixation would not be possible.

13.7.2 Phosphate Solubilization

The availability of phosphorus is very important for the cultivation of healthy crops to cope up with the universal requirement of food. Various metabolic and physiological processes like energy transfer, photosynthesis, respiration, signal transduction and nitrogen fixation in plants of the family leguminaceae require P as essential macronutrient. Although P is the most abundant macronutrient found in almost all types of soils, it acts as the major limiting factor for the plant growth because of its unavailability to plants. Inorganic P occurs mostly in insoluble mineral complexes in soil, some are present in chemical fertilizers, and the plants are unable to absorb the insoluble and precipitated forms. Soil microorganisms help in the transformation of phosphorus and make it easily available to plant roots as they possess the ability to solubilize and mineralize phosphorus from inorganic phosphorus (Rodriduez et al. 1999). P-solubilizing bacteria and fungi have been isolated from both rhizospheric and non-rhizospheric soils and phyllosphere (Zaidi et al. 2009). In addition to bacteria and fungi, various microbial species that exhibit P solubilization capacity are actinomycetes and algae. Examples of P solubilization microorganisms are *Pseudomonas species, Bacillus species, Rhodococcus species*, *Arthrobacter species, Serratia species, Chryseobacterium species,* etc. (Wani et al. 2005; Chen et al. 2006), *Azotobacter species* (Sharma et al. 2013), *Xanthomonas species* (Srinivasan et al. 2012), *Enterobacter species,* etc., (Zhu et al. 2012), and *Vibrio species and Xanthobacter species* (Babalola and Glick 2012). The *Rhizobium* species that fixes atmospheric nitrogen to the host plants also show P solubilization property. *Rhizobium species* and *Crotalaria* species (Jorquera et al. 2011) increase the P content in plants by making P easily available to plants. *Kushneria* species is a halophilic bacteria that was extracted from the soils of Daqiao saltern on the eastern coast of China, which have proved to be very beneficial for saline soils. Phosphate-solubilizing fungi include strains of *Fusarium, Alternaria, Sacchromyces,* etc. For better usage of amassed phosphorus in soils their use is very promising in the form of biofertilizers enhancing sustainable agriculture one step further (Richardson and Simpson 2011).

One of the enzymes that cause P solubilization is glucose dehydrogenase which is a membrane-bound enzyme and causes oxidation of glucose to gluconic acid. The gluconic acid is then converted to 2-ketogluconic acid and 2,5-diketogluconic acid by the action of enzymes. P is solubilized effectively by 2-ketogluconic acid as compared to gluconic acid. Although most of the studies on P solubilizing microorganisms were performed at mesophilic temperatures but some reports are also available of studies at low temperatures such as 10 °C (Vassilev et al. 2006).

P mineralization means debasement of the remaining portion of the molecule after solubilization of organic phosphorus which results in dissolution of Ca-P compounds. Phytase is another enzyme responsible for organic P mineralization. This enzyme causes the formation of phosphorus from organic materials which are stored in the form of phytate in soil (Yi et al. 2008). Other enzymes involved in P mineralization are NSAPs (non-specific acid phosphatases) which remove the phosphate group from phosphoester bonds of organic compounds. Various non-specific acid phosphatase (NSAPs) enzymes released by P-solubilizing microorganisms belonging to the family of phosphormonoesterases. The acid phosphatase enzymes play an important role in solubilization, though alkaline phosphatases are also present. Various solubilization and mineralization processes that involve different enzymes play an important role in recycling of phosphorus.

13.7.3 Stress Management

Cold-tolerant microbes permanently sustain low temperature in cryo-environments such as deep sea, mountains, and polar regions. These organisms are also known as psychrotolerant, psychrotroph, or psychrophiles as they grow better at very low temperatures (Morita 1975). Psychrophiles can overcome two main challenges because of their unique properties: First challenge is the survival of psychrophiles at very low temperatures because if there is decrease in temperature, the biochemical reactions are affected exponentially. Second, the viscous aqueous environments are considerably increased as temperature is decreased. Their growth rate is maximum between temperatures of 2 and 12 °C (Xu et al. 2003).

The membrane functions are also affected, which leads to decreased membrane fluidity and the loss of membrane functions. The physical properties of membranes are affected by fatty acid composition, and it changes with the environment of the microbes. In general, reduced temperature produces a higher content of branched fatty acids both saturated and unsaturated (Pandey et al. 2004). Another adaptation of psychrophiles is an increased content of big and more compact head groups of lipids, proteins, and carotenes (Deming 2002). In some psychrophiles, there is less non-polar carotenoid pigment synthesis (Chintalapati et al. 2004).

Microbial activity at temperatures around −20 °C occurs in normal water inside the ice. These contain increased concentrations of sodium chloride (NaCl) or other particulate matters which maintain the fluid flow. Different factors such as hydrostatic and osmotic pressure, solar radiations, availability of nutrients and stress also strongly affect the growth of psychrophilic microbes. Various specialized proteins are expressed in microbes when they are subjected to sudden change in temperatures. These proteins are involved in cellular processes like protein folding and the control of membrane fluidity (Russell 2000). In psychrophiles, Caps are expressed at low temperatures though they are similar to the Caps present in mesophiles. This shows that a sensory system that senses temperature is present in psychrophiles, and these thermosensors sense membrane fluidity as well (Arthur and Watson 1976).

Anti-freeze proteins (AFPs) or ice-restricting proteins have been recognized in microorganisms living in Antarctic lake (Gilbert et al. 2005) that can tie to ice precious stones in an expansive surface and brings down the temperature at which a life form can openly develop (Jia and Davies 2002). AFP from certain organisms is Ca^{2+}-reliant and hyperactive ice surfaces and control ice precious. AFPs bind to stone development and recrystallization by bringing down the point of solidification (warm hysteresis) (Krembs et al. 2002).

Other molecules that have an important role in protecting psychrophiles against cold conditions are disaccharide trehalose and exopolysaccharides. Trehalose binds the molecules together and helps in the prevention of protein denaturation and protein aggregation (Nichols et al. 2005). The trehalose disaccharide also rummage-free radicals and stabilize cellular membranes under cold climatic conditions. Increased concentrations of exopolysaccharides have been found in bacteria of sea of Antarctica water (Muryoi et al. 2004) and in sea ice of Arctic water (Nichols et al. 2005). These change the physiological environment of bacterial cells, participate in adhering of cells to surfaces and retain water, increase the nutrient concentration, retain and save extracellular enzymes against cold denaturation, and most importantly, it acts as cyoprotectant (Tosco et al. 2003). EPS have elevated amounts of polyhydroxyl which brings down the point of solidification of water. EPS can likewise trap water, supplements and metal particles and encourage surface grip, cell collection and biofilm arrangement and may likewise assume a job in securing extracellular catalysts against cold denaturation and autolysis. EPS have high levels of polyhydroxyl which lowers the freezing point of water (Campanaro et al. 2011). EPS influenced the species colonization and survival of the present organisms in the natural surroundings near the oceans ice by lowering the rate of development of ice because of higher saltiness (Mykytczuk et al. 2011).

The combination of cytoplasmic ice crystals is incited by cell solidifying. The accumulation of substances like sucrose, glycine, betaine and mannitol results in the bringing down of the point of solidification of cytoplasm consequently giving assurance against solidifying.

Ongoing transcriptome investigations have demonstrated that introduction to cold temperatures initiates a fast up-guideline of qualities engaged with layer biogenesis, for example, unsaturated fat and LPS biosynthesis, peptidoglycan biosynthesis, glycosyltransferases and outer membrane proteins (Deming 2002). Similar genomic studies have additionally uncovered that genes engaged with the synthesis of cell membrane are overexpressed in the genomes of psychrophilic microorganisms. General membrane transport proteins are elevated as seen by transcriptomic contemplates, against the lower dispersion rates over the cell layers experienced at colder temperature (Qiu et al. 2006). Specifically, the dimensions of peptide transporters are expanded which encourages cold and hyperosmotic stress which improves the take-up of supplements (Reva et al. 2006).

Another class of layer smoothness modulators are carotenoid pigments. Both polar and non-polar carotenoid pigments are delivered by different Antarctic microorganisms and have been proposed to keep up layer smoothness and aid in keeping up equalization amid changes in temperatures (Fig. 13.1). Wax esters additionally assume a significant job in cool balanced film ease. In *Psychrobacter urativorans*, they may represent up to 14% of the cell lipid content, and in *P. arcticus*, the wax ester synthase is constitutively communicated, paying little heed to the development temperature (Sung et al. 2011).

13.8 Industrially Important Cold Enzymes

Psychrophilic microorganisms produce enzymes that can sustain low temperature and other stresses of cold climatic conditions. These enzymes are used in paper, pulp, pharmaceutical and food industries (Whitman 1998). Psychrozymes or cold-adapted enzymes can sustain temperatures between 10 and 5 °C. There is an increasing demand of psychrozymes in industries because of their withstanding nature in adverse conditions. Nowadays more attention is paid on the use of proteins isolated from cold-loving microorganisms as they act at their optimum temperature enhancing the recovery of the products of enzymatic reaction (D'Amico et al. 2006).

The psychrozymes have the ability to degrade a wide range of polymeric substances and the substance that can produce enzymes like amylases, cellulases, pectinases, β-galactosidase, oxidases, protease and lipase. A huge amount of money is invested in psychrozymes worldwide due to their extreme potential. The industrially important psychrozymes are used in the fields of food industry (such as pectinase, β-galactosidase), bio-polishing of textile products and detergent formulation industries. Moreover, these psychrozymes are also used in bioremediation (such as oxidases), for biotransformations (methylases and aminotransferases) (Okuyama et al. 1999) and in biomedical applications. Psychrozymes are used in:

1. Industrial processes including food technology
2. Bioremediation and other pollution control technologies
3. Medical and other pharmaceutical uses

Psychrozymes have many benefits such as high specific activities at low temperature, they can offer many other advantages like saving energy, saving volatile compounds, contamination prevention and easy inactivation of enzymes. Most of the food industries treat the products with psychrozymes for maintaining the quality of food during their transportation and storage. Psychrozymes are also frequently utilized in detergent and textile industries. Similarly pectinases and cellulases are used in the clarification of fruit juices; proteases helps in the removal of fish skin.

Apart from food industry, psychrozymes are used for the low-temperature biodegradation, and they are best alternatives to physicochemical methods for the bioremediation of solids and waste waters polluted by hydrocarbons, oils and lipids (Violot et al. 2005). Biodegradation with psychrozymes have several advantages over other existing traditional methods. It has been observed that the treatment of contaminated soil with psychrozymes is much more cost-effective than traditional methods such as incineration, storage or concentration. In 1997, Brun et al. studied the recombinant Antarctic *Pseudoalteromonas haloplanktis* which secretes toluene-0-xylene monooxygenase (T0MO). This enzyme efficiently converts several aromatic compounds into their corresponding catechols in a broad range of temperature. It has been suggested that the genetically engineered Antarctic bacterium is used in the bioremediation of contaminated marine environments. The interest on psychrozymes have increased greatly because of their high activity at low temperatures which offers potential economic benefits (Margesin and Schinner 1994). For example, the "peeling" of leather by cold adapt protease can be done with normal water instead of at 37 °C. An important achievement in the field of cold-adapted enzymes has been the construction of a host-vector system that allows the overexpression of genes in psychrophilic bacteria even at low temperatures which prevents the formation of inclusion bodies and protects heat-sensitive gene products. A single PUFA is produced using psychrozymes, rather than the complex mixture which is yielded from fish or algal oils.

13.9 Conclusion

Microbes play a vital role in sustainable environment and affect both flora and fauna of any ecosystem. Cold environment has its own challenges which can be countered by using the strategies used by nature. Exploring the adaptations used by psychrophiles help us to mimic them in our day to day life. Many low-temperature microbes have a great role to play in all low temperature-based industries.

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Microbes for Cold Stress Resistance in Plants: Mechanism, Opportunities, and Challenges

14

Prity Kushwaha, Prem Lal Kashyap, and Pandiyan Kuppusamy

Abstract

Cold stress (CS) is one of the major hindrances for quality crop production and global food security. Under cold environment, different kinds of alterations in the biochemical, physiological, and molecular processes of plants have been observed. Hence, it becomes mandatory to develop eco-compatible, sustainable, and economically sound options for ensuring quality food grain production of high mountainous regions. The use of cold-tolerant microbes (CTM) enhances growth of agricultural crops under low temperature environment. Additionally, it provides an economically captivating and environment-friendly means for protecting agricultural crops from cold stress injuries. They can also trigger crop growth by improving nutrition acquisition, regulating release of plant hormone and siderophores in addition to the activation of antioxidant system under low temperature conditions. As a result, this plant–CTM interaction under cold environment is vital and CTMs may act as a principal cold stress engineer to answer global agricultural tribulations of high altitude. In this chapter, attempts have been made to explore about CTM and their mechanism of action to boost agricultural production in sustainable manner under low temperature environment.

Keywords

Agriculture · Cold · Microbes · Stress · Sustainability · Temperature

P. Kushwaha · P. Kuppusamy
ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh, India

P. L. Kashyap (✉)
ICAR—Indian Institute of Wheat and Barley Research (IIWBR), Karnal, India

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14.1 Introduction

Cold stress (CS), one of the most commonly occurring abiotic stresses, frequently threatens the sustainability of agriculture through wide range of impacts on plant growth and production. It is well known fact that about two third of the world's cumulative terrestrial area is annually affected by below freezing point temperatures and three fourth portion of the Earth's biosphere's temperature is below 5 °C (Awasthi et al. 2019; Margesin and Collins 2019; Larcher 2001; Baldi et al. 2011). In agricultural crops, CS affects several biological processes, including membrane damage and alterations in photosynthetic apparatus and starch metabolism of plant cells (Zhuang et al. 2019). Suzuki and Mittler (2006) observed cold stress-induced cellular changes in plants and mentioned rapid synthesis of reactive oxygen species (ROS) and disruption in cellular homeostasis as major alterations in plant system. ROS include singlet oxygen (O_2^-), hydrogen radical ($HO^·$), and hydrogen peroxide (H_2O_2) molecules that break large biomacromolecules (e.g., DNA, proteins, carbohydrates, and lipids) and responsible for complete death of plant tissue and cells (Gill and Tuteja 2010). Under such circumstances, traditional plant breeding tactics or genetic transformations are not observed as ideal options for improving agricultural crops by incorporating traits for frost hardiness (Kasuga et al. 1999). Fowler and Thomashow (2002) mentioned that property of cold resistance is not controlled by a single major gene, but involves multifaceted and convoluted mechanisms working simultaneously to guard plant cells from cold stress injury (Fowler and Thomashow 2002). Therefore, CTM (=psychrophiles and psychrotrophs) can play prominent role in enhancing plant resistance to chilling stress (Subramanian 2011). The maximum temperature for growth of psychrophiles lies at ≤15 °C, while psychrotrophic microbes showed their optimum growth at ≥15 °C (Srivastava et al. 2013; Moyer and Morita 2007). Utilizing such CTM can assuage cold stress in various crop plants, therefore emerging as a prospective and a sustainable option for solving chilling problem of high altitude agriculture. For instance, Mishra et al. (2009) inoculated cold-tolerant strain of *Pseudomonas* sp. derived from the rhizosphere of *Amaranthus* sp. and observed significant increments in the growth of wheat seedlings as a result of microbial inoculation (Mishra et al. 2009). Significant rise in nodulation and nitrogen fixation capabilities of soybean crop under low temperatures have been observed after plant inoculations with cold-tolerant strains of *Bradyrhizobium japonicum* (Zhang et al. 2003). Similarly, *Burkholderia phytofirmans* inoculated to grapevine seedlings have been reported to improve CS tolerance capacity by plummeting electrolyte leakage (Ait Barka et al. 2006). Additionally, plant inoculations with different types of plant growth-promoting rhizobacteria including *Azospirillum brasilense, Bacillus megaterium*, *B. subtilis,* and *Raoultella terrigena* have been reported as potential alleviator for minimizing the deleterious effects of chilling injury in barley and wheat seedlings by regulating freezing injury and antioxidant enzymes activity (Turan et al. 2013). Based on the available research findings, it seems that the possible mechanisms by which CTM could be advantageous to agricultural crops involve: (1) rapid synthesis of

1-aminocyclopropane-1-carboxylate deaminase (ACC) enzyme for minimizing cold stress triggered ethylene production (Shi et al. 2012; Subramanian et al. 2015); (2) enhanced efficiency of biological nitrogen fixation processes for ensuring sufficient available nitrogen (N_2) for plant under cold stress; (3) production of phytohormones [e.g., abscisic acid (ABA), gibberellic acid (GA), indole-3-acetic acid (IAA)]; (4) release of small and high affinity iron-chelating compounds by CTM; (5) activation of antioxidant enzymes machinery; and (6) solubilization and mineralization of nutrients, etc. Based on the existing research knowledge base on microbial mediated cold stress management, an effort is made to comprehend the current understanding of cold stress management of plants using microbes in this chapter. Additionally, attempts has been made to present a synthesis of budding trends in the field of crop stress microbiology and discuss plausible directions for future microbiological exploration that will offer more sound and erudite predictions regarding the role of cold-tolerant microbes in sustaining agriculture of high altitudes.

14.2 Cold Stress and Its Impact on Crop Growth and Yield

Cold stress (CS) is one of the biggest challenges of high altitude agriculture and resulted in significant hampering of plant growth and metabolism (Kashyap et al. 2018). Practically, CS influences entire cellular functions of the plants grown under low temperature. It has been well documented that CS negatively prejudices the normal crop growth and development (Patni et al. 2018; Rihan et al. 2017; Yadav 2010). Earlier published reports clearly highlighted the negative impacts of CS on quality growth of various agricultural crops including rice, cotton, tomato, potato, muskmelons, and sugarcane (Hussain et al. 2018; Ghadirnezhad and Fallah 2014; Zhu et al. 2013; Zhao et al. 2012; Thakur et al. 2010; van der Ploeg and Heuvelink 2005). Generally, low temperature stress severely affects the seedling vigor, lowers the seed germination rate, delays plant growth, and results in severe yield loss (Wang et al. 2016a, b; Ruelland et al. 2009; Oliver et al. 2007; Cruz and Milach 2004; Kang and Saltveit 2002). It also causes foliar necrosis, hinder leaf growth, protract cell cycle with diminishing cell production, wilting, and enhance susceptible level of plants against different kinds of pathogens and diseases (Rymen et al. 2007; Korkmaz and Dufault 2001). CS at reproductive stage of chickpea plant caused flower abortion and skimpy pod setting (Nayyar et al. 2005). Statistically, it has been predicted that CS in temperate regions is accountable for 30–40% drop in rice yield because of spikelet deterioration, meager spikelet fertility, and panicle deformation (Andaya and Mackill 2003). Similarly, Thakur et al. (2010) mentioned that CS occurred during reproductive phase of cereal grain crops lead to pollen tube deformation, pollen sterility, ovule abortion, flower abscission, meager fruit set, and drastic reduction in final grain yield. In case of legume crops, Junior et al. (2005) reported that chilled rhizospheric temperature drastically hinders root nodulation process and results in significant declination in nodule size, nodule number, and growth rate of nodule formation. Similarly, CS during wheat stem elongation stage resulted in spikelet death and reduction in biomass accretion and total grain yield (Whaley et al. 2004). Moreover, severe decline in the number of productive wheat tillers per plant has been noticed when CS occurred at jointing and booting stages of wheat (Li et al. 2015). CS can also attack photosynthetic machinery of plants and hence responsible for drastic decline of chlorophyll content, stomatal carbon-di-oxide (CO_2) concentration, net photosynthesis, and photosystem quantum yield (Karabudak et al. 2014; Mishra et al. 2011). This in turn can deteriorate plant robustness and reduce nutrient uptake efficiency from soil. Fernandez et al. (2012) noticed that exposure of grapevine plantlets to low temperature resulted in growth reduction and declination of photosynthetic rate of plantlets. Chinnusamy et al. (2007) mentioned that CS impairs membrane fluidity, disrupts genetic material (DNA and RNA) and protein structures, obstructs nutrient and water uptake, and causes notable modifications in the plant transcriptome. Additionally, it also severely affected cellular metabolism by declining the rates of biochemical reactions and reprogramming gene expression. At low temperature, cell membranes of cold susceptible plants become stiff and hard that further leading to significant level of disturbances in membrane-related processes, for instance, opening of ion channels and membrane-related electron transfer reactions (Uemura and Steponkus 1999). In turn, this can influence plant physiology negatively by reducing photosynthetic and growth rates (Ait Barka et al. 2006). Erdal (2012) observed that low temperature pessimistically influence the photosynthetic pigment production and thus cause etiolation in leaves. Cell signaling and gene expression alteration due to calcium (Ca^{2+}) ion influx from extracellular compartments triggered by cold stress in various plants has been well documented (Polisensky and Braam 1996; Monroy and Dhindsa 1995; Mahajan and Tuteja 2005). Exposure to low temperature alters cellular homeostasis status of the plant and as a consequence accumulation of reactive oxygen species (ROS) observed as a key product of CS triggered cellular modifications. ROS, such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2^{-}), and hydrogen radical ($HO^{\cdot}$), disintegrate biological macromolecules (e.g., DNA, carbohydrates, lipids, and proteins) and ultimately results in programmed cell death (Xu et al. 2011; Ruelland et al. 2009).

14.3 Diversity of Plant Growth-Promoting Cold-Tolerant Microbes

Low temperature environment dominates major portion of Earth's biosphere. It acts as a reservoir of CTMs with the ability to thrive with low metabolic activity at subzero temperature (Kumar et al. 2019). Generally, psychrophilic bacteria include *Arthrobacter*, *Bacillus, Flavobacterium, Micrococcus, Moraxella, Moritella, Pseudoalteromonas*, *Pseudomonas*, *Polaromonas*, *Psychrobacter*, *Psychroflexus*, *Polaribacter*, and *Vibrio*. Besides this, several other types of cold-tolerant microbial species representing Archaea, fungi, and microalgae have been reported (Feller and Gerday 2003). Johnson et al. (1987) documented the presence of *Trichoderma* strains in cold climatic conditions of Alaska and Tennessee. Later, McBeath

(1995) identified several strains *Trichoderma* spp. showing biocontrol activity towards different kinds of pathogenic fungi at low temperatures ranged from 4 to 10 °C. Ghildiyal and Pandey (2008) also isolated cold-tolerant strains of *T. harzianum, T. koningii* and *T. viride* displaying biocontrol abilities from glacial sites of Indian Himalayan soil. Prevost et al. (1999) identified cold-tolerant strains of *Mesorhizobium* sp. and *Rhizobium leguminosarum* from Canadian soils. Later, a superior strain of *Sinorhizobium meliloti* adapted for nodulation of alfalfa at low temperatures have been described by Prevost et al. (2003). Pandey and colleagues (2002) identified *Pseudomonas corrugata* strains as cold-tolerant phosphate solubilizer. Similarly, siderophore producing cold-tolerant mutant of *P. fluorescens* has been generated by Katiyar and Goel (2004). Negi et al. (2005) explored Garhwal region of Indian Himalayas and documented several strains of *Pseudomona*s with strong cold adaptation. Additionally, these strains were identified as siderophore producer with excellent plant growth-promoting features at wide range of temperature (4–25 °C). Similarly, microbial exploration of subalpine regions of central Himalayas of India by Pandey and colleagues (2006) resulted in the identification of cold-adaptive *P. putida* strains with phosphate solubilizing and antagonistic capabilities. Cold tolerance and plant growth-promoting features of *Serratia marcescens* strain SRM obtained from *Cucurbita pepo* have been described by Selvakumar et al. (2008a, b). Later, *P. corrugata* NRRL B-30409 mutants with increased potential of organic acid production, phosphate solubilization, and plant growth promotion at chilling temperature have been reported by Trivedi and Sa (2008). Gulati et al. (2009) described *Acinetobacter rhizosphaerae* from the cold deserts of Himalaya and established its high competence for crop growth promotion (Gulati et al. 2009). *Exiguobacterium acetylicum* strain 1P showing siderophores production and biocontrol capabilities from North Western Himalayas of India were reported by Selvakumar et al. (2009). Malviya et al. (2009) explored glacial sites of the Indian Himalayas and reported psychrotolerant *Streptomyces* strains with strong antagonistic and chitinolytic activity. Besides these features, *Streptomyces* strains were also found to curb the growth of multiple phytopathogenic fungi. Later, Selvakumar and associates (Selvakumar et al. 2009) also reported cold-adaptive *Pseudomonas fragi* strain with excellent phosphorous solubilization capability. Vyas and colleagues (2010) reported a series of phosphorous solubilizing fluorescent *Pseudomonas* strains displaying tolerance towards salinity, alkalinity, temperature, calcium salts, and desiccation-induced stresses from trans-Himalayan regions of India. Singh et al. (2011) found two very efficient strains of *Aspergillus niger* which demonstrated excellent solubilizing activity at 20 °C in the presence of tri-calcium phosphate. Similarly, Rinu and Pandey (2011) reported *Paecilomyces hepiali* as a cold-adaptive phosphate solubilizing fungus from rock soil of Indian Himalayas. Sati et al. (2013) identified several cold-tolerant strains of genus *Bacillus*, *Penicillium,* and *Pseudomonas* along with yeasts and actinomycetes from soil under potato farming in cold regions of Indian Himalayas. Further, they observed that the isolated microbes were endowed with strong antagonistic and multifarious plant growth-promoting attributes. Several cold-adapted nitrogen-fixing bacterial species were isolated from Himalayan soil, and proteome of psychrophilic diazotroph *Pseudomonas migulae* S10724 (Suyal et al. 2014) and psychrotroph *Pseudomonas palleroniana* N26 (Soni et al. 2015) was studied to document the protein profile under low temperature diazotrophy. Yadav and coworkers (2015) have mentioned that cold-adapted *Arthrobacter nicotianae, Brevundimonas terrae,* and *P. cedrina* can display diverse plant growth-promoting features. Later, it has been observed that the bacteria isolated from root nodule of pea crop cultivated under low temperature environment exhibited excellent plant growth-promoting attributes in addition to strong biofertilizer capabilities under CS conditions (Meena et al. 2015). Verma and associates (2015) identified cold-adaptive bacterial strains of genus *Arthrobacter*, *Acinetobacter Bacillus*, *Bordetella*, *Providencia*, *Pseudomonas,* and *Stenotrophomonas* associated with wheat seedlings in northern hill zone of India. Fungal diversity in soil at higher altitudes of Sikkim and Uttarakhand Himalaya has shown that *Penicillium*, *Aspergillus*, *Epicoccum*, *Fusarium*, *Myrothecium*, *Cladosporium*, *Paecilomyces*, *Gangronella,* and *Trichoderma* were the most abundant and diverse genus (Rai and Kumar 2015). *Amanita*, *Russula*, *Boletus*, *Lactarius*, *Suillus,* and *Hygrophorus* are the common ectomycorrhizal fungal genera associated with oaks and conifers in temperate forest of Western Himalaya (Wang et al. 2015). Kumar et al. (2018) documented *Dyadobacter* sp. as a potential growth-promoting potential psychrotolerant from Bhowali, which is a temperate region of Western Indian Himalaya. Qin et al. (2017) identified cold-adapted bacterium *Pseudochrobactrum kiredjianiae* from cave soil of Russia. Tiryaki et al. (2019) reported cold-tolerant bacterial isolates belonging to *Brevibacterium frigoritolerans, P. chlororaphis*, *P. fluorescens*, *P. fragi,* and *P. proteolytica* from foliage apoplast of *Colchicum speciousum*, *Draba nemorosa*, *Erodium cicutarium*, *Galanthus gracilis,* and *Scilla siberica* plants (Tiryaki et al. 2019). Gautam et al. (2019) identified a psychrotrophic *Virdibacillus arenosi* PH15 strain from rhizosphere of *Podophyllum hexandrum* Royle, a medicinal plant widely grown in Sangla valley of Himachal Pradesh, India, and characterized for various plant growth-promoting attributes. Similarly, *Arthrobacter humicola*, *Brevibacillus invocatus*, *Pseudomonas mandelii,* and *Pseudomonas helmanticensis* have been documented as psychrophilic diazotrophs from high altitude Gangotri soil ecosystem (Kumar et al. 2019). More recently, Awasthi et al. (2019) documented and characterized cold-adaptive *Pseudomonas koreensis* P2 strain from cold desert of Arunachal Pradesh (India).

14.4 Mechanism of Microbial Mediated Cold Stress Tolerance in Crop Plants

The beneficial effects of CTMs in determining specific plant responses that are connected with cold injury tolerance have been reported in several crops. Sun et al. (1995) highlighted that a major mechanism of CTMs under CS could be efficient synthesis and release of antifreeze proteins and their strong affinity with plant root growth promotion. For instance, cold-adaptive *B. japonicum* strain could enhance nodule formation and nitrogen fixation efficiency in soybean crop under low non-freezing temperature (Mishra et al. 2009), while *Burkholderia phytofirmans* inoculation could improve chilling resistance by tumbling electrolyte leakage in grapewine seedlings (Ait Barka et al. 2006). Zhu and associates (2010) indicated that arbuscular mycorrhizae (AM) inoculated maize seedlings had higher superoxide dismutase (SOD) and catalase (CAT) activities than nonmycorrhizal (NM) seedlings under CS. Abdel Latef and Chaoxing (2011) revealed that the AM fungus alleviates CS injury in tomato plants by plummeting membrane lipid peroxidation and enhancing accumulation of osmotic adjustment compounds, antioxidant enzyme, and photosynthetic pigments. Similar enhancements in the activities of SOD and peroxidase (POD) enzymes have been noticed by Zhou et al. (2012) in AM colonized *Tectona grandis* seedlings under CS. Later, it has been explained by Liu et al. (2014a) that under CS, AM colonization stimulates plasma membrane ATPase activities and ATP accumulation in *Cucumis sativus* plants that in turn accountable for regulation of intracellular pH and electrochemical gradient generation for active ion transport (Kim et al. 2013). The plasma membrane H^+-ATPase is active in AM and provides sufficient energy for plant–microbe exchanges at the symbiotic interface around arbuscule (Gianinazzi-Pearson et al. 1995). Further, it has been observed that AM inoculation is responsible for upregulated expression of proton pump and calcium-transporting ATPase-related genes (*CsHA2*, *CsHA3*, *CsHA4*, *CsHA8*, *CsHA9*, *CsHA10*, *CA1*, and *CA9*) in root zone of cucumber seedlings under CS. Chen and coworkers (2013) noticed that AM inoculation in cucumber plants triggered rapid build up of phenolics, flavonoids, and lignin compounds. Further, they also observed significant and high level activities of phenylalanine ammonia-lyase, shikimate dehydrogenase, glucose-6-phosphate dehydrogenase, cinnamyl alcohol dehydrogenase, polyphenol oxidase, guaiacol peroxidase, caffeic acid peroxidase, and chlorogenic acid peroxidase in AM inoculated cucumber plants than non-AM plants under CS. Parallel reports regarding the enhanced activities of sucrose phosphate synthase (SPS) in AM inoculated maize and rice plants have been demonstrated several researchers (Liu et al. 2013; Zhu et al. 2015), which further indicates the sucrose metabolism role in improving CS tolerance capability of plants due to AM symbiosis. Liu et al. (2014b) demonstrated that increase in the expression of trehalose phosphate phosphatase (TPP) and trehalose phosphate synthase (TPS)-related genes (OsTPS1, OsTPS2, and OsTPP1) could enhance trehalose biosynthesis and higher trehalose accumulation in the AM colonized rice plants under CS. Endophytic *Burkholderia phytofirmans* PsJN primed grape seedlings enhanced cold tolerance and adaptation process of plant via antioxidant scavenging process (Theocharis et al. 2012). Further, modulation of carbohydrate metabolism is involved in minimizing chilling stress in inoculated grapevine plantlets with *Burkholderia phytofirmans* PsJN (Fernandez et al. 2012). Some other physiological changes such as activation of gene machinery linked with C-repeat binding factor (*CBF*), alterations of sugar metabolism pathway, maintenance of plant photosynthetic ability, and significant rise in the total phenolic contents have been observed in grapevine plantlets inoculated with psychrotolerant bacteria (Fernandez et al. 2012; Mishra et al. 2011; Ait Barka et al. 2006). Fernandez et al. (2012) experimentally confirmed that trehalose metabolism is involved in

Burkholderia phytofirmans mediated cold resistance in grapevine plantlets. Similarly, in case of arbuscular mycorrhiza (AM), Chen et al. (2013) observed that under cold stress significant rise in the production of total phenols and flavonoids occurred in AM inoculated cucumber seedlings than non-AM seedlings. Turan et al. (2013) mentioned that inoculation of *Azospirillum brasilense, B. megaterium*, *B. subtilis*, and *Raoultella terrigena* in wheat and barley helped in recovering plants from cold injury by maintaining antioxidant enzymes activity as well as minimum freezing injury. Further, Subramanian et al. (2015) also reported the enhanced level of cold acclimatization gene(s) expression and antioxidant activity in *P. frederiksbergensis* OS261 and *P. vancouverensis* OB155 inoculated tomato (*Solanum lycopersicum*) plants is involved in shielding tomato from low temperatures stress. Subramanian et al. (2016) reported that *P. frederiksbergensis* OS211, *Flavobacterium glaciei* OB146, *P. vancouverensis* OB155, and *P. frederiksbergensis* OS261 conferred chilling resistance in tomato seedlings via activation of antioxidant enzymes, rapid proline synthesis, and membrane damage minimization under low temperature (15 °C) exposure. Kang et al. (2015) documented that application of *Serratia nematodiphila* enhances pepper growth under low temperature by maintaining the high level gibberellic acid (GA4) and abscisic acid (ABA) production and low levels of salicylic acid (SA) and jasmonic acid (JA). Chu et al. (2016) reported that like bacteria, arbuscular mycorrhizal fungi (AMF) inoculation also improves *E. nutans* seedlings tolerance to cold stress by modulating redox balance by activating ROS scavenging system and other stress-related defense mechanisms. Later, these observations have been supported by the studies of Pedranzani et al. (2016) that improved photosynthetic efficiency, shoot dry mass, and enzymatic activities of CAT, APX, and SOD with a decrease in H_2O_2 and MDA contents were noticed after inoculation of *Digitaria eriantha* plants with AM under low temperature conditions. Tiryaki et al. (2019) revealed that inoculation of bean (*Phaseolus vulgaris* L.) seedlings with bacterial (*B. frigoritolerans, P. fragi*, *P. chlororaphis*, *P. fluorescens,* and *P. proteolytica*) assisted in regulating freezing injury, ice nucleating activity, and lipid peroxidation content along with ROS generation. In addition, the inoculations of these strains improved the functionalizing of apoplastic antioxidant enzyme machinery [e.g., glutathione reductase (GR), SOD, CAT, and peroxidase] and therefore improve the cold resistance of bean under low temperature. On the basis of the above studies, it can be concluded that microbes enhance plant growth and resistance to chilling stress by adopting more than one mechanism of action (Fig. 14.1).

14.5 Microbial Mediated Cold Stress Management in Agricultural Crops

CTMs have been reported to possess multifarious plant growth-promoting attributes in addition to their strong relationship with series of agricultural crops and found to persuade several manifold advantages to plants grown under CS environment (Table 14.1) has been summarized in the following sections.

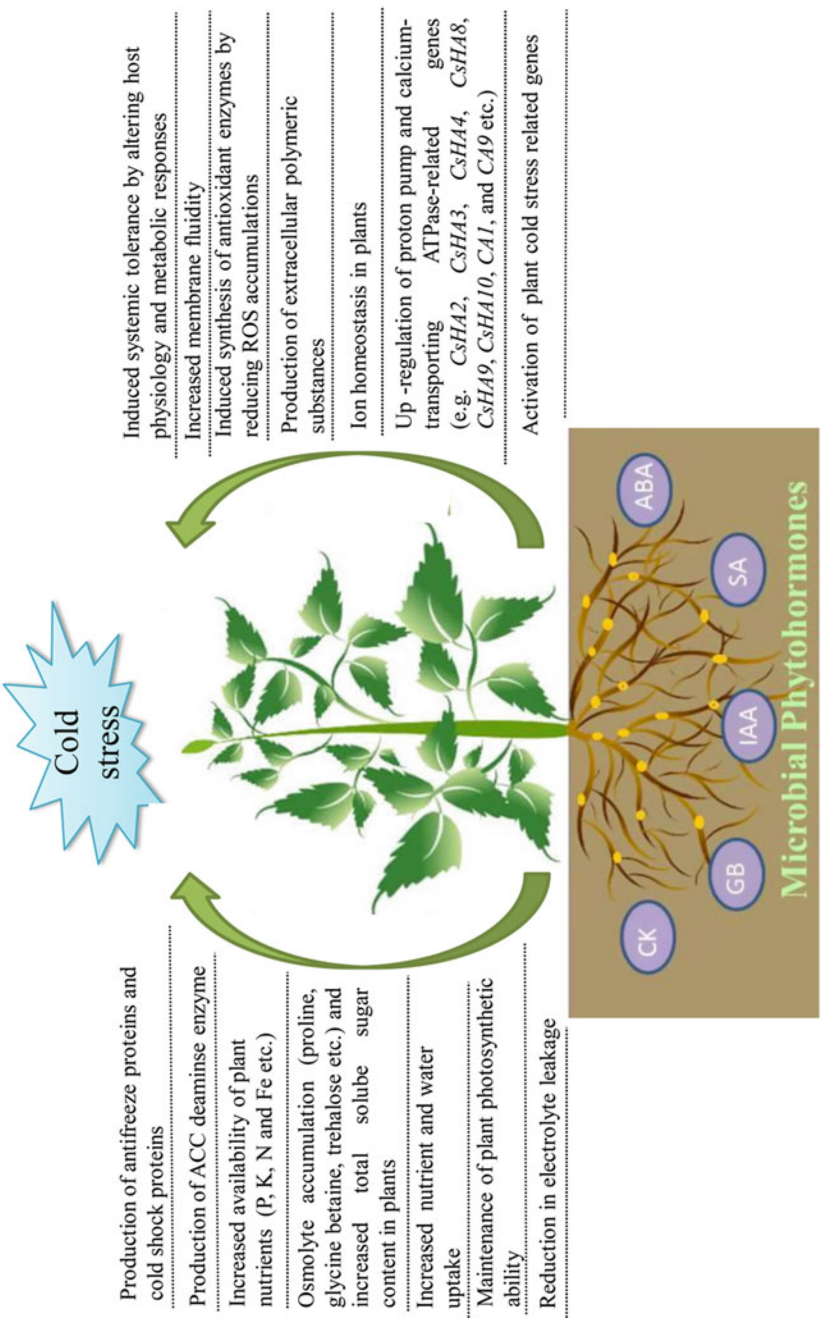

Fig. 14.1 Mechanism of microbial mediated cold stress tolerance in plants

Table 14.1 Principal examples of microbial mediated cold stress tolerance in plants

Microbe	Plant	Effect on plant growth	Mechanism of action	Growth conditions	Reference
Bacillus megaterium M3, *Bacillus subtilis* OSU142, *Azospirillum brasilense* Sp245 and *Raoultella terrigena*	Wheat (*Triticum aestivum* spp. *vulgare* cv 'Bezostiya') and barley (*Hordeum vulgare* cv 'Tokak')	Ameliorated the deleterious effects of stress condition on plant growth and increased biomass	Increased chlorophyll content, photosynthetic activity and relative water content, altering mineral uptake, and decreasing membrane damage	Pot trials	Turan et al. (2013)
Bradyrhizobium japonicum 532 C	Soybean	Improved nodulation and nitrogen fixation	–	Field trails	Zhang et al. (2003)
Burkholderia phytofirmans PsJN	*Vitis vinifera* L. cv. Chardonnay	Stimulates grapevine growth (root growth and plant biomass) and improves its ability to withstand cold stress	Significantly increased levels of starch, proline, and phenolics	Controlled plant growth chamber	Ait Barka et al. (2006)
Burkholderia phytofirmans strain PsJN	Arabidopsis thaliana	Reduces impact of freezing temperatures on photosynthesis	Differential accumulation of pigments, and reduced expression of RbcL and COR78 genes and prevent the plasmalemma disruption under freezing stress	Controlled plant growth chamber	Su et al. (2015)
Dyadobacter sp.	Chickpea (Cicer arietinum), black gram (Vigna mungo), green gram (*Vigna radiata*), pigeon pea (*Cajanus cajan*), and finger millet (*Eleusine coracana*).	Promote plant growth by fixing atmospheric N_2 and making it available to plant	Enhancements in agronomical parameters, leaf nitrate reductase activity and total chlorophyll content	Pot trial	Kumar et al. (2018)
Exiguobacterium acetylicum 1P	Wheat	Positively influenced the growth and nutrient uptake parameters	Phosphate solubilization, IAA, siderophore and HCN production	Pots in glass house	Selvakumar et al. (2010)

Flavobacterium sp. OR306 and *Pseudomonas frederiksbergensis* OS211	Tomato	Improve the cold resistance of seedlings under low temperature	Reduction of ethylene, increased expression of cold induced LeCBF1 and LeCBF3 genes in addition to reduced expression of ethylene-responsive transcription factor 13 (ETF-13) and ACO genes	Controlled plant growth chamber	Subramanian et al. (2015)
Glomus intraradices	*Phaseolus vulgaris*	Enhanced the cold tolerance of plants	Alterations in plasma membrane aquaporins (PIP)	Controlled plant growth chamber	Aroca et al. (2007)
Glomus mosseae	Tomato	Enhanced the cold tolerance of tomato plant, which increased host biomass and promoted plant growth.	Reducing membrane lipid peroxidation and increasing the photosynthetic pigments, accumulation of osmotic adjustment compounds, and antioxidant enzyme activity	Controlled plant growth chamber	Abdel Latef and Chaoxing (2011)
P. fragi, *P. chloropaphis*, *P. fluorescens*, *P. proteolytica* and *Brevibacterium frigoritolerans*	Bean (*Phaseolus vulgaris* L.)	Improve the cold resistance of seedlings under low temperature	Decreased freezing injury, ice nucleating activity and lipid peroxidation content in parallel with the decrease of reactive oxygen species level, stimulated the activity of apoplastic antioxidant enzymes	Controlled plant growth chamber	Tiryaki et al. (2019)

(continued)

Table 14.1 (continued)

Microbe	Plant	Effect on plant growth	Mechanism of action	Growth conditions	Reference
P. frederiksbergensis OS211, *Flavobacterium glaciei* OB146, *Pseudomonas vancouverensis* OB155, and *P. frederiksbergensis* OS261	Tomato	Improve germination, plant growth and induce antioxidant capacity in tomato plants	Reduction in membrane damage and activation of antioxidant enzymes along with proline synthesis	Controlled plant growth chamber	Subramanian et al. (2016)
Pantoea dispersa 1A	Wheat	Enhanced plant growth and nutrient uptake ability	Phosphate solubilization, IAA, siderophore and HCN production	Pot	Selvakumar et al. (2008a)
Pseudomonas corrugata NRRL B-30409	Wheat	Enhanced growth of wheat plants under low temperature	Increased phosphate solubilization, organic acid production	Greenhouse	Trivedi and Sa (2008)
Pseudomonas fragi CS11RH1	Wheat	Increased germination rate, plant biomass and nutrient uptake	Phosphate solubilization, indole acetic acid (IAA) and hydrogen cyanide (HCN) production	Pot	Selvakumar et al. (2009)
Pseudomonas jesenii MP1	*Cicer arietinum* (L.), *Vigna mungo* (L.) Hepper; *Vigna radiata* (L.) Wilczek., *Cajanus cajan* (L.) Millsp.	Stimulated growth of shoot length, root length, plant fresh weight and plant dry weight	Significant increase in chlorophyll content, nitrate reductase activity and phosphorous content	Pot trials	Kumar et al. (2014)
Pseudomonas lurida M2RH3	Wheat	Enhanced plant growth and nutrient uptake parameters	Phosphate solubilization, IAA and siderophore production	Pot	Selvakumar et al. (2011)
Pseudomonas putida GR12-2	Canola	Promote root elongation of both spring and winter canola at 5 °C temperature	Production of antifreeze protein	Petri dish	Sun et al. (1995)
Pseudomonas sp.	*Deschampsia antarctica*	Promoted root development	P-Solubilization	Petri dishes	Berríos et al. (2012)

Pseudomonas sp.	Lentil	Increased lentil shoot length, root length, root biomass, and shoot biomass	–	Pot trials in green house	Bisht et al. (2013)
Pseudomonas sp. NARs9	wheat	Enhancement in the germination, shoot and root lengths	IAA production and phosphate solubilization	Glass house	Mishra et al. (2009)
Pseudomonas sp. PGERs17	Wheat	Enhanced germination of wheat seedlings with higher root and shoot lengths	IAA production, tricalcium phosphate solubilization, HCN and siderophore production and antagonistic activity against plant pathogens	Pot	Mishra et al. (2008)
Pseudomonas spp.	Wheat	Significant increase in shoot length, root length, root biomass, and shoot biomass	IAA production, phosphate solubilization, HCN and siderophore production and increase in N, Fe and nutrient uptake	Pots in glass house	Mishra et al. (2011)
Serratia marcescens SRM	Wheat	Enhanced plant biomass and nutrient uptake	Phosphate solubilization, IAA, HCN and siderophore production	Pot	Selvakumar et al. (2008b)
Trichoderma gamsii NFCCI 2177	Maize, soybean, wheat and lentil	Positive influence plant growth as well as on rhizosphere based parameters	Phosphate solubilization, chitinase activity, and production of ammonia and salicylic acid	Green house	Rinu et al. (2014)

14.5.1 Cereal Crops

Kaushik and colleagues (2001) reported the role of *Azospirillum brasilense* inoculation in improving wheat crop growth at sub-optimal temperatures. Similarly, at 16 °C temperature, application of *Mycobacterium* sp., *Pantoea agglomerans,* and *P. fluorescens* isolated from German soil with typical semi-continental climate enhanced winter wheat growth and nutrient [Nitrogen (N), phosphorous (P), and potassium (K)] uptake efficiency but found less effective at 26 °C in loamy sand soil. Contrarily, Egamberdiyeva and Höflich (2003) reported highly efficient nutrient uptake capabilities of *Mycobacterium phlei* and *Mycoplana bullata* strains under both nutrient-rich and nutrient-poor soil at 16 °C and 38 °C, respectively. Saleem et al. (2007) highlighted that ACC deaminase enzyme produced by bacterial strains play significant role in reducing ethylene production under freezing temperatures and therefore assist in plant growth promotion. Selvakumar et al. (2008a, b) documented *Serratia marcescens* SRM as a promising cold-tolerant strain with potential capabilities to promote wheat growth under low temperature hilly terrains. Further, they also observed that *S. marcescens* has the ability to solubilize phosphorous and produce indole acetic acid, HCN, and siderophore at 15 °C and also reflected all the plant growth promotion attributes at 4 °C too. Moreover, seed treatment of wheat seedlings with *S. marcescens* strain showed improved wheat biomass and nutrient uptake under cold temperatures and therefore can be utilized as a prospective bioinoculant to protect wheat from cold injury. Later, *Pseudomonas* strains displaying excellent cold resistance as well as plant growth promotion attributes have been evaluated as seed bioinoculants under greenhouse conditions at 10 ± 2 °C temperature (Mishra et al. 2009). Experimental findings clearly concluded that seed bacterization significantly enhanced wheat biomass and found superior in comparison to non-bacterized seedlings. Further, a significant rise in total chlorophyll content, anthocyanin, free proline, total phenolics, and starch content along with rapid decline in Na^+/K^+ ratio, and electrolyte leakage have been recorded in *Pseudomonas* primed wheat seedlings. Selvakumar and workers (2011) also identified *P. lurida* M2RH3 as a potential psychrotolerant wheat growth-promoting bacterium owing to its inherent capacity to solubilize phosphate, produce siderophores, IAA, and hydrogen cyanide (HCN). These above-mentioned studies clearly indicate the potential of the bacteria to alleviate cold-induced stress in cereal crops. Turan et al. (2013) reported that *A. brasilense*, *B. megaterium*, *B. subtilis*, and *Raoultella terrigena* can enhance cold resistance in wheat and barley plants under chilling stress. Similarly, an endophytic psychrotolerant potassium solubilizing bacterium (*B. amyloliquefaciens* IARI-HHS2-30) have been isolated from North Western Indian Himalayas and identified as an excellent cold stress alleviator (Verma et al. 2015). Further, they noticed significant increment in plant biomass and chlorophyll "a" content under low temperature (4 °C) conditions, when wheat (cv. HS507) seed treated with (*B. amyloliquefaciens* IARI-HHS2-30). Recently, Qin et al. (2017) identified a cold-adapted bacterium with broad-spectrum biocontrol and wheat growth-promoting activity. They observed that under greenhouse conditions, *Pseudochrobactrum kiredjianiae* A4 strain improved physiological parameters as well as enhanced defense enzymes activities of wheat plants for effective mycelia growth suppression of phytopathogenic fungus *Rhizoctonia cerealis*. Overall above-mentioned studies clearly indicate that the cold-adapted microbes provide a promising solution to maintain crop growth and health under low temperature farming systems.

14.5.2 Leguminous Crops

Earlier studies of Prevost et al. (1987) indicated that rhizobia isolates of arctic region showed better nitrogenase activities at low temperatures than rhizobia isolates of temperate regions when tested their symbiotic association with *Onobrychis viciifolia*. Similarly, *R. trifolii* strains from subarctic regions of Scandinavia displayed better growth, fast nodulation, and enhanced nitrogenase activity with clover plant at 10 °C in comparison to *R. trifolii* strains isolated from southern regions (Ek-Jander and Fahraëus 1971). Further, they did not notice any significant differences in nitrogenase activity between strains of both regions at higher temperature (20 °C). Hume and Shelp (1990) found that inoculation of soybean with *B. japonicum* strain resulted in improved soybean yields in Ontario (Canada). Further, Zhang et al. (2003) also noticed that psychrotolerant strains of *Bradyrhizobium japonicum* also helpful in improving nodulation and nitrogen-fixing capability of soybean plants cultivated under low chilling temperature. Similarly, Mishra et al. (2011) found that cold-tolerant strains of *Pseudomonas* spp. and *R. leguminosarum*-PR1 protect lentil from CS as well as enhance iron acquisition, nutrient uptake, and plant growth. A study conducted by Katiyar and Goel (2004) revealed that *P. flouresens* ATCC13525 mutant strain is also able to produce significant amount of siderophores and able to promote *Vigna radiata* growth with significant increase in rhizosphere competitiveness. A psychrotrophic actinobacterium *Rhodococcus erythropolis* was also identified and described by Trivedi et al. (2007). During their investigation, they found that *R. erythropolis* was able to transform toxic and high concentration of chromium (Cr^{6+}) ions to less hazardous chromium (Cr^{3+}) ions and hence provide better growth of pea plants. A study made by Aroca et al. (2007) to decipher the role of AM symbiosis in improving *Phaseolus vulgaris* resistance to cold, salt, and drought stress revealed that in response to stress exposure, AM regulate root hydraulic properties, which were closely linked with the regulation of PIP2 protein levels and phosphorylation state. Gulati et al. (2009) identified *Acinetobacter rhizosphaerae* bacterium displaying IAA producing character from *Hippophae rhamnoides* plant, commonly found in the cold deserts of Himalayas. This strain was found promising in terms of chickpea growth promotion under controlled in vitro conditions and pea growth promotion under field conditions. Later, *Rahnella* sp. identified as a novel psychrotrophic enterobacteriaceae member by Vyas et al. (2010). This strain was found to augment plant growth of chickpea and pea under in vitro and field conditions by producing siderophores, phytohormones, organic acids, and enzymes like phytase and ACC deaminase (Vyas et al. 2010). Soybean plants inoculated with

Trichoderma gamsii NFCCI 2177 improved soybean growth under chilling temperature and used as a bioformulation (Rinu et al. 2014). Kumar and associates (2014) identified psychrotolerant *Pseudomonas jesenii* strain MP1 and tested for relative plant growth-promoting potential against *Cicer arietinum*, *V. mungo*, *V. radiata,* and *Cajanus cajan* pulse crops. They recorded significant stimulation in plant biomass of *Trichoderma gamsii* inoculated plants in comparison to their non-inoculated checks. Additionally, bacterium inoculated plants displayed significant rise in nitrate reductase activity, chlorophyll content, and phosphorous content too. Recently, Kumar et al. (2018) revealed the plant growth-promoting potential of psychrotolerant *Dyadobacter* sp. against four pulses (pigeon pea, green gram, black gram, and chickpea) and finger millet. They demonstrated that the bacterium was able to grow at nitrogen (N) deficient medium at both 10 and 28 °C and gave positive *nifH* amplification that confirms the diazotrophic nature of this psychrotolerant bacterium. Further, their pot trial-based study we concluded that psychrotolerant *Dyadobacter* sp. isolated from cold region of western Indian Himalaya has the potential to promote plant growth of pulses and finger millet by fixing atmospheric N_2 and making it available to plant. Overall, all the above-mentioned examples indicated that plant growth-promoting capabilities psychrotolerant bacterium and hence such microbes can be exploited to improve plant growth in cold climate agriculture.

14.5.3 Other Crops

Rapid augmentation of cold tolerance of *Burkholderia phytofirmans* strain PsJN inoculated grapevine plantlets have been documented by Ait Barka et al. (2006). During their study, they found significant and positive correlation of starch, proline, and phenolics in treated grapevine plantlets with plant ability to withstand CS. Halotolerant *P. putida* UW4 and GR12-2 were reported to enhance canola crop stand and growth in low chilling temperatures (Cheng et al. 2007; Sun et al. 1995). Kytöviita and Ruotsalainen (2007) observed that the biopriming of *Gnaphalium norvegicum* seeds with *Glomus claroideum* fungus significantly improved seed germination percentage, plant biomass, percent shoot nitrogen (N%), and root AM colonization at 8 °C than 15 °C. Similarly, Abdel Latef and Chaoxing (2011) indicated that *G. mosseae* fungus is proficient to enhance CS tolerance of tomato plant which increased host biomass and plant growth promotion by rapid production of osmotic adjustment compounds, increasing photosynthetic pigment productions, and regulating antioxidant enzyme activity and membrane lipid peroxidation. Theocharis et al. (2012) proved that *B. phytofirmans* PsJN primed grapevine plants exerted enhanced protection towards low non-freezing temperature (4 °C). They also observed a rapid and quick rise of both CS-related gene transcripts and metabolite levels in *B. phytofirmans* PsJN treated plantlets relative to non-bacterized counterparts. However, 1 week after CS exposure, more declination in the levels of stress-related metabolites in *B. phytofirmans* primed plants is observed. These results clearly indicated that the endophytic bacterium *B. phytofirmans* is involved in the cold-adaptive process via scavenging system. Later, Fernandez et al. (2012) experimentally revealed that trehalose metabolism is involved in *Burkholderia phytofirmans* induced chilling tolerance in grapevine. Subramanian et al. (2016) also established and confirmed the potential application of psychrotolerant bacteria (*P. frederiksbergensis* OS211*, F. glaciei* OB146*, P. vancouverensis* OB155, and *P. frederiksbergensis OS261*) in alleviating cold stress in tomato plants at low non-freezing temperature. Further, they found positive correlation of membrane damage reduction, rapid stimulation of antioxidant enzyme machinery, and proline synthesis with improved plant growth under CS conditions (15 °C). In another study, it was observed that 15.56% of survival rate of tomatoes achieved after *Bacillus cereus* AR156, B. *subtilis* SM21, and *Serratia* sp. XY21 application from 15.56 to 92.59% at 4 °C (Wang et al. 2016a, b). The experimental findings of Pedranzani et al. (2016) indicated that AM symbiosis can improve plant biomass, photosynthates, and enzymatic (CAT, APX, and SOD) activities of *D. eriantha* plants under CS condition and further play crucial role in supporting plants to with stand unfavorable CS environment. Similarly, studies of Matsubara et al. (2004) also demonstrated the advantageous role of inoculation of different species of AMF inoculation in growth promotion of strawberry plants. More elaborately, Chu et al. (2016) demonstrated that AMF inoculation improve CS tolerance level of *E. nutans* seedlings by scavenging ROS, modulating redox balance, and activating stress-related defense mechanisms.

14.6 Challenges and Opportunities

Cold-tolerant microbes (CTMs) play an important task as an ecological engineer to reduce low temperature stress problem of high altitude agriculture. On the basis of the information compiled in this chapter, microbial based approach is the most sustainable and prospective future plan for solving the biggest environmental challenges of CS to make global economy and food security intact. Therefore, considering current scenario of agriculture problems of high altitude agriculture, future research is required on the following directions to create more opportunities for sustainable growth of agriculture under cold stress environment:

- Development of highly efficient laboratory methodologies for preparation and formulation of cold-tolerant microbial inoculants on large scale. For instance, bioencapsulation and solid-state fermentation processes.
- Genetic and molecular interactions between soil, plants, and cold-tolerant microbes and their complexity should be explored deeply under field conditions in order to enhance the overall ecological relevance, economic value, and social importance of these microorganisms in a world that needs scientific solutions for higher crop production in low temperature environments.
- Genomic information of CTMs will facilitate to discover and experimentally validate novel secondary metabolism implicated in adaptation to cold ecological niches and promotion of useful attributes for low temperature resilient agriculture.

- Identification and evaluations of diverse types of microbial strains for cold stress tolerance should be carried out to formulate effective microbial consortia for overcoming the negative impact of changing environment problems.
- The psychrophilic microorganisms that can grow under cold environment as well as normal ambient temperature have huge impact on agriculture and allied sector under current scenario of climate change. In this context, attending the problem associated with successful field delivery of prospective CTM-based technologies and their appraisal is of utmost importance.
- Exploitation of effective microbial consortia comprised of multiple types of compatible CTMs displaying synergistic interactions with symbionts in addition to stable and multiple plant growth-promoting attributes for improving crop yields under low temperature is also a powerful strategic tool.
- Utilization of microbial elicitors to enhance plant cold stress tolerance capabilities.
- Incorporation of microbial genes into cold stressed plants or cultivars of high agronomical values.

14.7 Conclusions

Cold-tolerant microbes (CTMs) are widely present in the agro-ecosystem and participated in myriads of beneficial activities related to enhancement of CS tolerance level of various agricultural crops. So far, major emphasis of research work has restricted to the identification and functional characterization of limited groups of bacterial, fungal, and AM species. Therefore, focused and targeted research initiatives are required to decipher the mechanism of plant–CTM interaction, particularly taking care of extremities of high altitude agriculture. Another fascinating domain where microbiological research desires to be targeted is the identification and functional cataloguing of CTMs that can reduce environmental pollution of heavy metal toxicity and detrimental agro-waste because most decomposition processes appear to be faster under low temperature environment. If research efforts succeed in identifying a consortium of potential CTMs that retain their plant growth-promoting potential at lower temperatures, it would be a huge contribution for the resource poor farmers of high altitudes to enhance their livelihood income all over the globe.

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15 Nutrient Cycling at Higher Altitudes

Saranya Packialakshmi Jeyakumar, Biplab Dash, Anup Kumar Singh, Deep Chandra Suyal, and Ravindra Soni

Abstract

Altitude in simple terms is the height above the mean sea level, but it is not as simple as it sounds. The altitude has a significant influence on properties and processes of ecosystem. Altitude determines the biomes by determining the temperature and precipitation imposing an impact on the vegetation. As altitude increases, there is decrease in temperature leading to more diverse and sparse vegetation as well as at higher altitudes, the soils are frozen most of the time. The changing climatic conditions influence the plant and soil nutrient cycles along the increasing gradient. Nutrient cycling at higher altitude differs significantly from those in lower altitudes because of the changing climatic conditions, precipitation patterns, vegetation, and parent rock type. The key nutrients C, N, P, and K of soils at higher altitude differ significantly from those that are present on plains. There is a steady increase in soil organic C and microbial biomass. The total soil N and the microbial biomass N also increase. This implies that there exists a significant difference in the microbiome responsible for the turnover of nutrient cycling events. A comprehensive knowledge on higher altitude nutrient dynamics will bolster our understanding on how the nutrient cycling occurs, difference in

S. P. Jeyakumar · B. Dash
Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore, India

A. K. Singh · R. Soni (✉)
Department of Microbiology, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

D. C. Suyal
Department of Microbiology, Eternal University, Sirmaur, Himachal Pradesh, India

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the microbiome, organic carbon deposition, and microbial activity in permanently frozen soils.

Keywords
Soil nutrients · High altitude regions · Nutrient cycling · Microbiome · Microbial diversity

15.1 Introduction

Altitude is a measure of distance in terms of vertical or upward direction. It is often expressed as the relative distance between the object of consideration to the local ground surface. Earth's surface features several elevated regions with increasing or decreasing altitude. The lowest altitude on earth is −413 m which is the lowest exposed land on the earth, which is the Dead Sea and the highest elevation is the Mount Everest, which is 8850 m. Earth surface above 2400 m of mean sea level is considered high altitude. Very high altitude ranges from 3505 to 5486.4 m above MSL. As per the International Society of Mountain Medicine (ISMM), high altitude is defined into three categories: high altitude (1500–3500 m), very high altitude (3500–5500 m), and extreme altitude (>5500 m).

15.2 Ecosystem at Higher Altitudes

High altitudes directly point to the mountain systems and plateaus (Pauli 2016). Lowlands and altitudes differ by their climatic conditions, which affects the lifeforms residing there in different ways (Fig. 15.1). The altitude has a hostile combination of O_2, strong wind, and frigid temperature which limits life. The level of O_2 in the atmosphere is 21% which is the same at both sea level and higher altitudes. But as altitude raises, there is a drop in the air pressure. So, higher altitudes have low pressure. This is due to the gravity and density of the air. Gravity pulls the air molecules towards the surface of the earth. Air becomes denser with altitude. With increase in the altitude, the gas molecules in the air expand and the air becomes thinner than the air at the sea level. Hence, there is less oxygen available for any living organism to respire. At 12,000 ft (3658 m) the barometric pressure is only 483 mmHg, so there are roughly 40% fewer oxygen molecules. The temperature falls with the increasing altitude (considering the temperature of the sea level to be 15 °C under standard conditions). With every 1 km rise in the altitude, there is a decrease in 6.5 °C temperature. The raising altitude has a significant influence on the properties and processes of the ecosystem. Precipitation in higher altitude gets complex because higher altitude receives snow instead of rain as the temperature gets low. The highest peaks of mountains are capped with glaciers; hence, they are often mentioned as cold deserts. The reduction in air density, decreasing temperature, and increasing precipitate shape a unique ecosystem at higher altitudes. The

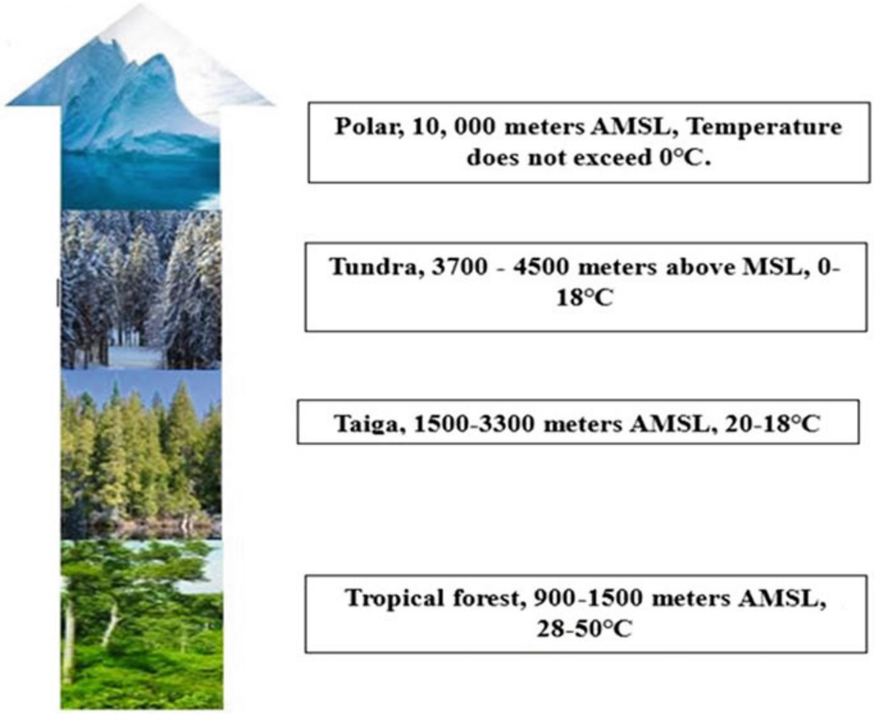

Fig. 15.1 Change of ecosystems along with altitude and the temperature

decreasing temperature changes the structure and composition of the biome. There is a major impact on the vegetation cover. The soil type and microbial processes changes with altitude which in turn has a major impact on the vegetation. With altitude there is different and sparse vegetation. The growth of trees responds indifferently to the changes of climate along the altitudinal gradient (Liang et al. 2019). The lowlands are characterized by moist tropical forests and dry deciduous forests. The moist, tropical forests are characterized by tall, straight evergreen trees with strong roots a dense canopy. The dry deciduous forests have dry evergreen trees whose canopy does not exceed 25 m. Contradictorily these forests are also characterized by thorny shrubs whose height does not exceed 10 m. These forests occupy the altitude 900–1500 above MSL. At altitude 2500–3300 m above MSL, the Taiga forests are dominant. These forests are characterized by pine forests and certain regions have broad leaved forests. These forests are found in areas where the temperature is 20–80 °C. As the altitude goes up above 3355 m above MSL, the temperature of this biome is 28–50 °C. It is located near the poles. Tundra vegetation is characterized by dwarf shrubs, sedges and grasses, mosses, and lichens, which makes it the treeless terrain. The biome covers one-fifth of the earth's surface. Above the tundra region, the temperature remains in such a way that it does not go above

0 °C. The altitude at this point reaches to 10,000 ft. These areas are permanently covered by ice and have no vegetation. The altitude determines the climatic and vegetational pattern.

15.3 The Physiochemical Properties of Soil at Higher Altitude

The physiochemical properties and the biodiversity of the soil ecosystems are highly influenced by higher altitudes (Kumar et al. 2019). The physical properties of the soil like soil texture, soil structure, soil weight, soil density, soil porosity, etc. and the chemical properties like pH, EC, organic C content, nutrient content quite vary with altitude (Table 15.1). There is a diverse variation of physiochemical properties among different altitudinal regions of different part of the world. The chemical properties of the soil are determined by the type of vegetation found at the altitudinal gradient. The soil of high altitude falls under the textural class sandy loam. There is a significant increase in the proportion of sand as we go up the altitude. The high altitude soils are immature and are originated from weathered rocks and therefore have a relative proportion of sand gravel and stones. The soils at high altitude have coarse texture, high water holding capacity due to increased pore spaces, and poor water and nutrient holding capacity (Dwivedi et al. 2005). As the altitude decreases, there is an increase in silt and decrease in sand (Ley et al. 2000). The pH of the soil decreases with altitude. This soil acidification is due to the increased precipitation

Table 15.1 Physiochemical properties of soil at high altitude

Physiochemical property	Trend with altitude	References
Soil texture	Sandy loam	Yuksek et al. (2013)
Soil porosity	High	Xu et al. (2006)
Water holding capacity	Low	Xu et al. (2006)
pH	Decreases	Qasba et al. (2017)
EC	Remains constant	Qasba et al. (2017)
Soil organic and biomass carbon	Decreases	Saeed et al. (2014)
Plant organic carbon	Remains constant	Saeed et al. (2014)
Soil nutrients	Decreases	Wu et al. (2013)
Microbial and enzyme activity	Decreases	Soethe et al. (2008)
Mineral nutrients (calcium, cobalt, nickel, boron, magnesium, sodium, potassium, iron, and copper)	Decreases	Kumar et al. (2019)

levels at the higher altitudes. High amount of rainfall leaches out the base forming cations like Ca^{2+}, Mg^{2+}, K^{+} and increases the ions like Al^{3+} and H^{+} (Northcott et al. 2009). The EC of the soils lowlands and high altitude does not have any significant variation proving that there is cumulative accumulation of salt along the altitude (Charan et al. 2013).

The soils at high altitude have low nutrient absorption capacity. The trees at higher altitude have selective nutrient absorption capacity. These nutrients are cycled back to the soil which bring about changes in the soil chemical properties (Singh et al. 1986). The decreasing temperature decreases the soil organic content at higher altitude. But there is an increase in soil organic carbon with altitude (Saeed et al. 2014). This may be owed to the soil texture, constant input of carbon with less loss, decrease in the microbial and enzymatic activity due to the decreasing temperature (Bolstad et al. 2001).

15.4 Nutrient Cycling at Higher Altitude

The circuits of nutrient circling at higher altitudes vary greatly from the lowlands. The quite unique ecosystem of the higher altitude imposes a greater thrust on the nutrient cycle. The low temperature at the high altitude will limit decomposition. The other well known reasons would be the soil texture, lesser microbial and enzymatic activity which can be owed to the unique ecosystem prevailing at higher altitudes. The soil organic carbon content increases with altitude but the availability of soil nutrients decreases with elevation (Koerselman and Meuleman 1996). The plant organic carbon content remains almost constant being insensitive to the environment, but the soil and microbial biomass C decrease. There is also decrease in organic matter degradation due to high C:N ratios of the leaf litter, roots, etc. increases the soil organic C (He et al. 2016). This may be owed to the soil texture, constant input of carbon with less loss, decrease in the microbial and enzymatic activity due to the decreasing temperature (Bolstad et al. 2001). The available N in the soil decreases in the altitude with soil depth. The rate of N_2 mineralization decreases with altitude. The same trend is adopted for soil P also. The P content of the soil decreases linearly with altitude. The available P content decreased with depth of the soil at higher altitude. Moreover, a large portion of P in the soil remains in unavailable form, which is contradictory to the fact that P is present in number of organic forms and is found in higher concentrations under tree cover (Johannessen 1958). There is a quick loss of P in the high altitude soils due to large elevation, different vegetation different zonalities and storage of P in biomass (Wu et al. 2013). Total soil potassium differs with altitude. The surface of the soil has high exchangeable potassium content which is later converted into soil solution (Dangwal et al. 2012). In the Alpine regions of tundra ecosystem, the total stocks of phosphorus, Ca^{2+}, and Mg^{2+} slightly increased with depth (Tian et al. 2017). There always exists difference in the foliar nutrient content and the soil nutrient content. In a montane ecosystem, the plant's access to available nutrients like N, P, K, and S decreased along the altitude (Soethe et al. 2008). There exists a well-balanced, tightly closed soil-plant-litter web in the high altitude ecosystem. The polar ice caps remain frozen for almost throughout the year. The nutrient levels are highly low due to the decreased temperature, consequently less vegetation and less microbial activity. In the Arctic regions, the psychrophilic microbes present in the soil will efficiently involve in immobilization leading to less mineralization of N, P, K (Jonasson et al. 1996).

15.5 Diversity of Plants and Microbes at High Altitudes

Nutrient cycling at high altitude soils is different from those at low altitude areas. Not only temperature plays an important role but also the presence of diverse micro and macro flora and fauna greatly influences the interconversion of these nutrients into one form or other. Nutrient cycling occurs in all known ecosystems; however, their rate varies of which presence of biotic components severs as a key factor. Plants and microbial diversity have a very influential role at high altitudes and therefore for better understanding of nutrient cycling, it is essential to have knowledge about these organisms.

15.5.1 Diversity of Plants

With decrease in temperature, the supply for nutrients decreases. This leads to decrease in growth of plants (Coomes and Allen 2007). Therefore forests that are found at low altitude areas are quite different from that of high altitudes. In high altitude regions, tree lines are found. These are basically the areas beyond which tree density ceases to zero due to prevalence of low temperature conditions (Elliott-Fisk 2000; Berdanier 2010). However, short and tall shrubs, sedges, mosses, and herbs are widely seen to be capable of growing in these harsh conditions. Shrubs like *Salix*, *Alnus*, *Betula* and grasses like *Carex*, *Eriophorum*, *Dupontia* are widely found in Tundra region (Bliss 2000) (Table 15.2). Recently researchers have discovered six vascular plant species at an elevation of 6150 m in Western Himalayas which defines the vast adaptiveness of plant species against all these harsh climatic conditions (Angel et al. 2016). The upper limit of forest belt is often referred to as tree line ecotone rather than only tree line (Korner and Paulsen 2004). It is reported that *Juniperus tibetica* Kom are the tress found to be growing at a height of 4850 m in Southeast Tibet which is said to be consisting the highest tree line of Northern hemisphere (Miehe et al. 2007). Given below are the tree species found in the mountain ranges.

15.5.2 Diversity of Microbes and Other Organisms

We all know about formation of clouds but seldom we may have imagined that microbes can someday help in their formation. In fact this is true, scientists have

Table 15.2 Tree species found in different mountain ranges

Mountain ranges	Elevation	Plant species	References
Himalayan	1700–3800 m	*Quercus semecarpifolia*	Tiwari and Jha (2018)
	2000–2600 m	*Pinus wallichiana*	
	2700–4300 m	*Betula utilis*	
	2800–4400 m	*Rhododendron campanulatum*	
	3000–4000 m	*Abies spectabilis*	
	3700–4400 m	*Juniperus* sp.	
Chiricahua	1950 m	*Pseudotsuga menziesii*	Sawyer and Kinraide (1980)
	2100 m	*Pinus ponderosa*	
Andes	3560–4680 m	*Polylepis* sp.	Kessler et al. (2014)
	3300 m	*Myrsine* sp., *Clusia* spp.	Bader et al. (2007)
	3600 m	*Weinmannia cochensis, Ilex colombiana*	
Alborz	2400–2850 m	*Quercus macranthera*	Noroozi and Korner (2018)
	2000–3000 m	*Juniperus excelsa*	
Alps	2200 m	*Alnus viridis*	Carnelli et al. (2004)
	2100 m	*Larix decidua, Pinus* sp.	Loranger et al. (2016)
	2250–2540 m	*Pinus cembra*	Jochner et al. (2017)

predicted that air borne microbes like bacteria, fungi, or microalgae influence the formation of clouds by serving as the agents for their condensation and can even grow at sub-zero temperature (Schiermeier 2008). This statement gives us an idea about presence of microbes even in upper atmospheric region but with respect to nutrient cycling, knowing about microbial diversity in high altitude soils is imperative.

Mostly due to low temperature range, microbes mostly thrive on these conditions which are known as psychrophiles. These organisms can grow at maximum 20 °C (Gounot 1986) and represent all three domains of life (bacteria, archaea, and eukarya). However, a wide range of microbes from bacteria, archaea, algae, and yeast have already been reported throughout the world (Feller 2013). These psychrophilic microbes are distributed extensively across all spheres of earth. Bacteria (*Planococcus halocryophilus*) growing at −15 °C have been isolated from artic permafrost (Mykytczuk et al. 2013), while psychrophilic bacterium have also their presence in marine environment (Dyrset et al. 1984). Similarly, psychrophilic bacterium have also been isolated by many researchers from high altitude soils (Gangwar et al. 2009; Kumar et al. 2019). Metagenomic studies reveal the predominance of *Proteobacteria*, *Acidobacteria,* and *Actinobacteria* bacterial phyla in high altitude soils (Kumar et al. 2019). In high altitude soils of Himalaya, *Proteobacteria* accounts for 73% of bacterial diversity, while *Betaproteobacteria* represents 31% (Margesin and Miteva 2011). Through 16S RNA gene sequencing, it was revealed that *Proteobacteria* like *Pseudomonas* and *Rhizobium* are dominant in Himalayan soils (Joshi et al. 2017). Fungi too are seen in high altitude conditions. These psychrophilic fungi have evolved themselves to adapt to these harsh climatic conditions. Fungi like *Phomasclerotioides* and *Pseudogymnoascus pannorum* are some of the predominant species found in alpine glaciers on Qinghai-Tibet Plateau (Wang et al. 2015). Photosynthetic microbes have also a rich diversity in high altitude soils. Abundant sunlight and moisture conditions are ideally suited for these microbes which allow them to grow in sub-zero temperatures. *Cyanophyceae*, *Chlorophyceae*, and *Bacillariophyceae* are some of the most dominant flora of algae capable of tolerating these extreme conditions (Toppo et al. 2016). Chlorophyceaen algae (*Euastrum oblongum, Penium cylindrus, Scenedesmus quadricauda*) are seen in water bodies of high altitudes (Ghimire et al. 2013).

Due to slow decomposition rates of organic matter, high altitude soils are often rich in soil organic matter (SOM) resulting in higher microbial activities (Siles et al. 2016). Reports of some psychrotrophic diazotrophs (*Dyadobacter psychrophilus* and *Pseudomonas jessenii*) found in high altitude soils are well-being documented through proteomic studies (Suyal et al. 2017). Similarly, lichens are also known to be growing abundantly in high altitude regions. Lichens like *Melanelia infumata* and *Xanthoria elegans* are found at an altitude of 3100–4000 m in central Himalayas (Singh et al. 2016).

15.6 Constraints for Nutrient Cycling at High Altitudes

High altitude soils are coarse textured, and have low nutrient and water holding capacity in them. These soils are exposed to extreme climatic conditions and have varied level of temperature, snowfall, and rainfall which ultimately affects the physiochemical properties of the soil (Charan et al. 2013). Low temperature is the main limiting factor of nutrient supply to plants at high altitude soils. Therefore, high altitude soils are infertile and crop production seems to be a major challenge in these soils. Plants are devoid of soil nutrients (N, P, S, and K) with increase in altitude (Soethe et al. 2008). Poor quality of soil and limited supply of water cease the growth of plants. Scientists have reported that though there is higher nitrogen content in leaves, still plant growth is limited as it is not properly utilized by the plant's body (Macek et al. 2012). With increase in altitude there is decrease in nitrogen uptake by plants which further limits plant growth. Besides top soil, soil bedrock also influences the nutrient content of plants and one such example is the phosphorous uptake by plants (Gerdol et al. 2017). Unlike in normal soils, nutrient cycling is a much more complex phenomenon in high altitude soils. Various biotic (plants and microbes) and abiotic factors (altitude, parent material, and vegetation community) regulate them. Concentration and ratio of carbon, nitrogen, and phosphorous of the four key ecological components *viz*., forest floor litter, fine roots, soil, and soil microorganisms depends on altitudinal gradients (He et al. 2016).

Soil ecology is an important aspect in mineral transformation at high altitudes. High altitude soils have more soil organic carbon and nitrogen because temperature limits the process of nutrient cycling. The C:N and C:P ratios of these soils are higher than normal soils mainly due to slow mineralization of forest litters at low temperature (Soethe et al. 2008; Qasba et al. 2017). As the altitude increases, there is a decrease in soil pH, base saturation, exchangeable potassium capacity, while C:N and organic matter increase (Dar et al. 2017). This seems to suggest that soil organic carbon is positively correlated with altitude while negatively correlated with temperature (Sevgi and Tecimen 2009). Microbial activity is always an essential component of nutrient cycling besides physical parameters. Decomposition process is mainly regulated by them. However due to low temperature, psychrophilic microbes are only able to survive; thus, at high altitude soils psychrophiles are the major players in nutrient cycling. Seasonal variation influences microbial activities which in turn determine the decomposition process. It has been reported that decomposition of litter is 40–60% greater in winter as compared to yearly rate (Taylor and Jones 1990).

Low temperature decreases the microbial and enzymatic activity of soil. Nitrogen fixation and nitrogen assimilating enzymes work differently for psychrophilic and mesophilic strains of the same organism. Psychrophiles exhibit maximum nitrogenase activity at low temperature, while mesophiles do not. However, at normal temperature, both mesophilic and psychrophilic strains showed equal enzymatic activity suggesting their similar nitrogen assimilating mechanisms (Thangaraj et al., 2017). Psychrophilic diazotrophs are exposed to dual stress conditions in high altitude soils, i.e., cold environmental stress and nitrogen starvation. In order to cope up with these situations, they devise unique adaptive mechanisms. Some of these diazotrophs (for example, *Pseudomonas migulae*) express two different proteins (NifU family SUF system FeS assembly protein and membrane protein, suppressor for copper-sensitivity B precursor) which is believed to be having a role in its nitrogen fixing ability under low temperature condition (Suyal et al. 2013). It is reported that altitude difference could significantly affect the diazotrophic counts (Kumar et al. 2019).

15.7 Conclusion

High altitude possesses altogether different sets of challenges when it comes to nutrient cycling as compared to low altitude plains. It is coupled with extreme climatic conditions, low vegetation, and broad microbial diversity. The soil physical and chemical parameters are very different as well as they are very unique to these habitats. All these parameters make nutrient cycling at high altitudes very slow. Moreover with growing climatic change across the globe, nutrient cycling is affected at these altitudes.

15.8 Our Efforts

Since the last several decades, our groups did a lot of sincere efforts to explore the rhizosphere bacterial diversity of Western Indian Himalaya specially belongs to Uttarakhand state (India) based on small ribosomal gene amplification of soil metagenome (Soni et al. 2010). We used several molecular tools like PCR-RFLP (Singh et al. 2010), DGGE and TTGE (Soni et al. 2010), and real-time PCR (Premalatha et al. 2009; Soni and Goel 2010; Suyal et al. 2015a, b) to explore the rhizosphere microbiome of high altitudes. Our group has earlier beautifully painted the prevalence of csp (Premalatha et al. 2009) and nifH genes (Soni and Goel 2010; Soni and Goel 2011; Soni et al. 2016) from Western Himalayan region of India. To the best of our knowledge, the first major metagenomic effort revealed the presence of diverse diazotrophic microbial assemblages in indigenous red kidney bean (RKB) rhizosphere (Suyal et al. 2014; Suyal et al. 2015a, b). Recently our group published a research where LC-MS/MS based gel less quantitative proteomics was employed to investigate the metabolic response of Western India Himalayan cold adapted nitrogen fixing *Pseudomonas palleroniana* N26 for nitrogen deficiency and cold stress (Suyal et al. 2018).

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16 Assessment of Genes and Enzymes of Microorganisms of High Altitudes and Their Application in Agriculture

Geeta Bhandari, Niki Nautiyal, and Mukund Sharma

Abstract

Extreme environments are considered the biodiversity hotspots especially in terms of microorganisms. Microbiomes of the extreme environment impart important information about the critical limits for survival and adaptability of microorganism. Hill and mountain agroecosystems demand distinct microflora which can endure in these extreme environments and simultaneously perpetuate their plant growth promontory properties. Microorganism native of the cold environment is widely distributed in the agroecosystem and has physiologically, metabolically, and biologically well adapted to such environments. Thus, microbial inoculants from these extreme conditions possessing PGP attributes can be efficiently utilized for promoting growth and yield of high altitude crops. Numerous plant growth-promoting rhizobacteria (PGPR) from high altitude soils containing vital enzymes involved in plant growth enhancement have been reported. These organisms can thus be employed as biofertilizers, biocontrol agents, and bioremediation for enhancing agricultural productivity.

Keywords

Microbial enzymes · High altitude regions · Agriculture · Microbial genes · PGPR

16.1 Introduction

Cold and high altitude consisting of permafrost soils, polar ice, glaciers, and snow cover are widespread on the earth and constitute up to 20% of the Earth's surface environments. High altitude environment is a strenuous habitat for the survival of various plants and microbes. However, agriculture at these ecosystems faces many

G. Bhandari (✉) · N. Nautiyal · M. Sharma
Sardar Bhagwan Singh University, Balawala, Dehradun, Uttarakhand, India

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challenges due to low temperature. Genetic modifications and transfer of low-temperature tolerance into commercially important plants is a complex and time-consuming process; therefore, a solution for the protection of plants from chilling and for their growth enhancement involves the application of cold-adapted PGPR. These are the beneficial microorganisms which reside on the plant's rhizospheric regions and enhance their growth directly and/or indirectly, viz. inhibiting plant pathogenic organisms (biopesticides), degradation of xenobiotics (bioremediation), or triggering induced systemic resistance (ISR) in plants; releasing plant growth-promoting substances (phytostimulation) and furnishing vital nutrients (biofertilizers) (Glick 1995). High altitude soils are of utmost significance since several ecosystems are subjected to low temperatures and therefore these environments have been broadly explored for the novel microorganisms (Kumar et al. 2016). High altitude microbiomes being hot spots of biodiversity are the habitat of various psychrophiles and psychrotolerant microorganisms, which have been reported by several authors (Miteva and Brenchley 2005; Pradhan et al. 2010; Sahay et al. 2013; Yadav et al. 2016). The psychrotrophic PGP microorganisms reported till date consist of *Bacillus, Flavobacterium, Janthinobacterium, Kocuria, Lysinibacillus, Methylobacterium, Microbacterium, Pseudomonas, Paenibacillus, Arthrobacter, Providencia, Brevundimonas Serratia, Citricoccus, Azotobacter, Clostridium, Exiguobacterium, Hydrogenophaga, Burkholderia, Enterobacter*, and *Azospirullum* (Mishra et al. 2011; Prasad et al. 2014).

16.2 The Necessity of Biofertilizers for Hilly Regions

Agricultural lands at higher altitudes are characterized by poor nutrient conditions, less fertility, and lesser soil moisture content besides extreme cold and frost in the winters. There are no improved technologies available for enhancing agricultural production or, even if available, they are not accessible by the small farmers. Thus, the condition of the soil in the hilly areas is becoming deteriorated, resulting in a decline of fertile soil (Jodha and Shrestha 1993). It is therefore needed to investigate other alternatives for improving crop production so as to upgrade the quality of living standard of hill population (Partap 1999). The nitrogen fixing microorganisms and P-solubilizing microorganisms are among the most studied group of the biofertilizers. However, the use of available commercial biofertilizers in hilly regions has demonstrated to be unsuccessful (Pandey et al. 1998) Temperate agro-ecosystems around the world also have short growing periods, which are interspersed by suboptimal temperatures, thus most microbial processes slow down or become standstill, thereby affecting the productivity adversely. The cold-adapted microorganisms are divided into psychrophiles and psychrotolerant. The psychrophilic microbes inhabit cold areas, such as polar areas, high altitudes, the deep sea having temperatures between subzero to 15 °C. The psychrotolerant microbes inhabit regions with a temperature between 4 and 42 °C with temperature optima above 20 °C (Morita 1975). In hill agriculture, the psychrotolerant microorganisms are of great significance due to better survival and adaptation at low temperature and ability to also grow optimally at a higher temperature. These microorganisms have been extensively studied and being developed as a potential biofertilizers nowadays (Table 16.1).

16.3 Plant Growth-Promoting Rhizobacteria

The rhizosphere is the surrounding region of the plant roots and is an extremely conducive environment for the growth of microbes. Rhizospheric bacteria greatly influence the soil fertility and their beneficial effect towards plant growth is known since the centuries (Tisdale and Nelson 1975; Beijerinck 1888). The terms "rhizobacteria" and "plant growth-promoting rhizobacteria" were coined by Kloepper and Schroth (1978, 1981). However, the term "plant growth-promoting bacteria" (PGPB) can also be used for such bacterial candidates (Andrews and Harris 2003). The mode of action of PGPR strains is divided into two major categories: direct and indirect (Fig. 16.1). The direct mechanism involves solubilization of phosphorus, nitrogen fixation, iron sequestration by siderophores and plant growth hormones synthesis, etc. (Hellriegel and Wilfarth; Glick 1995). The indirect mode includes antibiotic production, reduction of iron availability to phytopathogens, induced systemic resistance, and production of antifungal agents (Verma et al. 2015a, b, 2016). To utilize PGPR for growth promotion, it is inevitable that it must adapt in the plant's rhizosphere which is greatly influenced by soil temperature and type, predation by protozoa, production of antimicrobial compounds by other soil microorganisms, bacterial growth rate, and utilization of exudates.

16.4 Mechanism of Plant Growth Promotion at Low Temperature

Cold stress poses adverse impacts on plant growth by either limiting metabolic reactions or inhibited water uptake due to chilling, chlorosis, wilting, necrosis, damage of biomolecules, and reduction in osmotic potential of the cell. Under low-temperature stress, plant cells rigidify their cell membrane due to reduced fluidity of the cellular membranes, accumulation of cryoprotectants, and increased potential to tolerate oxidative stress. Plants employ several mechanisms for cold stress tolerance, however, a net decrease in plant growth and production is observed under low-temperature conditions (Haldiman 1998). PGPRs play an important role by helping plants to withstand cold tolerance, as several genes are induced by PGPR activities which allow plants to tolerate various abiotic stresses. PGPRs principally help in plant growth promotion in low-temperature condition by two major processes: phytostimulation and frost injury protection.

Table 16.1 Psychrotolerant plant growth promoting bacteria

Microorganism	Source	Function	References
P. syringae	Tomato and soybean	Increase in frost susceptibility by ice nucleating strains of *P. syringae*	Anderson et al. (1982)
Azospirillum brasilense	Finger millet, sorghum, pearl millet	Increase in yield	Subba Rao (1986)
Pseudomonas chlororaphis 2E3, O6	Spring wheat field	Increased seedling emergence	Freitas and Germida (1992)
Xanthomonas maltophila	Sunflower	Increased germination rate	Fages and Arsac (1991)
Azospirillum local isolates	Maize, wheat	Increase in yield	Okon and Labandera-Gonzalez (1994)
Pseudomonas putida R111, *Pseudomonas corrugate*	*Amaranthus paniculatus*	Plant growth and nitrogen content increased	Kropp et al. (1996)
Enterobacter cloacae CAL3	Mung bean tomato, pepper	Positive seedling growth	Mayak et al. (1999)
Bradyrhizobium japonicum	Soybeans	Improved nodulation and nitrogen fixation	Zhang et al. (2003)
Sinorhizobium meliloti	Alfalfa	Growth improvement under cold and anaerobic (ice encasement) stresses	Prévost et al. (2003)
Mycobacterium sp. 44 *Mycobacterium phlei* MbP18 *Mycobacterium bullata* MpB46	*Triticum aestivum* cv. Bussard	Increase root and shoot dry mass and enhance N, P, K uptake	Egamberdiyeva and Höflich (2003)
A cold-tolerant mutant of *Pseudomonas fluorescens*	Vigna radiata	Growth promotion at 25 and 10 °C and a 17-fold increase in siderophore production and increased rhizosphere colonization	Katiyar and Goel (2004)
Burkholderia phytofirmans PsIN	*Glomus vesiculiferum*—infected onion roots	Cold stress tolerance and increase in total phenolics, photosynthetic activity in Vitis vinifera	Barka et al. (2006)
P. putida UW4	Canola plant	Promotes plant growth at low temperature under salt stress and produces ACC deaminase	Cheng et al. (2007)
Serratia marcescens SRM (MTCC 8708)	Flowers of summer squash (Cucurbita pepo)	Increase root and shoot lengths and N, P, K uptake in *Triticum* sp.	Selvakumar et al. (2008b)

(continued)

Table 16.1 (continued)

Microorganism	Source	Function	References
Pseudomonas sp. NARs9	Rhizosheric soil Amarnath, NW Indian Himalayas	Increase germination rate, shoot and root lengths in *Triticum* sp.	Mishra et al. (2009)
Pseudomonas lurida	Rhizosphere of Himalayan plants	Protects plant from chilling stress	Bisht et al. (2014)

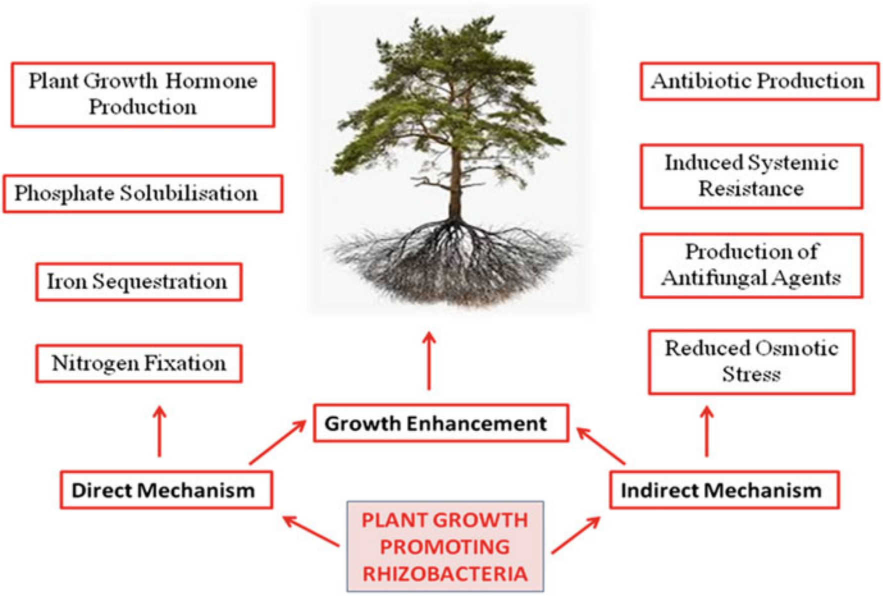

Fig. 16.1 Mechanism of plant growth promotion by plant growth promoting rhizobacteria

16.4.1 Phytohormones and Phytostimulation

Phytohormone production is one of the major ways of promoting plant growth (Glick et al. 1998; Spaepen et al. 2007). Phytohormones are organic molecules, which can impact the physical and metabolic processes of plants and act as chemical messengers (Fuentes-Ramírez and Caballero-Mellado 2006). Microbes producing the plant growth hormones are *Klebsiella pneumoniae, Proteusmirabilis, Pseudomonas vulgaris, Bacillus, Escherichia* produce auxin cytokinins, gibberellins and ABA (Bric et al. 1991; Griffith and Ewart 1995).

16.4.1.1 Indole Acetic Acid (IAA) Production

IAA is the major plant hormone which is responsible for cellular division and elongation in the plants (Tsavkelova et al. 2006). Many PGPRs have the capability to produce IAA (Timmusk et al. 1999). It can be synthesized using tryptophan or without it (Spaepen et al. 2007). Moreover, Selvakumar et al. (2008a, b) have isolated two IAA producing PGPRs, viz. *Serratia marcescens* SRM and *Pantoea dispersa* 1A from Himalayan regions. These microbes were found to increase weight and nutrient uptake by wheat plants growing at low temperature. Moreover, IAA producing *Pseudomonas* sp. PGERs17 and NARs9 strains have been isolated by Mishra et al. (2008, 2009) which were able to enhance seed germination rate and plant length of wheat seedlings growing at cold temperature.

16.4.1.2 ACC-Deaminase Production

1-aminocyclopropane-1-carboxylate (ACC) deaminase is an enzyme which stimulates plant growth positively. It helps in regulating ethylene levels in plants. Higher concentrations of ethylene inhibit plant growth (Cheng et al. 2007). The extent of ethylene and its production is tightly regulated by various transcriptional and post-transcriptional factors, which in turn are controlled by the environmental conditions (Hardoim et al. 2008). In low-temperature conditions, ethylene levels in plants result in decreased plant growth and development (Bottini et al. 2004). Microbes capable of ACC deaminase production, arrest plant ACC, and cleave it to form ammonia and a-ketobutyrate, which are readily metabolized by the bacteria. This results in a decrease in detrimental outcomes of ethylene which promotes plant growth (root, shoot and biomass) and stress tolerance (Glick et al. 2007). Barka et al. (2006) demonstrated enhanced cold resistance and ACC deaminase activity by *Burkholderia phytofirmans* in grapevine. Six psychrotolerant strains have been isolated from leaf apoplastic sap of cold-adapted wild plants by Tiryaki et al. (2019). The isolates were found to possess ACC deaminase activity and were able to secrete the different extracellular proteins under cold stress.

16.4.2 Frost Injury Protection

Various plant parts (stem, leaves, buds, and flowers) behave differently to freezing injury thus making it complicated. Ice nucleation in plants is due to induction by various catalytic sites available in microbes found in different plant parts (Lindow 1983). Plants are substantially damaged under the chilling conditions, not only of low nutrient availability or poor hormone production but majorly due to frost settlement on plants and ice crystallization within cells. Every year huge losses in the agricultural sector occur because of crops damaged by freezing injury. Microorganisms adapt various strategies to cope with this chilling stress.

16.4.2.1 Ice Nucleation Proteins

Ice crystal formation involves ice nucleation and ice growth. Each class of ice crystal controlling protein targets any one of these. Ice nucleation proteins (INPs) activate the development of ice crystals and successive freezing around high subzero temperatures (Kawahara 2008). However, ice nucleation maybe reduced by most PGPR strains, which produce either antifreeze proteins or ice-nucleating protein complexes that inhibit ice recrystallization or cold acclimation proteins. Ice nucleation proteins (INPs) mimic ice crystal surface and thus reduce supercooling and encourage freezing at temperatures higher than subzero. INPs are hydrophilic in nature and present as anchors on the cell membrane surface and have ice-binding sites (Xu et al. 1998). *Erwinia herbicola* INPs are huge multimeric proteins with subunits having a size from 120 to 150 kDa and belong to a structurally homologous class of proteins (Kawahara 2008). Its N-terminal domain has hydrophobic nature and is globular in shape and comprises 15% portion of the total protein. The N-terminal domain also binds to polysaccharides, lipids, and other INPs (Kajava and Lindow 1993) and thus this binding allows the INP to anchor to the cell membrane. This results in the formation of an organized assembly for higher activity of INPs (Govindarajan and Lindow 1988). CRD is assumed to be the site of ice interaction (Kawahara 2008). Innumerous microbes having ice nucleation property have been reported (Kawahara 2008). *P syringae* and are highly potent microbes possessing ice nucleation activity (Kozloff et al. 1983). Several other bacterial genera including Pseudomonas, Pantoea, Xanthomonas, and Erwinia have also been reported to possess ice nucleation property (Lindow et al. 1978; Obata et al. 1990). Bacteria possessing INPs are termed as "ice plus" bacteria (Maki et al. 1974; Lee et al. 1995). INPs help in ice crystallization at a temperature above subzero. The bacteria which don't have INPs are termed as "ice minus" bacteria and thus nucleate ice at low temperatures. Increase in frost receptiveness of soyabean and tomato was found, when ice plus *P. syringae* were sprayed on leaves of these plants in cold stress condition (Anderson et al. 1982). Ice nucleation genes in *P. syringae* have been identified, which has led to the formation of "ice minus" mutant. This mutant has been found to be inactive in ice nucleation of plants leaves (Xu et al. 1998). These mutants can further be used for controlling the ice nucleating activity of bacteria and thus helps plants to overcome freezing injury. Lindow (1983) identified the ice-nucleating factor from *P. syringae* by deletion mutation. A strain of naturally occurring *P. fluorescens* has been registered commercially as Frostban B for the protection of pear trees (Lindow 1997; Wilson and Lindow 1993). Lindow and Panopoulous (1988) carried out field experiments using *P. syringe* on potatoes and strawberries and concluded that the incidence of frost injury was significantly lower in inoculated potato plants than in uninoculated control plants in several natural field frost events. Tiryaki et al. (2019) have isolated several psychrotolerant microbes which were found to reduce freezing injury and ice nucleation and thus can be utilized for enhancing the cold tolerance in the crops.

16.4.2.2 Antifreeze Proteins (AFPs)

AFPs are the proteins possessing the capability to alter the structure of the ice crystal and restrict recrystallization of the ice (Raymond and DeVries 1977; Knight et al. 1988). The antifreeze proteins have two main activities: thermal hysteresis and restriction of ice recrystallization (Kawahara 2008). Thermal hysteresis involves a non-colligative reduction in the freezing temperature; this is called as freezing hysteresis. It also may involve slight elevation in melting temperature termed as melting hysteresis (Gilbert et al. 2005; Celik et al. 2010). Inhibition of ice recrystallization is the second antifreeze activity which makes small ice crystals by inhibiting ice recombination. These small ice crystals are energetically more favored than bigger ones. Antifreeze proteins when present in bound form reduce water movement between the ice crystals and don't allow the smaller ice crystal grains to destabilize small ice crystal grains, thus ice recrystallization is minimized (Yu et al. 2010). As compared to thermal hysteresis, comparatively smaller amounts of antifreeze proteins induce inhibition of ice recrystallization (Kawahara 2008). The presence of thermal hysteresis proteins in bacteria was reported by Duman and Olsen (1993) and a strain of Moraxella sp. was the first example of an Antarctic bacterium that was found to produce an AFP (Yamashita et al. 2002). AFPs are also assumed to help in the stabilization of biological membranes and preserve cell integrity (Collins and Margesin 2019). AFPs from *Pseudomonas putida* GR 12–2 were discussed by Muryoi et al. (2004). The antifreeze protein, AfpA, was isolated from *Pseudomonas putida* GR12–2 and found to have a size of 164 kDa. AfpA was found to consist of both sugar and lipid moieties. Muryoi et al. (2004) also isolated the gene responsible for encoding this AfpA. The greater similarity between AfpA and proteins associated with cell wall was found rather than between Afp A and INPs. AfpA protein sequence was found to be more hydrophobic in the region that is involved in the formation of ice template than INPs as disclosed by the hydropathy plots. This suggests the different nature of the interaction of AFPs and INPs with ice (Muryoi et al. 2004).

16.4.3 Biological N Fixation (BNF)

BNF involves the enzymatic reduction of atmospheric nitrogen to biologically available form. The available form of nitrogen: nitrate and ammonium have high biological demand but are found only in small amounts. Therefore biological nitrogen fixation is a significant process and acts as a source of fixed nitrogen (N) in many habitats (Vitousek and Howarth 1991; Arp 2000). Microorganisms are the living constituent of the ecosystem which plays an important role in the conversion of elements; including N_2 fixation (Atlas and Bartha 1998; Madigan et al. 2000) Innumerous microorganisms capable of fixing atmospheric nitrogen have been reported.

16.4.3.1 Nitrogenase

All the diazotrophs use nitrogenase enzyme for the process of nitrogen fixation. It catalyzes the reduction of atmospheric dinitrogen to ammonia coupled with the reduction of protons to hydrogen (Kim and Rees 1994). Nitrogenase is made of two multisubunit metalloproteins consisting of iron (Fe) protein (dinitrogen reductase) and the molybdenum-iron protein (MoFe), called dinitrogenase (Howard and Rees 1996). Nitrogenase is coded by the *nif*HDK genes; these are commonly present in contiguous array in the genome. Component I of nitrogenase is made of two hetero dimers and has a molecular weight of about 250 kDa. Component I contains the active site of N_2 reduction.

Component II is a homodimer and has molecular weight 70 kDa and is coded by the *nif*H gene. This unit integrates the hydrolysis of ATP to electron transfer. Component I and Component II proteins both contain Fe-S centers which are coordinated amongst the subunits. In the conventional enzymes, Fe-S centers also contain Mo, whereas in "alternative" and "second alternative" nitrogenases in place of Mo, V and Fe are present, respectively. The *nif*H genes present in all of these nitrogenase enzymes are highly conserved (Howard and Rees 1996). Both types of alternative nitrogenases include *nif*H, however also include a third protein in the place of the Mo protein that is coded by *nif*G (*nif*DGK) (Burgess and Lowe 1996; Eady 1996). The reduction carried out by nitrogenase requires 16 ATP and 8 electrons per molecule reduced and thus energetically quite costly. Nitrogenase under in vitro conditions is also quite sensitive to the presence of oxygen and becomes inactivated by its presence.

16.4.3.2 Diazotrophy in Low-Temperature Conditions

Cold temperatures condition impose several detrimental effects on nodulation effectiveness of rhizobia, delays root infection and may also suppress nodule function (Lynch and Smith 1994). Reduction in the synthesis of Nod metabolites by *Rhizobium leguminosarum trifolii* is also observed under low temperature thus suppresses nodulation and results in low yield of legumes (McKay and Djordjevic 1993). Prévost et al. (2003) selected cold-adapted rhizobia (*Mesorhizobium* sp. and *Rhizobium leguminosarum*) from Canadian soils, biochemical studies revealed higher production of CSPs in these strains. Eleven nodulation genes have been characterized from arctic *Mesorhizobium* strain N33, and the Nod factors involved in the specificity of nodulation have been identified by Prévost et al. (2003). The nodulation genes of rhizobia, *nod*ABCIJ genes are clustered into a single transcriptional unit. The *nod*ABCIJ genes are required for Nod factor's synthesis (Dénarié et al. 1992). The *nod*A gene of *Mesorhizobium* strain N33is not present adjacent to the *nod*B genes, unlike in other rhizobia. The *nod*BCIJ genes of *Mesorhizobium* strain N33 are found to be homologous in sequence to those of other rhizobia, except for the 3′-coding region of the *nod*C gene (Cloutier et al. 1996a). The presence of *nod*AFEG genes in *Mesorhizobium* strain N33 stipulates that the nod gene content of this arctic strain is analogous to that of *S. meliloti* (Cloutier et al. 1996b, 1997). The Nod factor of this arctic Mesorhizobium strain has been characterized by Poinsot et al. (2001). Its basic structure consists of a lipochito-oligosaccharide made up of oligomers of five N-acetyl glucosamine residues linked by β-1,4- glycosidic linkage and 6-O-sulfated at the reducing end.

16.4.4 P Solubilization

Soil phosphate is found mainly in organic and inorganic forms. Phosphates are generally found in its insoluble forms and therefore not accessible to plants. Inorganic P of soil mostly consists of insoluble mineral composites; most of these emerge after usage of chemical fertilizers. These mineral complexes are mostly precipitated and thus cannot be drawn by plants. Organic matter accounting for 20–80% of soil phosphate is the major pool of immobilized phosphate in soil (Richardson 1994). Phosphate solubilizing microbes (PSM) can convert bound form of phosphate to the available form and thus, contributes in the plant growth. PSM employ several mechanisms for P solubilization, which include: (1) producing organic acids, siderophores to dissolve bound P, (2) mineralization of inorganic P through enzymes (3) liberation of P by substrate degradation (McGill and Cole 1981). PSM also work as a sink of P, by immobilization of P even under very low concentration of soil P. Phosphate solubilizing microbes on starvation, predation, or death also act as a source of P to plants (Butterly et al. 2009).

16.4.4.1 Inorganic P Solubilization

Mineral phosphate dissolving ability in most microbes is attributed to the synthesis of organic acid (Whitelaw 2000; Maliha et al. 2004). These organic acids may lower the pH, enhance chelation of ions bound to P, and may form metal ion complexes (Ca, Fe, Al) which remain in association with insoluble P (Omar 1998; Zaidi et al. 2009). $H_2PO_4^-$, which is found mostly in low pH soils, is a soluble form of inorganic phosphate vitally present in the soil. Production and liberation of organic acid by phosphate solubilizing microbes results in acidification of the cells and the surroundings and the protons substitute the cations bound to phosphate thus leading to discharge of P ions from mineral P (Goldstein 1994). The important organic acids liberated by PSM include lactic acid, aspartic acid, and tartaric acid (Venkateswarlu et al. 1984), citric acid and oxalic acid (Kim et al. 1997), gluconic acid (Di-Simine et al. 1998). Subsequently, gluconic acid is thereafter transformed to 2,5-diketogluconic acid and 2-keto-gluconic acid (Goldstein 1995; Bar-Yosef et al. 1999). The 2-keto-gluconic acid thus formed is much more efficient in solubilizing phosphate than gluconic acid (Kim et al. 2002). Expression of the MPs gene in *E. coli* HB101 bestowed it with the potential to produce gluconic acid and thus solubilize hydroxyapatite (Goldstein and Liu 1987). Babu-Khan et al. (1995) cloned *gab*Y gene (also associated with gluconic acid production) and *MPs* gene from *Pseudomonas cepacia*. The results showed sequence similarity with membrane-bound protein rather that of GA synthesis. Gluconic acid is however made only if a functional glucose dehydrogenase (*gcd*) gene is expressed in *E. coli* strain.

16.4.4.2 Organic P Solubilization

Mineralization of organic P (Po) in the soil is a highly crucial process for phosphorus cycling in any agricultural system. Phosphorus may be liberated from its organic forms majorly by three groups of enzymes: (1) Nonspecific phosphatases dephosphorylate the phosphoester or phosphoanhydride bonds of organic P, (2) Phytases (3) Phosphonatases, responsible for cleaving C-P bonds in organophosphonates.

16.4.4.2.1 Nonspecific Acid Phosphatases (NSAPs)

NSAPs produced by bacteria are made by three molecular families (Kim et al. 1998). These enzymes work by scavenging phosphoester and thus provide the cell with vital nutrients (release inorganic P from sugar and nucleotides) (Beacham 1980;

Wanner 1996). Phosphomonoesterases are classified into alkaline and acid phosphomonoesterases, depending on the optimum pH range (Jorquera et al. 2008; Nannipieri et al. 2011).

16.4.4.2.2 Other Phosphatase Enzymes

The phytases can liberate P from the phytic acids. Phytic acid is the principal source of inositol and the prime form in which phosphate is stored in plants parts (seeds and pollens). Phytate is also the chief constituent of soil organic phosphate (Richardson 1994), however, plants have limited capability to procure this form of phosphate directly from phytate.

16.4.4.3 Cold-Tolerant PSB

Phosphate solubilization by microbes is a prominent process, due to the criticality of phosphorus in plant nutrition. A cold-tolerant mutants of *Pseudomonas fluorescens* was formed by Mishra and Goel (1999) which was capable of solubilizing phosphate. This capability was also determined by Mishra and Goel (1999). The Nitrosoguanidine treatment was used to construct the mutants of three different strains of *P. fluorescens* (ATCC13525, PRS9, and GRS1). Das et al. (2003) have also prepared P solubilizing *P. fluorescens* mutants. Katiyar and Goel (2003) also reported enhanced growth of wheat and mung bean by *P. fluorescens* mutants at low temperatures. Moreover, the P solubilizing mutants were also developed for psychrotrophic strain of *P.corrugata*, isolated from IHR (Trivedi and Sa (2008).

Native soil bacteria are found to be excellently acclimatized to the distinct climatic conditions of the particular regions and thus can be exploited (Paau 1989; Malviya et al. 2012; Kumar et al. 2013). The establishment of indigenous strains in the rhizosphere of crops is also comparatively more stable (Höflich et al. 1994; Selvakumar et al. 2009a, 2011). Till date, various bacterial species having the ability to solubilize inorganic phosphates and growth at low temperatures have been described from alpine and sub-alpine regions and are listed in Table 16.2. Several other bacterial species belonging to CT-PSB isolated till date include *Pseudomonas fluorescens, P. lurida, P. corrugate, Pantoea agglomerans, P. dispersal, Tetrathiobacter sp., Bacillus subtilis and Exiguobacterium acetylicum (*Pandey and Palni 1998; Egamberdiyeva and Höflich 2003; Pandey et al. 2006a, b; Selvakumar et al. 2008a, b). *Enterobacter ludwigii* PS1, a cold-tolerant phosphate solubilizing bacterial strain isolated from Seabuckthorn rhizosphere of Indian trans-Himalaya (Dolkar et al. 2018). The isolate was also produced auxin, siderophore, and hydrogen cyanide and was reported to enhance the growth of tomato on seed bacterization (Selvakumar et al. 2009b).

16.4.5 Siderophore Producing Bacteria

Iron works as a cofactor of several enzymes involved in oxidation and reduction reactions thus is a vital micronutrient for plants. Majority of Fe found in the soil occurs in insoluble forms (ferric hydroxide), thus is not easily accessible to plants

Table 16.2 Phosphate solubilization and growth promotion by psychrotolerant bacteria

Microorganism	Source	Function	References
Pseudomonas putida (B0)	Soil from central Himalayas	P-solubilization, antagonistic to *Alternaria alternaria, Fusarium oxysporum*	Pandey et al. (2006a, b)
Pseudomonas sp. PGERs17	Garlic root	P-solubilization, antagonistic to pathogen	Mishra et al. (2008)
Pantoea dispersa IA	NW Indian Himalayas	Involved in P-solubilization, IAA production, HCN production, increase in root and shoot lengths in *Triticum* sp.	Selvakumar et al. (2008a)
Acinetobacter rhizosphaerae BIHB 723	Rhizosphere of *Hippophae rhamnoides*	P-solubilization, IAA, ACC deaminase production *Hordeum vulgare*	Gulati et al. (2009)
Exiguobacterium acetylicum IP	Rhizosphere of apple tree	P-solubilization, IAA production, HCN production, increase root and shoot lengths and N, P, K uptake in *Triticum* sp.	Selvakumar et al. (2009a, b)
Pseudomonas lurida M2RH3	Rhizosphere of radish plant	P-solubilization, root and shoot length increased and N, P, K uptake	Selvakumar et al. (2011)

even in soils having high iron content. Iron accessibility to the plants is also restricted due to instantaneous oxidation of ferrous to ferric state (Neilands 1995). Several microbes have developed unique methods for the incorporation of iron, viz. synthesis of siderophores. Furthermore, siderophores can be divided into hydroxymates, catecholates, and their mixtures (Neilands 1981).

Two different pathways are involved in the biosynthesis of siderophores: (a) dependent on nonribosomal peptide synthetases (NRPS) (Gehring et al. 1997; Keating et al. 2000) (b) NRPs independent (Quadri et al. 1999; Challis 2005; Oves-Costales et al. 2009). Nonribosomal peptide synthetases are huge multienzyme complexes involved in the biosynthesis of several biologically important peptidic products without an RNA template (Crosa and Walsh 2002; Grünewald and Marahiel 2006). In general NRPS consists of three domains: (a) adenylation domain (b) peptidyl carrier protein domain (PCP or thiolation), and (c) condensation domain, responsible for the assembly of a wide range of amino, hydroxy, and carboxy acids in various combinations to produce polypeptides with high structural variability (Finking and Marahiel 2004). The adenylation domain is responsible for activating and recognizing the amino acid, which is thereafter bound by a cofactor in the thiolation domain and then is integrated into the growing polypeptide chain by peptide bond formation by the condensation domain. Eventually, the polypeptide chain is liberated from the synthetase by a cyclization process catalyzed by the C terminal thioesterase domain (Kohli et al. 2001). The genes responsible for coding the enzymes involved in the biosynthesis of aryl acids (2,3-dihydroxybenzoic acid (DHB) and salicylate) and NRPSs are controlled by the Fur repressor (Ratledge and Dover 2000; Quadri et al. 1999). In *E. coli* enterobactin biosynthesis, the product of genes *ent*B, *ent*C, and *ent*A are involved in the synthesis of DHB. Once the aryl acid (DHB) is synthesized, it together with amino acids (L-serine) leads to the assembly of enterobactin by the NRPSs. The enterobactin NRPS system consists of three enzymes EntE, EntB (C terminal), and EntF responsible for enterobactin assembly (Ehmann et al. 2000). Apart from the global repressor Fur, there are several transcriptional regulators that control siderophore biosynthesis and utilization. These generally function as activators by recognizing intracellular or extracellular iron-siderophore complex. These regulators are divided into several groups, which includes: (1) alternative sigma factors, e.g., the FecA-FecR-FecI regulatory proteins in *E. coli* (Enz et al. 2000; Braun and Mahren 2005), the FpvI/Pvd-FpvRFpvA system in *P. aeruginosa* (Mettrick and Lamont 2009) (2) the 2-component sensory transduction system (Dean and Poole 1993) (3) AraC-type regulators, e.g. the PchR in *P. aeruginosa* (Youard and Reimmann 2010), PdtC in *P. stutzeri* (Morales and Lewis 2006).

Siderophores production by microbes possesses an edge in the survival of both plants and bacterial species, due to the elimination of several pathogenic fungus and microbes present in the rhizosphere by reducing the available iron (Masalha et al. 2000; Wang et al. 2000). The siderophores produced in the rhizosphere arrest Fe in the rhizosphere and thus restrict the amount of iron needed by the various phytopathogens. Therefore the production of siderophores is also a biocontrol method against several soil borne plant pathogens. A cold-tolerant mutant of *Pseudomonas fluorescens* was developed by Katiyar and Goel (2003) which was able to produce siderophore. The mutant strain *Pseudomonas fluorescens* was reported to help in the growth of *Vigna radiata* at 25 °C and 10 °C (McBeath 1995; Negi et al. 2005). Several biocontrol agents against *Pythium*, *Sclerotium rolfsii, Rhizoctonia solani,* and *Fusarium oxysporum* have been isolated by Selvakumar et al. (2009a, b). Further, Mishra et al. (2008) have described HCN and siderophore producing cold-tolerant strain *Pseudomonas* sp. It also showed antagonistic properties against many phytopathogenic fungi (*S. rolfsii*, *R. solani*, *Pythium* sp. and *F. oxysporum*) (Mishra et al. 2008; Malviya et al. 2009).

16.5 Conclusion and Future Perspectives

Hill ecosystems are familiar with the exclusive agricultural as well as agro-forestry methods. Identification of immense tremendous capabilities of the microbial resource colonizing such ecosystems globally is making its mark. Development of cold-adapted bioinoculants is of utmost importance for increasing agricultural productivity at higher altitudes. Several cold-tolerant microorganisms have already been characterized for PGP ability. A detailed account of genes and enzymes involved in low temperature mediated plant growth promotion can assist in achieving the desired bio inoculants.

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Microbiological Advances in Bioactives from High Altitude

17

Suresh Chandra Phulara, Nazia Ahmad, Bidyut Mazumdar, and Vikrant Singh Rajput

Abstract

Owing to high altitude and extreme environmental conditions, Himalayas represents one of the biodiversity hotspot in India and home to several plants and microbial species. Thus, the plants and microbes present in these regions exhibit characteristic adaptations. The plants of high altitude Himalayan region are rich in varied secondary metabolites possessing several pharmacological activities. In this chapter, we focused on the antimicrobial and anticancer activities of the secondary metabolites present in the high-altitude plants and microbes, respectively, from the Himalayas. The ethnopharmacology of several plant species have been discussed which have been reported for antimicrobial activity by their essential oils. The major constituents of the essential oils that are responsible for such properties have also been explored. Later, the specific classes of secondary metabolites have been examined for their anticancer potential. However, the recognition of tremendous medicinal applications of Himalayan plants has resulted into their heavy exploitation in the past few decades. Due to this, several plant species like the Himalayan Yew have become endangered. To reduce their over exploitation and to obtain high-altitude bioactives in a sustainable manner, microbial production of such compounds have been investigated in past two decades. In the last section, we discuss about the biosynthesis of one of the largest class of bioactives i.e. terpenoids from microbial sources. Overall, the present

S. C. Phulara
Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India

N. Ahmad · V. S. Rajput (✉)
School of Biotechnology, Jawaharlal Nehru University, New Delhi, India

B. Mazumdar
Department of Chemical Engineering, National Institute of Technology Raipur, Raipur, Chhattisgarh, India

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chapter boasts the antimicrobial and anticancer potential of the bioactives from high-altitude and their sustainable production approach through microbial system.

Keywords
Antimicrobials · Higher altitude bioactives · Ethnopharmacology · Himalaya · Biodiversity

17.1 Introduction

The Himalayas are a mountain range in Asia that separates Indian subcontinent from Tibetan plateau and extend across 3500 km from Afghanistan to China. It comprises of many of the Earth's highest peaks. Owing to its high altitudes, extremely low temperature with high snow storms, wind velocity, scanty rainfall and blizzards and high ultraviolet (UV) radiation are common in Himalayan regions. Because of such extreme environmental conditions, the organisms that thrive in Himalayas possess characteristic survival adaptations. The principal component of such huge habitat multiplicity is the compression of thermal life zones and the fragmentation of the landscape into a various microhabitats. These microhabitats represent archipelagoes of peculiar life forms of therapeutic biodiversity. Even though Himalayas offer greater possibilities of having novel molecules and even largest quantities of active compounds (Dhawan 1997; Hazlett and Sawyer 1998), the information on medicinal biodiversity of high altitude regions in Indian Himalayan Region (IHR) is fragmentary. The chapter will discuss the antimicrobial and anticancer activities of some of the bioactivities from high altitudes and their sustainable production from microbial sources.

17.2 Major Families and Their Members Reported for Antimicrobial Essential Oils from Himalayas

Many microbial diseases are wreaking havoc on the world population. The greatest challenge is identifying potent novel entities which can be developed into novel antimicrobial agents. The section describes the major plant families and their aromatic plant species from Himalayas, with emphasis on Indian regions, known to be in used traditionally for their pharmacological applications, which are reported for antimicrobial potential of the essential oils extracted from them.

17.2.1 Asteraceae or Compositae Family

The family of flowering plants which is commonly referred as sunflower family, daisy family, or thistle family composed of almost 24,000–30,000 species and 1600–1700 genera distributed globally (Funk et al. 2005). The expression 'Aster' which means composite, refers to the typical inflorescence pattern—flower heads comprising many small flowers, encompassed by bracts gives the family its name. Almost 900 species under 167 genera represents the family in India. Forms such as annual, biennial or perennial herbs, shrubs, undershrubs, a few trees, aquatics and some scramblers can be observed in the taxa. Succulents, few spiny, and some plants possessing milky sap are a part of this family. The perennial species sustain the harsh winter season by the means of underground storage organs and production of annual stems in spring (Moreira-Muñoz and Muñoz-Schick 2007). The family is found globally, but usually distributed in the tropical mountains and temperate regions (Bisht and Purohit 2010). Different phytochemical entities such as polyphenols, flavonoids and diterpenoids have been extracted from many members of the family which have displayed diverse pharmacological activities viz. the antibacterial, antifungal, anti-inflammatory, insecticide, antitumor and wound healing (Ertürk and Demirbag 2003; Singh et al. 2002; Suntar 2014).

The species are applied in traditional system of medicine, Unani-tibb and Ayurveda and ethnobotanically used as incense owing to their sweet aromatic odour or as an offering to local deities. Numerous members of the genus exhibit varied medicinal properties like antibacterial, antifungal, antihepatotoxic, antioxidant and antimalarial. Out of about 500 species reported, 45 are found in India (Shah 2014). About 19 species of the family are known for their pharmacological applications in the Himalayas (Sah et al. 2010; Semwal et al. 2015). The following artemisia species have been reported for antimicrobial essential oil.

***A. dubia*:** The plant known to be used in Ayurvedic and folk medicine is commonly known as ‘dau’un’ or ‘dawn’ like, ‘nagdaun’, ‘nag damni’ and more precisely, in Marathi- ‘davan’, Gujratati- ‘damro’. ‘Dauna’ (Shah 2014). The leaf juice acts as an antiseptic and is used on cuts and bruises by the inhabitants of the Dolpa district (Kunwar and Adhikari 2005) and the Newar group of Kathmandu, Nepal (Balami 2004). Chrysanthenone (29.0%), coumarins (18.3%), and camphor (16.4%) are the chief elements of the essential oil (Satyal et al. 2012a). The leaf oil displayed cytotoxicity against human breast tumour cells and anti-fungal potential against *Aspergillus niger*. However the oil was inert against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia* coli and *Pseudomonas aeruginosa* (Satyal et al. 2012a, b). The non-volatile elements impart the antiseptic properties to the plant.

***A. indica*:** The aerial parts of the plant sample from Kashmir was used to extract the essential oil which displayed an minimum inhibitory concentration (MIC) of 16 μg/mL against *S. aureus* and *Penicillium chrysogenum* consisted of artemesia ketone (42.1%), germacrene B (8.6%) and borneol (6.1%) (Rashid et al. 2013). However, it exhibited cytotoxicity against many human tumour cell lines viz. THP-1 (leukaemia), A-549 (lung), HEP-2 (liver) and Caco-2 (colon) cells. Whereas the leaf oil from Nepal constituted of ascaridole (15.4%), isoascaridole (9.9%), trans-*p*-mentha-2,8-dien-1-ol (9.7%) and trans-verbenol (8.4%) did not display any antibacterial, antifungal or cytotoxic activities (Satyal et al. 2012a, b).

A. nilagirica**:** The tall aromatic shrub locally known as 'Indian wormwood' is a medicinal plant well known to be used for treating many microbial diseases, malaria, inflammation, diabetes and depression. The plant is applied in traditional along with Ayurvedic and Homeopathic applications (Mohanty et al. 2018). The paste derived from the plant leaves is traditionally used to control the bleeding through wounds by the residents of the Parvati valley, Himachal Pradesh, India (Stappen et al. 2014). With camphor (12.6%), artemisia ketone (10.2%), caryophyllene oxide (7.4%) and borneol (5.3%) as its major components, it demonstrated antifungal property against *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides*, the plant fungal pathogens; but no antibacterial effect on *S. aureus*, *E.* coli, *Salmonella abony*, *P. aeruginosa* or *Candida albicans* (Singh et al. 2012). The essential oil sample from the plants collected from Uttarakhand, India primarily contained α-thujone, which exhibited activity against plant pathogenic fungi *Rhizoctonia solani*, *Sclerotium rolfsii* and *Macrophomina phaseolina* (Jaitak et al. 2008). Another oil sample from Uttarakhand which displayed decent MIC values of 6.25 and 12.5 μg/mL on *S. aureus* and *P. aeruginosa,* respectively, suggesting strong antibacterial potential contained linalool (16.3%), α-thujone (13.9%), β-caryophyllene (7.5%), germacrene D (7.1%) (Semwal et al. 2015).

A. scoparia**:** The bitter aromatic herb commonly known as as 'Chauri Saroj' and 'Danti' in Bombay, 'Dona' and 'Jhan' in Punjab in India is known for various medicinal properties including antibacterial, insecticidal, antioxidant, anticholesterolemic, antipyretic, cholagogue, diueretic, purgative and vasodilatory effects (Shome et al. 1984; Bora and Sharma 2011). The paste obtained from the plant leaves are applied on cuts and wounds by the local population inhabiting in Nanada Devi, Uttarakhand (Rana et al. 2010). The leaf oil extracted from the sample collected from the Milam glacier, Uttarakhand, contained capillene (60.2%), γ-terpinene (11.1%), and 1-phenyl-2,4-pentadiyne (1.0%), while the root essential oil was rich in capillene (82.9%) and 1-phenyl-2,4-pentadiyne (2.6%) (Joshi et al. 2010a). The capillene (known for antibacterial and antifungal activities) rich oil is customary known to be used on cuts and wound in Uttarakhand as well as to relieve from colic, abdominal, cough and cold related issues (Rana et al. 2010; Yashina and Vereshchagin 1978; Christensen 2010). The essential oil displayed a MIC value of 12.5 μg/mL against *S. aureus* and *Bacillus subtilis*, demonstrating its significant antibacterial potential (Semwal et al. 2015).

Ageratum houstonianum **Mill:** The plant commonly referred to as floss flower, distributed globally in tropical and subtropical regions is applied in traditional medicine for skin diseases and wound healing. Bioactives such as alkaloids, steroids, flavones, pyrrolizidine, precocenes and benzofuran have been extracted from this plant. Antifungal, antibacterial and antimicrobial properties are well reported in the literature for this plant (Shin et al. 2017). The essential oil derived from the samples collected from Himachal Pradesh was rich in chromenes, precocene-I (22.45%) and precocene-II (52.64%) along with two other chromene derivatives, desmethoxyencecalin (0.78%) and androencecalinol (0.3%). The oil lacked monoterpene hydrocarbons but sesquiterpene hydrocarbons (15.97%) were abundantly present, with β-caryophyllene among the significant contributor. The chromene dominated essential oil earlier reported to possess acaricidal activity exhibited antibacterial effect on *Micrococcus luteus* and *Rhodococcus rhodochrous* (Kurade et al. 2010).

***Eupatorium adenophorum* Spreng.:** The plant commonly called as crofton weed is applied in folk medicines as, antiseptic, antimicrobial, antipyretic, analgesic, blood coagulant and enhancer of phenobarbitone induced sleep (Rai and Sharma 1994; Ansari et al. 1983; Mandal et al. 1981). The inhabitants of hilly areas of Kurseong and Darjeeling in Eastern Himalayas use leaves of the plant found at an altitude of 800–2050 m, for remedial purposes to treat oral and skin sores. The plant is reported for analgesic and anti-inflammatory properties (Chakravarty et al. 2011). The leaf juice of the plant in Nepal is believed to be antiseptic and is applied to treat injuries (Uprety et al. 2010; Uprety et al. 2011). The GC/MS investigation uncovered the major constituents to be 1-napthalenol (17.50%), α-bisabolol (9.53%), bornyl acetate (8.98%), β-bisabolene (6.16%), germacrene-D (5.74%) and α-phellandrene (3.85%). The essential oil samples of areal parts of the plant from Kangra valley, Himachal Pradesh exhibited moderate to high anti-bacterial activity against *Arthrobacter protophormiae*, *E.* coli, *M. luteus*, *S. aureus* and *R. rhodococcus,* respectively. The major constituents from the areal parts comprised of *p*-cymene (11.6%), α-phellandrene (5.7%), γ-curcumene (5.0%), δ-2-carene (5.0%), camphene (4.8%) and endo-bornyl acetate (4.4%) (Kurade et al. 2010).

***Blumea lacera*:** An annual herb called as kakronda in Hindi, bears a strong odour of turpentine is a common rabi weed which is described as antipyretic, anti-inflammatory, anthelminthic, astringent, acrid, thermogenic, errhine, styptic, ophthalmic, expectorant, liver tonic, febrifuge, deobstruant, diuretic and stimulant in ayurveda (Warrier and Nambiar 1993). The essential oil from *the plant* exhibits analgesic, hypothermic and tranquilizing properties (Pal et al. 1972). The plant also finds application as an important homoeopathic drug applied to treat enuresis, neuralgia, headache and cold borne cough (Oudhia et al. 1998). The plant is known to be traditionally used as antipyretic, antihelmintic, febrifuge and diuretic in the Sewa river region of Jammu and Kashmir, India (Khan et al. 2009). The oil derived from the areal parts of the plant collected from Baratnagar, Nepal majorly constituted (*Z*)-lachnophyllum ester (25.5%), (*Z*)-lachnophyllic acid (17.0%), germacrene D (11.0%), (*E*)-β-farnesene (10.1%), bicyclogermacrene (5.2%), (*E*)-caryophyllene (4.8%), and (*E*)-nerolidol (4.2%). The (*Z*)-Lachnophyllum ester has been reported to be antibacterial as well as anti-fungal (active against *S. aureus, C. albicans* and *A. niger*) and cytotoxic against MCF-7, MDA-MD-231 and 5637 human tumour cells (Satyal et al. 2015a).

***Inula cappa*:** Several reports in the literature exist for varied bioactivities for the genus Inula such as the reports against bronchitis and intestinal diseases; antipyretic, antihelmintic and antiseptic properties; or its combinatorial application with other plants for nausea, excessive sputum and its description in traditional Chinese medicine against tumour (Song et al. 2002; Bai et al. 2006; Kim et al. 2002; Kobayashi et al. 2002). The major chemical entities identified in the oil derived from areal parts of the plant (commonly known as sheep's ear) samples from Okhalkanda in Nainital district at an altitude of 1400 m (Uttarakhand, India) are sesquiterpene hydrocarbons (50.6%), oxygenated sesquiterpenoids (20.7%), oxygenated monoterpenoids (12.6%) and monoterpene hydrocarbons (4.3%). The oxygenated diterpenoids comprised of only 3.0% of the oil composition. Constituents such as β-Caryophyllene (27.3%), (*E*)-β-farnesene (5.6%), β-bisabolene (6.5%) and cis-dihydro-mayurone (6.7%) were the major components observed. The oil inhibited the growth of both Gram positive and Gram negative bacteria. The maximum zone of inhibition was observed against Gram-positive *Enterococcus faecalis* (16 ± 1.00 mm), *B. subtilis* (14 ± 1.00 mm) followed by Gram negative *Xanthomonas phaseoli* (15 ± 1.73 mm), *Klebsiella pneumoniae* (14 ± 2.00 mm), *Agrobacterium tumefaciens* (13 ± 1.73 mm), *E. coli* (13 ± 1.00 mm) and lowest (9 ± 1.00 mm) against *Erwinia chrysanthem*. The oil exhibited a MIC of 2 μL/mL against *K. pneumoniae*, and 125 μL/mL for *E. coli*, *X. phaseoli*, *Salmonella enterica*, *Pasteurella multocida* and *B. subtilis* (Priydarshi et al. 2016).

***Matricaria recutita* L.:** The annual plant commonly called as chamomile possesses thin spindle-shaped roots only penetrating flatly into the soil (Singh et al. 2011). In ancient Egypt, Greece and Rome, it is known for herbal applications for many years (Issac 1989). It is well known as 'star among medicinal species'. for its various pharmacological properties such as antimicrobial, antiviral, antioxidant, anti-inflammatory, antispasmodic and sedatory effects. It is used tropically to treat skin and mucous membrane inflammation, bacterial infections; anal and genital disorders and respiratory irritations (Bruneton 1999; Satyal et al. 2015b). Terpenoids, flavanoids, coumarins and spiroethers are the major constituents responsible for the varied pharmacological activities. The GC/MS results of the oil extracted from the areal parts of the plant samples from Nepal revealed the composition to be (*E*)-β-farnesene (42.2%) and α-bisabolol oxide A (22.3%), (*E*,*E*)-α-farnesene (8.3%), cisbicycloether (5.0%), α-bisabolol oxide B (4.5%), and α-bisabolone oxide A (4.0%). The oil exhibited no notable toxic effects for larvicidal activity against glassworm (*Chaoborus plumicornis*), brine shrimp (*Artemia salina*) lethality, insecticidal activity against fruit fly (*Drosophila melanogaster*) and nematicidal activity against *Caenorhabditis elegans*, however displayed only moderate activity against *S. aureus, P. aeruginosa* and *C. Albicans* with a MIC of 313 μg/mL (Satyal et al. 2015b).

***Tanacetum longifolium* Wall. Ex DC:** The genus Tanacetum, commonly referred to as tansy comprises of six species viz. *T. nubigenum, T. tibeticum, T. longifolium, T. arteminiodes, T. gracile* and *T. senecionis* found in Kumaun and Garhwal regions (India) at an altitude of 3600–4300 m (Strachey and Duthie 1974; Polunin and Stainton 1984). These plants are reported for anthematic, carminative, stimulant, antispasmodic and anti-migrant properties (Joshi and Bisht 2012). The Gaddi shepherd community of Kashmir use the root powder of the plant for stomach pain and the leaves are also applied for stomach ache and indigestion by residents of Kedarnath Wildlife Sanctuary, Uttarakhand (Ballabh and Chaurasia 2007; Singh and Rawat 2011). The essential oil derived from areal parts from Milam glacier, Uttarakhand is dominated by trans-sabinyl acetate (43.2%) and trans-sabinol (12.7%) was found to be antifungal (*C. Albicans* and *C. glabrata)*. The oil also displayed good antibacterial effect against *E. coli* with 22 mm zone of inhibition and low to moderate killing potential against *S. aureus* (9 mm), *B. subtilis* (9 mm), *K. pneumonia* (11 mm) and *Streptococcus mutans* (14 mm) when compared with standard antibiotic chloramphenicol (21–30 mm) (Joshi 2013).

17.2.2 Lamiaceae

The mint family of flowering plants mostly shrubs and herbs represents around 236 genera and 6900–7200 species is known to be distributed in temperate forests of India and elsewhere (Tamokou et al. 2017; Gairola et al. 2010). The square and opposite leaves are typical characteristics of the species of this family. Most species are aromatic and are known to possess essential oils (Lawrence 1992). The oils find their applications in cosmetic, flavouring, fragrance, perfumery, pesticide and pharmaceutical industries (Özkan 2008). Due to ease of cultivation and propagation through stem cuttings, many species of the family are widely cultivated (Raja 2012). Many phenolic classes of compounds such as simple phenols (e.g. eugenol), tannins, quinones, flavonoids, lignans and some terpenoids are observed in the family possessing diverse biological activities such as antimicrobial, anti-inflammatory, analgesic, anti-tumour, antioxidant, immunostimulant, antitussive, expectorant and cytotoxic. Owing to the existence of thymol and carvacol which interfere with the cellular metabolism after penetrating the cell wall in many members of the family are reported to possess antimicrobial properties (Carović-StanKo et al. 2016).

***Anisomeles indica* (L) Kuntze:** Commonly called as Kala bhangra in Hindi is a camphor-scented large perennial woody shrubby herb, prevalent in tropical and subtropical regions of India. It possesses aromatic astringent, carminative and tonic characteristics and is known to be applied in traditional medicine as an antidote to gastric catarrh and fever and essential oil found in the herb is employed in urine infection (Patel and Patel 2013; Murthy et al. 2015). The plant is reported to be applied for various disorders such as colic dyspepsia in children, liver issues, rheumatism, stomach ache, fever, abdominal pain, psoriasis and many others. The plant extract is reported to be antibacterial and antifungal (Patel and Patel 2013; Kundu et al. 2013). The oil form Toranmal forest, Satpuda valley in Maharashtra, India at an elevation of 1800 m is reported to be antibacterial. The GC/MS investigation of the oil extracted from aerial parts and roots of the plant revealed the presence of monoterpenes and sesquiterpenes compounds such as isobornyl acetate (64.6% and 55.36%), isothujone (6.01% and 12.37%), nerolidol (3.17% and 7.19%), camphene (3.54% and 5.52%), decanal (2.29% and 1.61%) and eugenol (3.25% and 4.15%). The root oil in general was more potent than the oil extracted from the aerial parts. The oils displayed antibacterial effects with decent MIC values against *E. coli* (125–250 μg/mL), *S. aureus* (125–250 μg/mL), *P. aeruginosa* (62.5–125 μg/mL) and *Bacillus pumilus* (31.25–62.5 μg/mL). The oils were also found to exhibit bactericidal effect against these pathogens (Ushir et al. 2010).

***Leucas aspera* (Wild) Link:** Commonly referred as 'Thumbai', is a species of annual branched herb spread throughout South Asia (India, Bangladesh and Nepal), Malaysia and Mauritius. In India the plant is applied traditionally to cure headache, asthma and bronchitis. The plant is also useful against scabies psoriasis, snake bite, toothache and is applied tropically as insect repellent and the leaf extract is employed to soothe toothache (Das et al. 2012; Rajakumar and Shivanna 2010). The plant is known for its various pharmacological activities viz. antimicrobial, antioxidant, antinociceptive and cytotoxic activity (Prajapati et al. 2010). The essential oil of *L. aspera* collected from Biratnagar, Nepal analyzed by GC-MS contained 1-octen-3-ol (30.6%), β-caryophyllene (23.4%) and caryophyllene oxide (24.4%). The oil was tested for antimicrobial effect against *B. cereus*, *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans*, and *A. niger*. It was moderately hostile to *S. aureus* (MIC = 625 μg/mL), B. *cereus* (MIC = 313 μg/mL), and *A. niger* (MIC = 313 μg/mL), most probably the sesquiterpenes present in the oil accounts for the effect (Satyal et al. 2015b).

Nepeta genus: The genus containing around 280 annual and perennial species, mostly aromatic plants is also called as Glechoma and Cataria. The genus is widely spread in temperate Asia, Europe, North Africa and North America and in the Mediterranean region. Almost 30 species are reported in India, mainly recorded in the temperate Himalaya. The members are characterized by the presence of terpenoids, flavonoids and phenolic constituents which are responsible for various biological properties such as antimicrobial, antispasmodic, diuretic, febrifuge, diaphoretic, insecticidal, larvicidal, cytotoxic, anticancer, analgesic, anti-inflammatory, anticonvulsant and antiseptic (Süntar et al. 2018; Bisht et al. 2010). Besides these activities various species are also employed for tooth troubles; as laxatives to treat dysentery, and for liver and kidney ailments. Various Nepeta species are applied in traditional medicines for digestive disorders, cold, influenza, diarrhoea, fever and malaria (Dutt et al. 2015; Bano et al. 2014; Sharma et al. 2015; Bisht et al. 2012). Ethnobotanical investigations reveal the use of many species such as *N. Indica* L. infusion as tonic and for treating bronchitis in Turkey; *N. betonicifolia* C.A. Mey application against cough, wound healing, cancers and rheumatism; *N. Cataria* L. use for relieving menstrual problems in Serbia; *N. lagopis* Benth. extract application on wounds and the decoction obtained from the aerial parts of *N. praetervisa* Rech. f. is utilized to treat helminth infections in Pakistan (Süntar et al. 2018). The existence of pharmacologically potent iridoids, monoterpene neptalactones in Nepeta species accounts for the diverse pharmacological effects. The essential oil from various *Nepta* species viz. *Nepeta leucophylla, Nepeta discolour, Nepeta govaniana, Nepeta* *clarkei, Nepeta elliptica* and *Nepeta erecta* collected from regions of Uttarakhand are reported to be antimicrobial with moderate to high effect against five Gram negative bacteria viz. *P. aeruginosa*, *E. coli*, *P. multocida*, *Proteus vulgaris* and *Serratia marcescens*; one Gram positive bacterium *S. aureus* and fungal pathogens *C. albicans* and *Trichophyton rubrum*. The essential oils of the *Nepeta* species notably exhibited the high inhibition zones varying from 18.2 to 28.4 mm against *P. aeruginosa* with the observation that oils of *N. elliptica* and *N. Erecta* were most potent (28.4 mm, MIC = 0.31 μL/mL and 28.0 mm, MIC = 0.62 μL/mL) followed by *N. leucophylla* (27.4 mm, MIC = 0.42 μL/mL) and N. clarkei (22.0 mm, MIC = 0.15 μL/mL). The essential oils from *N. elliptica* and *N. erecta* were very active against *S. marcescens* (20.2 mm, MIC = 0.43 μL/mL and 18.3 mm, MIC = 1.59 μL/mL) as well. The oil from *N. Leucophylla* also showed notable hostility to *P. vulgaris* and *S. aureus* (21.2 mm, MIC = 3.21 μL/mL; 16.4 mm and MIC = 1.78 μL/mL). The oils also displayed antifungal activities with *N. leucophylla* displaying killing potential against both *C. ablicans* (20.0 mm, MIC = 0.78 μL/mL) and *T. rubrum* (19.2 mm, MIC = 0.19 μL/mL). The oils from *N. elliptica, N. erecta* and *N. govaniana* also exhibited noteworthy activity against both the fungal strains; however, *N. clarkei* and *N. discolor* demonstrated poor activity against both the strains (Bisht et al. 2010). The oils extracted from above mentioned Nepeta species majorly contained iridodial derivatives (*N. Leucophylla*), viz. iridodial β-monoenol acetate, dihydroiridodial diacetate and iridodial dienol diacetate; β-caryophyllene, 1,8-cineolep-cymene (*N. discolor*); isoiridomyrmecin and pregeijerene (*N. govaniana*); germacrene D, β-sesquiphellandrene, α-guaiene and diastereomeric iridodial esters (*N. clarkei*); and 7(R)-trans,trans-nepetalactone and isoiridomyrmecin (*N. elliptica* and *N. erecta*) (Bisht et al. 2010).

***Origannum vulgare* L.:** The plant commonly known as 'oregano' and 'Himalayan Marjoram' in India is an aromatic perennial herb which is vastly applied in traditional medicine for treating ulcers, bronchitis, diarrhoea, dysentery, wounds, weeping eczema, insect bites, cough and cold, stomach ache and many others (Sharma et al. 2004; Rana et al. 2010; Uniyal and Shiva 2005; Pezzani et al. 2017). Various medicinal bioactives, such as phenolic glycosides, sterols, flavonoids, tannins, and large amounts of terpenoids are reported from its aerial parts which accounts for its varied biological properties like antimicrobial, anti-inflammatory, anticancer and antioxidant (Pezzani et al. 2017). Monoterpenes (contributing 90.5% of the total oil constituents) forms the chief component of *Origanum vulgare* essential oil. The main fraction of the monoterpene hydrocarbon component was *p*-Cymene (10.3%), whereas thymol (53.2%) and carvacrol (3.9%) served as the most abundant oxygenated monoterpenes. The thymol rich oil from Uttarakhand was found to be antifungal against *Aspergillus flavus* and *A. niger*. The huge amount of oxygenated compounds recovered may explain for the antifungal activity observed (Bisht et al. 2011).

***Thymus serppyllum* L:** The plant commonly called as the wild thyme is extensively distributed in Jammu and Kashmir. The aromatic plant is widely applied in traditional folk medicine for years as antiseptic, diaphoretic, analgesic and diuretic (Aziz et al. 2010). The plant juice is taken to treat cough and asthma in Almora district of Uttarakhand (Kumari et al. 2012). It is also an emmanagogue, carminative and stimulant. The plant is helpful in digestive, genito-urinary system and respiratory problems. The major fractions of the essential oil extracted from the plant in regions of Muzaffarabad, Pakistan at an elevation of 1200 ft are thymol (30%), carvacrol (20%), *p*-cymol, linalool and other terpenes. The oil displayed significant activity (inhibition 60%) against *Trichophyton longifusus* and *Fusarium solani* (inhibition 70%), but was inactive against *C. albicans, A. flavus* and *C. glaberata.* The presence of thymol and carvacrol are thought to account for the activity (Aziz et al. 2010).

17.2.3 Lauraceae

The woody plants family (barring Cassytha, the herbaceous parasite) representing 50 genera and about 2500–3000 species is spread around the tropical to subtropical latitude are deciduous or evergreen shrubs or trees often with aromatic bark and leaves (Oliveira-Filho et al. 2015). Globally, most of its members bear great economic value because they not only provide timber but are extensively used in the pharma and food industries, with prominent applications of the genera Aniba, Nectandra, Licaria and Ocotea (Silva et al. 2009). The members are reported to display varied biological properties such as antibacterial, fungicidal, antiviral, antipyretic, antispasmodic, antitumour, anticonvulsant, cytotoxic, cruzain inhibitory activities and antioxidant effects (Oliveira-Filho et al. 2015). Bioactives like monoterpenes and sesquiterpenes, triterpenes, alkaloids, and sterols, 2-pyrones, benzophenones, flavonoids and arylpropanoids are known to be a part of various members of the family (Chin et al. 2010). On analyzing the terpenoid diversity in the family through biochemical investigations, the Himalayan Lauraceae species are categorized into two groups, viz., furan-carrying genera (Neolitsea, Lindera, and Dodecadenia), mono- and sesquiterpenoid-rich genera (Persea and Phoebe); and oxygenated monoterpenoids-dominating genus (Cinnamomum) (Joshi et al. 2009).

***Cinnamomum* species:** The genus Cinnamomum contains over 250 aromatic evergreen trees and shrubs, primarily spread in Asia and Australia. The cinnamon bark is known to be applied in Ayurvedic medicine as an antiemetic, antidiarrheal, antiflatulent and general stimulant (Barceloux 2009). Different plant species are employed as antiviral, antiseptic, bactericidal, anti-inflammatory, diuretic, counterirritant, expectorant, stimulant, vermifuge, rubefacient, decongestant, analgesic and cough suppressant (Agarwal et al. 2012; Hamidpour et al. 2013). Many traditional uses of the species are observed such as *Cinnamomum glanduliferum*is is used to treat toothache and wounds in Nepal; *Cinnamomum glaucescens* is applied for kidney problems in Manipur, India and *Cinnamomum tamala* is utilized for gastric issues in far western Nepal. Cinnamomum species are commercially valuable source of camphor, cinnamaldehyde and safrole oil in the world (Kumar and Kumari 2019). The essential oil extracted from the *Cinnamomum camphora* collected from various places viz. Pantnagar and Naukuchiatal, Uttarakhand and Mekwanpur, Nepal majorly contained camphor along with linalool, 1,8-cineole, nerolidol, borneol, camphene, limonene, sabinene, and β-pinene. The leaf oil from Uttarakhand displayed antibacterial effects against *P. multocida* whereas the sample from Nepal was observed to be antifungal against *A. niger* (Agarwal et al. 2012; Satyal et al. 2013). The Nepal sample also exhibited cytotoxic activity against MCF-7 human breast tumour cells, allopathic properties and insecticidal activity as well (Satyal et al. 2013). The leaf oil extracted from *C. glanduliferum* from northern India contained 1,8-cineole (41.4%), α-pinene (20.3%), α-terpineol (9.4%), germacrene D-4-ol (6.1%) and α-thujene (5.10%) (Singh et al. 2013). The plant leaves are traditionally used in northern India against cough and cold. The high content of 1,8-cineole in the oil is responsible for its potency against coughs and cold (Kumar and Kumari 2019). The oil showed antibacterial activity against Gram-positive bacteria and Gram-negative bacteria viz. *Micrococcus luteus*, and *E. coli, P. aeruginosa* and *Aeromonas salmonicida* with MIC values of 6.86 and 3.40, 3.43 and 1.72 μg/mL, respectively (Singh et al. 2013). The leaf oil extracted from *C. tamala* collected from Munsyari and Lohaghat Uttarakhand displayed antibacterial activity against *S. enteric*, *E. coli*, *P. multocida.* The oil constituted of linalool, (*E*)-innamaldehyde, 1,8-cineol and (*E*)-cinnamaldehyde (Agarwal et al. 2012).

***Dodecadenia grandiflora* Nees:** The plant commonly referred as Tailiya is dispersed in India, Myanmar, Bhutan and Nepal. Traditionally, it is known to be applied for diabetes. The compounds phenylpropanoyl esters of catechol glycosides and two lignane bis esters account for the antihyperglycemic effect (Kumar et al. 2009). The aqueous decoction of the leaves is used by the traditional practitioners in the parts of Uttaranchal, India to check the blood sugar level in humans (Kumar et al. 2010). The chief constituents of the leaf essential oil from the plant sample collected from Uttarakhand were furanosesquiterpenoid furanodiene (13.7%) and germacrene D (26.0%). The oil displayed antibacterial property against *S. aureus* and *P. multocida* along with potent free radical scavenging and impediment of lipid peroxidation activities (Joshi et al. 2010b).

***Lindera neesiana* (Wall. Ex Nees) and *Linedera pulcherrima*:** *Lindera neesiana* is commonly called as ‘Siltimur’ in Nepal. It is a medium-sized tree known to grow in temperate regions of Himalayas. The leaves of this aromatic and spicy plant are applied as a carminative. In Nepalese traditional medicine, the fruit is usually chewed to treat diarrhoea, tooth pain, gastric disorders and headache; while its paste is tropically applied as a remedy to boils and scabies (Uprety et al. 2010; Rokaya et al. 2010; Comai et al. 2010). The plant is also used to obliterate intestinal parasites, and to medicate against plant poisoning in cattle (Comai et al. 2010). The essential oil proposed to be used tropically displayed antimicrobial activities against *S. aureus* and *C. albicans* with no cytotoxic properties. *Linedera pulcherrima*, an evergreen shrub is spread in temperate Himalayan regions. The bark and leaves are applied as spice to treat cold, cough and fever. The essential oil majorly contained furanodienone (46.6%), curzerenone (17.6%) which are reported to possess antimicrobial, insecticidal, analgesic and anti-inflammatory properties (Joshi et al. 2009; Joshi et al. 2010b). The essential oil manifested activity against *S. aureus and S. enteric*. It also displayed potent free radical scavenging and retardation of lipid peroxidation activities (Joshi et al. 2010b).

***Persea duthiei* (King) Kosterm. and *Persea gamblei* (King ex Hook. F.) Kosterm.:** In India the leaves are utilized for fodder and the fruits are consumable, however the plants are not reported to for any medicinal applications (Negi 2005; Rijal 2011). The essential oil extracted from leaf sample *of P. duthiei* and *P. gamblei* collected from Uttarakhand contained (*E*)-nerolidol (13.2%), limonene (10.1%), α-pinene (10.0%), β-pinene (10.0%), epi-cubebol (5.8%), b-caryophyllene (5.8%) and b-eudesmol (4.0%); and sesquiterpene hydrocarbons (62.8%) viz. b-caryophyllene (22.1%), c-gurjunene (16.8%) and b-cubebene (7.2%), respectively ([b]Joshi et al. 2009). The essential oil of *P. duthiei* was effective against Gram negative bacteria viz. *E. coli* and *P. multocida*. On the other hand, *P. gamblei* essential oil demonstrated potency against Gram positive bacteria *S. aureus* only (Joshi et al. 2010b).

17.2.4 Cupressaceae

The well-known gymnosperm family that produces allergenic pollen is most widely distributed worldwide. The family represents approximately 30 genera, and about 160 species of monoeciuos, subdioeceous (rarely) or dioecious trees and shrubs (Bartel 1994). Many members of the family are known for their antimicrobial, anti-inflammatory, antifungal, analgesic, hepatoprotective, antidiabetic and antihyperlipidemic activity, antioxidant activity, antihypercholesterolemic, anticataleptic and cytotoxic activities, and are also reported to stimulate cancer cells towards apoptosis (Bais et al. 2014). Traditionally different members are applied to relieve pain, cough and cold, digestive issues, haemorrhoid, varicose veins and venous circulation disorders, and urinary problems (Pirani et al. 2011; Al-Snafi 2016).

***Thuja orientalis* L.:** The traditional medicines and homeopathy use of the evergreen, monoecious plant commonly referred as morpankhi in many ways is well known. Traditionally it finds its application to treat cystitis, bronchial catarrh, enuresis, psoriasis, amenorrhea, uterine carcinomas and rheumatism (Srivastava et al. 2012). The boiled decoction of the bark of the evergreen species which is extensively cultivated ornamental plant is consumed orally to treat leucorrhea in the Garhwal belt of India (Ghildiyal et al. 2014). The powdered seeds are known to be applied for tooth ache in the Khyber Pakhtunkhwa, Pakistan (Khan et al. 2015). The GC and GC/MS results of the essential oil extracted from leaf sample of the plant collected from Kangra, Himachal Pradesh revealed α-Pinene (29.2%), Δ-3-carene (20.1%), α-cedrol (9.8%), caryophyllene (7.5%), α-humulene (5.6%), limonene

(5.4%), α-terpinolene (3.8%) and α-terpinyl acetate (3.5%) as its chief constituents. The sample exhibited antifungal potential against *Alternaria alternata*. One of the bioactive compounds in the oil was identified as α-cedrol (Guleria et al. 2008).

***Juniperus macropoda* Boiss.:** The evergreen tree commonly called as the Indian juniper or Himalayan pencil cedar with its origins in the Indian subcontinent (India, Pakistan) along with western and middle Asia is spread in Himalayas at an elevation up to 4500 m above sea level. The plant berries are applied to treat diarrhoea, skin diseases, cough, colic, indigestion, while the resin is applied on ulcers in Himachal Pradesh and the leaf paste works as incense in Kashmir (Bhattacharyya 1991; Rather et al. 2012) and Tibet (Choedon and Kumar 2012). The berries along with other fruits are consumed for kidney ailments (Ballabh et al. 2008). Variation in oil constituents was observed in samples collected from various regions of Himalayas. The leaf samples from Himachal Pradesh contained mainly sabinene (27.5%), Cedrol (14.1%), terpinen-4-ol (9.4%) and pcymene (4.2%) (Stappen et al. 2015). The leaf oil derived from plants grown in Garhwal areas of Uttaranchal, India contained β-elemene (42.5%), trans-sabinene hydrate (8.8%) and α-cubebene (7.9%). Whereas α-thujone (22.6%), biformene (7.7%) and sabinene (5.8%) was prominently found in the leaf sample from Mussorie, Uttarakhand (Srivastava et al. 2005). The oil exhibited moderate activity against *C. albicans* (MIC 250 μg/mL) but a low antibacterial potential against *S. aureus*, *E. coli* and *S. abony* (MICs of 1000 μg/mL, each). Previous reports of weak antifungal activity by other species containing sabinene as major constituent indicates that the effect displayed by *J. macropoda* is due to terpinen-4-ol (Stappen et al. 2015).

17.2.5 Caprifoliaceae

The family is cosmopolitan in distribution, primarily found in temperate regions The members of the family mostly shrubs and vines, rarely herb reside at cold and inaccessible high altitudinal realms of lofty mountain ranges of the Himalaya and the Western Ghats (Clarke 1880). The family is known to include 260 species of 12 genera. The members are known to be used to treat fevers, bacterial dysentery, stomach disorder, enteritis, conjunctivitis, laryngitis, inflammations of the urinary tract and reproductive organs, flu, cardiac disorders, rheumatism and pain (Acharya 2016).

***Morina longifolia* Wall. Ex DC:** The plant commonly known as Whorlflower, Biskandra or Somrus spread over the temperate and alpine areas of the Himalayas from Kashmir to Bhutan at an elevation of 2400–4200 m is a perennial aromatic herb of medicinal value. There are several reports of the plant's medicinal applications in traditional system of Indian and Tibetan medicines. The root powder is used on boils and wounds in Parvati valley of Himachal Pradesh; fresh leaves are applied to treat boils and injuries in Chamoli district of Uttarakhand and the root juice is utilized for dysentery and diarrhoea by the local population of Kaverpalanchowk district of central Nepal (Sharma et al. 2004; Phondani et al. 2010; Malla and Chhetri 2009). Morinoursolic acids A and B, *n*-triacont-3-one, 8-methylditriacont-7-ol and β-sitosterol 41, 2,6-dihydroxy-5-methoxy-(3-C-glucopyranosyl) benzoic acid, β-sitosterol, *p*-hydroxybenzoic acid, caffeic acid and oleanolic acid have been reported in the plant. The leaf essential oil demonstrated higher antibacterial potential against Gram positive *S. subtilis* and *S. aureus*, than Gram negative *E. coli*, *P. aeruginosa* and *P. vulgaris*. The leaf oil exhibited antifungal activities against *F. solani*, *A. alternata*, *A. flavus* and *A. fumigates* as well (Kumar et al. 2013).

***Nardostachys grandiflora* DC.:** The plant is widely applied in folk medicine system in Nepal. Some of its medicinal applications are, the use of rhizome and its oil is for headaches and epilepsy, respectively in far western Nepal (Kunwar et al. 2009); the consumption of juice from whole plant during high altitude sickness and headache in central region of Nepal (Uprety et al. 2010); utilizing the root powder for treating food poisoning, cough, cold, fever, intestinal worms, stomach disorder, headache due to high altitude sickness in north western Nepal; taking the root decoction early morning is supposed to be a tonic; the plant is also applied as an incense and few others (Rokaya et al. 2010). The dried rhizome from sample collected from Jaljale, Nepal was used to extract the essential oil which constituted of calarene (9.4%), valerena-4,7(11)-diene (7.1%), nardol A (6.0%), 1(10)-aristolen-9β-ol (11.6%), jatamansone (7.9%), valeranal (5.6%), and cis-valerinic acid (5.7%). The oil exhibited antimicrobial potential against *B. cereus*, *E. coli* and *C. albicans* (MIC = 156 μg/mL), but displayed cytotoxicity against MCF-7 cells (Satyal et al. 2015c).

17.3 Anticancer Bioactives from High Altitude

Cancer is one of the leading causes of deaths worldwide. One in eight people die because of cancer (WHO 2005; Mathers and Loncar 2006; Lopez et al. 2006). And researchers claim that by 2030, the number of people dying from cancer will escalate from 7.1 million to 11.5 million (Mathers and Loncar 2006). Most preferred treatment of cancer is chemotherapy but it has its own intrinsic problems. It results in a variety of toxicities e.g. chemotherapeutic agent 5-fluorouracil cause myelotoxicity, cardiotoxicity and in rare cases may also act as a vasospastic agent (Rastogi et al. 1993). Therefore, most of the times use of chemotherapeutic drugs lead to serious problems for the patient. Various microbial/ plant-derived products based alternate therapies have also been proposed. Among these, utilising microbes for cancer treatment appears to be the most basic one. Even though it is not used commonly anymore, but it may assist our fight against cancer. Some of the anticancer agents obtained from Himalayan microbes are mentioned in Table 17.1.

Table 17.1 Anticancer agents from microorganisms that are found in Himalayan region

Anticancer agents	Organism	Type	Mode of action	Target	References
Staphylococcal superantigens-like (SSL)	*Staphylococcus aureus*	Proteins	Binds overexpressed eukaryotic receptors in cancer cells	Lymphoma and cervical carcinoma cells.	Walenkamp et al. (2009); Mokta et al. (2015)
Amino acid-degrading enzyme arginine deiminase (Ma-ADI)	*Pseudomonas* sp.	Enzyme	Deplete arginine	Hepatocellular carcinoma, melanoma, leukemia, renal cell carcinoma and prostate cancer, fibrosarcoma, breast cancer and leukemia cells.	Lind (2004)
Exotoxin A (PE) fused immunotoxins	*Pseudomonas* sp.	Protein	Binds overexpressed cell-surface receptors, arrest protein synthesis and induce apoptosis	Leukemia and bladder cancer	Frankel et al. (2000); Michl and Gress (2004)
Manumycin A	*Streptomyces* sp.	Metabolite	Farnesyltransferase inhibitors	Human pancreatic tumor, thyroid carcinoma, leukemias, myeloma and hepatocellular carcinoma	Ito et al. (1996); Pan et al. (2001); ENVIS (2015)
Prodiginines	*Serratia marcescens*	Secondary metabolite	Induce or correct DNA damage; arrest cell cycle	Liver, spleen, blood, colon, gastric, lung, breast and chronic myeloid leukemia	Manderville (2001); ENVIS (2015)

(continued)

Table 17.1 (continued)

Anticancer agents	Organism	Type	Mode of action	Target	References
Antibiotics (Doxorubicin, Bleomycin)	*Streptomyces peucetiusvar.*	Proteins/ peptides	Intercalation between the base pairs of the DNA strands and inhibition of the synthesis of DNA and RNA, generation of iron-mediated free radicals, causing oxidative damage to cellular membranes, proteins and DNA	Neuroblastoma, soft tissue and bone sarcomas, breast carcinoma, ovarian carcinoma, transitional cell bladder carcinoma, thyroid carcinoma, gastric carcinoma	Botlagunta et al. (2016); Vittorio et al. (2018)
Bacteriocins. (Laterosporulin 10, Nisin A, Bovicin HC5)	*Streptococcus* sp., *Lactococcus* sp., *Brevibacillus* sp.	Proteins/ peptides	Induction of apoptosis, stopping of cell cycle and reduction of HNSCC cell proliferation	Embryonic kidney cancer (HEK293T), fibrosarcoma (HT1080), lung carcinoma (H1299) breast cancer (MCF-7	Paiva et al. (2012); Baindara et al. (2017); ENVIS (2015)
Toxicins (Diphtheria toxin, Diphtheria toxin)	*Pseudomonas* sp.	Proteins/ peptides	Induces caspase-3 and -7 dependent apoptotic processes in the breast cancer cell line	Glioblastomas (U118MG, U373MG, U87MG), cutaneous T cell lymphomas (CTCL), breast carcinoma (MCF 7), cervical adenocarcinoma (HeLa)	Vallera et al. (2002); Lutz et al. (2014)

17.4 Other Himalayan Compounds Having Potent Anticancer Activity

17.4.1 Polyphenols

Polyphenols are secondary metabolites that play crucial roles in the growth and metabolism of organisms. Apart from these, polyphenols have also been shown to possess various biological activities that make them a target of a number of research studies to investigate its health benefits; like protection against neurodegenerative disease, diabetes, cardiovascular disease and even aging (Scalbert et al. 2005). They are also shown to possess anticancer properties (Ramos 2008).

Polyphenols are divided into phenolic acids, stilbenes, lignans and flavonoids; depending on the number of phenol rings and other structural elements (Table 17.2).

Polyphenols affect carcinogenesis by modifying various biochemical pathways and processes involved in its progression, by strengthening immune system, and also by safeguarding cells against oxidative shock. The anti-proliferative effect of polyphenols is dose and time-dependent. Polyphenols exert their anti-carcinogenic effect by preventing oxidation, detoxifying xenobiotic compounds, inducing apoptosis, affecting immune system, affecting nuclear factors. These processes in turn modulate cell signaling cascades, DNA transcription, gene expression, cell proliferation and survival (Shen et al. 2007; Chen et al. 2000).

17.4.2 Flavonoids

Flavonoids are secondary metabolites produced by plants. They are safe to be consumed by human beings and do not cause any toxicity to living organisms. Flavonoids have shown to have remarkable anticancer properties. They are classified

Table 17.2 Classification of polyphenols

Class	Source	Target	References
Lignans	Algae, and certain vegetables	Carcinogenic tumors, in particular hormone-sensitive ones such as breast, endometrium and prostate tumors	Buck et al. (2010)
Phenolic acids	Blueberries, kiwis, plums, apples, and cherries	Murine leukemia cell line (l1210), human promylolytic cell line (hl-60), human breast cancer cell line (mcf-7), parenteral human acute lymphoblastic cells (ccrf-cem)	Nandi et al. (2007)
Flavonoids	Milk thistle, acai palm, grape juice, kale, cherries	Lung cancer, leukemia, thyroid, stomach, laryngeal, colon	Batra and Sharma (2013)
Stilbenes	Mulberries, peanuts, grapes, red wine	Breast, lung, colon, skin (nonmelanoma skin cancer and melanoma), prostate, ovarian, liver, oral cavities, thyroid, and leukemia	Sirerol et al. (2016)

into various sub classes depending on their structure and functions. Subclasses of flavonoids along with their specific compounds, their sources and their target cancer types are listed (Table 17.3). Fruits and vegetables have enough flavonoid content to fight cancers in human beings. Some flavonoids are able to fight against breast cancer (Jahanafrooz et al. 2017) e.g. compounds under the subclass flavones have the ability to regulate macrophage function in cancer cell elimination and act as a potential inhibitor of cell proliferation resulting in anti-proliferative activity.

Flavonoids are secondary metabolites produced by plants. They are safe to be consumed by human beings and do not cause any toxicity to living organisms. Flavonoids have shown to have remarkable anticancer properties. They are classified into various sub classes depending on their structure and functions. Subclasses of flavonoids along with their specific compounds, their sources and their target cancer types are listed (Table 17.3). Fruits and vegetables have enough flavonoid content to fight cancers in human beings. Certain flavonoids are anti-proliferative against breast cancer (Jahanafrooz et al. 2017).

Many *in vitro* and *in vivo* studies and some intervention trials have confirmed that flavonoids have good activity against various cancer cell lines (Rossi et al. 2010; Batra and Sharma 2013). They cause apoptosis after blocking the cell cycle in cancer cells (Chahar et al. 2011). They have been utilized for the remedy of various cancers

Table 17.3 Classification and anti cancer properties of Flavanoids

S. No.	Subclasses	Compounds	Sources	Target	References
1.	Flavanols	Catechin, Gallocatechin, Epicatechin, Epigallocatechin	Strawberries, apple, chocolate, cocoa, beans, cherry, green, and black tea	Human oral, rectal, and prostate cancer	Rossi et al. (2010); Batra and Sharma (2013)
2.	Anthocyanidins	Cyanidin, Malvidin, Petunidin	Blueberries, blackberries, blackcurrant and aubergine	Colorectal cancer	Lagiou et al. (2008)
3.	Flavones	Apigenin, Chrysin, Luteolin	Siberian larch tree, onion, milk thistle, acai palm, lemon juice, pepper, broccoli, capsicum, parsley, and celery	Breast cancer, lung cancer, leukemia, thyroid, stomach, laryngeal, colon, and oral cancer	Batra and Sharma (2013)
4.	Isoflavonoids	Daidzein, Genistein, Glycitein, Equol	Soybeans, soy flour, soy milk, beer, and tempeh	Prostate cancer, breast cancer, and thyroid cancer	Batra and Sharma (2013)

including ovarian, cervical, prostrate, breast and pancreatic cancers. Cancer cells have been shown to cease or at least downgrade variety of cancer pathological factors such as cyclin dependent kinases (CDKs) (Bueno et al. 2012), protein kinases (Kupcewicz et al. 2013), epidermal growth factor receptors (EGFRs), COX (cyclo-oxygenase), LOX (lipoxygenase), (Kim et al. 2017) etc.

Flavonoids affect cancer cells only and not normal cell; therefore can prove to be useful in finding newer and better drugs for treatment of cancer (Chahar et al. 2011).

17.4.2.1 Flavonoid Dual Modes Against Cancer

17.4.2.1.1 Anti-proliferation

In cancer cells, the mechanism of proliferation, among other things, involves the retardation of pro-oxidant processes which results in tumor advancement. The reactive oxygen species (ROS) and growth promoting oxidants like xanthine oxidase (Chang et al. 1993), COX or LOX55 are the major catalysts that assist cancer cells to proliferate. Flavonoids are known to inhibit them and hence retard tumor cell proliferation (Kim et al. 2017). Also, the inhibition process of polyamine biosynthesis mechanism may assist the anticancer activities of flavonoids. An enzyme in the biosynthesis of polyamines (Ornithine decarboxylase) is associated with the rate of DNA synthesis in several tissues. It has been shown that flavonoids inhibit ornithine decarboxylase resulting in corresponding decrease in polyamine and inhibition of DNA and protein synthesis (Ünlü et al. 2017; Kumar et al. 2017; Radan et al. 2017).

17.4.2.1.2 Induction of Apoptosis

Apoptosis is a tightly regulated form of cell death. It removes damaged and unwanted cells and thus has a crucial role in their development and survival. It is under the control of a number of genes and a network of interacting protease and their inhibitors. Dysregulation of apoptosis plays a critical role in oncogenesis. Flavonoids induce apoptosis only in certain cancer cell lines and not in normal cells. Even though exact mechanism is not known, certain processes like inhibition of DNA topoisomerase I/II activity, (Okoye et al. 2016; Kaleem et al. 2016) decrease of ROS, (Aslan et al. 2016) regulation of heat shock protein expression, (Toton et al. 2016) modulation of signaling pathways (Mansour et al. 2016) down-regulation of nuclear transcription factor kappa B (NF-κB), activation of endonuclease and suppression of Mcl-1 protein (Panat et al. 2016; Venkatesan et al. 2016) are involved.

17.4.2.1.3 Alkaloids

Alkaloids refer to the class of compounds that consists of a ring structure along with a nitrogen atom. Most of them possess cytotoxic activity and some of them have been commercially developed as chemotherapeutic drugs like camptothecin (CPT) and vinblastine, which interacts with tubulin (Morin 2003). As the structure and the activities of alkaloids are quite varied and complex, it is a challenge to conventionally classify alkaloids. Therefore, a classification of alkaloids is attempted here depending on the strategies (mode) employed by them to terminate cancer progression (Table 17.4).

Table 17.4 Mode and mechanism of anticancer properties in alkaloids

Mode	Alkaloid	Mechanism	References
DNA damage	Hirsutine	PI3k/Akt signal transduction cascade	Yang et al. (2014)
Apoptosis	Subditine and scutebarbatine A	Cleavage of caspases-3,8 and -9 as well as the down-regulation of Bcl-2 protein expression	Liew et al. (2014); Yang et al. (2014)
Cell-cycle arrest	Noscapine	Induces G2/M arrest in breast cancer lung cancer, and colorectal cancer	DeBono et al. (2015)
Alteration of the MAPK pathway	Rohitukine, beta caboline and hirsutine	Activate p38 MAPKs leading to a dose-dependent cytotoxicity against breast cancer, ovarian, and lung cancer	Safia et al. (2015); Lou et al. (2015); Fan et al. (2015)
Suppression of the NF-κB pathway	α-tomatine, taxol, Hirsutine	Inhibit NF-κB pathway activation thereby abolishing cancer progression	Lee et al. (2011); Lou et al. (2015); Kampan et al. (2015)
Formation of G-Quadruplexes	β-carboline	Regulate genes on oncogenes, act as antitumor agents against human promyelocytic leukemia, prostate cancer, and gastric cancer	Okamoto and Okamoto (2010); Neidle (2016)
HER2 targeting	Hirsutine	Impede growth signals leading to cell death in cancer cells	Lou et al. (2015)
Inhibition of the p-Glycoprotein ABCB1	Pretazettine	Inhibit p-glycoprotein (P-gp) (ABCB1-member of the ABC proteins) in breast cancer, cervical cancer, and skin epidermoid carcinoma	Zupkó et al. (2009)

17.4.2.1.4 Terpenoids

Terpenoids are the largest class of natural products comprising of 25,000 compounds that are mainly used in flavour, pharmaceutical and chemical industries (Gershenzon et al. 2007). Depending on their structures, they can be divided into multiple subclasses i.e. monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids and tetraterpenoids. Some derivatives with prominent anti-cancer activity are discussed below (Table 17.5).

17.5 Microbial Advances in Production of Bioactives from High-Altitude

In the previous sections, we have discussed the antimicrobial and anticancer potential of the several classes of bioactives including polyphenols, alkaloids, flavonoids and terpenoids. Among these terpenoids are the largest sources of bioactives having vast array of application. To date, plants are the major source of terpenoid and due to this, their exploitation has been increased since ever. To overcome such challenges,

Table 17.5 Different subclasses of terpenoids with mode of action against cancer cells

Subclass	Compounds	Target	Mode of action	References
Monoterpenoids	D-limonene	Pancreas, stomach, colon, skin, and liver cancers in animal models and human gastric cancer	Inhibit HMGCoA reductase resulting in inhibition of protein isoprenylation of small G proteins, including p21, and its membrane localization	Clegg et al. (1982)
	Cantharidin	Broad spectrum of cancer cells, including leukemia, colorectal carcinoma	Target serine/threonine protein phosphatase 1 (PP1) and 2A (PP2A)	Chen et al. (2002); Huan et al. (2006); Huh et al. (2004)
Sesquiterpenoids	Artemisinin and its derivetives	Leukemia, breast cancer, ovarian cancer, prostate cancer, colon cancer, hepatoma, gastric cancer, melanoma, and lung cancer	Mediate G1 cell cycle arrest by affecting cyclin D, cyclin E, CDK2, CDK4, p21, etc., induce apoptosis in various cancer cell types via activation of p38 MAPK, enhancement of Fas expression and activation of caspases	Hou et al. (2006)
Diterpenoids	Tanshinone IIA	Leukemia, breast cancer, colon cancer, and hepatocellular Carcinoma	Binds to DNA minor groove resulting in DNA structure damage	Sung et al. (1999); Liu (2006); Wang et al. (2005); Su et al. (2008); Wu et al. (1991); Tang et al. (2003); Yuan et al. (2004)
	Triptolide	60 US National Cancer Institute cancer cell lines	Affect transcriptional machinery of cancer cells	Liu (2011)
	Pseudolaric acid B	lung, colon, breast, brain, and renal cell lines	Destabilize microtubules	Pan et al. (1990)
	Andrographolide	Colon cancer	NF-kB signaling blockage, inhibition of JAK-STAT and PI3K, suppression of HSP90, cyclins, and cyclin-dependent kinases, metalloproteinases and growth factors, and induction of tumor suppressor proteins p53 and p21	Lim et al. (2011)

(continued)

Table 17.5 (continued)

Subclass	Compounds	Target	Mode of action	References
	Oridonin	Liver cancer, skin carcinoma, osteoma, and colorectal cancers	Inhibit DNA binding activity of NF-kB thereby blocking the NF-kB signal pathways	Ikezoe et al. (2005)
Triterpenoids	Celastrol	Breast cancer	Directly inhibits the IKKA, b kinases and proteasome function	Pang et al. (2010); Yang et al. (2006); Sethi et al. (2007); Salminen et al. (2010)
	Cucurbitacins	Lung, breast, pancreatic cancer lines	Induce cell cycle arrest,mainly G2/M, S phase arrest	Chen et al. (2010a); Chen, et al. (2010b); Lui et al. (2009); Rivat et al. (2005); Tang et al. (2010)
	Alisol	Human epithelial colorectal adenocarcinoma cells, (Caco-2)	Induces endoplasmic reticulum stress, autophagy, and apoptosis in several cancer cell lines by targeting sarcoplasmic/endoplasmic reticulum Ca2 + ATPase	Chou et al. (2003); Huang et al. (2006)
	Pachymic Acid	Lung cancer A549 cells, prostate cancer DU145 cells and colon carcinoma HT29 cells	Activates PARP, caspases-9, and caspases-3. It also shows inhibitory activities on both DNA topoisomerase I and II	Gapter et al. (2005)
Tetraterpenoids	Lycopene	Lung cancer	Modulates the expression of a broad range of proteins, including cell cycle proteins and heat shock proteins	Vaishampayan et al. (2007)

microbial production of terpenoid has been investigated in past two decades (Phulara et al. 2016). In the later sections, we will discuss about the biosynthesis of terpenoids, challenges in their production from natural sources and advances in the microbial production of terpenoids.

17.5.1 Terpenoids: Current Status and Future Opportunities

Terpenes or terpenoids, or isoprenoids form the largest class of secondary metabolites existing in almost all living organisms. It is the highly diverse group of natural products available on the earth and comprised of over 50,000 known compounds (Phulara et al. 2016). Terpenoids are used as a source of fragrances, flavours, and medicines in several traditional systems due to their tremendous structural and functional diversity (Breitmaier 2006; George et al. 2015a). To date, plants are the major resources of isoprenoids. Several plant varieties including the high altitude plants such as, conifers, balm trees, citrus species, eucalyptus, lemon grass, peppermint species, thyme, or plant parts of those have characteristic smell, taste and pharmacological activities (Breitmaier 2006). This is due to the presence of terpenoids. In addition to their role as secondary metabolites, terpenoids also play vital role in plants such as, quinones ubiquinone and plastoquinone as electron transport components; carotenoids and side chains of chlorophyll as pigments; gibberellins, and abscisic acid as hormones; cholesterol and ergosterol as sterols; and limonene, pinene and menthol, as other characteristic plant compounds (Ajikumar et al. 2008).

Due to their diverse nature, this tremendous class of biomolecules possesses several other biological activities, such as regulation of cation channels (Roullet et al. 1997), suppression of tumor proliferation (Yu et al. 1995; Burke et al. 1997; He et al. 1997), clampdown free radical generation (Ludwiczuk et al. 2017), protective effects against several cancers (Huang et al. 2012; Ludwiczuk et al. 2017) etc. Using animal model *C. elegans* we have also found that isoprenoids such as iridoids and isopentenol can be helpful to improve longevity, health-span and stress tolerance (Shukla et al. 2012; Pandey et al. 2019). In addition, to their remarkable activities against life-threatening disorders, isoprenoids have also been foreseen as potential alternate to diesel and gasoline fuel (Phulara et al. 2016). This is due to their poor hygroscopic nature, higher energy density and good fluidity at low temperatures (Gupta and Phulara 2015).

17.5.1.1 Biosynthesis

The wide range of terpenoids, which includes branched-chain/cyclic alkanes, alkenes and alcohols, are produced from two common five-carbon common precursor, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Gupta and Phulara 2015) (Figs. 17.1 and 17.2). The first discovered pathway for the biosynthesis of isoprenoids was the mevalonate (MVA) pathway (Katsuki and Bloch 1967; Lynen 1967) (Fig. 17.1). It is present in almost all eukaryotes and in few prokaryotes. The MVA pathway synthesizes isoprenoids from acetyl-CoA in six

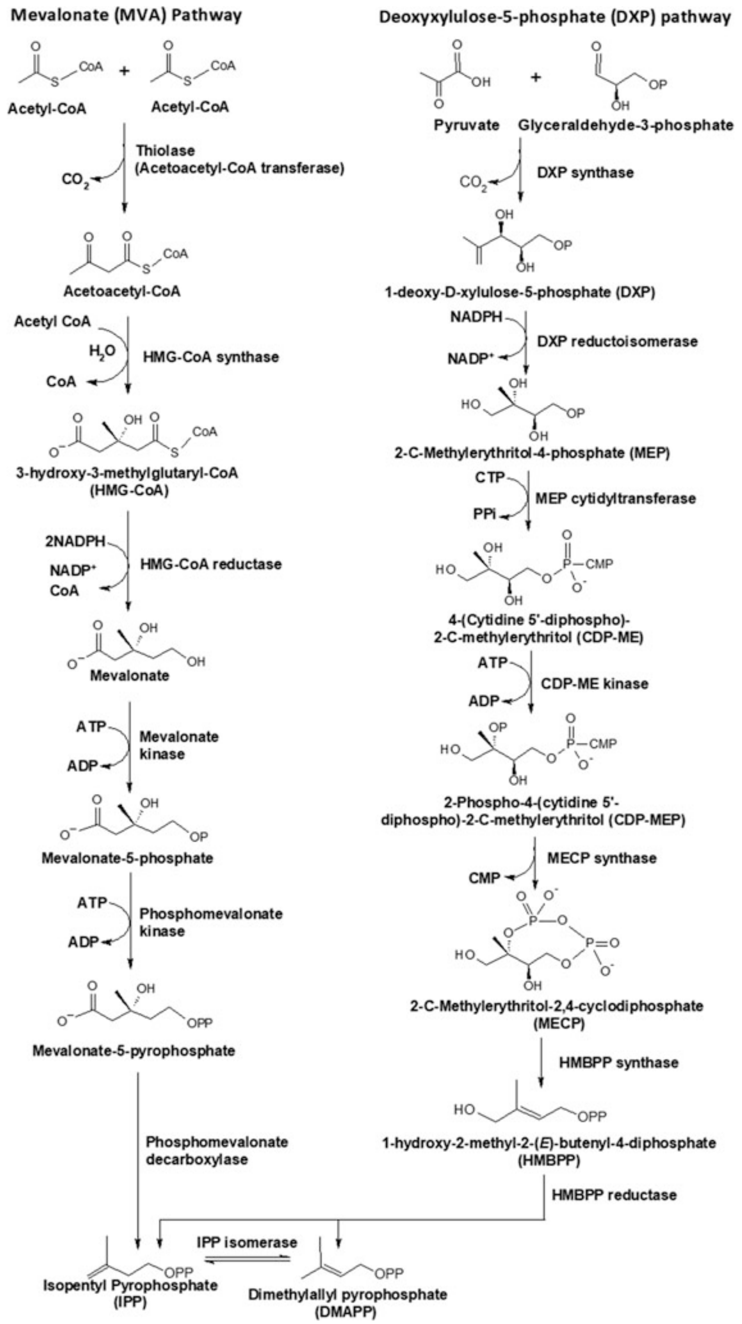

Fig. 17.1 Terpenoid biosynthesis pathway (upper/upstream module)

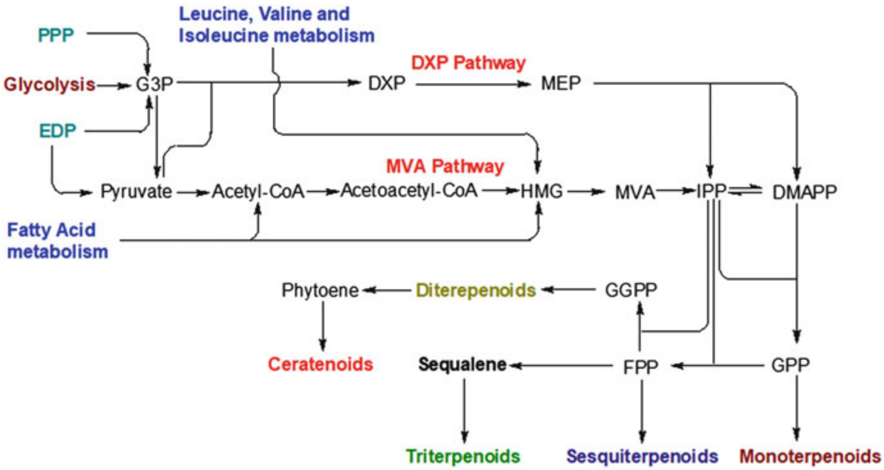

Fig. 17.2 Biosyntheic routes for isoprenoids production. *PPP* pentose phosphate pathway, *EDP* Entner–Doudoroff pathway, *G3P* glyceraldehyde-3-phosphate, *DXP* deoxyxylulose-5-phosphate, *MEP* 2*C*-methyl-D-erythritol-4-phosphate, *HMG* 3-hydroxy-3-methylglutaryl-CoA, *MVA* mevalonate, *IPP* isopentenyl pyrophosphate, *DMAPP* dimethylallyl pyrophosphate, *GPP* geranyl pyrophosphate, *FPP* farnesyl pyrophosphate, *GGPP* geranylgeranyl pyrophosphate. The PPP and EDP along with glycolysis supply precursors (G3P and pyruvate) to DXP pathway for the biosynthesis of isoprenoids. Whereas, fatty acid metabolism and amino acid metabolism provide precursors (acetyl-CoA and HMG) to MVA pathway

enzymatic steps (Miziorko 2011). The first three steps of the MVA pathway are committed to transform three molecules of acetyl-CoA into MVA. First, two molecules of acetyl-CoA are condensed to acetoacetyl-CoA via a raction catalyzed by the acetoacetyl-CoA thiolase (atoB). The HMG-CoA synthase (HMGS) then convert acetoacetyl-CoA into hydroxymethylglutaryl-CoA (HMG-CoA) (Ferguson and Rudney 1959), which is further converted to MVA by HMG-CoA reductase (HMGR) (Durr and Rudney 1960). The later three steps transform MVA to IPP through successive phosphorylation and decarboxylation steps. First, the mevalonate kinase (MK) phosphorylates MVA into mevalonate-5-phosphate (MVAP) by utilizing ATP (Tchen 1958). The MVAP is then again undergone phosphorylation by phosphomevalonate kinase (PMK) to from mevalonate-5-diphosphate (MVAPP) (Helling and Popjak 1961). In the last step, MVAPP is decarboxylated to IPP by phosphomevalonate decarboxylase (PMD) in an ATP dependent manner (Bloch et al. 1959). The conversion of IPP to its isomer DMAPP is catalyzed by IPP isomerase (IDI) through a stereospecific isomerization reaction (Wilding et al. 2000) (Fig. 17.1).

For several decades, MVA pathway was believed to be responsible for the biosynthesis of terpenoids. However, in past two decades, existence of an MVA independent pathway has been elucidated in several eubacteria and plant organelles such as, chloroplast etc. due to extensive genomics studies (Rohmer et al. 1993; Eisenreich et al. 1996). This pathway was recognized as 1-deoxy-D-xylulose-5-

phosphate (DXP) pathway that recruits seven enzymatic steps to convert glyceraldehyde-3-phosphate (G3P) and pyruvate into IPP and DMAPP (Rohdich et al. 2002b; Hunter 2007) (Fig. 17.1). The DXP pathway starts with the condensation of G3P & pyruvate into DXP that is catalyzed by DXP synthase (DXS) enzyme (Lange et al. 1998), followed by reduction to 2*C*-methyl-D-erythritol-4-phosphate (MEP) by DXP reductoisomerase (DXR or IspC) (Lange and Croteau 1999). MEP is then cytidilyzed to 4-diphosphocytidyl-2*C*-methyl-D-erythritol (CDP-ME) by MEP-cytidyltransferase (IspD) (Rohdich et al. 1999), which is further phosphorylated to 4-diphosphocytidyl-2*C*-methyl-D-erythritol-2-phosphate (CDP-MEP) by CDP-ME kinase (IspE) (Lüttgen et al. 2000). Conversion of CDP-MEPto 2-*C*-methyl-D-erythritol-2,4-cyclopyrophosphate (MEcP) is catalyzed MEcP synthase (IspF) (Herz et al. 2000). Finally, MEcP is converted to IPP and DMAPP by two successive reduction and elimination steps catalyzed by 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate synthase (HMBPP synthase or IspG) and HMBPP reductase (IspH), respectively (Rohdich et al. 2002a) (Fig. 17.1).

Several downstream enzymes such as, geranyl pyrophosphate synthase (GPPS, C_{10}), fernesyl pyrophosphate synthase (FPPS or IspA, C_{15}), geranyl-geranyl pyrophosphate synthase (GGPPS, C_{20}), etc. utilize IPP and DMAPP to from the precursors for the synthesis of the largest class of secondary metabolites i.e. terpenoids (bisabolene, pinene, limonene etc.) by enzymes such as bisabolene synthase (BS), pinene synthase (PS), limonene synthase (LS) etc. Based on carbon atoms present, terpenoids can be further divided into following sub-categories like, hemiterpens (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}) and carotenoids (C40) (Fig. 17.2).

17.5.1.2 Concerns in the Production of Commercially Important Terpenoids from High-Altitude

Conifers, the most abundant higher plants of high-altitudes, are the rich source of oleoresin (a mixture of different classes of terpenoids and phenolics). Monoterpenes and diterpenes compose about almost all of the resin produced by conifer species, while sesquiterpenes occur in small amounts (Michelozzi 1999). Pinene and paclitaxel (Taxol) are amongst the commercially important isoprenoid present in high amounts in various Himalayan *Pinus* sp. and *Taxux* sp. plants respectively. Pinene, a major component of resin, is produced by conifers and is a potential antimicrobial agent (da Silva et al. 2012). On the other hand Taxol is extensively utilized as a chemotherapeutic agent for the treatment of various cancers (Ajikumar et al. 2010).

International Union for conservation of Nature (IUCN) has reported a 90% decline in *Taxus wallichiana* (Himalayan yew) population across most of its range through the Indo-Nepal Himalayan region (Thomas and Farjon 2011). Due to the heavy exploitation of Himalayan Yew for its leaves and bark (to produce paclitaxel or similar chemicals), the IUCN has classified it as endangered (Thomas and Farjon 2011). Though, the *Pinus roxburghii* or chir pine that is exploited for its resin, has been classified as least concern by IUCN (Farjon 2013); however, the resin extraction process sometime cause other serious issues such as forest fire, which is damaging to high-altitude ecosystem and biodiversity. There are several other concerns, which limit the natural production of these commercially important terpenoid from high-altitude plants, such as (1) slow growth rates of plants, (2) low-level and tissue-specific synthesis of the terpenoids, (3) soil and land requirements for the propagation of such plants and (4) difficulties in harvesting of plants and extraction of such terpenoid (Tippmann et al. 2013; Phulara et al. 2016).

17.5.1.3 Alternative Strategies for the Production of Terpenoids

Because of the concerns related with plant based extraction of pinene and Taxol, researchers have explored other ways for the production of these chemicals, such as chemical route and/or microbial route. Though, there are some stereochemical complexities in the synthesis of Taxol by chemical route; however, the present process for the commercial production of Taxol relies on plant-based semi-synthetic routes (Chandran et al. 2011). Despite of success, the semi-synthetic route has some limitation of scale and cost as the process reliant on plants (Chandran et al. 2011). Use of hazardous solvents in chemical synthesis is another environmental and health concern that restrains the production of Taxol and pinene in large amounts from this route (Tippmann et al. 2013; Gupta and Phulara 2015). These barriers have led researchers' to investigate the alternative strategies for the production of Taxol and pinene through biotechnological approaches. To achieve this goal, several metabolically engineered microbial cell factories have been explored yet for the large-scale production of taxadiene, taxadiene-5α-ol (both are Taxol precursors) and pinene (Ajikumar et al. 2010; Sarria et al. 2014; Gupta and Phulara 2015) (Fig. 17.3).

17.5.1.3.1 Microbial Production of Terpenoids

It is well known that microbes have several advantages over plants such, (1) it is easy to culture and handle microbe in less space, (2) their growth rates are far higher than plants, (3) growth medium requirements are also low and (4) their genetic traceability and tractability (Keasling 2008). In addition, the success rates of genetic modifications in microbes are greater. The high-throughput synthetic biology tools and techniques make it more easier either to introduce an entire novel pathway or to silence the existing pathway in microbial hosts (Li and Pfeifer 2014). Recent years have seen nonnatural production of terpenoids from genetically modified microorganisms either by tuning endogenous pathways or by engineering heterologous pathways/genes in suitable hosts (Tippmann et al. 2013; George et al. 2015a; Wong et al. 2017). To bypass the endogenous regulations of native pathways, components of the non-native pathways have been incorporated in microbial host for increasing metabolite titres (Carlsen et al. 2013; Sarria et al. 2014). Most of the studies, which are concentrated on the heterologous production of higher terpenoids such as pinene and Taxol have been conducted on genetically tractable hosts like *E. coli* and *S. cerevisiae* (Engels et al. 2008; Ajikumar et al. 2010; Sarria et al. 2014).

Manipulation of a host's genetic code always comes up with several challenges, which includes competition between the host and foreign enzymes for substrate, feedback inhibition by product or intermediates, and accumulation of undesired or

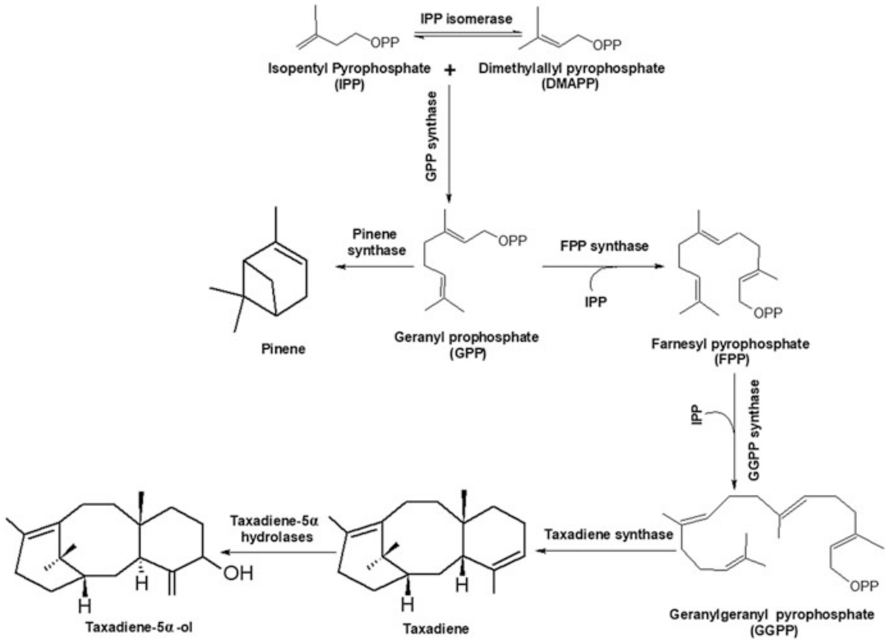

Fig. 17.3 Pinene, taxadiene and taxadiene-5α-ol biosynthesis

toxic metabolites (Gupta and Phulara 2015). However, microbial system provides more opportunity to overcome such bottlenecks over the plants system. Therefore, past decade has seen several advances in terms of computational, rational and combinatorial approaches to boost up the nonnatural production of higher terpenoids in microbial system (Tippmann et al. 2013; George et al. 2015a). To enhance the precursor or cofactor supply for terpenoid production, alternate pathways of a microbial host can also be tuned using such approaches (Zhao et al. 2013; Liu et al. 2014). It was reported that activating Entner-Doudoroff (ED) pathway and silencing Embden-Meyerhof-Parnas (EMP) pathway in *E. coli* could increase G3P and NADPH supply towards DXP pathway (Liu et al. 2014). Construction of fusion proteins by linking two successive enzymes of a pathway by a linker has been utilized to overcome the feedback regulation of isoprenoid pathway and to enhance terpenoid production in microbes (Wang et al. 2011; Sarria et al. 2014). Several other approaches, such as localization of intermediates into micro-compartments, harnessing efflux pump and protein tagging have been utilized to improve terpenoid production (Martin 2010; Chen and Silver 2012; Foo and Leong 2013; Niu et al. 2018).

17.5.1.3.2 Cyanobacteria as a Microbial Host for the Production of Terpenoid

Metabolic engineering together with microbial technology have provided the opportunity to grasp advantages of the less commonly utilized microorganisms for producing economically valuable metabolites (Phelan et al. 2014; Davies et al. 2014). Cyanobacteria or blue-green algae are amongst such microbial hosts that have gained a considerable interest in past decade. This is because they can produce metabolites directly utilizing CO_2 (the least complex carbon source on the plant) and solar energy. These cyanobacteria are also an integral part of high-altitude ecosystems. Several studies have found them as a dominant microbial community in extreme environments of high-altitude, such as hot springs, bare rocks, cold-desert environments (Čapková et al. 2016; Singh et al. 2018). Due to their ability to withstand with extreme environments, cyanobacteria have established themselves as one of the most widely utilized alternate microbial hosts to develop microbial cell factories for the large-scale production of terpenoids (Nozzi et al. 2013; Halfmann et al. 2014a). In addition, other advantages such as fast growth, genetic traceability and tractability, high photosynthetic rates, easy genetic manipulations compared to plants and sequenced genome enable cyanobacteria to compete with heterotrophic microbes (capable of metabolizing lingo-cellulosic biomass) in the field of microbial metabolite production.

Despite their respective advantages, a microbial host also have some limitations, which need to be rectified prior their commercial utilization. To achieve maximum photosynthetic efficiency light exposure is a prerequisite in case of cyanobacteria. This limitation can be overcome by providing saturating amount of light (Iwaki et al. 2006) and by avoiding shelf shading via continuous monitoring of cell mixing rate and depth of the culture (Qiang et al. 1998). Another limitation associated with use of cyanobacteria is the generation of heat as large amount of sunlight is not utilized during photosynthesis. Use of hyperthermophilic cyanobacteria from hot springs on high-altitudes could effectively solve this problem. Alternatively, the non-photosynthetically active radiations could be converted into usable wavelengths (Wondraczek et al. 2013), that is a tedious and energy consuming task. Diurnal running condition is also a constraint that is faced while developing a photo-bioreactor. Metabolic engineering efforts have given a platform to overcome such limitation by incorporating sugar transporter system in cyanobacteria (McEwen et al. 2013). It allows cyanobacteria to grow in the absence of sunlight and one could obtain chemical production throughout a day i.e. for 24 h from cyanobacterial species (McEwen et al. 2013).

Apart from these technical issues, one major issue that could put an additional strain on fertilizer industry while using cyanobacteria, is their competition with plants for similar nutrients (nitrogen and phosphorous). These nutrients are essential for the survival of both and the world-wide fertilizer industry is facing a heavy demand due to the current agricultural practices. (Nozzi et al. 2013). Despite the discussed advantages and disadvantages, utilization of cyanobacteria for the production of high-altitude terpenoids (Halfmann et al. 2014b; Davies et al. 2014) is still in the primary phase of research and there is a long way to go.

17.5.2 Microbial Advances for the Nonnatural Production of Commercially Important High-Altitude Terpenoids: Pinene and Taxol

As discussed earlier, metabolic engineering efforts have enhanced the nonnatural production of a wide-range of terpenes from microbes by either endogenous or heterologous genes/pathway expression. Both the DXP and MVA pathway have been engineered in microbes to achieve this goal (Zheng et al. 2013; Liu et al. 2014). Despite the stoichiometrical superiority of DXP pathway over MVA pathway (Dugar and Stephanopoulos 2011), the terpenoid titers achieved to date by the optimizing DXP pathway (Liu et al. 2014; Kang et al. 2014) are far lesser than the titers obtained by the expression of heterologous MVA pathway (Sarria et al. 2014; George et al. 2015b). Among the terpenoid molecules that are abundant in high-altitude plants, pinene and Taxol have gained a considerable interest in recent years due to their commercial potential.

17.5.2.1 Advances in Microbial Production of Pinene

Pinene is a monoterpenoid compound that possesses antimicrobial activity (da Silva et al. 2012) and widely used as flavouring and fragrance agent (Sarria et al. 2014; Kang et al. 2014). Recently, it is projected as a potential jet-fuel (Yang et al. 2013; Kang et al. 2014) due to the similar physico-chemical properties of pinene dimers to that of missile fuel J-10 (Harvey et al. 2010; Meylemans et al. 2012). Bokinsky et al. (2011) have achieved the first microbial production of pinene from an engineered *E. coli*. To enhance precursors (IPP and DMAPP) supply, the heterologous MVA pathway was incorporated in *E. coli.* Since pinene is a monoterpene and *E. coli* is lacking GPPS enzyme responsible of the conversion of IPP and DMAPP into monoterpene precursor GPP, a GPPS enzyme from *Abies grandis* was also incorporated. Finally, to convert GPPS into pinene, a downstream pinene-specific enzyme pinene synthase (PS) from *Pinus taeda* was incorporated to convert GPP into pinene (Bokinsky et al. 2011). This study did not focus upon improvement of pinene production, as its primary aim was to engineer *E. coli* for the conversion of biomass-derived sugars into advanced biofuels. However, it unlocked the opportunities to take advantages of the metabolic engineering approaches for the enhanced production of pinene from microbial hosts. It also provided the opportunity to explore alternate microbial hosts capable of digesting biomass-derived sugars for the low-cost production pinene.

Culture condition modulations together with metabolic engineering have shown improved terpenoid production productions in microbes (Zhou et al. 2013; Phulara et al. 2018). Utilizing similar strategy, Yang et al. (2013), improved pinene production from engineered *E. coli*. For metabolic engineering of the host (*E. coli*), *A. grandis* GPPS and *P. taeda* PS genes were co-expressed with heterologous MVA pathway. To optimize culture conditions temperature, IPTG and nitrogen source were taken as parameters in a shake flask experiment. It was found that the engineered strain produced highest titre in a shake flask supplemented with 0.25 mM IPTG and MD beef extract (organic nitrogen source) at 30 °C (Yang et al. 2013),

Table 17.6 Summary of titer, engineered microbe, and production time of high-altitude terpenoid production from engineered microbes.

Isoprenoid	Engineered microbes	Titer	Time	References
Pinine	E. coli	1.7 mg/L	N.A.	Bokinsky et al. (2011)
		0.97 mg/L	32 h	Yang et al. (2013)
		32.4 mg/L	N.A.	Sarria et al. (2014)
		140 mg/L	24 h	Tashiro et al. (2016)
		166.5 mg/L	N.A.	Niu et al. (2018)
	C. glutamicum	~27 μg/g DCW	48 h	Kang et al. (2014)
	Synechocystis spp.	~80 μg/L	N.A.	Tashiro et al. (2016)
Taxadiene	E. coli	1.3 mg/L	N.A.	Huang et al. (2001)
		1 g/L	120 h	Ajikumar et al. (2010)
		26.77 mg/L	120 h	Boghigian et al. (2012)
	S. cerevisiae	1 mg/L	65 h	DeJong et al. (2006)
		8.7 mg/L	N.A.	Engels et al. (2008)
taxadiene-5α-ol	E. coli	58 mg/L	100 h	Ajikumar et al. (2010)
	S. cerevisiae	~25 μg/L	65 h	DeJong et al. (2006)

which was ~threefold higher than the previous report (Bokinsky et al. 2011) (Table 17.6).

To date, the pinene titers (Tashiro et al. 2016; Niu et al. 2018) from engineered microbes do not surpass the levels that have been achieved for hemiterpenes (George et al. 2015b) and sesquiterpenes (Peralta-Yahya et al. 2011; Wang et al. 2011). This might be due to the (1) toxicity of pinene, (2) competition for the GPP between PS and IspA, (3) inhibition of GPPS by GPP or high Mg^{2+}, (4) inhibition of PS by GPP and pinene or lower enzymatic activity due to the use of Mg^{2+} as a cofactor rather than Mn^{2+} and (5) low-level expression of PS in engineered hosts (Sarria et al. 2014; Niu et al. 2018).

Fusion protein product of two consecutive enzymes of terpenoid pathway has shown improved production of terpenoids from engineered microbes (Wang et al. 2011; George et al. 2015b). Utilizing similar strategy Sarria et al. (2014) expressed various fusion combinations of GPPS/PS to improve GPP availability for PS. It was found that expression of fusion product significantly increased the pinene titers over co-expression. Highest pinene production was achieved from the fusion product of *A. grandis* GPPS/PS (Sarria et al. 2014) that boosted pinene production by ~sixfolds than the previously reported by Yang et al. (2013). Later, Tashiro et al. (2016) screened different mutant variants of PS after a single round of mutagenesis and then utilizing a high-throughput screening approach to elevate the consumption of GPP. Expression of PS_{mut}, a mutant variant of PS (PS_{mut}), in engineered *E. coli* not only outperformed the wild-type PS (PS_{wt}) enzyme, but also significantly altered its metal dependency. A typically conifer monoterpene synthase requires Mn^{2+} as a cofactor (Savage et al. 1994) and the activity of wild type PS drops by 1/25 to 1/20 in the absence of Mn^{2+} (Tashiro et al. 2016). Interestingly, the screened mutant PS_{mut} demonstrated 60% activity in buffer deficient in Mn^{2+} ions (supplemented only with Mg^{2+}), which enables it to retain its activity in Mg^{2+}-rich and Mn^{2+}-deficient cytosol of *E. coli* (Outten and Halloran 2001; Tashiro et al. 2016). Co-expression of PS_{mut} with heterologous MVA pathway enzymes, IDI, and GPPS yielded 140 mg/L pinene in a shake flask (Tashiro et al. 2016) that is fourfolds over the previous report of Sarria et al. (2014).

In a recent study, improved nonnatural production of pinene has been achieved by improving pinene tolerance and utilizing a modular coculture system of the whole-cell biocatalysis (Niu et al. 2018). Overexpressing efflux pump in *E. coli* improved its pinene tolerance to 2%. Further, utilizing the error-prone PCR and DNA shuffling approach, a more active variant of GPPS ($GPPS^{D90G/L175P}$) was obtained to improve GPP flux by competing with endogenous IspA enzyme for DMAPP. The previously evolved PS_{mut} ($Pt1^{Q457L}$) (Tashiro et al. 2016) was used to efficiently convert GPP into pinene. Incorporation of a tunable intergenic region (TIGR) between $GPPS^{D90G/L175P}$ and $Pt1^{Q457L}$ stabilized the expression of multiple genes. Using chemically induced chromosomal evolution, the TIGR-mediated gene cluster was incorporated along with the MVA pathway into the genome of the pinene tolerance *E. coli* strain. Finally, a 166 mg/L pinene titer was achieved via *E. coli*-*E. coli* modular coculture system of whole-cell biocatalysis (Niu et al. 2018).

As discussed earlier, less commonly used microbial host can also be employed for the production of valuable chemicals by utilizing metabolic engineering efforts. Recent years have also seen such advancements in the production of pinene from alternate microorganisms, such as *Corynebacterium glutamicum* (Kang et al. 2014) and cyanobacteria *Synechocystis* spp. (Tashiro et al. 2016). To enhance precursor flux towards pinene production in *C. glutamicum*, the host's DXP pathway was altered by overexpressing endogenous DXS and IDI enzymes along with *P. taeda* GPPS and *A. grandis* PS (Kang et al. 2014). On the other hand, to obtain pinene form *Synechocystis* spp., Tashiro et al. (2016) separately introduced PSs (PS_{wt} or PS_{mut}) into *Synechocystis* at a silent locus on its chromosome. The pinene production from alternate microbes is far less than that obtained from engineered *E. coli*; however, it provides an opportunity for the low-cost production of such chemicals from variety of carbon sources.

17.5.2.2 Advances in Microbial Production of Taxol

Taxol is a complex, diterpene-based, highly effective, less toxic, and broad-spectrum natural antineolastic drug that has been used against a wide range of cancers, such as breast, uterine, colon, ovarian and other cancers (Li et al. 2009; Zhou et al. 2010). The international market for Taxol is fast paced and rapidly growing (~12.3% average growth rate) with a global revenue of ~$80 that establishes Taxol at the forefront of worldwide best-selling anticancer drug (https://www.reportsweb.com/reports/global-paclitaxel-market-growth-2019-2024). The bark of Yew (*Taxus* spp.) is the major natural source of Taxol, where it exists in small quantities ranging from 0.01–0.05% (Zhou et al. 2010). The *Taxus* species are very slowly growing and the present extraction techniques for Taxol are less efficient. To extract 1 kg of taxol that is sufficient to treat just few hundred patients requires 10 tons of bark or 300 trees (Zhou et al. 2010). Thus, the current extraction process of Taxol from its natural source is environmentally and economically costly due to the heavy exploitation of

Yew (which is now endangered). In addition, like several other natural products, the structural complexity of taxol limits its synthetic production due to the requirement of multiple steps. This also complicates its economic production through chemical routes as there is a subsequent loss in yield at every step (Li et al. 2009). Cell cultures of yew however have come up with some hope and contributed notably to manage the Taxol supply. Yet, the lengthy culture duration, sensitivity to shear stress poor yield and high cost involved restrict its production and extraction via cell culture (Kusari et al. 2014). Therefore, in an immense need to seek new ways for obtaining Taxol to protect its natural reserves (for maintaining ecological balance and saving high-altitude biodiversity) and to reduce the cost of drug therapy, microbial production of Taxol has been explored in past two decade (Li et al. 2009; Ajikumar et al. 2010; Kusari et al. 2014).

As discussed earlier, microbes can be a potential alternate to obtain plant-based terpenoids due to their ease of culture, fastidious growth, easy genetic manipulations and less costly media requirement. The current research for Taxol production is based on either exploring Taxol producing entophytic fungi (Li et al. 2009; Kusari et al. 2014) or engineering microbial hosts like *E. coli* or *S. cerevisiae* (Engels et al. 2008; Ajikumar et al. 2010). It is well established that endophytic fungi have fast growth, can be isolated easily from the ex-plants and can be cultured with a comparative ease. The Taxol producing endophytic fungi (TPEF) have gained considerable interest in the past few years. These TPEF can be isolated from several tissues and organs of yew trees, namely, leaves, stems, roots, and fruits (Zhou et al. 2010). Majority of the researches that have been carried out to obtain Taxol from endophytic fungi includes screening of endophytic fungi with high primeval taxol yield, strain improvement by mutation and/or modern biotechnological methods, and advanced fermentation methods (Zhou et al. 2010).

Mutations are known to induce novel genetic characteristics in microorganisms and commonly used to screen superior microbial strains. Both chemical, such as ethyl methyl sulfomar (EMS), nitrosoguanidine (NTG), etc. and physical mutagens (ultraviolet, χ-ray, γ-rays, fast neutron, laser, microwave, etc.) can induce genetic variation in a host. In fungi, the production of bioactives can be enhanced by altering their mycelium via mutations. It has been reported that induction of mutations in TPEF from *Taxus cuspidate* improved Taxol production over 2.5 folds in mutant strain compared to wild-type (Zhou et al. 2001). In another study, it was found that among treatments UV, NTG, and UV + NTG, the treatment of UV + NTG to Taxol-producing endophytic fungi increased taxol yield ~1.4 folds over the wild type.

Advanced molecular biology techniques, such as gene manipulation, genetic recombination, protein engineering, and modulation of the inherent metabolic pathways have provided important breakthrough and direction to improve final titers of terpenoids from microbial sources. The biosynthesis of Taxol is a complex process and yet to determine fully. It consists of 19 enzymatic steps that include 8 cytochrome P450-mediated oxygenations (Croteau et al. 2006). Using recombinant technology Huang et al. (2001) laid the foundation for further development in the area of microbial Taxol production. Taxadiene, a key intermediate of Taxol biosynthesis has been produced enzymatically in engineered *E. coli* by overexpressing IDI, GGPPS, and taxadiene synthase (TS) genes (Huang et al. 2001). Similarly, in *S. cerevisiae*, a eukaryotic host, eight taxoid biosynthetic genes were expressed to produce Taxol precursors and related taxoids (DeJong et al. 2006).

Presently, researchers primarily focusing on three main aspects of microbial Taxol production: (1) enhanced supply of GGPP; (2) overexpression of TS for the efficient conversion of GGPP into taxadiene (Engels et al. 2008); and engineering cytochrome P450-mediated oxygenations for the conversion of taxadiene to taxadien-5α-ol (Ajikumar et al. 2010). In an attempt to obtain Taxol precursor, taxadiene, from engineered *S. cerevisiae*, GGPP synthase from *Sulfolobus acidocaldarius* and a codon optimized taxadiene synthase from *Taxus chinensis* were overexpressed (Engels et al. 2008). To overcome sterol steroid-based negative feedback a truncated HMG-CoA reductase (tHmg1) was overexpressed. In addition, upc2-1, a mutant allele of the transcriptional sterol regulator was expressed to allow steroid uptake under aerobic conditions. The resultant strain was able to produce 8.7 mg/L taxadine after 48 h incubation (Engels et al. 2008) (Table 17.6). Ajikumar et al. (2010) raised up taxadiene levels in engineered *E. coli* utilizing an approach termed as 'multivariate-modular pathway engineering (MMPE)'. They have demonstrated that splitting DXP pathway into two modules and fine-tuning each module distinctly could improve the titers of desired metabolites. By utilizing this approach, they were able to identify a correct balance between upper and lower DXP pathway modules and obtained a combination that produced 1.0 g/L taxadiene (Ajikumar et al. 2010) (Table 17.6). In the next step, to convert taxadiene into Taxol, a chimeric protein was expressed in taxadiene producing strains that was obtained by fusion of the CYP450, taxadiene-5α-hydroxylase from *Taxus cuspidate* with its CYP450 reductase (CPR) counterpart. The resultant strain produced 58 mg/L taxadiene-5α-ol (Ajikumar et al. 2010), which was ~2400-fold higher than the previously reported titers in S. cerevisiae (DeJong et al. 2006) (Table 17.6). Later, a computational approach was also applied to enhance taxadiene production in *E. coli* (Boghigian et al. 2012). A variation of the minimization of metabolic adjustment (MoMA) algorithm was utilized to identify gene targets for the improved production of taxadiene. Though the study could not surpass the previously achieved taxadiene levels (Ajikumar et al. 2010); however, it was able to identify four genetic engineering targets outside of the native DXP pathway, which could be utilized further with MMPE to enhance cofactor supply for increasing taxadiene accumulation.

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18 Bioprospecting of Endophytic Microbes from Higher Altitude Plants: Recent Advances and Their Biotechnological Applications

Vinay Kumar, Lata Jain, Ravindra Soni, Pankaj Kaushal, and Reeta Goel

Abstract

Endophytes are plant beneficial microbes that inhabit inside the plants and can enhance plant growth and development and tolerance to various biotic and abiotic stresses. Endophytes ubiquitously present in almost all the plant species grown worldwide and these microbes are known to provide direct benefits to the host plant by improving nutrient uptake by plant and controlling plant growth by synthesis of phytoharmones. In addition, they also produces wide range of natural compounds namely antibiotics, hydrolytic enzymes, peptides, alkaloids, etc. These novel compounds/metabolites can be prospective candidates for agriculture and pharmaceutical industries. In this chapter, efforts were made to comprehend the microbial diversity at high altitude plants and their functional traits in the plant hosts with regard to their significance and impacts on environment and humans.

Keywords

Bioprospecting · Endophytes · Enzymes · Secondary metabolites · Himalayan region

V. Kumar (✉) · L. Jain · P. Kaushal
ICAR-National Institute of Biotic Stress Management, Baronda, Raipur, Chhattisgarh, India

R. Soni
Department of Agril. Microbiology, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur, Chhattisgarh, India

R. Goel
Department of Microbiology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

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18.1 Introduction

The plant microbiome have been considered as key determinants of plant growth, health and productivity for over the decades (Berg et al. 2016). Endophytes can be defined as the microorganisms which colonize the plant tissues and live inside without causing any apparent harm to the host plant (Petrini 1991). The association between bacterial endophytes and their host plants has been established very early in evolution (Kawaguchi and Minamisawa 2010). For maintaining stable symbiosis, endophytic microbes produce various natural compounds which helps in promotion of plant growth and better adoption in the environment. In turn endophytes are well protected from microbial competition and extreme environmental conditions by the host plants. Endophytic microbes has been isolated from numerous plant species including both monocotyledons and dicotyledons and plant tissues viz., roots, leaves, stems, flowers, seeds and fruits (Kobayashi and Palumbo 2000). The holobiont theory opened a newer source of genetic variation, produced by plant microbiome and mainly from the endophytic compartment which is heritable in nature (Nogales et al. 2016). The plant (host) genome and cluster of the genes of the microbial communities (microbiota) which inhabited in various tissues of the host plant (plant microbiome form the holobiome also known as 'the holobiont'. A holobiont refers to as an aggregation of the organism and its microbial symbionts live and functions together as unit of biological association and have the ability to duplicate and transfer its genetic information/composition and the collective genomes of the holobiont form a 'hologenome' (van Opstal and Bordenstein 2015; Theis et al. 2016). Further advancement in the knowledge of microbiome revealed that the genetic diversity in the parents required for breeding of varieties for tolerance to various biotic and abiotic stresses is not only contributed by the parental genome but also by the associated microbes. The multifaceted symbiotic and beneficial interactions of the plant holobiont is governed by its holobiome (composed of the genomes of plant host their microbiota. Interaction of plant and microbial endophytes have demonstrated to have a crucial impact on maintaining integrity, sustainability and proper functioning of agro-ecological systems (Nagarajkumar et al. 2004).

The Himalayas and its foothills represents as the main hot spot for the biodiversity in the world (Hanson et al. 2009). Himalayan region of India along with alpine, sub alpine zones of the temperate, glaciers and cold deserts providing an unexploited spots for the isolation and characterization of novel microbial diversity adapted at cold or low temperature (Pandey et al. 2018).

Bioprospecting of microbial endophytic resources mainly bacteria (Rodrigues et al. 2018), fungi (Kumar et al. 2019) and actinomycetes (Shan et al. 2018) and their capability to inhabit in the various environments, genetic diversity and discovery of novel bioactive compounds research needs more attention to understand functional diversity, species richness and response under changing biotic and abiotic stress conditions. Exploitation and proper management of microbial diversity inhabiting crop/plants, plays a significant role in sustainable development towards industrial applications. Majority of endophytes particularly in extreme habitats still remains hidden and they must be explored for better use of humankind and the environment across the world.

18.2 Significance of Endophytes

The endophytic microbes have various significant contributions in environment and agroecosystems. The following paragraphs elaborate the various bioprospectings of endophytic microbes. The various properties of these microbes are also depicted in Fig. 18.1.

18.2.1 Agriculture

Endophytic microbes (bacteria and fungi) have recently drawn attention as a group of prospective plant growth promoters (biofertilizers) and controlling the biotic stresses caused by the pathogens (biocontrol agents). An opportunity of exploring and application of endophytes for development of microbial bioinoculants is evolving as a realistic move towards sustainable agriculture (Rai et al. 2014). Endophytes are well-known to play a crucial role in promoting plant growth and suppressing and/or inhibiting pathogen growth and eliciting induced systemic resistance (ISR) or defence against pathogenic microbes and herbivore insects (Van Oosten et al. 2008). Endophytic bacteria have been explored as possible microbial inoculants for increasing plant productivity (Hallmann et al. 1997). Secondary

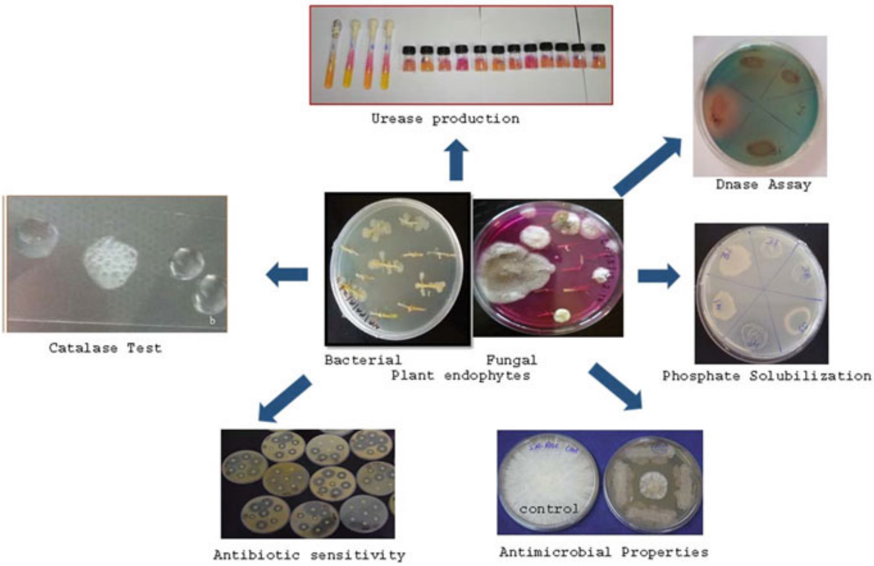

Fig. 18.1 The various properties of the microorganisms

metabolites or natural compounds synthesized by the endophytes play a vital role in various metabolic interactions between microbes and their host plants namely regulation of the symbiosis, signalling and defence mechanism (Schulz and Boyle 2005). Plants colonized with specific endophytes are often growing faster due to the synthesis of phytohormones and as a result they dominate in a specific environment hence they are also considered as chemical and metabolite synthesizers within the plants (Owen and Hundley 2004). Information about the microbial communities accompanying with seeds is crucial, because seed is considered as the basis or unit for the transmission (vertical) of the microbes to next generation. The seeds also accumulate the plant microbiome that have beneficial effect on growth and development of the plant (Hardoim et al. 2015).

Fusarium oxysporum, a fungal endophyte has been recovered from a mangrove plant *Rhizophora annamalayana* and analysed for the production of taxol. Endophytes synthesize secondary metabolites via several metabolic pathways like isoprenoid, amino acid origin and polyketide (Tenguria et al. 2011). Endophytic microbes are being considered as a key constituent of the biodiversity, as the distribution of endophytic microflora varies with the host. There is an increasing requirement of novel and effective chemotherapeutic agents, agrochemicals (insecticides and pesticides) and antibiotics to cope up the emerging agricultural, environmental and medical problems faced by mankind, turns the interest for understanding chemistry of endophytic microbes.

The discovery of *Taxus andreanae* (endophytic fungi) from yew plant (*Taxus brevifolia)* which produces anticancer drug paclitaxel (Stierle et al. 1993, 1995) has established platform for future research areas in other plants for presence of paclitaxel and other important drugs producing endophytes, to use such them in industrial production of drugs. Microbial communities associated with plant such as plant growth promoting (PGP) bacteria and mycorrhizal fungi that enhances the growth and development of plants and improving plant health under multiple (biotic and abiotic) stresses (Vimal et al. 2017; Dash et al. 2019) and also helpful in improving environmental stability and sustainable agriculture (Kumar and Verma 2018). Bacterial endophytes can enhance uptake of nutrient, accumulation and metabolism by producing phytohormones involved in growth regulation of host plants (Afzal et al. 2019).

18.2.2 Peptide Production

A number of endophytic microbes reported for production of peptides demonstrating that endophytes can act as valuable source and exploited for manufacturing of peptide-based drugs. The antimicrobial peptides produced by microbes have huge attentiveness and are indispensable field for intensive research worldwide (Christina et al. 2013). Molecular screening of endophytes for non-ribosomal peptides which are produced by one or more specific non-ribosomal peptide synthetase enzymes exhibiting peptide producing capability. The major group of complex and cyclic lipopeptides with antifungal activities synthesized by *Bacillus subtilis* (Dunlap et al. 2015). The non-ribosomal peptide synthetase enzymes have ability to synthesize several peptide derivatives through one enzyme complex and genetic modifications in NRPS genes also offers the possibility to produce peptides with higher/better pharmacological activities (Abdalla and Matasyoh 2014). Lipopeptide fengycin was first reported as to have antifungal properties (Vanittanakom et al. 1986), recently cyclic lipopeptide, bacillomycin F was detected and isolated from *B. subtilis subsp. Inaquosorum* (Knight et al. 2018). Iturins produces several isomers which have potential broad spectrum antifungal activities, while surfactin is known as the most potent bio-surfactant lipopeptide (Falardeau et al. 2013).

18.2.3 Antimicrobial Activities

Fungal endophytes has been reported as a key source for novel and bioactive antimicrobial compound production namely alkaloids, peptides, steroids, flavonoids and phenol that possess numerous applications in medical sciences and agricultural industries (Strobel et al. 2004). Bioactive compounds synthesized by the fungal endophytes viz*., Fusarium* spp. and *Acremonium spp* exhibited potential antimicrobial property (Powthong et al. 2012), Fungal endophyte, *Pestalotiopsis species* was isolated from leaf tissues of *Pinus caneriensis* exhibited broad range of antibacterial properties against both gram negative and positive bacteria (Bagyalakshmi et al. 2012).

Bacterial endophytes inhabiting the plant tissues reported to provide defence/ protection of host plant from plant pathogenic microbes namely bacterial, fungal and virus and virus like organisms. Endophytes provide protection to the host plant by triggering the induced systemic resistance (ISR) mechanism (Alvin et al. 2014). Endophytic microbes can also initiate induced systemic resistance by using phytoharmones (salicylic acid, jasmonic acid and ethylene) mediated pathways. Bacterial endophytes of genus *Serratia, Bacillus sps, Bacillus pumilus* and *Pseudomonas sps,* reported to protect their hosts through ISR (Pieterse et al. 2012; Kloepper and Ryu 2006). Plant defense mechanisms primed by ISR, protect unexposed/ healthy plant tissues against future attack by insect pests.

18.2.4 Antibiotics Production

Antibiotics are the chemical substances/compounds synthesized by the microorganisms which have toxic/lethal effects on other microbes. They are mainly used to kill and or check growth of pathogenic microorganism and mode of action of antibiotics is highly specific and affect the vital biological processes such as synthesis of DNA, RNA, cell wall and proteins. In order to overcome the pressure of ever-increasing drug resistance in human and plant pathogens, substitutes are urgently required to be explored for isolation and identification of more effective novel natural products and secondary metabolites. The antimicrobial compounds are grouped in numerous structural classes namely alkaloids, flavonoids terpenoids, steroids, phenols, quinones peptides. New antibiotics are essentially needed to treat microbial pathogens which are becoming gradually resistant to various antibiotics available in the market. Endophytic microbes serve as an important source of novel bioactive compounds which can be used as an important source for antitumor, antiarthritic, antidiabetic drugs and other pharmaceutically important compounds to treat various diseases. They also produce various compounds and peptides which have antifungal, antibacterial and insecticidal activities to control pests and phytopathogens in agricultural sector (Jalgaonwala et al. 2011; Godstime et al. 2014).

18.2.5 Bioactive Compounds and Other Metabolites

Microorganisms are the rich and important source of biologically active compounds and able to produce >20,000 compounds, which are influencing the survival and performance of other organisms (Demain and Sanchez 2009). Endophytic bacterial microbiome exhibited beneficial effects and promotes plant growth and health in several cases mediated by metabolic interactions (Nter Brader et al. 2014). It was reported that microsymbionts associated with plants may produce an array of diverse metabolites that may play a crucial role in defence, required for precise communication and interaction with the plant host. Microbial endophytes mainly grouped as bacterial, fungal and actinobacterial and are considered as highly metabolically active than their counterparts (present in phylosphere or free) due to their specific nature of living and functions inside the plant tissue in turn activation of various metabolic pathways (Strobel 2006; Riyaz-Ul-Hassan et al. 2012).

Plants are highly dependent on its microbiome present in the rhizosphere and phylosphere for their functioning. In some cases, plants were unable to culture as transplants and/or unable to induce seed germination in the absence of specific endophytic microbes (Hardoim et al. 2008). Several functions of the plant microbiome is crucial for the host for instance seed germination, is the first and most critical stage of a plants' life cycle and it was noticed that in several plant species, seed germination is not possible without microorganisms. Plant produces a range of phytohormones including auxins, cytokinins, abscisic acid, ethylene, gibberellin, salicylic acid and jasmonic acid plays an essential role in the growth development, signalling and stress responses. Besides providing benefit the plants and environment; endophytes represent a huge natural resource to be explored for human welfare. They are also known to communicate with their host plants via chemical messengers/phytohormones signalling which play a crucial role in promoting plant growth (Kusari et al. 2012). Endophytes have capability to synthesize a wide range of secondary metabolites/natural compounds which are involved in the plant-endophyte interaction. They are probable sources of novel and diverse natural compounds to be discovered for pharmaceutical and agricultural industry (Bacon and White 2000).

18.2.6 Enzymes Production by the Endophytes

The enzymes produced by the endophytes are of two types namely intracellular and extracellular enzymes. Intracellular enzymes are those which produced inside the microbial cell and remain inside whereas extracellular enzymes produced by the growth and multiplication of microbial cells, they have ability to perform their functions outside the cell in numerous biological pathways and/or environmental processes. Endophytes (fungi and bacteria) have been described to produce certain enzymes viz., chitinase, cellulases, xylanases, pectinases, proteases, amylases, hemicellulases, gelatinase, phytases, tyrosinase, etc. Majority of the reported enzyme produced by plant endophytes have been screened or detected using agar-based methods. The chapter summarized the type of enzymes and bioactive compounds produced by the endophytes and their source and their application for further studies and biotechnological applications (Table 18.1). *Acremonium zeae,* endophyte recovered from maize exhibited the production of hemicellulase enzyme extracellularly (Bischoff et al. 2009). Production of various enzymes by endophytes and their biotechnological applications has been mentioned in earlier report of Dhanya and Padmavathy (2014). In the last two decades, numerous chemical entities have been obtained from such microorganisms that are potential leads for pharmaceutical, industrial and agricultural applications.

18.3 Endophytic Microbes Isolated from High Altitude Plants

Microbial diversity is the key and functional backbone of any ecosystem and is essential for life because they perform several functions which are vital for the biosphere. About 3,00,000 plant species exists on earth, and every individual plant hosts of one or more endophytic microorganisms (Strobel and Daisy 2003). Plants capable of growing at high altitudes about 5000 m or more have their highly specialized physiological processes which modulate the biochemical response of the plant, ranging from modifying cell membranes for water permeability and flexibility and synthesis of lipids molecular, anti-freeze carbohydrates, strong antioxidants and scavengers for free radical which are generally not observed in the plants grown at low altitude (Alonso-Amelot 2008). The Indian Himalayan region (IHR) represents great diversity, particularly with respect of geographic, topographic, and climatic conditions and supports a wide range of habitats involving the colonization of microorganisms. Thus plant grown at high altitude region offers an opportunity to identify and explore novel and useful endophytes among diverse plants in the diverse locations and ecological systems.

The *Dysosma versipellis*, plant species is endemic to China and spread at high altitudes and contains fungi belongs to three different orders Pleosporales, Capnodiales and Venturiales comprising eight generas namely *Cladosporium, Alternaria, Phoma, Ochroconis, Microsphaeropsis, Pyrenochaeta*, *Pseudocercospora* and *Ramichloridium* (Tan et al. 2018). Kaul et al. (2013) reported that about 35 fungal endophytes were present in asymptomatic parts of *Digitalis*

Table 18.1 List of important secondary metabolites/bioactive compound produced by the fungal endophytes

S. No.	Secondary metabolites/ natural compound	Endophytic fungi	Host plants	Application/uses	Reference
1.	Taxol and Taxane	*Taxomyces andreanae*	*Pacific yew*	Anticancer	Stierle et al. (1993)
2.	Camptothecin	*Fusarium solani*	*Apodytes dimidiata*	Antitumor	Shweta et al. (2010)
3.	Camptothecin	*Entrophospora*	*Nothapodytes foetida*	Antitumor	Rehman et al. (2008)
4.	Paclitaxel	*Taxomyces andreanae*	*Taxus brevifolia*	Antitumor	Stierle et al. (1995)
5.	Paclitaxel	*Fusarium solani*	*Taxus chinensis*	Antitumor	Deng et al. (2009)
6.	Paclitaxel	*Cladosporium*	*Taxus media*	Antitumor	Zhang et al. (2009)
7.	Paclitaxel	*Aspergillus niger*	*Taxus cuspidata*	Antitumor	Kim and Ford (1999)
8.	Paclitaxel	*Phyllosticta dioscoreae*	*Hibiscus rosasinensis*	Antitumor	Kumaran et al. (2009)
9.	Podophyllotoxin	*Monilia species*	*Dysosma veitchii*	Antitumor	Yang et al. (2003)
10.	Podophyllotoxin	*Penicillium implicatum*	*Diphylleia sinensis*	Antitumor	Zeng et al. (2004)
11.	Podophyllotoxin	*Fusarium oxysporum*	*Juniperus recurva*	Antitumor	Kour et al. (2008)
12.	Quercetin	*Aspergillus oryzae* and *Aspergillus nidulans*	*Ginkgo biloba*	Anti- inflammatory	Qiu et al. (2010)
13.	Diosgenin	*Cephalosporium species*	*Paris polyphylla*	Antitumor, anti-inflammatory	Cao et al. (2007)

14.	Hypericin	*Chaetomium globosum*	*Hypericum perforatum*	Anti-depressant	Kusari et al. (2008)
15.	Chlorogenic acid	*Sordariomycete species*	*Eucommia ulmoides*	Antitumor and antimicrobial	Chen et al. (2010)
16.	Piperine	*Colletotrichum gloeosporioides*	*Piper nigrum*	Anticancer, anti-inflammatory and antimicrobial,	Chithra et al. (2014)
17.	Cajaninstilbene acid	*Fusarium oxysporum, F. solani, F. proliferatum and Neonectria macrodidym*	*Cajanus cajan*	Antioxidant and hypoglycemic	Zhao et al. (2012)
18.	Ginkgolide B	*Fusarium oxysporum*	*Ginkgo biloba*	Anti-inflammatory and antiallergic	Cui et al. (2012)
19.	Borneol	*Cochliobolus nisikadoi*	*Cinnamomum camphora*	Anti-inflammatory, antioxidant	Chen et al. (2011)
20.	Griseofulvin	*Xylaria sp.*	*Abies holophylla*	Antimicrobial	Park et al. (2005)
21.	Graphislactone A	*Cephalosporium sp.*	*Trachelospermum jasminoides*	Antioxidant compounds	Song et al. (2005)

lanata from Gulmarg, Jammu and Kashmir, India. They reported that the *Penicillium, Aspergillus* and *Alternaria* species of fungal endophytes were more abundant in comparison to other genera. Furthermore, an endophytic fungus was isolated from *Juniperus procera* from high altitude regions of Saudi Arabia and screened for its antibiotic activities (Gherbawy and Elhariry 2016). Similarly, Verma et al. (2015) screened 41 bacterial endophytes recovered from wheat culm and roots grown in north-west, Indian Himalayan region. These isolates have PGPR features with cold adaptability which suggest that these endophytes need to be exploited as beneficial bioinoculants for sustainable agroecosystem of high altitudes. A list of some important fungal endophytes isolated from the crop plants of high altitude is given in Table 18.2.

18.3.1 Importance/Functional Significance of Endophytic Microbes

Exploring the plants microbiome and its diversity is of great significance to identify new and effective microorganisms which can be used as biological control agent (BCAs) and production of bioactive substances/compounds. Microbial endophytes have ability to naturally colonize and live within the plants and plant tissues without showing any apparent damage or negative effect to their host. Endophytes attuned to their host in a balanced antagonism manner between both the associates (Schulz and Boyle 2005). Bacterial endophytes may be considered as potential candidates for BCAs, as they provide additional advantages over the other microbes owing to their ability to colonize plant tissues and able to enter inside the plant tissues through wounds and natural openings and also able to survive as epiphytes (Porras-Alfaro and Bayman 2011). It was confirmed that microbes initially isolated as endophytes may possess higher capabilities to colonize and enter in to the plant or seed when endophytes inoculated on plant or seed surface than the non-endophytes, thus escaping from UV rays, moisture and temperature fluctuations confronted on the plants (Hallmann et al. 1997). The endophytes exhibiting antagonistic activities, majority of them associated with the process of antibiosis, through the production of bioactive compounds, peptides and metabolites (Ulloa-Ogaz et al. 2015), and exploring endophytes may provide a better opportunity to isolate and identify BCAs as endophytes have been reported as better producers of antimicrobial compounds, metabolites than the plant epiphytes (Nongkhlaw and Joshi 2015) or soil isolates (Schulz et al. 2002). A recent report revealed that the plants inoculated by overnight soaking of their seeds or roots in plant growth promoting bacterial culture showed huge resistance to multiple biotic stresses (Ngumbi and Kloepper 2016).

Table 18.2 List of endophytic microbes isolated from the crops/plants grown at high altitude

S. No.	Name of host plant/ crop	Name of the fungal endophytes	Location	Plant parts used	Reference
1.	*Picrorhiza kurroa*	*Chaetomium globosum, Valsa sordid, Thielavia subthermophila* and *Diaporthe phaseolorum*	Jammu & Kashmir, Western Himalaya	Stems or twigs of the plants	Qadri et al. (2013)
2.	*Cannabis sativa*	*Alternaria alternata, Schizophyllum commune, Alternaria* sp. and *Alternaria brassicae*			
3.	*Withania somnifera* (Ashwagandha)	*Gibberella moniliformis, Cochliobolus lunatus, Fusarium* sp., *Fusarium equiseti, Gibberella moniliformis, Hypoxylon fragiforme, Nigrospora sphaerica, Cercophora caudate* and *Cladosporium cladosporioides*			
4.	*Rauwolfia serpentine* (Sarpgandha)	*Alternaria brassicae, Cladosporium cladosporioides, Alternaria alternata, Fusarium proliferatum, Lasiodiplodia theobromae, Glomerella acutata* and *Diaporthe helianthi*			
5.	*Cedrus deodara*	*Sordaria humana, Talaromyces trachyspermus, Cochliobolus spicifer* and *Scleroconidiomas phagnicola*			
6.	*Abies pindrow*	*Daldinia fissa, Penicillium oxalicum, Polyporus arcularius* and *Apiosordaria otanii*			
7.	*Pinus roxburgii*	*Petriella* sp. *Bipolaris tetramera, Trichophaea abundans, Penicillium expansum* and *Ulocladium* sp.			
8.	*Nothapodytes nimmoniana*	*Phomopsis* sp. and *Petriella setifera*			
9.	*Platanus orientalis*	*Fusarium tricinctum, Fusarium solani* and *Gibberella* sp.			
10.	*Artemisia annua* (Artemisia plant)	*Fusarium tricinctum, Fusarium flocciferum, Sordaria superba, Fusarium redolens, Chaetomium* sp., *Alternaria alternata, Alternaria brassicae, Paraphoma* sp. and *Gibberella avenacea*			

(continued)

Table 18.2 (continued)

S. No.	Name of host plant/ crop	Name of the fungal endophytes	Location	Plant parts used	Reference
11.	*Ocimum sanctum* (Indian medicinal plant)	90 endophytic fungi were isolated and 23 different endophytic fungal isolates characterized as *Meyerozyma guilliermondii, Colletotrichum sp., Penicillium crustosum, Fusarium proliferatum* and *Chaetomium coarctatum*	Mukteshwar, Uttarakhand		Chowdhary and Kaushik (2015)
12.	*Pinus roxburghii*	*Alternaria alternata, Geotrichium albida, Penicillium frequentans* and *Thielaviopsis basicola*	-do-	Spike	Bhardwaj et al. (2015)
13.	*Eugenia jambolana* (*Schizium cumini*) (Jamun)	24 fungal species isolated, Ascomycetes (20) and two each of Basidiomycetes and Zygomycetes *Aspergillus niger* and *Alternaria alternata* were most dominant and *Chaetomium globosum, Aspergillus japonicus*, *Aspergillus niger* strain, *Aspergillus fumigatus* strain were very rare species	-do-	Leaf, stem and petiole	Yadav et al. (2016)
14.	*Withania sominifera* (Ashwangandha)	6 fungal endophytes	Dehradun	Leaf tissue	Kapoor et al. (2018)
15.	*Ocimum basillicum* (Tulsi)	2 fungal endophytes	-do-	Stem and internal stem tissues	Kapoor et al. (2018)
16.	*Syzygium aromaticum* (Jamun)	6 fungal endophytes	-do-	Leaf, stem and internal stem tissues	Kapoor et al. (2018)
17.	***Dysosma versipellis*** (Podophyllum)	224 fungal endophytes were isolated were 4 belonged to at least 29 genera of 15 orders of Ascomycota (93%), Basidiomycota (6%), and Zygomycota (1%)	High altitudes region (200–2400 m) above sea level in China	Root, rhizome, stem and leaves	Tan et al. (2018)
18.	*Juniperus procera*	A total of 144 isolates were obtained and identified into 6 distinct operational taxonomic units *Aspergillus fumigatus, Penicillium oxalicum, Preussia*	Taif region (Saudi Arabia)	Twigs	Gherbawy and Elhariry (2014)

		sp., Peyronellaea eucalyptica, Peyronellaea sancta and Alternaria tenuissima			
Bacteria					
19.	Sugarcane varieties of Himalayan region	Seven different species of *Gluconacetobacter spp.*	Hilly areas of Uttarakhand		Singh et al. (2013)
20.	*Mussaenda roxburghii* Akshap	*Pseudomonas sp., Klebsiella sp. Acinetobacter sp.*	Arunachal Pradesh, eastern Himalayan province	Stem, leaf and root	Pandey et al. (2015)

18.4 Conclusion

In the past few years, significant increase in research on microbial endophytes related to the isolation and characterization of novel endophytic microbes and their bioactive metabolites have been noticed. Endophytic microbes hold an enormous potential to be deployed for product development to practical applications. They may be used as plant biofertilizer and biocontrol agents and for production of antimicrobial compounds. Looking into the importance of endophytes in the various sectors, it is high time to explore endophytes from extreme and diverse habitats and identify their natural products like antibiotics which can be effective against multi-drug resistant (MDR) bacteria. Molecular studies is required for understanding of plant and endophyte interactions in a more comprehensive way and identification of potential endophytes for bioenergy crops, biodegradation of xenobiotics and bioremediation of toxic metals, etc. Unravelling the microbiome associated with plants offer genetic variability to the plants thus opening up novel possibilities for breeding of next-generation crops for developing high yielding, pest and disease resistant plants and resilient to climatic change.

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Biosensor: A Boon for Heavy Metals Detection in Natural Water Reservoirs at Higher Altitudes

19

Shraddha Chauhan and Lata Sheo Bachan Upadhyay

Abstract

The gradual increase in human population and civilization has affected the nature cycle and negatively affects the environment. These cause the occupation of more land even at higher altitudes. The population growth accelerates the use of resources to maintain the comfort that eventually lead to unbalancing of atmosphere. To fulfil the need of the population new advanced and more technologies are applied in the field of agriculture to improve yields, thus increasing the use of chemicals, pesticides, synthetic fertilizers, etc. Eventually hazardous chemicals are released in the rivers and other water bodies. The natural water reservoirs present at higher altitude are the major source of water supply in every region. The contamination of these water resources causes infection in the whole chain of water distribution. Therefore, the detection at source of origin becomes a need. The identification of heavy metals contamination at higher altitude require tools that can provide easy and on-site detection as setting a sophisticated lab setup is difficult and requires heavy investment. Biosensors are the most suitable device for detecting heavy metals at higher altitude zone in an economical way. Basically, biosensor is an analytical device that comprises the biological recognition element (protein, DNA, antigen, antibody or a living organism) which shows the specific response in the occurrence of a specific analyte. This chapter summarizes the ground knowledge of biosensors and its need to detect heavy metals.

Keywords

Biosensor · Higher altitude · Heavy metal · Whole cell biosensor

S. Chauhan · L. S. B. Upadhyay (✉)
Department of Biotechnology, National Institute of Technology Raipur, Raipur, Chhattisgarh, India
e-mail: lupadhyay.bt@nitrr.ac.in

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19.1 Introduction

Water is the most precious source of life and needed to sustain the lifecycle on earth. Today heavy metal contamination in water is a major threat. Heavy metals (HMs) are metals with high density and atomic weight. Iron, copper, tin gold, silver and platinum are known as heavy metals. These metals and their ions are ubiquitous in nature. As their name indicates, the HMs have higher molecular weight that ranges between 63.5 and 200.6 g mol^{-1} and a particular magnitude more than 5 g cm^{-3} (Srivastava and Majumder 2008).

It is not compulsory that all HMs pose harmful effect to us, some of them are necessary nutrients for example iron, zinc and cobalt. The toxicity of the heavy metal depends on the amount of dosage, larger amount or toxic forms of heavy metal can affect the human health. Few metals including lead, arsenic, mercury, cadmium are highly poisonous. When these metals are taken up by living organism it can cause serious issues as they cannot be discharged and remained inside the body.

Chemical and physical characterizations of significant metals should be used carefully, because the metals concerned don't seem to be continuously systematically outlined. In addition as being comparatively dense, significant metals usually have lesser reactive rate than metals with light molecular weight and lower rate of sulfides and hydroxides. Whereas it's comparatively easy to differentiate an important metal like tungsten with the metal like sodium with is light, some significant metals, like zinc, lead and mercury, have a few similar features of lighter metals, and some low molecular weight metals like glucinium, scandium, and metal, have similar characteristics like heavy metals. Heavy metals are comparatively limited within the crust however they occur in several aspects of recent life. They are being used to make cars, cutlery, cell phones, etc.

Nowadays due to the rapid increase in the population wide, variety of human activities are causing harm to the environment at extreme environmental places like at higher attitude. The Table 19.1 indicates the study of heavy metal contamination at different water bodies present at higher altitude.

As these metals are abundantly present in environment they are used by human in agriculture, tannery, metal working, pulp/paper and textile industries. Naturally they are emitted from the earth's crust and enter into the atmosphere. In the developing world the industrial waste and consumption of non-renewable energy is also a major source of heavy metal contamination. Heavy metals exert the toxicity by causing alteration in biological and biochemical system of life. The secretion and release of these toxic heavy metals to the water source cause the contamination (Fig. 19.1). As all life on earth depend on water, and these heavy metals have the capacity to bind to the surface of the microorganism, they tend to transport into the cell (Fig. 19.1).

Identification of small amount of heavy metals in various biological and environmental samples like serum, wastewater, and ground water has become very important because the pollution in these media can cause direct harm to the human life (Srivastava and Majumder 2008; Thompson et al. 1998; vel Krawczyk et al. 2000). The detection of these heavy metals usually requires analytical techniques that include inductively coupled plasma mass spectroscopy, absorption and emission

Table 19.1 Heavy metal contamination at higher altitude

Contaminated site	Pollutants	References
Summit of Mont Blanc	Pb, Cd, Cu, Zn, Na, Mg, K, Ca	Batifol and Boutron (1984)
Ganga (Rishikesh)	As, Cd, Cu, Zn	Haritash et al. (2016)
Ganga (Haridwar)	Cr, Cu, Fe, Mn, Ni, Zn	Rai et al. (2012)
Col du Dome	Pb, Cd, Cu, Zn, Na, Mg, K, Ca	Batifol and Boutron (1984)
Col du Midi	Pb, Cd, Cu, Zn, Na, Mg, K, Ca, Fe, Al	Batifol and Boutron (1984)
Jungfraujoch	Cd, Cu, Na, K, Ca, Al, Mn	Batifol and Boutron (1984)
Gurgler Ferner	Pb, Cd	Batifol and Boutron (1984)
Tsanfleuron Glacier	Pb	Batifol and Boutron (1984)
TsoMoriri	Ca, Mg, Na, K, Li, Sr	Gopal et al. 2002
TsoKar	Li, Ca, Mg, Na, K, Sr	Gopal et al. (2002)
StartsapukTso	Ca, Mg, Na, K, Sr	Gopal et al. (2002)
TazangkuruTso	Li, Ca, Mg, Na, K	Gopal et al. (2002)

Pb lead, *Cd* cadmium, *Cu* copper, *Zn* zinc, *Na* sodium, *Mg* magnesium, *K* potassium, *Ca* calcium, *Sr* strontium, *Li* lithium, *Ni* nickle

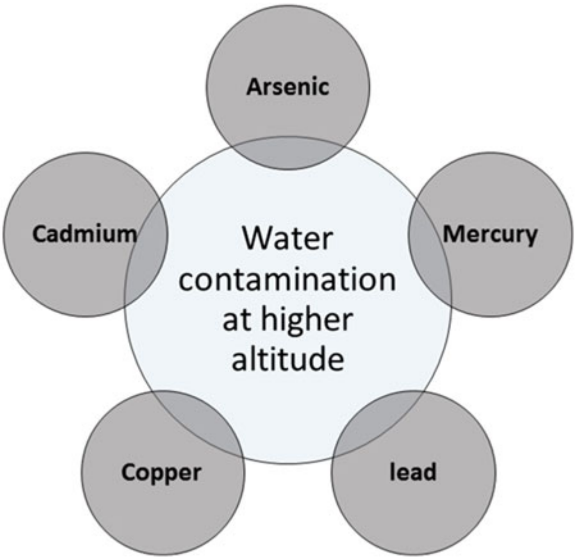

Fig. 19.1 The major heavy metals cause water contamination at higher altitudes

spectroscopy and many other chromatographic techniques. The detection from these analytical techniques provide very accurate, sensitive and reliable results but they are time consuming, costly and require trained personnel for operation. This makes this method complicated (Thompson et al. 1998; Bontidean et al. 1998; Han et al. 2001). Consequently, with the comparable sensitivity and property, the electrochemical methods like ion-selective electrodes, biosensors, qualitative analysis, and different voltammetric methods also are widely used as easy option to the traditional methods, because of their less advanced instrumentation and shorter measure time (Han et al. 2001). Also, easy, cheap and transportable devices are enticing and fascinating for concurrent monitoring of samples and constant analysis of environmental samples (Turdean 2011).

19.2 Biosensor

Many lab based methods are available to detect various pollutants and metals in water that can cause serious threat to the human health. However these techniques utilize sophisticated instruments, long analysis time, advance laboratory and skilled personals. Thus is not feasible for regular and on-site monitoring. Biosensors provide simple and quick identification analysis of analytes. Because of these qualities this field of designing biosensor has been emerged as multidisciplinary research area.

According to the IUPAC definition, "biosensor is a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals". This gives an insight towards the concept of biosensor. The biosensor is mostly made up of three components (1) biological recognition element that reacts with specific analyte, (2) transducer to change biological signal to measurable signals and (3) analyzer that detects and quantify the change in signals. The schematic arrangement of biosensor with its components is mentioned in Fig. 19.2. This device utilizes a biological entity to provide quantitative and semi-quantitative information about a particular analyte (Thompson et al. 1998). According to the signals received the transducer can be fabricated and based on its mechanism the biosensor can be electrochemical, optical, electrical, piezoelectric and thermal (Pearson et al. 2000; Thévenot et al. 2001; Velasco-Garcia and Mottram 2003).

In biosensor the recognition element that is a biomolecule (enzyme, DNA/RNA, protein, antibody) interacts with the analyte and their interaction generates a signal that can be due to the formation or uptake of gases, transfer of electron, heat transfer, product formation, color change, etc. These signals were converted into measurable signals by transducer. The signal processing system converts these signals into the digital values based on the effect of analyte concentration present (Graziella 2011). It is a synergistic combination of two different fields of science: biotechnology and microelectronics. It has been termed as the offspring produced by the marital tie-up

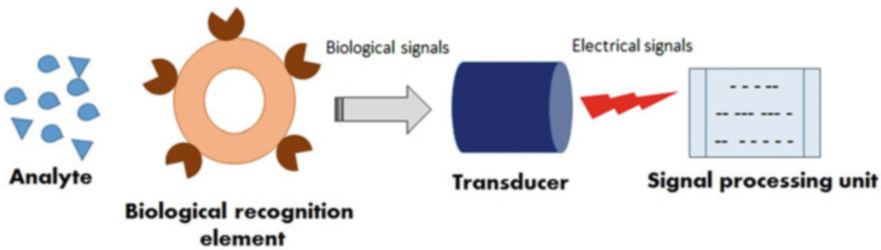

Fig. 19.2 The schematic arrangement of a biosensor

between two fields of biotechnology and electronics (Verma and Singh 2005). Biomolecules are the major responsible component for the identification of the particular analyte, while the transducer provides a measurable form of signals that is further amplified (Scheller and Schubert 1991). Enzymes are very specific in nature that makes them a perfect element for biosensor. Mostly, these enzymes are isolated from microorganisms so these microorganisms can also be used as a potential catalyst for biosensing (Harding 2009). As they reside inside the microorganism retaining their functionally active condition, that increases the stability of the enzyme (Guilbault 1984).Other than microorganisms, many cell membranes and organelles are also used to construct the biosensor (Verma and Malaku 2001). Immunosensors are designed that utilizes the interaction between antigen and antibody. These sensors that are based on specific interaction are very useful to detect very low concentration of analytes such as for the detection of drugs or any other toxins (Prasad et al. 2004). Immobilization is a technique that advances the fabrication of biosensors. Numerous procedures for immobilization have been known for designing the biosensor. The immobilization procedure depends on the type of biomolecule, its nature as well as the property of analyte. To consider all the parameter before immobilization is necessary to achieve high activity and specificity of the bioelement. Most common methods to immobilization microbes and enzymes are entrapment, covalent binding, encapsulation and adsorption (Scheller and Schubert 1991). Likewise the biomolecule, different transducers are also different to capture different biological changes such as thermal, electrochemical, piezoelectric and optical (Tauriainen et al. 1998). The biosensor should be specific, portable, affordable, sensitive and user-friendly; it should be useful for real-time analysis. These qualities make the system a good sensing tool.

19.3 Working Principle of Biosensor

Biosensors are important analytical devices used for real time analysis strategies (Mulchandani and Bassi 1995). The biosensor is based on the principle that relies on the specificity of the analyte with biological recognition element (Mulchandani and Bassi 1995). The biological recognition element is immobilized either by physical, covalent, non-covalent or membrane entrapment. These biological materials are closely interacted to the transducer. The binding of analyte with the bioelement produce an electronic signal that can be further analyzed. Mostly the analyte is transformed into the product in a form of gas, heat or electron ions formed due to their reaction. The transducer then converts the biological signals into electrical which is amplified for further measurements (Fig. 19.3).

The design of biosensor incorporates much interdisciplinary research area like chemistry, engineering and biology. The biological recognition elements can be a DNA/RNA, aptamer, enzyme, amino acid, antibodies or microorganism (Chambers et al. 2008; Iqbal et al. 2000). As like a biological recognition element transducer is also a major part that is responsible for the classification of the biosensor. The transducer can be categorized into five types as follows: (1) electrochemical (2) optical

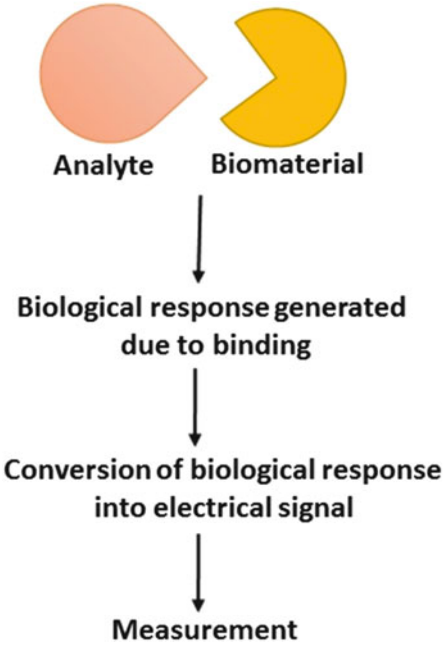

Fig. 19.3 Working principle of the biosensor

(3) thermometric (4) piezoelectric (5) magnetic (Newman and Turner 1992). Most common biosensor that is extensively used is a glucose biosensor that is an example of electrochemical biosensor (Saurabh Bhatia 2018). Electrochemical biosensors are further categorized into potentiometric, amperometric and conductometric sensors (Newman and Turner 1992; Habermüller et al. 2000; Pearson et al. 2000).

19.4 Biosensors for Heavy Metal Analysis

As described earlier heavy metals are the major cause of the environmental pollutions in today's world. The toxicity level of the heavy metals is so high that even in small concentration they are too lethal to environment and living beings (Peavy et al. 1988). Exposure to heavy metals present in the environment is being a constant source of worry for people.

Utensils used for everyday household needs are also a source of metallic contamination. Metallic components released from pesticides and therapeutic agents add the hazardous threat to the environment. Release of toxic gases from industries, tannery and textile factories are the main source of heavy metals pollution in the environment. Chromium, arsenic, lead, zinc, cadmium and mercury are common heavy metal pollutants (Liu and Lu 2003). Identification of these toxic metals in minute concentrations can help to regulate the level of contamination. Nowadays awareness among the people towards the environmental pollution is also growing. Thus a reliable, specific, economic method is required for pollution monitoring to avoid the harmful health effect on human. Available analytical methods for metals monitoring are atomic absorption spectrometry, UV-visible spectrophotometry and inductively coupled plasma mass spectrometry (Upadhyay et al. 2018; Breuil et al. 1998). These technique provide accurate measurements but suffers from bunch of deficiencies, either they are costly, require trained persons for handling or they are bound to the laboratory analysis. At higher altitudes it is impossible to use these conventional methods for water monitoring. To avoid these issues, biosensors have taken up the responsibility to monitor heavy metal contaminants. The biological element present in the biosensor makes them more unique and specific for measurement (Dennison and Turner 1995; Riedel et al. 2002). Biosensors are generally classified on the basis of either transducer or its biological recognition element. Here is the classification of biosensor that has been described on the bases of transducer and biological recognition element (Fig. 19.4).

19.5 Types of Biosensors

19.5.1 Electrochemical Biosensors

They are simple and easy to make sensors that provide the signals based on generation of electric current or change in conductance. Mostly electrochemical biosensors are enzyme based that are generally being used in our daily lives for detection of environmental pollutants and identification of diseases. Medical field is a major area that utilizes biosensor for example glucose biosensor for monitoring blood sugar level, determination of urea, etc.

Amperometric biosensors: Amperometric biosensors generate current as a response when a potential is projected between two electrodes. The response time and sensitivity of amperometric biosensor work similar to the potentiometric sensors. When combined with the enzyme the system is known as enzyme-based amperometric electrochemical biosensor and it provides a highly stable selective and sensitive response. As compared to the conventional analysis methods like nuclear magnetic resonance (NMR) spectroscopy, atomic absorption spectroscopy (AAS), the biosensors can analyze the presence of analyte faster and provide real-time analysis (Wang 2008). The amperometric biosensor works on the principle of movement of electrons; this movement is measured in the form of current that is usually generated due to the redox reaction catalyzed by enzyme. The voltage that has been passed amid the two electrodes can be measured easily. While the enzymatic reaction takes place the substrate when converted into a product is either oxidized or reduced and can transfer electrons. This transfer of electrons generates the flow of current that can be easily identified. The concentration of analyte is proportional to the intensity of the current. The simplest example of amperometric biosensor is Clark oxygen electrode which demonstrates the oxygen (O_2) reduction, and determination of the concentration of glucose by glucose oxidase (Wang 2008; Weltin et al. 2016).

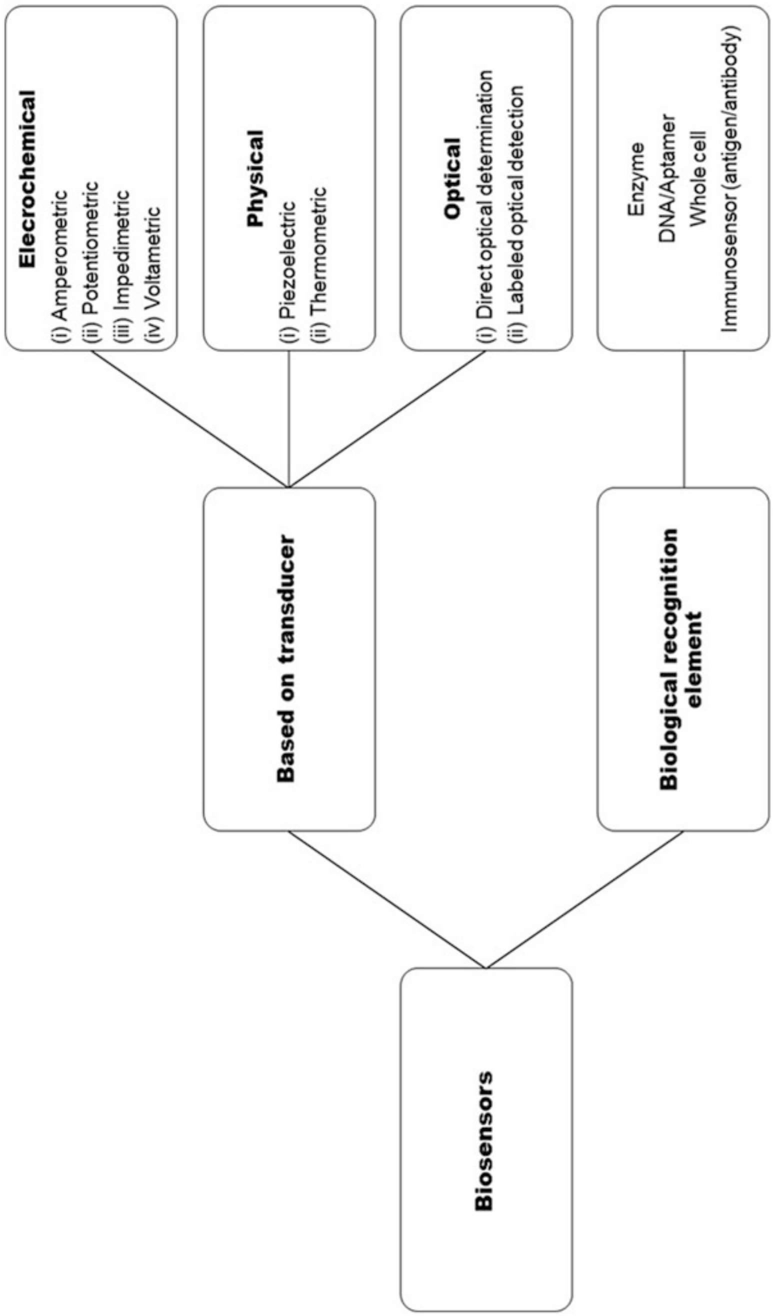

Fig. 19.4 Classification of the biosensors

19.5.2 Potentiometric Biosensors

A potentiometric device determines the build-up of a charge potential on the working conductor in comparison to the reference conductor in a chemical science cell when negligible current flows between them (Eggins 2008; Chaubey and Malhotra 2002; D'Orazio 2003).

In explicit, potentiometry offers data regarding the particle functional capacity in an electrochemical reaction. The principle of this sensor relies on variations in the concentrations of ions that are analyzed by electrodes that are ion selective. The pH conductor is a normally used ion-selective conductor, as several protein reactions embraces the discharge or absorption of hydrogen ions. Alternative important electrodes square measure ammonia-selective and CO_2-selective electrodes. The potential found between the potentiometric conductor and therefore the reference conductor will easily be determined. It's relative to the concentration of the substrate. One of the main limitations of potentiometric biosensors is that the sensitivity of enzymes towards the ionic concentrations like H^+ and NH^{4+}. This biosensor is also used as a method to electrically analyze the biological and chemical reactions at which same concentration of contrasting solutions are in equilibrium state.

This is often referred to as determining a volumetric analysis endpoint; the procedure is thought as a potentiometric titration. Through completing a volumetric analysis at negligible or constant current, the end-side is recognized from the changes in potential of the electrode that are created by variations in the concentration of the solution of the potential determining ion. Numerous potentiometric tools support several sorts of electronic transistor devices to determine pH variations, ion concentrations of selective and therefore the dynamics of biocatalytic reactions encompassing enzymes (Bakker and Pretsch 2005). An additional example of a novel hybrid methodology of optical/electrochemical sensor is named the light-addressable potentiometric sensor (Bakker and Pretsch 2005; Caras and Janata 1980; Hafeman et al. 1988; Mourzina et al. 2001; Poghossian et al. 2001; Xu et al. 2005; Kloock et al. 2006). Which is a silicon-based detector that measures the distribution of electric potential. By scanning with a focused source of illumination, it will quantify the spatially resolved distribution of surface potential on the interface of the model and on the surface of substrate (Stein et al. 2004).

19.5.3 Conductometric Biosensors

Conductometric biosensors were initially designed in the year 1961 to detect urea. The procedure is predicated on electrical conduction variation. Aldehyde, pesticides, insecticides and nitrate biosensors using conductometry were additionally designed (Ghourchian et al. 2004). The urea biosensor was upgraded employing a platinum electrode as a matrix for enzyme immobilization (Mulchandani and Rogers 1998). A conductometric biosensor identifies little variations within the conductivity of an answer by using a conductometric electrical device, i.e., a conductivity meter.

Conductivity determination depends on the catalyst reaction of the sample on an electrode. The ions will be generated due to the reaction which is able to result in the variation in conductivity (Spink and Wadsö 1976). It includes two electrodes one reference and another working coated with bacterial cellulose nata de coco membrane. Biological element (enzyme) is immobilized on the working electrode. All the reactions take place in the working electrode and no reaction occurs on reference electrode. The movement of ions in both the electrodes is different; thus the conductivity is changed. The biological element is immobilized on the electrode causing various reactions that creates changes in ionic concentration. This ionic variation alters the electrical conductivity which can be identified. The most common type of conductometric biosensor is a urea biosensor that consists of urease immobilized on the working electrode.

19.5.4 Thermometric Biosensors

Thermometric biosensors are also known as calorimetric biosensors that works on the principle of emission or absorption of heat generated during the reaction (Spink and Wadsö 1976). This absorption or emission of heat causes the variation in the temperature of a reaction mixture. The change in heat is the measure of the reaction that takes place in the system by biomolecules (Bari et al. 2019).This approach leads to the designing and advancements of thermometric devices (Bondavalli 2019). This type of sensors primarily identifies the change in the fluid temperature flowing during the reaction course of substrate and enzyme. Thermometry is the term to define the measure of heat/temperature. The simplest device based on the above phenomena is thermometer that is commonly used to measure the body temperature. Similarly thermistors have been designed to determine the heat and to make them more sensitive enzyme have been immobilized and called enzyme thermistors (Danielsson et al. 1981). The production coat of regular calorimetric is usually high that restricts the use of these devices for regular practice. To overcome this issue, the immobilized enzyme-based thermistors have been designed with heat sensing element (Danielsson and Mosbach 1986). For past two decades, the devices have been designed by combing the phenomena of enzyme catalysis and immobilization with calorimetry. It also includes a heat insulator and heat exchanger. All the reactions take place in a packed bed reactor system with enzyme that transforms the substrate into product and generates the heat. The difference in temperature before and after catalysis is recorded by thermistor. The thermal biosensors are so sensitive that they can even measure the minor changes in the temperature. The thermometric biosensors are generally used for the determination of cholesterol level. The system detects the heat change created when cholesterol oxidase oxidizes the cholesterol. Similar approach has been used to measure the glucose, urea, antibiotics, and uric acid. When the thermometric biosensors have been used as a subunit of enzyme linked immunoassays (ELISA), it is known as thermometric ELISA.

19.5.5 Optical Biosensors

An optical biosensor is an analytical device that contains a biological recognition component coupled with an optical transducer. It produces the signal that is equivalent to the amount of the measured analyte. The basic principle of the optical biosensor is based on the measurement and identification of the optical properties i.e. absorbance, fluorescence, etc. to fabricate the optical biosensor fiber optics and optoelectronic transducers are used. The term 'optoelectronic' stands for the word optical and electrode. In the optical biosensor, bioelement like enzyme and antibodies acts as a transducing element. Optical biosensors are simplest sensors to design and provide sensing platform that doesn't need an electrical setup. They do not involve a reference sensor.

19.5.6 Piezoelectric Biosensors

A piezoelectric sensor is a means to measure piezoelectric effect that can be a change in pressure, temperature, etc. these piezoelectric effects are further converted into electrical signals. The biosensor works on the principle of recording the affinity interaction of the bioelements. Since nineteenth century piezoelectric effect based biosensors had been studied. Well-known physicists Jacques Curie and Pierre Curie identified the first piezoelectric effect with anisotropic crystal. Anisotropic crystal is a crystal that lacks central symmetry but still produce electrical dipole when subjected to mechanical stress. Mainly the piezoelectric biosensors record the sound vibrations generated. The working principle of piezoelectric crystals is the base of these biosensors. The crystals have different positive and negative charge with variant frequencies. The frequency is changed with the different biomolecular reactions achieved on the crystal surface where the recognition element is adsorbed. The resonance is recorded by electrical unit attached with the biosensor. Any biological component like enzyme, antibody, and DNA can be attached to the crystal. Enzyme acetylcholine esterase has been used to develop the piezoelectric biosensor for monitoring organophosphorus insecticide. Similar approach has been used to design the biosensor for formaldehyde by conjugating formaldehyde dehydrogenase.

19.5.7 Enzyme Biosensor

Enzymes biosensors combine enzymes to the transducer, the interaction between enzyme and analyte generates a signal that indicates the concentration of analyte. These signals can be a change in proton, generation of gas, heat or light emission, etc. Enzymes are very specific in nature, various variety of enzymes have been reported for heavy metal analysis. The diffusion path in case of enzyme is very short thus they provide faster reaction when the heavy metal is present. But the isolation and maintenance of the enzyme to keep them active is expensive. Glucose biosensor is the most common biosensor that is used more extensively among people. Due to the specificity of the enzyme system there has been huge attention given to the use of enzyme to design electrochemical sensors. The enzyme coupling with sensor provides simple detection of heavy metal. Enzyme-based biosensors either rely on enzyme activation or inhibition by the analyte. When the metal ions act as an essential part for the functioning of enzyme like a cofactor, it causes the activation of the enzyme. This is the most common recognition system in which an enzyme that can be either mono or multienzyme, is immobilized on surface of transducer by different immobilization techniques. This immobilized enzyme utilizes substrate or analyte along with a co-substrate and form a product. The biosensor reaction can be attained by either measuring the co-substrate depletion or yield of the product. This is called direct monitoring of analyte, instead indirect monitoring refers to the assessing of substances or inhibitors that specifically interacts with immobilized enzyme and inhibits its biocatalytic properties. Such inhibitors bind either to the enzyme or enzyme–substrate complex, and further interfere with the enzymatic reactions. The beneficial aspect of indirect monitoring is that most of the enzymes are affected by a very low concentration of inhibitors, thus increasing the sensitivity of biosensor. Biosensor response is generally identified by assessing the changes in enzyme activity; after it is exposed to the inhibitor concentration, it is usually quantified by determining the percentage of inhibition, before and after exposure to the inhibitors. The percentage inhibition is calculated by the Eq. 19.1:

$$I\% = \frac{A_0 - A_i}{A_0} \times 100 \tag{19.1}$$

where

$I\%$ = Percentage of inhibition;
A_0 = The activity of the immobilized enzyme before the contact to inhibitors;
A_i = The activity of the immobilized enzyme after the contact to inhibitors.

19.5.7.1 Enzyme Inhibition

A variety of substances can cause a reduction in the rate of an enzyme catalyzed reaction in which some of the substances are the non-specific protein denaturants, while other are specific and are known as inhibitors. The inhibition in the activity of enzyme can be classified as either reversible or irreversible, however the difference between the two is sometime difficult, if the inhibitors binds intensely to the enzyme, and if it is released very gradually (Turdean 2011). Reversible inhibition is characterized by a high rate of association and dissociation of inhibitors with the enzyme, while irreversible inhibition refers to the permanent inhibition of enzyme activity. It directed the formation of an extremely responsive inhibitor product that binds to the enzyme irreversibly, and thus inhibits its activity.

For the detection of zinc ions, a colorimetric biosensor has been designed in which the enzyme alkaline phosphatase has been activated due to the presence of metal ion which acts as a cofactor in the reaction (Satoh 1991). But mostly the

Table 19.2 Enzyme based biosensor for heavy metal detection

Pollutants	Enzymes used for biosensor	References
2,4-Dichloro-phenoxy acetic acid	Acetyl cholinesterase	Sassolas et al. (2012)
Mercury, cadmium, and arsenic	Urease	Pal et al. (2009)
Mercury	Urease	Volotovsky et al. (1997)
Phosphate	Alkaline phosphatase	Upadhyay and Verma (2015)
Cadmium, copper, and lead	Urease	Ilangovan et al. (2006)
Silver, mercury	Invertase, mutarotase and glucose oxidase	Soldatkin et al. (2012)
Mercury	Glucose oxidase	Malitesta and Guascito (2005)
Cadmium	Urease	May and Russell (2003)
Zinc	Alkaline phosphatase	Satoh (1991)
Mercury	Pyruvate oxidase	Gayet et al. (1993)

enzyme-based biosensors are based on the inhibition mechanism of metal ion in due to the interaction of enzyme or protein amino acid with metal ion (Corbisier et al. 1999; vel Krawczyk et al. 2000). This inhibitory behavior of metal ions is used to design the biosensors. The enzyme is usually immobilized on the suitable matrix to avoid the loss of function of enzyme during the process. The biosensor was reported by Gayet et al. that utilizes the L-lactate dehydrogenase for the detection of heavy metal (Gayet et al. 1993).

Same enzyme has also been utilized in the combination with hexokinase and pyruvate kinase for the identification of chromium (Cr). For the determination of Hg (II), Ag(I), Pb(II), Cu(II) and Zn(II) an inhibition based enzyme sensor was developed by immobilizing L-lactate dehydrogenase with L-lactate oxidase. The detection of these metals depends on the inhibition received in the enzyme activity due to the presence of metal ions (Fennouh et al. 1998). To enhance the durability and reusability the immobilization modification has been done in the biosensors. Shekhovtsova et al. have reported the inhibition of peroxidase immobilized on the chitosan film due to the presence of mercury. The sensor gives high specificity and detection limit of 0.02–1000 μM (Shekhovtsova et al. 1997). Among all the reported enzymes urease was the most studied enzyme. Urease has been immobilized on glass surface to design a fiber optic biosensor for mercury determination. Urease has also been reported to design an optical biosensor for fluoride identification. The reported enzyme used for detecting heavy metal contamination is mentioned in Table 19.2.

19.5.8 Whole Cell Biosensor

In a whole cell biosensor, the cell itself acts as a recognition device. Microorganisms either eukaryotic or prokaryotic cells, mammals, plant tissues or a whole cell can be used as a biological recognition element.

They are very specific and sensitive towards really small analyte concentration (Upadhyay et al. 2018). It can be used for multipurpose monitoring. The cell-based biosensors generate results similar to enzyme-based sensors. Still microbial biosensors have several advantages over enzyme-based biosensors. The enzymes are costly as compared to microbial strains and enzyme inside the cell work better as they achieve the optimal active conditions inside the cell (Corcoran and Rechnitz 1985; D'souza 2001).

A wide variety of microbes are suitable for a designing a whole cell biosensor and they can easily culture in a normal growth media. The culturing of microbes is easier than the isolation and purification of enzymes. They can withstand wide variety of pH and temperature conditions. The study of the changes in the metabolic pathway of the microbial strain leads to the development of sensitive and selective whole cell biosensors for analyzing analyte of interest (Riedel et al. 2002). The inhibition in the growth of cell in the presence of an ion or metal is an indicator towards its concentration. The biosensor for Cu(II) determination was developed that analyzes the negative effect of Cu(II) on the bacterial growth that results in the change in the biomass (Yamasaki et al. 2004). Whole cell based biosensor are also useful where multiple cofactors are required for the reaction. Either live or dead microbes can be utilized for designing of this sensor. These sensors have higher life span and are more economical. But the major drawback of this system is that the time of catalysis is higher than isolated enzymes.

19.5.9 DNA-Based Biosensor

From last few years the use of nucleic acids as a recognition element has been increased to monitor analyte of interest, as these nucleic acids show affinity towards heavy metals. The interaction of metals ions with DNA can bring either favorable results or may damage the biochemical process (replication, transcription) (Tencaliec et al. 2006), that generally cause changes in the function and structure of the genetic material (Oliveira et al. 2008). Therefore the interaction between DNA and analyte is widely explored to detect toxic metals like Pb, Cd, and Ni (Wong et al. 2007). These metals ions can be detected in different type of samples, either food samples or samples collected from higher altitude region. The International Agency for Research on Cancer (IARC) has listed some toxic heavy metals like Pb, Cd that are carcinogens and can cause the cancer and promote tumor growth (Oliveira et al. 2008) to design a DNA-based biosensor; the DNA has been used in the native form of denatured like as a single stranded form (Wong et al. 2007; Babkina et al. 2004). To convert the interactive biological signals caused due the interaction of DNA with metal, the electrochemical techniques like chronopotentiometric, conductometric or voltammetric are suitable for developing biosensors and for monitoring heavy metals as they provide selectivity and sensitivity response with low signal-to-noise ratio (Tencaliec et al. 2006; Oliveira et al. 2008).

An electrochemical DNA biosensor is a device that combines receptor-transducer system in which DNA is used as a biological recognition element that quantitates particular binding courses of analyte with DNA through electrochemical transducers (Tencaliec et al. 2006). To design an efficient DNA based electrochemical biosensors, DNA and its components including purine pyrimidine is immobilized on electrode surface. For immobilizing DNA different immobilizing techniques has been utilized such as adsorption or covalent binding (Oliveira et al. 2008). The use of DNA as a recognition element has a variety of advantages as it has four binding sites; phosphate oxygen atoms which are negatively charged, the ribose hydroxyls, base ring of nitrogens, and the exocyclic base of keto groups (Oliveira et al. 2008). In the DNA based biosensors the detection takes place in two ways: one is to monitor the changes occurred due to interaction of pollutants with immobilized DNA or to detect the hybridization of DNA sequences from infectious microorganisms (Rodriguez-Mozaz et al. 2004).The detection of some heavy metals that interacts to the DNA with more than one site is complex (Turdean 2011).

19.6 Conclusion

The chapter has described the idea along with working principles of different categories of biosensors designed for the study of heavy metal concentration in the sample. Protein or enzyme based biosensors are the most commonly studied biosensors as they provide fast results but they usually lack the specificity as the enzyme generally inhibits the activity in the presence of toxicants. Instead of enzymatic biosensors non-enzymatic metal binding protein can be used because they have potential sensitivity towards the detection of low level of contaminants. Immunosensor based antibodies are also very sensitive as well as specific. Microbial biosensors are cheap and provide certain advantages and provide higher enzymatic activity inside the cell. Therefore genetically modified bacterial strains are designed to generate more quantifiable signals generated due to the contact of heavy metals. The reason to study the biosensor is to facilitate the contamination monitoring at higher altitude where the facility of lab testing is not available and this eventually cause the negligence towards the primary identification level. Due to lack of the sources available at higher altitudes the identification is not possible, thus this contaminated water enters into the food chain and cause hazardous effects to the environment and living beings.

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Frankia: A Promising N-Fixing Plant Growth Promoting Rhizobacteria (PGPR) Improved Drought Tolerance in Crops at Higher Altitude

20

Sanjay Mohan Gupta, Kamal Kumar, Rajat Kumar Joshi, Supriya Gupta, and Madhu Bala

Abstract

The high-altitude Himalayan regions are unique micro-climatic and geographic separations that have precise agro-technical needs, which is quite different from other agro-climacteric zones of India for vegetable production. In such marginal degraded regions mainly drought stress causes significant harmful effect on survival, biomass production and yields of vegetable crops up to 70%. Since drought tolerance is quantitative and multigenic in nature, a massive challenge exists to solve this problem. The use of N-fixing growth promoting rhizobacteria (PGPR) in place of agrochemicals (pesticides and fertilizers) is a promising approach to increase the plant productivity by naturally increasing nitrogen content of soil and also not leads to deterioration of the soil micro-flora and environment. In this attempt, this chapter highlights the importance of promising indigenous *Frankia sp*. strains (potential N-fixing PGPR) isolated from the root nodules of Casuarina plant of Kumaon region of Uttarakhand, India. Also, the effect of bio-inoculation of isolated Frankia sp. strains has been demonstrated to improve PEG-mediated drought stress tolerance in tomato (*Solanum lycopersicum* L. cv. Pusa ruby) for improving sustainable hill agriculture in rain fed conditions. This successful model may be useful for improving the productivity of other crops grown in drought prone regions of Himalaya.

Keywords

Frankia · Plant growth promoting rhizobacteria · N-fixation · Drought stress · Tomato · Actinorhizal plants

S. M. Gupta (✉) · K. Kumar · R. K. Joshi · M. Bala
MBGE Laboratory, DIBER, DRDO, Haldwani, India

S. Gupta
Department of Molecular Biology and Genetic Engineering, CBSH, GBPUA&T, Pantnagar, India

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20.1 Introduction

Nitrogen is an important macro-element and one of the main factors limiting plant growth and productivity worldwide. Although nitrogen being the most rich element in the atmosphere (~78%), the majority of plant species are unable to directly utilize atmospheric nitrogen and mainly rely on poor nitrogen sources in soils for their nutrition (Carole et al. 2013; Kumar et al. 2015). Since, plants only uptake ammonia (NH_3) form of nitrogen for synthesis of nucleic acids, amino acids, proteins and other essential nitrogen-containing compounds necessary for their survival (Robertson and Vitousek 2009). The use of agrochemicals to increase the nitrogen content of soil is not promising since it leads to deterioration of the soil micro-flora and environment and also financial considerations preclude this as a solution for the majority of farmers in many developing countries (Kalia and Gosal 2011; Kumar et al. 2012). Therefore, it is crucial to explore alternative integrated soil nutrient-management methods that have minimum risk of environmental deterioration (Khatri et al. 2012; Senthil et al. 2012). In such circumstance, the use of N-fixing plant growth promoting rhizobacteria (PGPR) for rehabilitating soil nutrients and maintaining fertility as a replacement of agrochemicals (pesticides and fertilizers) is a most promising sustainable agriculture approach nowadays (Arora et al. 2012; Shridhar 2012; Ghodhbane-Gtari et al. 2019).

The fixation of nitrogen is biologically mediated in nature by many N-fixing bacteria that mainly include cyanobacteria, the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Sinorhizobium*, *Mesorhizobium, Frankia, etc.* (Chanway et al. 2014; Hocher et al. 2019). Among these, the symbiosis between Rhizobium and legumes has been studied intensively and lately the symbiosis between Frankia and non-leguminous (so-called actinorhizal) plants has been receiving increased attention (Nouioui et al. 2019). Unlike the Rhizobium-legume symbiosis, where most of the host plants belong to a single large family, Frankia *sp.* (Gram positive N-fixing bacteria) members of actinomycete family that can form root nodules in symbiosis association with a broad spectrum of angiosperm plants (~200 genera) belonging to eight families collectively called actinorhizal plants (mainly woody shrubs and trees like *Alnus, Elaeagnus, Hippophae, Casuarina, etc.* except for the genus *Datisca*, which is herbaceous) (Sayed 2011; Carole et al. 2013; Gtari et al. 2019a, b).

Frankia sp. helped in plant growth promotion by various mechanisms, which involve decomposition of organic matter, recycling of essential elements, soil structure formation, solubilization of mineral nutrients, producing numerous plant growth hormones, soil pollutants degradation, root growth promotion, improving soil fertility and bio-control of plant soil pathogens for promoting plant growth (Beneduzi et al. 2012). In addition to these, it has been also reported that symbiotic association of Frankia (nitrogen-fixing PGPR bacteria) with the actinorhizal plants can help in biotic and abiotic stresses tolerance (Udwary et al. 2011; Gupta et al. 2012, 2013; Kucho et al. 2019). Since Frankia is resistant to drought conditions, it can be efficiently used as biofertilizers in land affected by drought stress (Sayed 2011). Many workers showed that Frankia improves plant growth in drought stressed conditions (Tani and Sasakawa 2003; Srivastava et al. 2012; Zhong et al. 2019).

This book chapter reports the isolation and characterization of promising indigenous *Frankia sp*. strains from the root nodules of Casuarina plant of Kumaon region of Uttarakhand, India to explore its potential to improve PEG-mediated drought stress tolerance in tomato (*Solanum lycopersicum* L. cv. Pusa ruby) for improving sustainable hill agriculture in rain fed conditions. This successful approach can be used for improving the productivity of other crops grown in drought prone regions of Himalaya.

20.2 *Frankia*: Potential N-Fixing PGPR

The members of the genus Frankia *sp*. gram positive bacteria form symbionts root nodules in dicotyledonous plants that contribute in nitrogen fixation. These nitrogen-fixing soil actinomycetes are capable of forming actinorhizal symbiosis and help in utilization of atmospheric nitrogen for growth and nutrition (Ghodhbane-Gtari et al. 2019). Till date on the basis of host infectivity, four groups of Frankia strains have been identified, namely (1) strains, which nodulate *Casuarina*s and Myrica; (2) Strains, which nodulate Alnus and Myrica; (3) Strains, which nodulate *Elaeagnaceae* and *Myrica*; and (4) Strains, which nodulate only *Elaeagnaceae* (Sellestedt and Mattson 1994). However, studies also showed that many members of Frankia genus can also remain infective in soils and survive in absence of host plants (Hahn et al. 1999). The range of host plants of Frankia includes ~194 species of mainly ~24 genera of ~8 different families (Benson and Silvester 1993). Non-leguminous actinorhizal woody plants can also have develop capacity for nitrogen fixation due to Frankia root nodulation. These nodulated species can grow and improve soil fertility, used in the recolonization and reclamation of degraded lands, soil erosion control and in agro-forestry (Wheeler and Miller 1990; Hocher et al. 2019). Frankia vegetative hyphae can grow deep in arid soil environments to survive under drought conditions (Burleigh and Dawson 1994). Also, one of the outstanding features of Frankia strains is ability to develop spores and vesicles, which are the main site for the actinorhizal nitrogen fixation. Several nitrogenase enzymes are localized in these vesicles that can be proven by acetylene reduction assay method (Burggraaf and Shipton 1983). The reproductive spores of Frankia are also powerful tool for their genetic studies (Krumholz et al. 2003). However, the ability to cultivate Frankia in in vitro condition causes a rapid increase in knowledge of its physiology, related enzyme and nitrogen fixation (Muller et al. 1991). The most promising species of Frankia are *Frankia alni*, *Frankia elaegni* and *Frankia brunchostii* (Lechevalier and Lechevalier 1989). The exceptional ability of *Casuarina*s plants to flourish in nitrogen deficient soils is due to their symbionts association with Frankia bacteria that help to enrich the soil nitrogen status (Gauthier et al. 1985; Zhong et al. 2019). However, all reported Frankia strains have the capability of establishing an effective endosymbiosis relation with many woody plants (Bond 1983).

20.2.1 Taxonomy of *Frankia*

As per morphological features and cell wall type, Frankia has been taxonomically classified under the taxon "Actinomycetes with multilocular sporangia" along with Dermatophilus and Geodermatophilus (Goodfellow 1986; Gtari et al. 2019a). However, all reported Frankia having filamentous, branched ones morphological features are puts under the order Actinomycetales under *Frankiaceae* family (Becking 1970; Ribeiro-Barros et al. 2019). However, on the basis of physiology, all Frankia strains are classed into mainly two groups. The "Group A" includes heterogeneous diverse groups, while "Group B" includes homogenous microaerophilic strains of Frankia (Lechevalier and Lechevalier 1984). On the basis of Frankia host specificity, cytochemical studies have separated all Frankia strains into *Alnus* and *Elaeagnus* major groups (Laurent and Lalonde 1987). Several workers have also used quantitative analysis of the total fatty acid composition of Frankia for taxonomical investigations (Lalonde et al. 1988).

Molecular methods have been also used to show the genetic diversity among Frankia strains. On the basis of DNA base content, total nine genomic species of Frankia strains were identified, namely three genomic species from *Alnus* compatible Frankia strains, five from *Elaeagnaceae* compatible Frankia strains and one from *Casuarina*s compatible Frankia strains (Fernandez et al. 1989). At gene level, it has been proven that Frankia "nif genes" have conserved regions that could also be used to demonstrate large phylogenetic relationships between different Frankia strains. However, the presence of considerable morphological and physiological variation within the genus Frankia makes exact species identification and characterization difficult at global level. Therefore, the strains have been designated by a combination of assigned letters and numbers (Lechevalier and Lechevalier 1989). Usually, a combination of initial three letters that stand for the name of laboratory from where the strains first reported followed by two numbers indicating the plant host genus and up to eight further allotted numbers to be used at the prudence of the person designate the strains (Lechevalier 1983). Therefore, a catalogue of reported Frankia strains is constantly updated and maintained at global level for helping new researcher as reference Frankia database.

20.2.2 Genetics of *Frankia*

It has been proven that the total genomic size of Frankia is approximately twice that of *E. coli* genome (Gueddou et al. 2019). The total G + C content of Frankia DNA content is about 66–72%, which is in accordance with other members of actinomycetes. Frankia contains plasmids of approximately 7–190 Kb in size that contains many structural genes viz. nif K, nif D, nif H, *etc.*, which encode for nitrogenase enzyme. The gene encodes for Frankia nitrogenase has been sequenced (Simonet et al. 1984; Ligon and Nakas 1987). However, it has been found that even individual cultures obtained from single root nodule may have mixed Frankia genotypes, therefore, extreme care should be taken while Frankia purification through single spore lines (Sarma and Misra 2002).

20.2.3 Morphological Characteristics of *Frankia*

The Frankia colonies showed mainly three distinct morphological characteristics viz. (1) hyphae, (2) spores and (3) vesicles (Taveres and Sellstedt 2000). Frankia is a slow growing gram positive actinomycete that takes approximately 4–8 weeks to mature. The mature colonies showed diversity in pigmentation that depends upon the content of growth medium. Usually they are colourless or pigmented but may also be reported as dark red, grey, black, green, brown or yellow by many workers (Lechevalier and Lechevalier 1989). The shape of Frankia colonies showed polymorphism in solid media ranging from starfish, diffuse or compact in shapes (Rao 1998). The colonies are mucilaginous in the centre in solid medium while in liquid medium, colonies showed ellipsoidal or spherical shape. Mostly the hyphae of Frankia are thin, poorly branched and form compact dense thalli structure (Rao and Rodriguez Barrueco 1995). As per extent of spore formation by Frankia within host root nodules, they are mapped into spore negative and spore positive types (Hahn et al. 1999). Usually their sporangia are found round, compartmentalized, cylindrical or highly irregular, seen intrahyphally or terminally in position (Rao 1998). These sporangia develop as swelling of hyphal tips or within hyphae. The spores are produced by diversion of swollen hyphae along numerous axis and are reported in pairs or in tetrads (Akkermans et al. 1991). However, mature sporangia are present as club shaped that contain polygonal, non-motile and thick walled mature spores. Also, many Frankia strains are able to produce specialized hyphae with thick double layered cell wall called as reproductive torulose hyphae (RTH), which were observed mainly in *Casuarina*s host (Diem and Dommergues 1983). However, the presence of vesicles in the form of swollen types of hyphae is a unique feature reported in Frankia genus. These are the actual site of nitrogen fixation present in culture media as well as in root nodules. They are formed mainly in nitrogen-free media and have a diameter of approximately 2.5–5.0 μm in size (Zhang and Benson 1992). All effective nitrogen-fixing Frankia strains contain vesicles; however, an exception to this is observed in *Casuarina*-Frankia symbiosis (Baker et al. 1981). They showed clusters of Frankia hyphae without presence of any vesicles (Gauthier et al. 1981). On the basis of vesicle morphology, the actinorhizal nodules can be classified into four major categories, viz. (1) spherical vesicles found in *Alnus sp.* and *Elaeagnaceae* (Newcomb et al. 1987); (2) lanceolate or finger like vesicles found in *Myrica* and *Datisca sp*. (Henry 1977); (3) Pear shaped found in *Ceanothus sp*. (Newcomb 1981); and (4) without any structure resembling distinct vesicle has also been observed in *Casuarina*s, even though the micro-symbionts fix large amounts of N_2 and produce vesicles when grown in in vitro condition. However, it is present in filamentous or non-vesiculate form (Tyson and Silver 1979; Pesce et al. 2019). But whatever cellular entity is showed as the site of nitrogenase in *Casuarina*s, it would constitute under fourth morphology category

(Akkermans et al. 1991). Usually all vesicles are borne on short Frankia hyphae arising directly from mycelium.

20.2.4 Physiology of *Frankia*

Generally all Frankia strains are slow growing and showed variations in their multiplication time ranging from 1 to 4 days. Mostly all Frankia strains are mesophilic in nature with optimal growth temperature range of 25–37 °C. Physiologically they are aerobic, microaerophilic, chemoorganotrophic and catalase positive in nature (Lechevalier and Lechevalier 1989). They are capable of utilizing propionate and other fatty acids, glucose and other monosaccharides; pectin; carboxylic acids like succinate and lipid Tween-80; and cellulose (Blom et al. 1980; Simonet et al. 1984; Sen et al. 2019). However, β-oxidation of short chain fatty acids and glyoxalate cycle were also observed in some Frankia strain grown on Tween–80 or acetate substrates (Blom and Harkink 1981). The enzymes responsible for degradation of monosaccharide have been reported in some Frankia strains grown on glucose substrate (Lopez and Torrey 1985). On physiological basis all Frankia strains are classified into two groups “A” and “B”. “Group A” is a heterogeneous collection of morphologically and chemically diverse strains. They are mostly aerobic, soil saprophytes, have pigmented cells and can be cultured and maintained on slants. Usually these strains are having their specific original host plants and can form vesicles of various size and shapes on complex substrate media. They can utilize carbohydrate (0.5%) as substrate and showed rapid growth. They are genetically and serologically diverse strains (Baker et al. 1981). On the other hand, Frankia strains of “Group B” are more homogenous and are genetically and serologically related together (Lechevalier and Lechevalier 1984). Usually they are colourless cells and microaerophilic, cannot be cultured and maintained on slants and also cannot take up 0.5% carbohydrate as substrate. Most of these strains can develop effective (N_2-fixing) nodules on the roots of host plant. The vesicles are produced on nitrogen-free media along with succinate. “Group B” Frankia are sensitive to streptomycin and penicillin and to various plant phenolics such as *P*-coumaric acids and ferulic acid (Perradin et al. 1983). Their growth is strongly inhibited by caffeic acid and juglone. Mostly they are non-pathogenic and known to produce indole acetic acid (IAA) (Bell 1979; Wheeler et al. 1981).

20.2.5 Biochemical Properties of *Frankia*

Mostly all Frankia strains are having a chemotype III cell wall that contains glutamic acid, alanine, meso-diaminopimelic acid, glucosamine and muramic acid (Van Dijk 1978). However, no mycolic acid is present in its cell wall. Frankia can possess xylose (Baker et al. 1981), fucose, rhamnose and 2-methyl-D-mannose (Mort et al. 1982) as whole cell sugars as substrate. However, Frankia strains of group-A show protease and amylase activity, whereas group-B strains lack protease activity (Sarkar et al. 2019).

Besides atmospheric nitrogen they can also utilize various amino acids, urea and NH_3 as nitrogen source. Beta-glucosidase activity (Horriere 1984) and glutamate oxaloacetate transaminase activity (Akkermans et al. 1982) have also been reported in Frankia by many workers. Trehalose has been isolated and purified from Frankia (Lopez and Torrey 1985). Wheeler et al. (1981) reported the occurrence of polyamines from various cultured Frankia strains. These polyamines play a specific role in vesicle initiation and development. Cellulase enzyme activity has also been reported in some cultured Frankia strains. Calcium crystals composed of calcium oxalate have been formed by Frankia in the *Alnus glutinosa* root nodules (Jones et al. 1989). Bifunctional catalase peroxidase and monofunctional catalases have also been reported in Frankia strain R43 mainly during the stationary growth phase of Frankia (Taveres et al. 2003). Also, an uptake of hydrogenase associated with N_2 fixation was detected in Frankia (Sellestedt and Mattson 1994). Productions of hydrolyzing enzymes, iron chelating siderophores, indoles, benzonapathacene quinone, *etc.* metabolites have been observed in Frankia (Sen et al. 2019). These metabolites help Frankia to survive during non-symbiotic conditions. Calcimycin antibiotics that comprise a group of natural antibiotics capable of transporting monovalent and divalent metal cations across biological membrane are also reported in Frankia (Sarma et al. 2003). Nitrogen fixation in Frankia is dependent on the availability of free Ca^{2+} ions (Tisa and Ensign 1987). Nitrate reducing activity has been demonstrated in *Alnus* root nodules (Li et al. 1972). Nitrate reductase is a key enzyme having an important role in the nitrate metabolism in higher plants (Campbell 1988). Acid phosphatase and alkaline phosphatase activity was also observed in Frankia and it was found to be highest when phosphate is omitted from the substrate culture (Yang et al. 1997).

20.2.6 Nitrogen Fixation by *Frankia*

Mostly all Frankia strains are capable to fix atmospheric N_2 (Noridge and Benson 1986). The presence of nitrogenase enzyme in pure culture of Frankia was demonstrated by acetylene reduction method (Tjepkema et al. 1980). It has been proven that nitrogenase enzyme is located in the Frankia vesicles (Noridge and Benson 1986). The structural genes "nif K", "nif D" and "nif H" encoding for nitrogenase have been already sequenced and reported in various Frankia strains (Ligon and Nakas 1987). The nitrogenase enzyme of Frankia is quite similar to other nitrogen-fixing organisms that possess an active hydrogen uptake system to maintain low redox potential (Sellestedt et al. 1986; Bomar et al. 1989). Frankia converts ammonia to glutamine with the help of glutamine synthetase enzyme (Blom 1981). However, till date no glutamate dehydrogenase has been reported from Frankia (Blom 1981). Frankia contains Fe^{2+} and Mn^{2+} ion containing SOD enzymes that protect nitrogenase from oxygen (Steele and Stowers 1986). The nitrogenase activity has been reported at 0.3% oxygen concentration but in this case no vesicles are formed in host root nodules (Murry et al. 1985). Nitrogen fixation is generally seen in Frankia during the lag phase of growth, which increases during early logarithmic phase and after that it slightly declines. This decrease in nitrogen fixation is related with the decelerating phase. The nitrogen-fixing activity in root nodules of non-leguminous plants was firstly demonstrated by using 15 N and C_2H_2 reduction experiments. Also, haemoglobin content was also observed in the root nodules of *Alnus*, *Myrica* and *Casuarina* plants, which protects nitrogenase enzyme from oxygen inactivation (Tjepkema 1984). Likewise, Ganesh (1997) reported that some novel vesicles of Frankia contain vanadium nitrogenase in place of normal nitrogenase, which contains molybdenum that plays a major role during non-symbiotic mode of survival. However, an uptake of hydrogenase, which utilizes the hydrogen gas evolved during nitrogen fixation, was found mainly in root vesicles, but to some extent it was also observed in hyphae of free living Frankia stains (Sellestedt and Mattson 1994; Zhong et al. 2019).

20.3 Isolation of Indigenous *Frankia* Strains From *Casuarina*

The root nodules of mature *Casuarina* plant (~2 years old) was used for isolation and characterization of *Frankia sp*. The sampling area was a biodiversity rich Kathgodam roadside (~554 m asl; 29.27° N and 79.53° E) of Uttarakhand state, India. Fresh looking roots of *Casuarina* species were used for nodule collection. The roots were surface sterilized according to Diem and Dommergues (1983). Surface sterilized *Frankia* nodules lobes were then aseptically cut into small pieces (~2 × 2 cm) and homogenized with 2 mL molecular grade sterile water using mortar-pestle. Nodule extract was centrifuged at 6000 rpm for 5 min and clear supernatant was collected in a fresh aseptic tube. One millilitre of this supernatant was added with 100 mL of nitrogen-free Samoset's media used for *Frankia* bacterium enrichment at 28 °C for 1 week. After the observation of optimum turbidity of the broth culture, the inocula were re-suspended in the Samoset's media to adjust 1.0 OD at 600 nm. Finally, 100 μL of bacterial cell enriched broth culture (about 108 cfu/mL) were evenly spread onto MHA (Muller Hinton agar) plates (90 mm diameter) using a sterile disposable spreader, respectively. The plates were kept at 28 °C for 1 week. Same procedure was repeated until the single cell colony was not observed. Single colony *Frankia* cultures were used for mass multiplication on MHA media plates and freshly stored at 4 °C in Petri dishes that were closed with parafilm, and they were stored for about 2 weeks followed by DNA extraction (Fig. 20.1).

20.3.1 Biochemical and Molecular Characterization of *Frankia* Isolates

Isolated Frankia strain was biochemically (gram staining, ornithine utilization, urease activity, nitrate reduction, *etc.*) characterized by using Himedia ready prepared KB001 and KB002 HiAssorted™ Biochemical test kit that showed positive results (Figs. 20.2 and 20.3) Total genomic DNA was isolated from cultures of

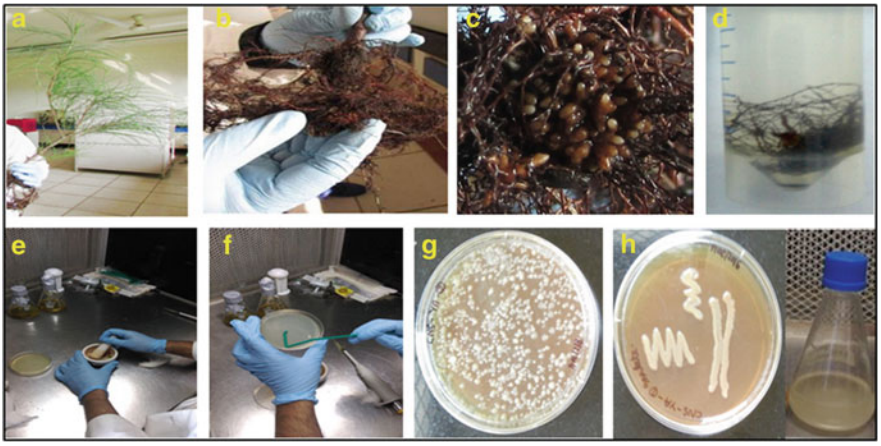

Fig. 20.1 Isolation of *Frankia* strain from *Casuarina* plant (~2 years old) grown naturally at Kathgodam (~554 m asl; 29.27° N 79.53° E), Uttarakhand, India. (**a**) *Casuarina* plant (~2 years old); (**b** & **c**) root nodules of *Casuarina*; (**d**) nodule in sterile water; (**e**) preparation of water extract of nodule in laminar air-flow; (**f**) planting of clarified nodule extract; (**g**) grown colonies of putative *Frankia* strains on FTW media and (**h**) isolated pure cultures of *Frankia* strain

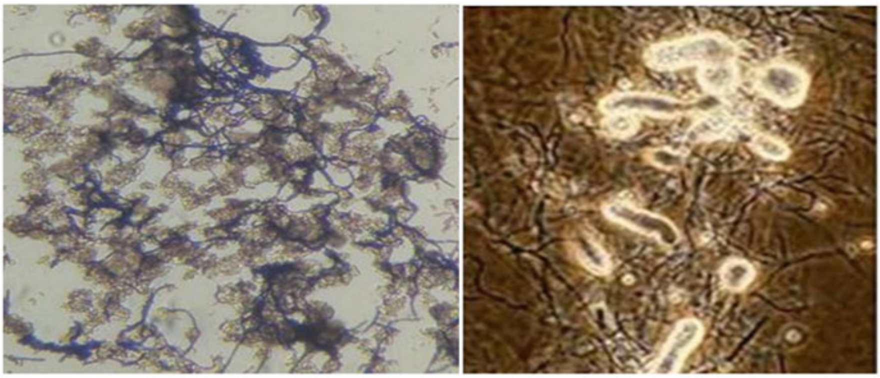

Fig. 20.2 (**a**) Gram staining of *Frankia* isolates under simple microscope, (**b**) *Frankia* under phase contrast microscope

Frankia isolates by following Himedia kit instructions. The quality of isolated genomic DNA was checked and qualified by agarose gel electrophoresis (1%) using standard DNA marker (Himedia, Pvt. Ltd). The Frankia isolates was molecular confirmed by PCR by using Frankia-specific *nif* H gene primers (forward primer F1-5′ GAAGACGATCCCGACCCCGA 3′ and reverse primer R1-5′ GGTCGGGACCTCATCCTCGA 3′) (Simonet et al. 1991) (Fig. 20.4).

Fig. 20.3 Various biochemical tests (1: galactosidase activity; 2: lysine utilization; 3: ornithine utilization; 4: urease activity; 5: phenylalanine deamination; 6: nitrate reduction; 7: h2s production; 8: citrate utilization; 9: Voges–Proskauer's test; 10: methyl red test; 11: indole test; 12: malonate test) performed on isolated *Frankia* strains

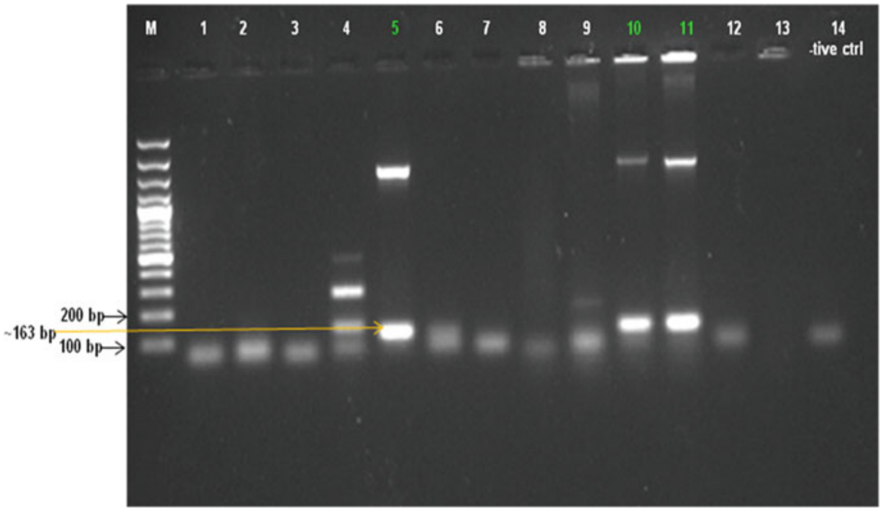

Fig. 20.4 Amplified ~163-bp PCR amplicon of *Frankia*-specific *nif* H gene by using F-*nif*-H-F and F-*nif*-H-R primers that showed among 13 *Frankia* isolates: 5, 10 and 11 gave positive molecular PCR confirmation

20.4 Bio-inoculation of *Frankia* Isolates on Tomato Seeds Under Drought Stress

Tomato seeds (*Solanum lycopersicum* L. cv. Pusa ruby) were used for priming with *Frankia* cultures under PEG 6000-mediated drought stress condition. The PEG 6000 solutions (0, 5, 10, 15, 20, 25 and 30%) were used for creating drought stress condition in tomato seed (~20 per plate) germination as well as pot experiments. All experiments are carried out under Completely Randomized Design (CRD) with four replicates. Tomato seeds without bio-inoculated with *Frankia* cultures were treated as control.

Freshly stored *Frankia* cultures were allowed to grow in nitrogen-free Samoset's media for 1 week at 28 °C, after which culture density was determined using the colony-forming unit (CFU) method. Priming was performed by soaking the tomato seeds in Frankia solutions containing 10^7 bacteria mL^{-1} for 2 days at 28 °C with shaking at 150 rpm. Another set of tomato seeds was soaked in water to be used as a control. Twenty primed or non-primed tomato seeds were sown in disposable Petri plates containing different concentration of PEG 6000 solutions and allowed to grow in controlled aseptic environment growth chambers (*LT-105*) (*Percival Scientific Inc*., *Perry*, Iowa, USA) equipped with 22/16 °C (day/night), 16/8-h photoperiods at 450 μmol m^{-2} s^{-1} and 70 ± 2%. humidity. Plants were watered every other day for 1 month. After 3 days of seed sowing, the protrusion of the radicle was observed in control and other PEG 6000 treatments. Percentage of germination was measured by ISTA (International Seed Testing Association) standard method (Table 20.1). At the end of 1 month, the percentage of germination, germination rate, the length of root

Table 20.1 Effect of bio-inoculation of *Frankia* isolate on seed germination in terms of radicle protrusion and opening of cotyledonary leaves and growth responses of tomato (*Solanum lycopersicum* L. cv. Pusa ruby) under PEG 6000-mediated drought stress

PEG treatment (%)	Radicle protrusion (%)		Opening of cotyledonary leaves (%)		Fresh weight (FW) (g)		Dry weight (DW) (g)	
	P	NP	P	NP	P	NP	P	NP
0	81.25[a]	77.38[a]	73.88[a]	60.75[a]	8.28[f]	6.02[a]	0.79[f]	0.54[a]
1	79.50[a]	65.88[b]	57.63[b]	30.13[b]	10.94[e]	3.93[b]	1.06[e]	0.35[b]
5	60.00[b]	55.88[c]	50.38[c]	0	13.76[d]	2.2[c]	1.36[d]	0.20[c]
10	43.75[c]	40.13[d]	39.13[d]	0	16.69[c]	0	1.61[c]	0
15	21.87[d]	9.63[e]	1.75[e]	0	19.52[b]	0	1.88[b]	0
20	4.63[e]	4.25[f]	0	0	23.44[a]	0	2.31[a]	0
25	2.00[f]	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
LSD	5.02	5.21	4.31	4.63	1.10	0.63	0.20	0.07
SE	1.71	1.77	1.47	1.57	0.36	0.21	0.07	0.02

Different letters in each column indicate significant differences at $P \leq 0.05$, as per Least Significant Difference (LSD) test. SE of each parameter are given in the last row. *P* tomato seeds primed with culture of *Frankia* isolate, *NP* not-primed tomato seeds

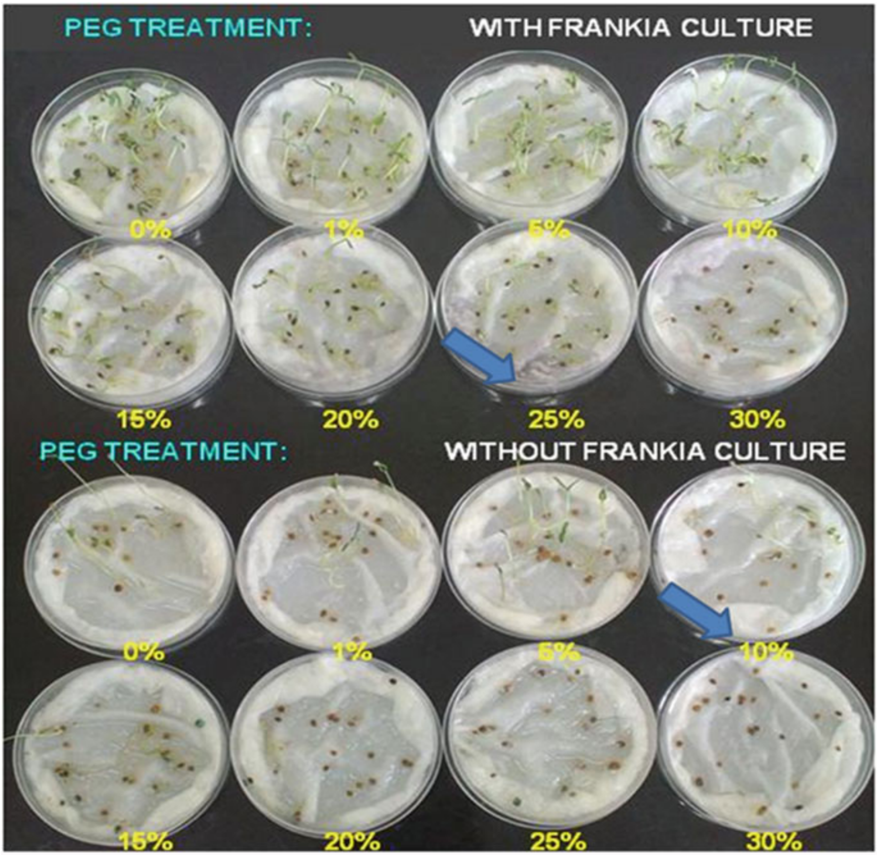

Fig. 20.5 Effect of bio-inoculation of *Frankia* isolate on growth and seed germination in tomato (*Solanum lycopersicum* L. cv. Pusa ruby) under PEG 6000-mediated drought stress. Frankia primed seeds showed higher degree of drought stress tolerance up to 25% PEG 6000 as compared to control (10% PEG 6000)

and shoot of seedlings and dry matter weight of root and shoot were also measured that indicates higher degree of drought stress tolerance up to 25% PEG 6000 as compared to control (Fig. 20.5). Similar results were obtained in pot experiments (Fig. 20.6).

20.5 Physiochemical Analysis of Frankia Primed Tomato Seedlings

Various physiochemical tests (TSS, Proline, MDA, total phenol content) were performed on Frankia primed tomato seeding to check the effect of Frankia bio-inoculation during PEG-mediated drought tolerance (Table 20.2). All data

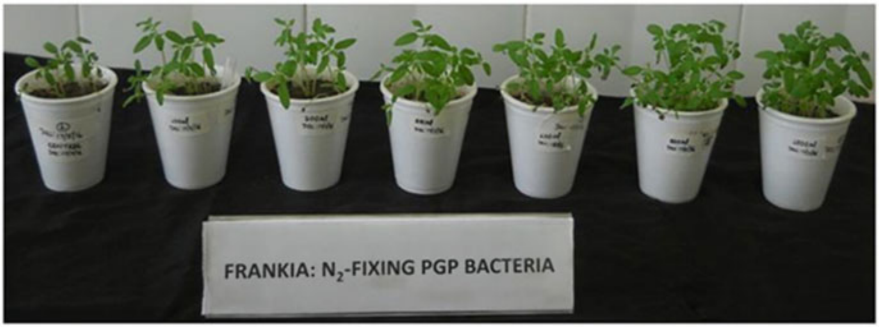

Fig. 20.6 Pot experiments of tomato seeds primed with *Frankia* cultures showed significant increase in growth in soil environment

Table 20.2 Effect of bio-inoculation of *Frankia* isolate on physiochemical responses (accumulation of total soluble sugars (TSS), proline content, MDA content and total phenol accumulation) in tomato (*Solanum lycopersicum* L. cv. Pusa ruby) under PEG 6000-mediated drought stress

PEG treatment (%)	Total soluble sugars content (μg g^{-1} FW)		Proline (μg g^{-1} FW)		MDA (mM g^{-1} FW)		Total phenol (mM g^{-1} FW)	
	P	NP	P	NP	P	NP	P	NP
0	32.08[a]	25.46[a]	30.03[e]	37.00[c]	0.03[c]	0.03[b]	109.31[b]	174.65[c]
1	34.33[a]	20.08[b]	109.03[d]	304.19[b]	0.20[b]	0.39[a]	248.68[a]	384.58[b]
5	36.00[a]	0	156.98[c]	357.94[a]	0.63[a]	0.40[a]	275.64[a]	446.19[a]
10	28.75[b]	0	211.36[b]	0	0.81[a]	0	296.26[a]	0
15	23.45[c]	0	221.77[b]	0	0.82[a]	0	322.22[a]	0
20	8.13[d]	0	291.44[a]	0	0.72	0	342.22[a]	0
25	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
LSD	2.90	4.85	25.95	26.96	0.29	0.22	31.79	20.51
SE	1.73	1.33	8.82	9.17	0.1	0.08	10.81	6.97

Different letters in each column indicate significant differences at $P \leq 0.05$, as per Least Significant Difference (LSD) test. SE of each parameter are given in the last row. *P* tomato seeds primed with culture of *Frankia* isolate, *NP* not-primed tomato seeds

were analysed by unidirectional analysis of variance (ANOVA) test to determine the significance between the different treatments using CropStat for Windows (7.2.2007.2 module), developed by the Biometrics unit, IRRI, Philippines. The treatment means were compared by Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$. Duncan's multiple range test (DMRT) was also performed to evaluate the significance of differences between mean values.

20.5.1 Estimation of Total Soluble Sugars (TSS)

The TSS was estimated by following anthrone method (Watanabe et al. 2000) with slight changes. Leaf samples (200 mg) were homogenized in 10 mL of ethanol (80% v/v) and the extract was centrifuged at 8000 rpm for 15 min at 4 °C. After that, supernatant (1 mL) was mixed with freshly prepared anthrone (3 mL) and the tube was kept at 100 °C for 15 min. The reaction was terminated by quick cooling of reaction tube on ice for 5 min. The absorbance was measured at 620 nm and TSS content (μg g^{-1} FW) was quantified by standard curve made by using D-glucose. Our results suggest that Frankia gives significant increase in TSS content up to 20% PEG that may facilitate tomato seedling to tolerate drought stress (Table 20.2).

20.5.2 Proline Estimation

Free proline content was determined by following Bates (1973) methods. Leaf samples (200 mg) were homogenized in aqueous sulfosalicylic acid (3% w/v, 2 mL) and the filtered homogenate (2 mL) was added with equal volume of acid ninhydrin and acetic acid. The reaction tube was kept heated at 100 °C for 1 h with occasional shaking. The reaction terminated in an ice bath for 5 min. After that, the reaction mixture was extracted with 2 mL toluene and mixed vigorously with a stirrer for 10–15 s. The chromophore containing toluene was aspirated from the aqueous phase and warmed to room temperature and absorbance was recorded at 520 nm by using toluene as blank. Proline content (μg g^{-1} FW) was determined from a standard curve made by using L-proline as substrate. Table 20.2 suggests the significant increase in proline content in Frankia primed tomato seedling as compared to control as an alternative strategy to tolerate PEG-mediated drought stress.

20.5.3 Determination of Malondialdehyde (MDA) Content

Lipid peroxidation was estimated by measuring the concentration of TBARS by following Heath and Packer (1968) method. The fresh leaf was ground in liquid nitrogen and homogenized in 4 mL of 0.25% TBA in 10% TCA. The mixture was heated at 100 °C in a hot water bath for 25 min, after that the reaction was terminated in an ice bath and allowed to cool to room temperature. The mixture was centrifuged at 12,000 rpm for 15 min and absorbance of the supernatant was measured at 532 and 600 nm. The level of lipid peroxidation was expressed as 1 mol of MDA formed g^{-1} FW using an extinction coefficient of MDA-TBA complex (155 mM^{-1} cm^{-1}). Our results indicate higher MDA content in Frankia primed tomato plants as compared to control that may help to tolerate PEG-mediated drought stress (Table 20.2).

20.5.4 Estimation of Total Phenol Content

Total phenol content was estimated by using Folin–Ciocalteu method (Ainsworth and Gillespie 2007) with slight changes. The fresh leaf samples (100 mg) were homogenized in liquid nitrogen and extracted with 2 mL of ice cold 95% (v/v) methanol followed by incubation in dark for 48 h. The methanolic extract was centrifuged at 12,000 rpm for 10 min and the supernatant (100 μL) was mixed with 200 μL of 10% (v/v) Folin–Ciocalteu reagent and 800 μL of 70 mM Na_2CO_3. The reaction assay tubes were incubated at room temperature for 2 h and absorbance was measured at 765 nm. Total phenol content (mM g^{-1} FW) was calculated from a standard curve made by using gallic acid as substrate. Our results clearly indicate that the total phenol content was significantly higher in Frankia primed tomato as compared to control that speculate the role of Frankia in providing antioxidant activity under PEG-mediated drought stress tolerance (Table 20.2).

Our results clearly suggest that Frankia significantly improved the seed growth and germination and physiochemical responses of the tomato plant grown under PEG 6000-mediated drought stress. The degree of tolerance of PEG 6000-mediated drought stress is significantly increased after bio-inoculation of Frankia primed tomato seedlings as compared to control. In our study, the degree of tolerance of PEG 6000-mediated drought stress up to 20% clearly suggests the potential role of Frankia. Since, the low availability of water in high-altitude Himalayan border regions is facing serious drought conditions that cause loss of plant productivity. Therefore, this successful approach may be adopted with other crops to overcome the problem of drought stress in the high-altitude marginal degraded lands of Himalayan border area not only for improving plant productivity but also to mitigate the harmful effect of chemical fertilizers for sustainable agriculture. Nowadays efforts are being made to develop various biofertilizers based on potential N-fixing PGPRs and some products are already commercialized in the market (Table 20.3).

20.6 Conclusion

The use of N-fixing growth promoting rhizobacteria (PGPR) in place of agrochemicals is a promising approach to increase the plant productivity. Since, it will naturally increase nitrogen content of soil and also not leads to deterioration of the soil micro-flora and environment, maintaining fertility as a replacement of agrochemicals (pesticides and fertilizers) and essential to explore alternative integrated nutrient-management (INM) approaches with minimum or zero risk of soil and environmental degradation. This present research reports the isolation and characterization of promising indigenous *Frankia sp.* strains from the root nodules of Casuarina plant of Kumaon region of Uttarakhand, India to explore its potential to improve PEG-mediated drought stress tolerance in tomato (*Solanum lycopersicum* L. cv. Pusa ruby) for improving sustainable hill agriculture in rain fed conditions. This successful model could be applied for improving the productivity of other crops under drought stress.

Table 20.3 List of commercial biofertilizers

<table>
<tr><th>Crops</th><th>Microorganism</th><th>Biofertilizer name</th><th>Picture of products</th><th>Dose concentration</th></tr>
<tr><td>Pulses</td><td>Frankia</td><td>N-fixer, IFGTB, Coimbatore</td><td></td><td>5 mL/ seedling</td></tr>
<tr><td>Casuarina and tomato</td><td>Azotobacter</td><td>Sashant biofertilizer, Jaysingpur, Maharashtra</td><td>
</td><td>1 kg/500 mL in 50 L of water</td></tr>
<tr><td>Jowar, bajra, wheat, maize, paddy, cotton, sugarcane, vegetables, fruit trees</td><td>Azospirillum</td><td>Maharashtra bio fertilizers India Pvt. Ltd., Latur</td><td>
</td><td>1 kg/ 25–30 kg seeds</td></tr>
<tr><td>Leguminous crops like soybean, groundnut, gram, etc.</td><td>Rhizobium</td><td>Maharashtra bio fertilizers India Pvt. Ltd., Latur</td><td>
</td><td>4 g/kg of seeds</td></tr>
<tr><td>Groundnut soyabeans, subabul, shisam, shinsh</td><td>Nitrogen biofertilizer</td><td>National fertilizer Ltd., New Delhi</td><td>
</td><td>50–300 kg/ ha</td></tr>
</table>

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Preservation of Fungal Culture with Special Reference to Mineral Oil Preservation 21

Pooja Kannojia, Abhijeet S. Kashyap, Nazia Manzar, Divya Srivastava, Udai B. Singh, Sushil K. Sharma, and Pawan K. Sharma

Abstract

It is very important to preserve fungi so that they can be studied and utilized in future. Fungal resource centres play a key role in conservation of fungi for research pertaining to diversity, taxonomy, epidemiology, biotechnology, biosafety, biosecurity and IPR issues. Preserved cultures of fungal strains are utilized by all stakeholders associated with agriculture, pharmaceutical, brewery and industry for developing new technologies and products for all the sections of the society. Various preservation methods of fungi are given in this chapter with special reference to mineral oil preservation method which is easy, simple and cost-effective as it does not require any sophisticated tool and material. This is the simplest method for preservation of both sporulating- and non-sporulating fungi in any small laboratory and small-scale industry where infrastructure is less.

Keywords

Fungi · Biosafety · Mineral soil preservation · Culture collections · Microorganisms

21.1 Introduction

Fungi, integral component of the Earth, are associated with various ecosystem functions and services. Over the years, our understanding about fungi has developed for their utilization as biological 'tools' in biotechnology. Fungal-based fertilizers,

P. Kannojia
National Centre of Organic Farming, Ghaziabad, Uttar Pradesh, India

A. S. Kashyap · N. Manzar · D. Srivastava · U. B. Singh · S. K. Sharma · P. K. Sharma (✉)
ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau Nath Bhanjan, Uttar Pradesh, India

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growth stimulators and bioinsecticides have the potential to enhance production and to ameliorate barren and degraded lands. In medical sciences, novel pharmaceutical products, blood proteins, hormones, interferon, cell growth stimulators, insulin, therapeutic products, etc. are being produced by employing fungi and other microbes (Blanch et al. 1985). Gases, power-alcohols, petroleum substitutes and other renewable energy sources can be produced using fungi. Recycling processes relying on fungal based biotechnology are cost-effective by means of disposing effluent (Robinson and Howell 1985). Besides, authentic and viable fungal cultures are needed to use as reference for identification of human, plant and animal pathogens and also for carrying out taxonomic and ecological studies. Scientific development in the field of mycology is incumbent upon the availability of authentic cultures defined in various publications and patent applications for independent study. Fungal studies are based on the availability of quality cultures that are well defined and this can be taken care of by their long-term storage. That is why culture collections are important for the preservation of diverse fungi and also serve as a source from which material can be had for teaching, research and other purposes (Onions 1971). The upkeep of fungal cultures in collections is a tardy task. It is advantageous to have a procedure that ensures the viability of valuable strains. One of the methods which is very simple and widely used for extending the ordinary stock cultures is mineral oil method (Buell and Weston 1947; Hartsell 1953). It is, therefore, of paramount importance to preserve cultures of high quality for use in agriculture, medicine, industry, etc. and also in the area of taxonomy (Malik and Claus 1987). This chapter will discuss about fungal culture collection or resource centres as well as methods for preservation of fungi.

21.2 Fungal Culture Collections

Fungal culture collections have an important place in microbiology and biotechnology. Their primary role is to preserve cultures and competent to provide information on the availability, identification, maintenance, nomenclature of cultures and regulations pertaining to transportation and patent regulations. Specialized culture collections give advice and provide services to industry. To characterize and identify fungal cultures is a time-consuming task. These cultures are invaluable and should be preserved and deposited at Culture Collection Centres at national and international level (Tables 21.1 and 21.2). At these centres, the experts properly maintain the cultures that are subjected to rigorous rules and regulations to safeguard the intellectual property rights of the depositors. The culture can be procured from these centres on payment. Therefore, these cultures are very valuable from commercial point of view (Anonymous 2008). As per World Data Centre for Microorganisms, total 3,119, 654 microorganisms comprising1,341,588 bacteria, 824,696 fungi, 38,622 viruses and 32,220 cell line are preserved in 781 culture collections in 76 countries and regions are registered in CCINFO (http://www.wfcc.info/ccinfo/. Accessed as of 13th June, 2019).

Table 21.1 National status of fungal culture collections: 15 out of 32 culture collections in India are fungal collection centers

Culture collection center	No. of fungal strains
1. Culture Collection, Department of Microbiology, Bose Institute	40
2. Culture Collection, Microbiology and Cell Biology Laboratory, Indian Institute of Science	78
3. Food and Fermentation Technology Division, University of Mumbai	20
4. Goa University, Fungus Culture Collection and Research Unit	1000
5. Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute	3800
6. MACS Collection of Microorganisms, Agharkar Research Institute	08
7. Microbial Culture Collection, National Centre for Cell Science	15,338
8. Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH)	1245
9. National Agriculturally Important Microbial Culture Collection, National Bureau of Agriculturally Important Microorganisms (NBAIM), Indian Council of Agricultural Research (ICAR), Kushmaur, Uttar Pradesh	3828
10. National Collection of Dairy Cultures, National Dairy Research Institute (Karnal)	15
11. National Collection of Industrial Microorganisms National Chemical Laboratory (NCL) (CSIR)	950
12. National Fungal Culture Collection of India MACS' Agharkar Research Institute, Pune	3050
13. NII Microbial Culture Collection NIICC National Institute for Interdisciplinary Science and Technology (CSIR) Trivandrum, Kerala	78
14. North Maharashtra Microbial Culture Collection Centre, Jalgaon	
15. Fungal Culture Collection, New Delhi	60

Status of Fungal Culture Collection Centers. (http://www.wfcc.info/ccinfo/collection/by_country/i/ dated 10/04/2019)

21.3 Preservation Techniques

Various techniques are available to preserve microorganisms (Table 21.3). These can be classified into three categories:

1. Continuous growth.
2. Dehydration.
3. Frozen storage.

The preservation methods aim to keep the viability and genetic stability of the culture through reduction in metabolic rate of microbes. This helps to prolong the period between subcultures. Continuous growth is achieved through various

Table 21.2 Global status of fungal culture collections: 4 out of 5 regions in world have fungal culture collections as follows

Name	Acronym	Country
I. Africa		
1. National Collections of Fungi: Culture Collection	PPRI	South Africa
2. Suez Canal University Fungarium	SCUF	Egypt
II. America		
3. ARS Collection of Entomopathogenic Fungi	ARSEF	U.S.A.
4. Culture Collection of Fungal Pathogens Strains from the Basic Mycology Laboratory of the Department of Microbiology and Parasitology, Faculty of Medicine, UNAM	BMFM-UNAM	Mexico
5. Entomopathogenic Fungal Culture Collection of Argentina	CEP	Argentina
6. Invertebrate-Associated Fungal Collection of Embrapa	CG	Brazil
7. Chilean Fungal Collection	CHFC-EA	Chile
8. Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes (Colecao de Culturas de Fungos Fitopatogenicos Prof. Maria Menezes	CMM	Brazil
9. Canadian Collection of Fungal Cultures	DAOMC	Canada
10. Culture collection of biomedical interest fungal	DMic	Argentina
11. Fungal Genetics Stock Center	FGSC	U.S.A.
12. Colecao de Culturas de Fungos Filamentosos	Fiocruz/CCFF	Brazil
13. Coleção de Fungos da Amazônia	Fiocruz/CFAM	Brazil
14. Colecao de Fungos Patogenicos	Fiocruz/CFP	Brazil
15. Coleção de Fungos de Referência em Vigilância Sanitária	Fiocruz/CFRVS	Brazil
16. FUNCTIONAL FUNGI	FUNCTIONAL FUNGI	U.S.A.
17. IIB-INTECH Collection of Fungal Cultures	ICFA	Argentina
18. Pathogen Fungi and Actinomycetes Collection	INDRE	Mexico
19. INIFAT Fungus Collection	INIFAT	Cuba
20. International Culture Collection of Arbuscular Mycorrhizal Fungi	INVAM	U.S.A.
21. Culture Collection of Histoplasma capsulatum Strains from the Fungal Immunology Laboratory of the Department of Microbiology and Parasitology, Faculty of Medicine, UNAM	LIH-UNAM	Mexico
22. The Fungus Culture Collection of the Northern Forestry Centre	NoF	Canada
23. UAMH Center for Global Microfungal Biodiversity	UAMH	Canada
III. Asia		
24. Center for Fungal Genetic Resources	CFGR	Rep. of Korea
25. First fungal culture bank of Pakistan	FCBP	Pakistan
26. Fungal Molecular Biology Laboratory Culture Collection University of Agriculture, Faisalabad	FMB-CC-UAF	Pakistan

(continued)

Table 21.2 (continued)

Name	Acronym	Country
27. Goa University Fungus Culture Collection and Research Unit	GFCC	India
28. Iranian Fungal Culture Collection	IRAN	Iran
29. Kasetsart University Fungus Collection, Department of Plant Pathology, Faculty of Agriculture	KUFC	Thailand
30. Mongolian Cultur Collection of Fungal	MCCF	Mongolia
31. mycolo fungal culture collection	mccf	Israel
32. Marine-derived Fungi Collected from South China Sea	MFCSCS	China
33. National Culture Collection of Pathogenic fungi	NCCPF	India
34. National Fungal Culture Collection of India	NFCCI	India
35. National Fungal Culture Collection of Pakistan	NFCCP	Pakistan
36. Research Center on Entomogenous Fungi	RCEF	China
37. Fungal Pathogens of Hevea Rubber in Sri Lanka	RRIASR	Sri Lanka
38. Fungal isolates	RRISL	Sri Lanka
39. Fungal Culture Collection	TAUFCC	Israel
40. Fungal Culture Collection	VPCI	India
41. Yew Endophytic Fungus	YEF	Iran
IV. Europe		
42. ATHens University Mycetotheca—Culture Collection of Fungi	ATHUM	Greece (Hellenic Rep.)
43. BCCM/IHEM Fungi collection: Human and Animal Health	BCCM/IHEM	Belgium
44. Centraalbureau voor Schimmelcultures, Filamentous fungi and Yeast Collection	CBS	Netherlands
45. Culture Collection of Fungi	CCF	Czech
46. EX Culture Collection of extremophilic fungi	EX	Slovenia
47. Culture Collection of Fungi at Kyiv University	FCKU	Ukraine
48. Fungal Cultures University of Goteborg	FCUG	Sweden
49. IBT Culture Collection of Fungi	IBT	Denmark
50. Fungal Strain Collection, Laboratory of Cryptogamy	LCP	France
51. Belgian Coordinated Collections of Microorganisms/MUCL Agro-food and Environmental Fungal Collection	MUCL	Belgium
52. National Collection of Pathogenic Fungi	NCPF	U.K.
53. Swiss Collection of Arbuscular Mycorrhizal fungi	SAF	Switzerland
54. Tartu Fungal Culture Collection	TFC	Estonia
55. UOA/HCPF University of Athens/Hellenic Collection of Pathogenic Fungi	UOA/HCPF	Greece (Hellenic Republic)
56. Uppsala University Culture Collection of Fungi	UPSC	Sweden

Status of Fungal Culture Collection Centers. (http://www.wfcc.info/ccinfo/collection/by_country/i/ dated 10/04/2019)

Table 21.3 Methods of preservation of fungal cultures

Method of preservation	Preservation procedure
Periodic transfer	Transfer of microbial culture to new media at periodic interval depend on several factors viz. medium used, periodicity of transfer, temperature at which cultures are stored. These factors determine the rate of mutation and appearance of variants
Mineral oil slant	The fungal culture slant is immersed in sterilized mineral oil and stored at low temperature
Distilled water or water agar	Water is used to preserve cultures under refrigeration. This helps to maintain the viability of cultures for three to five months or longer
Freezing in growth media	Microbial structures can be damaged by freezing cultures in growth media and, therefore is not a good storage method. However, it can be used for maintenance of cultures
Drying	Sterile filter paper disks, sterile soil or gelatin drops are used to dry microbial cultures which can be frozen or stored at low temperature to enhance their viability
Lyophilization	The water content in spore-forming fungal cultures is reduced by sublimation in the presence of cryoprotectant. The cultures are sealed in an ampoule. They can remain viable for up to 30 years
Ultrafreezing	Fungal cultures can be stored in vapour phase of liquid nitrogen at −156 °C. By this method, the viability of cultures can be maintained for more than 15 years

techniques that permit the fungi to grow and metabolize during period of storage. Various elements, namely, controlling growth conditions by restricting carbon, nitrogen and energy sources, decreasing the temperature, preventing dehydration, etc. step up the time period between subcultures (Anonymous 2008). Fungi can also be preserved by dehydration or drying techniques that comprise air-drying, drying in a vacuum either from the liquid or frozen phase, desiccation in or above a desiccant. In frozen storage, the fungal culture is stored at a temperature which freezes the culture, its metabolic rate is reduced and there is no physical change in it. The success of the preservation depends on medium and cultivation method used and the age of the culture at the time of preservation. There are two preservation methods: Short term and long term. Short-term methods of preservation are serial transfer of fungi to fresh medium followed by low temperature storage and keeping spores in dry sterile soil. Long-term methods include freeze-drying or ultra-freezing in vapour phase liquid nitrogen (−156 °C). There is no one method that can be used to preserve all types of fungi (Nakasone et al. 2004). Different fungal taxonomic groups react differently to different methods of preservation. Biological properties of fungi and their reaction to changes in their environment determine the type of methods for success of their preservation (Fennell 1960; Lloyd 1994; Simione and Brown 1991).

Different preservation methods withhold availability of nutrients, water and oxygen to fungi leading to reduction in their metabolic rate. This is achieved by reduction in storage temperature or by a combination of these. The method of preservation to be used is determined by a number of factors, viz. nature of fungi, objective of preservation, availability of equipment and skilled manpower, probable preservation time frame, culture number and their use in future, ease of carrying them, frequency of use of cultures and keeping costs (Collee et al. 1996). All preservation methods have more or less similar protocol with distinct stages, viz. checking culture purity, preparing ampoules, growing the culture, cells suspension in preservation medium, putting cell suspension into ampoules, preservation, storage of ampoule stocks, updation of ampule stock records and testing viability, purity and genetic stability of preserved culture stocks.

21.3.1 Serial Transfer

Fungal cultures can be maintained by periodic transfer on fresh and sterile medium. Alternate cycles of active growth and storage periods are achieved by a series of subcultures. Periodicity of transfer differs with the kind of fungi.

21.3.2 Storage in Soil

Dry, sterile soil or sand is a very good medium for storage of some fungi for many years. This method is cheap and suitable for fungi such as *Rhizoctonia* (Sneh et al. 1991), *Septoria* (Shearer et al. 1974) and *Pseudocercosporella* (Reinecke and Fokkema 1979). Dryness induces dormancy over a period of time. Changes in morphology of some fungi due to it have been reported. The available moisture is used by the fungi during their growth and then they reach dormant state. The bottles are kept in refrigerator. The culture can be regrown by putting few soil particles on a suitable medium.

21.3.3 Storage in Silica Gel

Perkins (1962) developed this method for preservation of *Neurospora* species. He observed that viability of sporulating fungi can be maintained by using skimmed milk to protect them and then storing on silica gel. Silica gel powder can be used to store bacteria and yeast for a period of 1–2 years at low temperature. In this method, fine powder of silica is made followed by heat sterilization, cooling and then mixing with a thick suspension of cells and storing at a low temperature. This technique relies on quick desiccation at low temperature, and this helps to keep the cells viable for a long period.

21.3.4 Liquid Nitrogen

Liquid nitrogen can be used to preserve many fungi. The fungi which are not amenable to lyophilization can be preserved in liquid nitrogen. It is a costly method of preservation because continuous supply of liquid nitrogen has to be maintained. Dictyostelids (Raper 1984), amoebae (Davis 1956; Evans et al. 1982), zygomycetes including Entomophthorales (Humber 1994), oomycetes (Nishi and Nakagiri 1991), phytopathogenic fungi (Dahmen et al. 1983) and yeasts (Kirsop 1991) can be preserved using liquid nitrogen. Cell division and metabolic rate determines the mutation rate of cultured fungi. Therefore, any storage method that prevents division of cells and slows down metabolism while retaining viability is considered as the best method. Freezing fungal cultures at or below −139 °C prevents growth of ice crystals and slows down biophysical processes considerably, thereby ensuring cell survival.

21.3.5 Storage by Freeze-Drying

Spore-forming fungal cultures can be preserved by freeze-drying or lyophilization. In this method, cultures are rapidly frozen and a cryoprotectant is added to dissolve ice crystals and minimize their growth. Skimmed milk powder (sterile 5% or 10% solution) and filter-sterilized bovine serum are the commonly used cryoprotectants besides the proteinaceous materials.

21.4 Mineral Oil Overlay

A mineral oil is a petroleum distillate and contains a light mixture of higher alkanes. It is colourless and odourless. Mineral oil is also obtained as a liquid by-product while refining crude oil to make gasoline and other petroleum products. This liquid by-product is transparent, colourless and contains alkanes and cycloalkanes, related to petroleum jelly. It has a density of around 0.8 g/cm^3. It is also known as liquid paraffin and white oil.

This method is simple, cost-effective and preserves fungal cultures for long duration time at ambient temperature (Fig. 21.1). Slant culture of microbes is immersed in mineral oil and the tubes are stored in upright position at room temperature.

Points to be taken into account while preserving the microbial cultures in oil**:**

1. The medium dries out and separates from the tube wall and floats to the wall surface if the medium is not completely immersed in oil leading to death of fungal culture.
2. The oil must be of good quality. The fungi are harmed if it is rancid or contains any toxic substance.

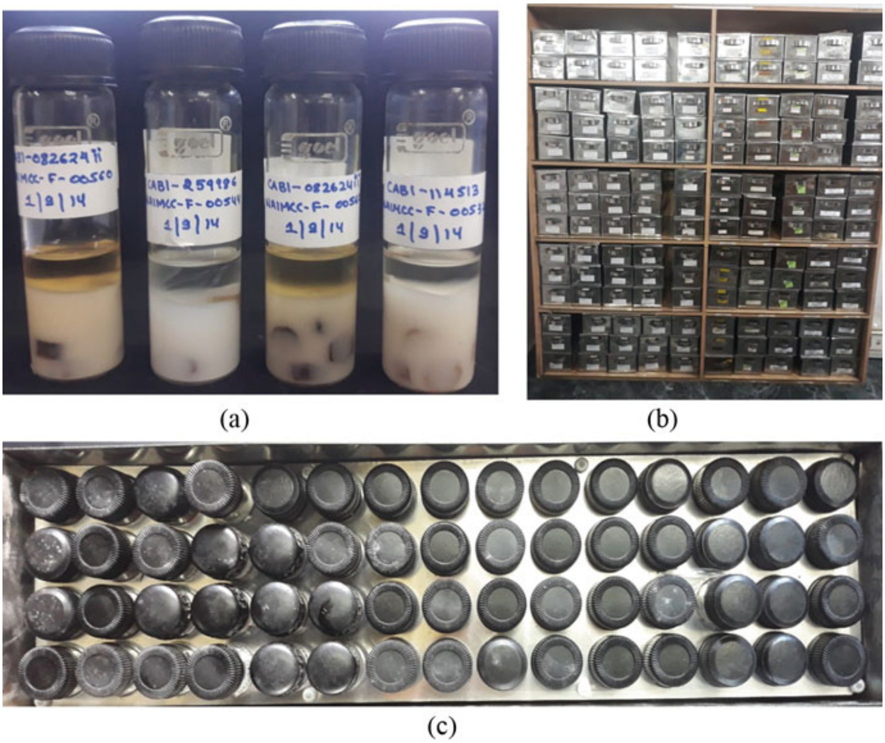

Fig. 21.1 Mineral oil storage of fungal cultures at NAIMCC, ICAR-NBAIM, Maunath Bhanjan, U.P. (**a**) Tubes containing fungal culture in mineral oil; (**b**) Rack to place the box in store room; (**c**) Box containing tubes

3. During autoclaving, the oil becomes milky due to mixing of moisture with it. Therefore, it is advisable that the oil be sterilized in the hot air oven at 150–170 °C for 1 h to remove milkiness.

21.4.1 History of Mineral Oil Preservation

Professor Frantisek Kral (1846–1911) of Prague was the first person to understand the importance of Culture Collections. He collected cultures and made these available to other workers by charging a fee. Professor Ernst Pribram later shifted this Collection to the University of Vienna in 1915. The other very old Collection, Centraalbureau voor Schimmelcultures (CBS), was founded in 1906 and is still in existence at Baarn, the Netherlands (Malik and Claus 1987). Mineral oil was used to preserved bacterial cultures about 100 years ago by Ungermann in 1918. He conserved bacterial cultures in dilute sera by overlaying them with oil. M. Michelle in (1921) used this method to preserve *gonococci, meningococci* and *pneumococci* in broth (Krasilnikov 1967). He slightly modified the method by using solid media. Morton and Pulaski (1938) used this method to maintain 45 cultures of bacteria.

He compared this method with other storage methods and found mineral oil method to be useful to maintain viability of bacterial cultures for longer duration (Uzunova-Doneva and Donev 2005; Hartsell 1953).

Sherf (1943) used this method to conserve filamentous fungi, viz. *Fusarium* and *Alternaria,* and found cultures to be viable even after 6 months. In Australia, Norris (1944) preserved fungi by the said method and published a note on reporting cultures of seven genera of plant pathogens still viable after 18 months under oil (Hartsell 1956).

There are reports mentioning that fungi depending on their properties can be conserved without cultivation for 1–12 years under Vaseline oil. Optimal time limits have been worked out for cultivation of different taxonomical groups (Uzunova-Doneva and Donev 2005).

21.4.2 Process of Preservation of Fungi in Mineral Oil

1. The culture slants in glass tubes should be fresh and vigorously grown.
2. Take good quality mineral oil of low specific gravity, i.e. 0.830–0.890 g/cm^3.
3. Heavy mineral oil should be autoclaved twice. First autoclaving may cause activation of bacterial spores followed by keeping the vials at room temperature for 24–48 h for their germination. Second round of autoclaving would kill germinated spores presented in oil.
4. Mineral oil is dried in oven at 170 °C for 1–2 h to remove entrapped moisture. It is an important step to remove water molecules from oil water.
5. The culture slant should be covered with sterile oil up to the depth of 1 cm.
6. Tightly cap tubes and put paraffin film to act as a vapour barrier.
7. Tubes should be stored in upright position. The cultures remain viable for a longer time if stored at low temperature as compared to ambient temperature.
8. Periodically check oil level in the tubes and add oil if need be.

21.4.3 Process of Culture Revival

1. Remove a part of culture immersed in mineral oil with a sterile needle/loop.
2. Excess oil should be drained from the explants by keeping on sterile filter paper and then placing it on fresh medium.
3. Afterwards, the tube should be resealed and returned for long-term storage.
4. Cultures should be monitored for viability and contamination.
5. The culture may be required to be subcultured several times to remove oil from it.
   Mode of action of mineral oil preservation.

The mode of action of mineral oil is yet to be fully unravelled. The evidence points to its action via checking dehydration of the cultures, retarding metabolic activity and growth of the fungi and by slowing down the gases exchange within fungi and surrounding (Table 21.4).

Table 21.4 Maximum survival time of *Aspergillus* species under mineral oil (Christina 1989)

Name of *Aspergillus* species	Duration of survival (years)
Aspergillus fumigatus	11.9
A. niger	11.9
A. candidus	11.8
A. flavus	11.8
A. clavatus	6.8
A. versicolor	11.9
A. sydowii	11.7
A. nidulans	11.8
A. unguis	11.10
A. melleus	13.2
A. ustus	11.6
A. terreus	11.9

21.4.4 Advantage and Disadvantage of Mineral Oil Preservation

The mineral oil method has merits such as:

1. The procedure is simple.
2. Preserved cultures can be easily transferred.
3. The medium does not dry due to presence of paraffin oil.
4. All fungi including non-sporulating ones can be preserved.
5. The cultures do not get contaminated with mites.
6. Cultures remain in dormant state due to aerobic conditions.
7. Is useful for laboratories with limited amenities.

The mineral oil method has some disadvantages also

1. Changes in characterization of fungal cultures still occur due to growth of fungi and there can be selection of mutants capable of growing under adverse conditions.
2. Slow growing on retrieval.
3. Contamination of microbial spores from air.

21.5 Conclusion

Utmost care has to be exercised while preserving and maintaining fungal cultures in fungal culture collections. A number of fungal collections are available in India and abroad. Quality control is also important so that the revived cultures are true to type when compared with the original cultures. One has to be conversant with various methods of preservation so that suitable preservation method may be chosen keeping in view the characteristics and requirements of the microorganisms. Various factors, namely, the strain, culture medium, storage temperature, the sub-culturing medium, and the periodicity and technique of transfer determine viability duration and the maintenance of cultural characteristics of the strain. Of these methods, the mineral oil method appears to be an easy, convenient, economical and effective tool for the preservation of fungal strains for their long-term preservation under low temperature.

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22 Applications of Advanced Omics Technology for Harnessing the High Altitude Agriculture Production

Apoorv Tiwari and Gohar Taj

Abstract

The farmers of hill regions are mostly trivial farmers, practicing little external effort based production system. Agriculture, livestock, horticulture, and forestry collectively with animal husbandry provide opportunities for best possible consumption of the resources available in the system. Microorganisms are fundamental components to maintain the ecological integrity of any ecosystem, and the identification of native microorganisms as potent bioinoculants for plant growth promotion will definitely increase the production of agriculture products. Omics tools are capable to enhancing the nutritional quality of crops; growing agricultural production with a significant function in microbial-plant association by understanding the genomic secrets of microbes which significantly affecting the growth of agricultural economics. Genomics, transcriptomics, proteomics, and metabolomics, the major branches of omics, along with the microbiology are widely used to understand the complexities of microbial genomes, and these combined approaches are explored for high altitude regions also to produce resistant and improved quality crops but still reveal a high nutritional worth. Systems biology approach of omics enables to understand the multifarious interactions between genes, proteins, and metabolites within the expected phenotype. These approaches are a set of bioinformatics and computational analysis as well as chemical analytical methods including many more disciplines of biology

A. Tiwari
Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Department of Computational Biology and Bioinformatics, Jacob Institute of Biotechnology and Bio-Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

G. Taj (✉)
Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

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for betterment of higher-altitude socioeconomic condition by producing quality agriproducts. Omics can allow advanced development of agricultural research for food, well-being, energy, feedstock, and chemicals while helping to protect, improve, and remediate the environment of high altitude regions.

Keywords
Omics · DNA · RNA · Bioinformatics · Genomics · Proteomics

22.1 Introduction

Microorganisms are one of the architects of the natural world—able, despite their minute size, to "build" massive mountains. It is notified by several studies that taxon richness and evolutionary diversity decline monotonically from the lowest to highest altitude while plants followed a unimodal prototype, with a height in richness and evolutionary diversity at mid-elevations. At all altitude, microbial community had a propensity to be evolutionarily clustered and holding closely linked taxa. The microbial ancestry were not arbitrarily distributed, but rather revealed spatial pattern across the gradient, while plant ancestry did not exhibit a significant evolutionary signal. Putting a figure on the influence of model scale in inter-taxonomic assessment what's left a challenge (Bryant et al. 2008). Each year, hundreds of millions of metric tons of dust, water, and pollutants made by humans create their mode into the atmosphere, repeatedly travelling between continents on jet streams. It was confirmed by a new study that some microorganisms put together the trip, seeding the skies with many bacteria and potentially affecting the weather. What's additional, some of these high-flying organisms may actually be able to feed while travelling through the clouds, figure out an active ecosystem over the surface of the Earth. Microbiology is the investigation of tiny living beings, for example, microscopic organisms, archaea, parasites, and protozoa. This order incorporates key research on the organic chemistry, physiology, cell science, nature, advancement and clinical parts of microorganisms, including the host reaction (Gupta et al. 2015). The major difference between information produced in epigenomics study and expression study is the utility of the data of test area in the genome. It is recently suggested that all the biological process are dependent on the geometry of little atoms and the atomic proteomics states about the protein mass, atomic nature of a protein, and so forth (Deans and Maggert 2015).

22.2 Microbial Genomics for the Improvement of Natural Product Discovery

The breakthrough of new natural products has reached a fresh era by way of massive computational and genetic data being produced which is now accessible. This data was used to detect biosynthetic pathways for earlier unknown metabolites with the help of whole-genome sequence mining (Bachmann et al. 2014). Alternatively, genomic information has been fished out of genetic material libraries using established paradigms for secondary metabolite biosynthesis, leading to the finding of fresh natural products by isolating gene clusters for identified metabolites. New natural products were found in heterologous hosts by articulating genetic data from uncultured organisms or strains that are hard to influence (Liujie et al. 2019). In addition, precision in heterologous expression not only helped to recognize clusters of genes, but also facilitated the manipulation of these genes to produce new compounds. Finally an important phase of the effective and successful use of abundant genetic information, new enzyme chemistry remains to be found and helped us to understand how natural products are de novo biosynthesized and allowed us to research on the present paradigms for biosynthesis of natural products (Nidhi and Yi 2014).

22.2.1 Omics Approach for Biodiscovery of Microbial Natural Products in Antibiotic Resistance Era

The requirement for powerful antibiotics pipeline is the need of hour to stand up against resistant pathogens and has turned into a noteworthy worldwide worry for human well-being. To stand up with these situations, there is a requirement for revelation and advancement of novel set of antibiotics (Prestinaci et al. 2015). Nature is viewed as fortune trove, and there is re-risen enthusiasm for investigating undiscovered microbes to give way of novel molecules because of their good impacts related with manufactured medications. Researchers have created numerous new systems in the course of recent years for creating various biopotential compounds. Taking edge in the progress of genomics, proteomics, molecular drug designing, pharmacophores, and target-based modelling is important for the research point of view in the higher-altitude regions microbes. These procedures have been financially feasible and furthermore demonstrated effective natural product discovery. In this chapter, it is trying to focus on the advancement in different areas like bioinformatics techniques, computational and systems biology, and medical sciences for potential applications for hill agriculture (Chandra et al. 2018) (Fig. 22.1).

22.3 Next-Generation Sequencing and Genome Assembly

The innovative growth in the computation memory storage and speed potential has fuelled with a new era of biological data analysis. Many microbial and eukaryotic genomes, including one with a draft human genome, were sequenced and raised expectations of superior regulation of microorganisms (Bansal 2005). The objectives are as wide as the advancement of balanced medications and antimicrobial specialists, improvement of new upgraded strains of bacteria for bioremediation and contamination control, improvement of superior and simple to regulate immunizations, the improvement of biomarkers discovery for different bacterial maladies, and better comprehension of host-microorganisms collaboration to avert

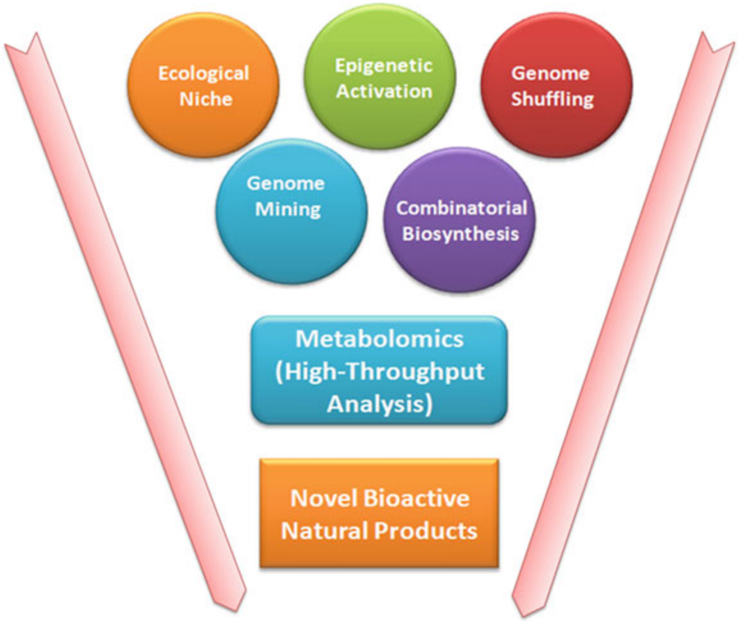

Fig. 22.1 Omics based approach for biodiscovery of microbial natural products

bacterial diseases. In the most recent decade, the advancement of numerous new bioinformatics strategies and databases has encouraged to fulfillment of these objectives (Koboldt et al. 2013).

Bioinformatics research can be done and ordered into some major categories, (1) genomics: genome sequencing and deep analysis of genomes to annotate the genes responsible for functional process, (2) proteomics: characterization of protein properties with their regulatory metabolic pathways, (3) simulation studies of cell to study behavior of cell, and (4) application to the advancement of drug design in gas antimicrobial agents. Bioinformatics research can be characterized under three noteworthy methodologies: (1) Research depends on the accessible trial of wet-lab information, (2) the utilization of numerical demonstrating using mathematical modelling to determine new biological information, and (3) a multidisciplinary approach that coordinates search strategies with scientific data (Guingab et al. 2013). Bioinformatics study has made important attempts to mechanize the genome sequencing, robotized improvement of coordinated genomics with proteomics information by computationally analyzing the genomes to characterize the gene regulatory network and metabolic pathways. Protein–protein and DNA–protein interactions, protein structure modelling and molecular docking with chemical molecules for rational design of drug, pathogenic and non-pathogenic strain assessment play a significant part in the identification of candidate genes for antimicrobial agents as well as for vaccines design and the entire genome comparison to comprehend microbial evolution (Tie et al. 2004).

The advancement of bioinformatics methods has improved the pace of natural disclosure via computerized annotation of enormous microbial genomes. This information can be utilized to comprehend cell environment at the fundamental dimension (Wise and Wanner 2011). The created bioinformatics methods can possibly encourage (1) the disclosure of reasons for illnesses, (2) antibody and judicious medication plan, and (3) improved financially savvy specialists for bioremediation by pruning out the impasses (Struk et al. 2019).

22.3.1 Functional Genomics

Whole-genome sequencing of microorganisms has become a significant way for mapping genes and QTLs (quantitative trait loci) related to important traits (Land et al. 2015). Sequencing the whole microbial genome is significant for creating precise reference genomes for related microbial genome analysis and other near genomic ponders. Dissimilar to PCR-based methodologies, cutting edge sequencing (NGS) enables microbiology specialists to grouping many living beings with the intensity of multiplexing. In contrast to conventional techniques, NGS-based microbial genome sequencing does not depend on work concentrated expensive cloning steps, sparing time and streamlining the work process (Lasken and McLean 2014). NGS can distinguish low recurrence variations and genome revisions that might be missed or are too costly to even consider through employing different strategies (Abel and Duncavage 2013).

The change in the DNA sequencing advancements has empowered all the more dominant and complete hereditary profiling of microorganisms. The sheer number of instructive loci given by genome sequencing permits the annotation and novel bits of knowledge into hereditary sources, development, and epidemiological history (Pareek et al. 2011). Microbial genomes can be sequenced as a group at high inclusion yet have related difficulties of high change rates and low coverage of genome. Thus, recognizing changes in DNA requires a nuanced approach explicit to the living being, accessibility of comparative genomes, and sorts of variety (Ozlem 2014). The high intensity of genome sequencing is used to distinguish a wide extent of polymorphism classes. For unrelated species, a framework of genome arrangement require combined techniques that can be upgraded by dynamic strategies for development (Ekblom and Wolf 2014). Polymorphism distinguishing verification relies upon genome structure, and error rates in related examples can be diminished by modifying the data. Strong variation screening encourages progressively delicate identification of population structure and a more profound comprehension of the particular procedures that shape microbial phenotypes. Genome annotation is the way towards identifying the significant highlights contained inside a genome grouping and connecting important natural data to those highlights (Mudge and Harrow 2016). Commonly one of the initial steps is to be connected in the wake of sequencing another genome by utilization of an assortment of programming devices

Table 22.1 List of genomics tools frequently used for genome analysis

S. No	Software/tool	Tool description
1.	LOVD 3.0	Free tool for gene-centered collection and display of DNA variations
2.	SpliceCenter	The tools on SpliceCenter help evaluating the impact of gene splicing variation on specific molecular biology techniques
3.	GIGA	GIGA is an efficient tree building program. It supports phylogenetic reconstruction of very large gene families
4.	Phred	Phred is a base-calling program for DNA sequence traces
5.	CodonCode Aligner	CodonCode Aligner is a program for sequence assembly, contig editing, and mutation detection
6.	DIALIGN	DIALIGN aligns multiple sequence with optional user-defined constraints
7.	Off-Spotter	Off-spotter helps in the design of optimal “guide” RNAs (gRNAs)
8.	CrispRVariants	CrispRVariants resolves and localizes individual mutant alleles with respect to the endonuclease cut site
9.	CRISPOR	CRISPOR assists with guide selection in 120 genomes, including plants and many emerging model organisms, and pre-calculated result
10.	Breaking CAS	Breaking CAS is a versatile system for detecting putative sgRNA off-targets in CRISPR/Cas applications
11.	BioGPS	Breaking CAS is a versatile system for detecting putative sgRNA off-targets in CRISPR/Cas applications
12.	Genome browser	Breaking CAS is a versatile system for detecting putative sgRNA off-targets in CRISPR/Cas applications.

and annotation procedures. A comprehension of the instruments, databases, computational strategies, and accessible pipelines used to produce genome annotation is important to evaluate their exactness and their appropriateness for downstream applications (Table 22.1) (Mungall et al. 2002).

22.3.2 Proteomics

Proteome alludes to the whole supplement of protein, communicated by a genome, cell, tissue, or life form which is examined by proteomics (Chandramouli and Qian 2009). Proteomics to propel information with regard to the area of natural biotechnology, together with the study of physiology of bacteria, digestion, and environment is broadly utilized (Lacerda and Reardon 2009). In ecological biotechnology, microbes are usually linked to their ability to catalyze methanogenesis, dehalogenation, and denitrification among others and their resilience to radiation and harmful mixture is additionally of significance. Proteomics has a significant job in revealing the pathways of cell forms and natural examples are frequently exceeding perplexing, which makes proteome ponders in this field particularly testing (Verrills 2006). A portion of these difficulties are the absence of genome groupings for most by far of ecological microscopic organisms, troubles in secluding microorganisms and proteins from specific situations, and the nearness of complex microbial networks (Table 22.2). In spite of these difficulties, proteomics offers a

Table 22.2 List of proteomics tools frequently used for proteome analysis

S. No.	Software/tool	Tool description
1.	PredictProtein	PredictProtein have several features to characterize proteins such as prediction for secondary structure, transmembrane helices, protein–protein and protein–DNA binding sites, etc.
2.	PEAKS CMD	The de novo sequencing software PEAKS can be used to study unsequenced organisms and to identify novel peptides
3.	PEAKS studio	PEAKS studio combines de novo sequencing, protein identification, multi-engine protein identification, PTM search, sequence homology searching and quantification
4.	PANTHER-PSEP	PANTHER-PSEP identifies single nucleotide polymorphism and estimates the likelihood of a particular amino acid changing coding SNP to cause a functional impact on the protein
5.	SIFT	SIFT predicts based on sequence homology and the physical properties of amino acids whether an amino acid substitution affects protein function
6.	MutationTaster	MutationTaster estimates disease-causing potential of sequence alterations
7.	MuPIT	MuPIT visualizes mutations on 3D structures
8.	LS-SNP	LS-SNP/PDB provides information useful for identifying amino acid changing SNPs (nsSNPs) that are most likely to have an impact on biological function
9.	PeCop	PSI-BLAST your sequence to find Persistently Conserved Positions
10.	Rosetta	The main features of the Rosetta Software include modelling and analysis of protein structures

unique view of cell working environment. Metaproteomics (microbial network proteomics) can possibly give bits of knowledge into the capacity of a network without secluding life forms (Hettich et al. 2013; Victor and Marc 2007).

Proteome is the entire set of proteins govern by a genome, cell, tissue and the analysis of complete arrangement of proteins created at a given time is described as 'proteomics' (Jungblut et al. 2008). In the most recent decades, portrayal and separation of microbial proteome was performed by gel-based or without gel based protein separation techniques. Various procedures for the examination of proteome exist and they can be isolated in two noteworthy gatherings: (1) spectrophotometric strategies, which contain HPLC (High-execution fluid chromatography) and LC/MS (Liquid chromatography-mass spectrometry); (2) antibodies-based techniques, which incorporate ELISA (enzyme-connected immunosorbent measure), immunoprecipitation, immunoelectrophoresis, and western blotting (Cosette et al. 2012). Even though today metaproteomics gives significant data about the bacterial biological system under thought, just the reconciliation between metaproteomics, metagenomics, metatranscriptomics, and metabolomics can develop the annotation and interpretation of microbiota, featuring its uniqueness towards the human well-being and its relationship with the host. In addition, it is of crucial significance to coordinate all these omics approaches with explicit bioinformatics methodologies that can give a unique information to the incredible measure of profitable information we get. Microbial proteomics is getting to be fundamental in giving a useful asset to translational applications, improving clinical determination and antimicrobial treatment, just as developing the portrayal of bacterial pathways and the relationships existing among microorganism and the human well-being (Chen et al. 2016).

22.3.3 Metabolomics

Microbial metabolomics establishes an incorporated part of frameworks of systems biology with considering the entire set of metabolites inside a microorganism and inspection of the results between growth and development. Metabolomics may offer a gradually accurate picture of the actual physiological condition of the microorganisms (Tang 2011). Ongoing researches of advances and post-genomic improvements empower the investigation and assessment of metabolome. This novel pledge brought about numerous logical orders consolidating metabolomics as one of their “omics” stages. Metabolomics of microorganisms can choose as points to show its effect on the comprehension to frameworks microbiology (Beger et al. 2016).

Microorganisms produce and emit numerous essential metabolites to the encompassing condition during their development (Pinu and Villas-Boas 2017). Additionally, the pledge of these metabolites is almost simpler than intracellular metabolite extraction and analysis, as there is no necessity for cell rupture. Many explanatory methods are now available and have been used throughout last two decades to annotate extracellular metabolites from microorganisms. In this chapter, we explain the function and advantages of microbial metabolite and their role for human welfare by utilizing the metabolomics information in the metabolic demonstration of various significant microorganisms (Farhana et al. 2017).

Usually, metabolomics techniques are not well prepared to test a wide assortment of situations or ecological elements but played an important role in the analysis of culture microenvironments, irregular natural solvents for metabolite confinement and microbial extracts (Barkal et al. 2016). Using Aspergillus, a parasitic family recognized for its extensive secondary digestion, the impacts of the culture geometry and development lattice on optional digestion, featuring the probable utilization of microscale frameworks to open obscure or secretive optional metabolites for characteristic items revelation (Layla et al. 2016). Microbial metabolomics establishes a coordinated part of frameworks for science. By considering the total set of metabolites inside a microorganism and checking the results in a collaborative manner, metabolomics can conceivably give an increasingly precise depiction of the genuine physiological condition of the cell (Liu et al. 2013). Ongoing progression of advances and post-genomic improvements empower the examination and investigation of metabolome. Omics stage contributed significantly in numerous logical orders joining metabolomics (Jane 2011).

22.3.4 Glycomics

Lectins are proteins other than enzymes that widely used in glycan determinants and these are showed on surface of the cell as cell grip proteins. Numerous surface lectins of microbes start host microbe or organism microorganism communications prompting commensal or infection states. In fact, lectin binding can start pathways that impact cell capacities, including intrinsic and obtained insusceptible reactions (Habibi et al. 2016; Kuhn and Vyas 2012). It is a time taking and laborious trial way from the distinguishing evidence of lectin to assurance of its role in signalling of cell, cell separation, and host communications. Initial action for recognizable proof of ligands and this progression have turned out to be substantially more orderly with glycochip innovation. In the first place, the created programming with the glycochip results to question UniCarbKB (lectins and their ligand glycans database). The database can be accessible by structures, scientific categorization, proteins, tissues, or related ailment (Diderrich et al. 2015; Donohue et al. 2011).

22.3.5 Lipidomics

"Lipidome" first appeared in the literature in 2001 is an increasing area of biomedical research involving complicated lipidome testing. A lipidome is basically a thorough and meaningful depiction of many of the lipid groups found in living beings. Lipidomics involves processes range that distinguishes and quantifies thousands of cell lipid atomic species processes and structures and their communication with other in vitro lipids, proteins, and various molecules (Wenk 2005). Lipids are tiny atoms that give periodic physical and concoction characteristics as a category important to a certain amount of metabolic regulation, from subcellular spaces to whole organ vitality monitoring and sensing. It is an increasing arena for the detection, depiction, and evaluation of lipids of systems scale in clinical research. Lipidomics has a significant function to play within metabolomics and at the cell dimension, lipidomics can constitute all lipids and their capabilities quantitatively. Lipidomics is integrated with omics approaches, which adds the ability of lipids to function and provide an essential asset in understanding the element of disease based on lipids, biomarker testing, and pharmacological control (Dennis 2009).

22.3.6 Microbiomics and Meta-Omics

People, similar to all other complex living creatures, support trillions of microbiological cells inside, and on the outside of, their bodies which they obtain from their condition directly from their time in the belly, to their entrance into their tomb (Richardson 2017). These living cells are named as our microbiota, and their totality, when found in their genotypical and phenotypical forms, is known as the microbiome. The present worldview of customized drug is vigorously dependent on the variety in human host genomics (Bordenstein and Kevin 2015). Research has demonstrated that human microbiome changes from individual to individual to a lot higher degree contrasted with the host genome, rendering it increasingly individualized. This has become causing another move in the customized drug methodology from its present concentration to a multi-omics approach, which incorporates not only microbiomics as one of its vital reinforces, yet in addition metabolomics and proteomics (Sender et al. 2016). The resultant insight in the prediction of medicine for a disease is to be seen in the years to come. Complex microbial networks are a basic piece of the earth's biological systems. Over the most recent decades, the culture-autonomous methodologies provide newer bits of knowledge for its composition and ability with tremendous growth of high-throughput sequencing bringing about comprehensively accessible devices for microbial reviews (Sender et al. 2016). However, the domain remains a lengthy way from attaining a mechanical level as both computing technologies and the phases of nucleotide processing for microbial genomic and transcriptional substances continue to improve. Current microbiome examinations are in this manner beginning to receive various and integral meta-omic approaches, prompting remarkable chances to thoroughly and precisely describe microbial networks and their communications with their surroundings and hosts. This assorted variety of accessible measures, examination techniques, and open information is starting to allow microbiome-based perceptive and demonstrating tools (Qin et al. 2010).

22.4 Machine Learning Techniques Applied for Microbial Data Analysis

Artificial intelligence is an information processing procedure that prepares computers to do what's going on for individuals and animals without any problems. AI calculations use computing approaches to genuinely "know" data from information without relying as a model on a preordained situation. Microorganisms are widespread and strongly associated with people every day. As the amount of test dataset is available for learning increments, the calculations adaptively enhance their performance (Liu et al. 2016). Since they were first found in the nineteenth century, analysts have indicated incredible enthusiasm for microorganisms. Individuals contemplated microorganisms through development; however, this strategy is costly and tedious. Notwithstanding, the development technique cannot keep up to speed with the advancement of innovation in the sequencing with high performance. To handle this problem, AI (machine learning) techniques are broadly connected with microbiology. Research surveys have demonstrated that ML (machine learning) can be utilized in numerous parts of microbiology investigation, particularly grouping issues, and for investigating the collaboration among microorganisms and their encompassing condition and thus we can abridge the utilization of ML in microbiology (Fig. 22.1).

High-throughput sequencing innovation has brought about age of an expanding measure of microbial information. Customary techniques utilizing magnifying lens and organic societies are costly and work serious; consequently, AI strategies have

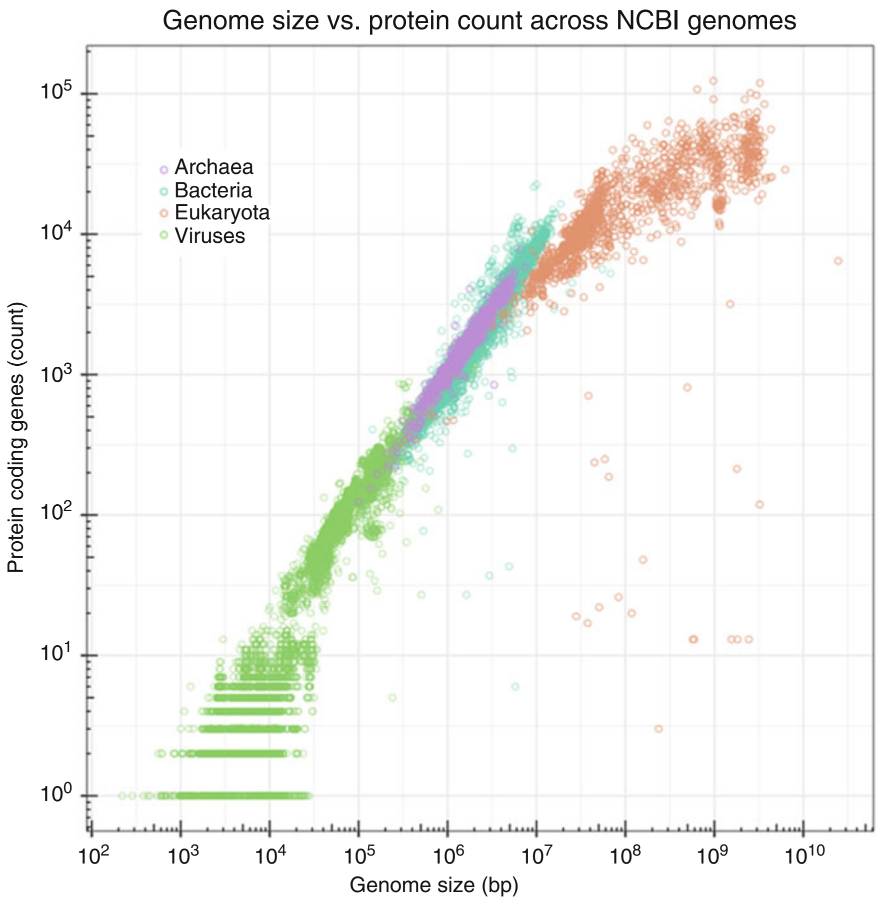

Fig. 22.2 A log-log plot of the total number of annotated proteins in genomes submitted to GenBank as a function of genome size. (Adopted from NCBI)

been step by step connected to microbial examinations (Huang et al. 2017) (Fig. 22.2). ML is broadly utilized in microbiological investigation, and that it has concentrated on analysis issues and examination of interaction data analysis. Numerous issues stay uncertain and will require the participation of scientists from various fields, for example, science, informatics, and prescription, to mutually advance the improvement and advancement of microbiological observations. Then again, the ongoing created interface expectation (Zeng et al. 2017) and computational insight strategies (Song et al. 2018; Cabarle et al. 2017) could be helpful to find the connection among maladies and microorganisms.

22.5 Applications of Bioinformatics in High Altitude Agriculture

Bioinformatics is the utilization of data innovation to oversee natural information that aids in deciphering plant genomes and yet it is emerging in each field of Science. The interdisciplinary divisions of the science made out of biological sciences, arithmetic and software engineering generates new opportunities in the biological researches. Bioinformatics has turned out as an instrument to smoothing the ways for artificial discovery with the advancement of genome projects (Victor and Casimir 2003). As it has its application in the drug by giving the genome data of different life forms, comparably the field of agribusiness has likewise take advantage of this field on the grounds that small-scale creatures assume a significant trade in farming and bioinformatics gives full genomic data of these living beings (Dennis et al. 2007). The genome sequencing of the plants and creatures has additionally given advantages to horticulture. Utilization of different bioinformatics instruments in natural research empowers stockpiling, recovery, investigation, explanation and representation of results and advances better comprehension for agriculture (Wani et al. 2018). In addition, bioinformatics is by and large alluded to as the utilization of innovation to the handling and dealing with the information produced in natural findings. The term bioinformatics was initially begotten for the utilization of data innovation to enormous volumes of natural, especially, genomic information (Dahiya and Lata 2017). The quick progression of sequencing technologies combined with a new version of bioinformatics algorithms to deal with enormous scale information analysis is giving energizing chances to researchers to comprehend microbial networks (Radford et al. 2012). Information annotation is critical for a more profound learning of organisms and their environments, and for some applications of microbiology based on understanding premise of host pathogen communications to configure compositions and create immunizations, to numerous other implementation of biotechnology, such as microbial bioremediation, barcoding, as well as bio-fuel production, etc. (Kumar and Kumar 2017) (Fig. 22.3).

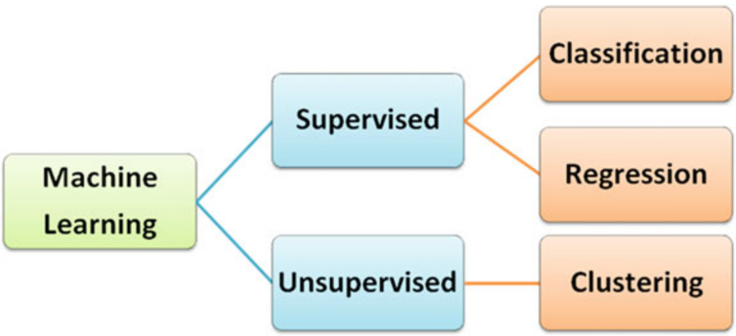

Fig. 22.3 Machine learning approaches for omics analysis of microbial genome

22.5.1 Plant-Microbe Systems Biology

Systems biology goes for an extensive and computational comprehension for annotation of biological issues. A produced model is utilized to construct new theories to be tentatively confirmed, and the tentatively ensured data expands goals of a next-generation analysis (Ukai and Ueda 2010). Plants are intently connected with microorganisms including pathogens and mutualisms that impact plant growth, development and relate with the yield attributes. Sub-atomic hereditary methodologies have revealed various signalling parts from the plants and organisms and their method of activities. Nonetheless, signalling pathways are exceedingly interrelated and affected by differing sets of natural variables. Accordingly, it is critical to have frameworks so as to comprehend the genuine idea of plant–microorganism communications. In fact, fundamental computational science methodologies have uncovered recently disregarded or confounded properties of the plant insusceptible signalling system. Latest progress in metagenomic networks furthermore focused on the importance of crops linked of frameworks methodologies and open the discovery area of microbial network recreation (Kitano 2002). The initial step of systems and computational biology is accomplished in numerous researches of plant–microorganism association by methods for useful and similar genomics utilizing model life forms (Cunnac et al. 2011 and Tsuda et al. 2009). Reconstruction of microbial networks encourages our comprehension of choice components and elements of plant root-occupying microbiota of bacteria (Bulgarelli et al. 2012). Every one of these investigations gives bases to improving future agrarian profitability and sustenance security. For example, it is a basic level ready to create synthetic aggravates with an objective of center effectors to control pathogens successfully (Kim et al. 2012 and Cunnac et al. 2011).

22.6 Future of Omics Technology for High Altitude Regions

Complex microbial networks are a vital piece of the Earth and of living beings but over the most recent two decades, culture-free methodologies have given new bits of knowledge in its composition and ability, exponentially with the diminishing expense of sequencing by high-throughput techniques bringing about comprehensively accessible apparatuses for microbial studies. Current microbiome analysis is along with these techniques beginning to receive numerous and integral meta-omic approaches, prompting to exhaustively and precisely portray microbial networks and their associations with their surroundings and hosts. Microorganisms and their biochemical exercises are a fundamental segment for all intents and biological systems on earth, forming situations going from profound hill regions to our very own body. For instance, microbial networks are in charge of half of the oxygen delivered on this planet (Rocap et al. 2003) and unpredictable microbiome of human supplements us with multiple times a greater number of qualities than those of in our genome (Qin et al. 2010).

22.7 Conclusion

Despite the fact that advances and examinations are always improving, sequencing is presently achieving development as in approved, institutionalized trial and bioinformatics strategies are accessible to respond to inquiries of biological problems. These include evaluation of microbial populations, taxonomy and evolutionary structure at a resolution stage beyond even individual marker genes, but also the quantification of biomolecular characteristics, including gene families, functions, metabolism, and structural modules. It is then possible to perform statistical methods for the discovery of biomarkers and, in some cases, prediction of phenotypes. Other meta-omic methods, such as metatranscriptomics, metaproteomics, and metabolomics, are still under fast growth, with no laboratory or simulation pipelines yet achieving a similar standardized level. These will be essential for the effective investigation of transcriptional regulation of microbial community, dynamics of metabolites, and protein signalling. The chance to incorporate and meta-analyze various information sets will be an interesting next phase in the biology of microbial system (Qin et al. 2010). The initial microarray, genome-wide association and meta-analysis attempts, systemic variations between systems and protocols of various initiatives cause powerful technical distinctions between information collections, but these are gradually resolved (Bittner 1999). Little such study has been pursued in microbial communities and the notion of "knock-out" or "knock-in" is still not well investigated. Synthetic communities provide a particularly effective way to systematically add or remove bacteria or add or remove single microbial genes. Bioinformatics is an essential key area in today's microbiological studies that includes biological resources and high-end computing abilities to unravel compressed and symmetric encryption of biological information within living beings. Combined with these all omics based advanced computational models, meta-omics in these contexts will proceed to enhance our knowledge of microbial community development technologies to enhance higher-altitude agriculture.

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Nanotechnology for Agricultural and Environmental Sustainability at Higher Altitudes

23

Mamta Bisht, Deepika Rajwar, and M. Raju

Abstract

The distribution of hill and mountainous areas in India are vastly located in the Himalayas region. In recent years, the protection of ecology in this region has become a national concern due to the fast deterioration of the ecosystem. The disturbances in climatic conditions including recurring floods, droughts, low temperature, less water content, high precipitation, and solar intensity possess non-sustainable production of agricultural crops. These ecological stress imbalances natural resource management and agricultural sustainability in this region. The major problem in hilly areas is less water storage in soil. Most of the water occur in the form of precipitation and infiltrate with the high magnitude depending upon the soil texture and degree of slopes. Therefore, the cropping productivity is low due to moisture stress and poor soil conditions. The farmers in these areas are using higher dose of fertilizers and pesticides in order to acquire higher agricultural production. It is well recorded that the excessive and improper use of fertilizers, pesticides, and toxic metals causes environmental problems. In this chapter, we have discussed the role of nanotechnology in higher altitudinal agriculture that emphasized the different application in various sectors of agriculture and environment. Nanotechnology has the ability to increase the application efficiency of pesticides at a lower dose and helps in the removal of contaminants in polluted soils/groundwater through adsorption, absorption, chemical reactions, and filtration. Thus, nanotechnology has the potential to minimize these impacts on agriculture and the environment.

M. Bisht (✉) · M. Raju
Groundwater Hydrology Division, National Institute of Hydrology, Roorkee, Uttarakhand, India

D. Rajwar
Central Soil Salinity Research Institute, Karnal, Haryana, India

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Keywords
Himalayan region · Nanotechnology · Agriculture · Environmental sustainability

23.1 Introduction

Mountains are considered to be one of the most important geographical zones in the world. They cover 24% of the earth's continental surface (Bridges 1990) and support 22% of the world's population (UNEP-WCMC 2002). In case of India, Himalayan region constitutes a geological feature embracing cultural, social, and environmental diversity. Indian Himalayan region is spread over the states of Jammu and Kashmir, Himanchal Pradesh, Nagaland, Uttarakhand, Arunachal Pradesh, Manipur, Mizoram, Sikkim, along with some parts of Uttar Pradesh and West Bengal (Sharma and Acharya 2004). The high altitudinal region shows the various geographical topography including alpine meadows, cold deserts, inter-montane valley, snowfields, deep gorges, alluvial plains, and glaciers. The variations in altitude play a crucial role in determining the climate and physiography of the region. This region shows geological instability resulting from population pressure, deforestation, landslide, soil erosion, and water scarcity. Mountain people are facing great physical demands, significant natural hazards, and constrained agricultural production. Only about 3% of land is suitable for rain-fed agriculture that can restrict the livelihood opportunities of mountain people (UNEP-WCMC 2002). Agriculture potential in mountain regions is completely dependent on Himalayan River and its environment (Dhar 1997).

The Himalayan ranges are the home of various kinds of rivers. The three perennial rivers are originated from Himalayan range including the Indus, the Ganga, and the Brahmaputra. Out of them, the Ganga basin is one of the largest basins in the world and covered by thick alluvial sediments (Sharma and Acharya 2004). These rivers carry an enormous amount of silt, debris, and fertile soil that influences agro-economy of this region. As per the estimates of IPCC (2001), the amount of total water available to Indian plains from Himalaya is $8.6 \times 106\ m^3$ per year, out of which snow in Western and Eastern Himalaya contributes to more than 60 and 10% of water, respectively (Sharma and Chettri 2005).

The mountain ecosystem exhibits environmental stresses and climatic variations including frequent floods, droughts, hypoxia, low temperature, and high solar intensity possess unsustainable production of agricultural crops. Unsustainable environmental conditions have transformed agriculture into liability of farmers (Shukla et al. 2018). Unseasonal rain and snowfall is the main reason for agriculture failure in Himalayan region. In Lahaul, a district of Himachal Pradesh, located at around 4270 m of elevation, snowfall in late September was disastrous for the summer crops such as potatoes, cauliflower, cabbage, apples, pears, plums, and cherries (Sharma and Acharya 2004). United Nations University reported that the pressure arises from development, tourism, deforestation, climate change, and pollution, and other external forces are responsible for landscape eroding in various mountain regions. Moreover, climate change affects the food production in mountainous regions. The Wildlife Institute of India (Dehradun) observed that the farmers in Pithoragarh, Uttarakhand have stopped growing 30 traditional crops, including millets and local vegetables, due to climate change and emphasizing to grow off-season vegetables to maintain farmer's livelihood.

23.2 Distribution of Himalayan Range

The term Himalaya is composed by two Sanskrit words 'hima' (snow) and 'alaya' (abode). The Indian Himalayan range (IHR) is the young fold mountain system on the Earth (Gopi et al. 2016) and extended between latitudes 26° 20′ and 35°40′ North, and between longitudes 74°50′ and 95°40′ East (Ives and Messerli 1989; Ives 2004). The geographical area of this region ranges about 530,795 km^2 and is inhabited by 31,593,100 people, representing 3.73% of the total population of India (Valdiya 1998). As reported by Geological Survey of India, glaciers in the Indian Himalaya cover around 38,000 km^2 areas. The IHR supports nearly 816 different tree species, 675 edibles plants, and around 1743 endemic types of medicinal plant (Samant et al. 1998). Generally, the IHR stretches about 2500 km from west to east and 200–400 km from south to north, which results climatic variation (Shreshtha et al. 2012). They provide territorial security over Indian subcontinent via preventing from cold and dry winds which come from Central Asia. The mean annual precipitation and temperature ranges between 150 and 4000 mm and 8–22 °C (Yadav et al. 2012). The eastern Himalayan region constitutes eight states including Arunachal Pradesh, Manipur, Assam, Mizoram, Meghalaya Nagaland, Tripura, Sikkim, and Darjeeling hills of West Bengal and western Himalayan region constitutes Jammu and Kashmir, Uttarakhand, and Himachal Pradesh (Zobel and Singh 1997). The IHR shares the international borders with China, Myanmar, Nepal, Bangladesh, and Bhutan. The Himalayas are divided into three parallel zones: (1) the Great Himalaya, (2) the Middle/Lesser Himalayas, and (3) the Sub-Himalayas foothills (Fig. 23.1).

The Great Himalaya is the highest peak and completely covered by snowy peaks with an average elevation around 20,000 ft. In southern slopes, the snow line varies from 14,700 ft (in Nepal and Sikkim) to 17,000 ft (in Punjab). It carries lots of higher peaks such as Mount Everest (29,028 ft), Kanchenjunga (28,145 ft), Nanga Parbat (26,620 ft), Nanda Devi (25,645 ft), and Namcha Barwa (25,445 ft). The maximum number of highest peaks is exhibited in Sikkim and Nepal region followed by Kumaon, Punjab, and Bhutan region. It has several ranges, namely Zanskar, Ladakh, and Karakoram ranges. This region is occupied by small and dispersed cluster settlements due to the limited development. The short growing season and extremely cold winter in IHR restrict the farmers to grow only one crop per year.

The lesser Himalayan ranges are mainly composed by rocks and altitudinal variations are laid between 12,139 and 14,763 ft. The average width of this region is nearly about 50 km. This region shows moderate population due to dense forest and fertile land. This region forms rugged mountain such as the Dhaula Dhar range, Pir Panjal and Mahabharat ranges. These ranges consist famous valley of Kashmir, the Kullu and Kangra valley of Himachal Pradesh.

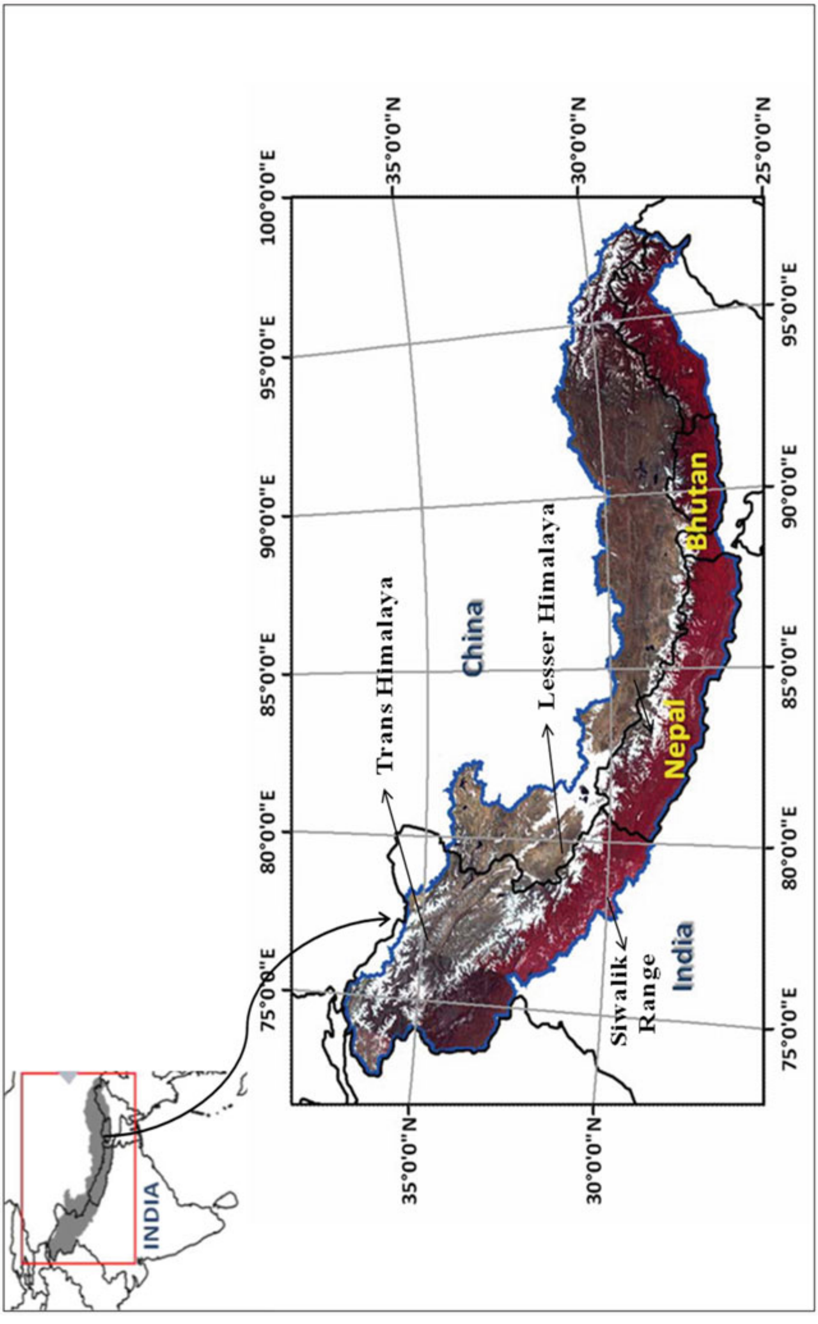

Fig. 23.1 The three parallel zones of the Himalayas

Siwalik range which forms the outermost hills represents the youngest range comprising fragile siltstones and sandstones. They intervene between the middle Himalayas and the plains of the Indus-Ganges. The width of this region is about 48.2 km in west and completely disappears in Bhutan and Assam. This region shows a remarkable feature such as "Duns." Duns are longitudinal valleys with flat bottom, occurring between the siwalik and lower Himalaya range. South facing slopes are covered with scrubs, whereas north facing slopes are covered by Sal forest. In South, the forested foothills are known as Terai and Duars that contain densely cultivated belt of agricultural land.

23.3 Agriculture at Himalayan Region

The agroclimatic conditions of the IHR for crop production are quite uncertain. A large section of Himalayan population is completely depended on primary sector such as agriculture, forestry, and livestock for sustaining their livelihood. Agriculture of this region has witnessed dynamic shifts and acute crises due to scanty water, undulating terrain, sloppy area, gravel soil, little use of modern technology, low productivity, agro-pastoral agriculture, scattered land holding capacity, etc. (Dhar 1997). Rabi season is observed from December–January to May–June, and the major crops are grown in this season, namely barley, wheat, mustered, gram, pea, and masur. Although all type of crops are grown in the entire region, their yield vary from one area to other. Kharif season is observed from June–July to October–November, and the major crops are grown in this season, namely rice and millets. Table 23.1 shows the appropriate type of farming practices are being done with respect to altitudinal division. Wide spectrums of farming systems are being practiced in IHR such as mixed cropping, crop rotation, organic fertilizer, shifting and subsistence agriculture rather than commercial value of agriculture (Tiwari 2004; Zobel and Singh 1997).

More than 90% of the farmers in the Himalayan region are small land holders, cultivating less than 1 ha of land each. In Uttarakhand, more than 75% people are engaged with the traditional and allied agricultural farming (Bahadur 2004; Singh and Singh 1992; Samal et al. 2003). Singh (2002) reported shift in the Himalayan

Table 23.1 List of altitude-specific mountain farming systems in Indian Himalayan Region (IHR)

S. No.	Altitude in above msl[a]	Farming systems
1	High altitude 2500–3500 m	Livestocks, fruits and vegetables, pseudo-cereals and medicinal and aromatic plants based
2	Mid-high altitude 1750–2500 m	Livestocks, fruits and vegetables, pseudo-cereals and medicinal and aromatic plants based
3	Middle altitude 1500–1200 m	Food crops and vegetables based
4	Low altitude 1000–1200 m	Field crops, livestock, and fruits based

[a]Mean sea level

Table 23.2 Economic arrangement in IHR (Khawas 2006)

Region	Arable land (in percent)	Sources of economic activity
Jammu and Kashmir	5	Agriculture and allied activities, forestry, fishery, livestock, manufacturing, and tourism
Himachal Pradesh	10	Agriculture and allied activities, forestry, fishery, livestock, hydropower, and tourism
Uttarakhand	14.4	Agriculture and allied activities, forest, hydropower, tea, tourism, small-scale and cottage industry
Arunachal Pradesh	2.5	Agriculture and allied activities, forest, hydropower, tourism, cottage industry, and handicrafts
Darjeeling and Sikkim	36	Agriculture and allied activity, tea, tourism, forest, and hydropower

agriculture from traditional cereal crops to commercial farming. This modification towards commercial farming is led to new challenges in Himalayan region. Table 23.2 shows the human and livestock population in relation to the available arable land in Himalayan region.

However, agriculture is the primary livelihood but agriculture land use patterns are varied from region to region. In central region and northwest region, settled and intensive agriculture practices are being dominant, whereas in northeast region Jhum cultivation is an alternative way of securing their livelihood.

23.3.1 Jhum or Shifting Cultivation

The Jhum cultivation in northeast India is vigorous in nature and is known as “slash and burning” cultivation (Verma 1998). This region is highly vulnerable to a number of biophysical, ecological, and economic problems. Rice is the main crop and other crops are finger millet, beans, banana, maize, foxtail, sweet potato, ginger, chilies, and cassava are grown in this region. In Jhum cultivation, farmers burn as well as clear the vegetation, and abandon the land to fallow and move to a new place. This fallow land leads to considerable soil erosion due to heavy intense rainfall and decreasing the fertility of soil (Tiwari 2004). When the fallow area is considered to gain fertility, the cultivators cultivate the fallow land and repeat the same process again. Ramakrishan and Saxena (2006) have observed that loss of rich biodiversity in northeast Indian region is due to large scale of deforestation and the continuing impact of Jhumming cultivation. In Sikkim, soil erosion is caused by un-terraced farming on the slopes and by canal irrigation system without protective cover to the channel. In northeast region, there are so many constraints at environmental level such as rugged terrain, steep slopes, poor soil conditions, and dry terrace, etc. These environmental constraints do not provide an ideal and suitable condition for growing crops. Less soil moisture in flat valley leads to abundant growth of weeds and insect pests which are detrimental to the domesticated crops.

23.3.2 Challenges in Himalayan Agriculture

The diversification is found in mountain farming but the fragility of mountainous region does not permit intensive agricultural practices. The following circumstances have been observed to be generally responsible for challenging agriculture activities in IHR.

- Deforestation and forest fire is the other problem to loss of vegetation and wildlife biodiversity and generate a path for secondary problems such as erosion, landslide, and poor crop production.
- Soil erosion which prevents crop production is a natural process and gets accelerated under influence of man-induced disturbances.
- Hydrological imbalances are currently observed in Himalayan region, which is considered the major cause of vegetation loss.
- Inherent inefficiency of traditional methods of farming due to increase in the population density along with needs (ICIMOD 1996).
- Expension of pine forests are the biggest problem which leads to diminishment of the groundwater recharge and discharge of springs.
- Technologies developed for plain crops are implemented into mountain agriculture without appropriate modification.
- Lack of complementary and coordinate efforts for research and development.
- Lack of varietal improvement in crops, restoration of degraded land, water resources management, soil conservation, and processing of foods and vegetables.

To overcome all these circumstances, the idea of sustainable agriculture has obtained prominence through Brundtland report in 1987 (World Convention on Environment and Development 1987) that introduced the concept of sustainable development for the first time (Tait and Morris 2000). The appropriate definition of sustainable agriculture comprises "management procedures that work with natural processes to conserve all resources, minimize waste and environmental impact, prevent problems and promote agroecosystem resilience, self-regulation, evolution and sustained production for the nourishment and fulfillment of all" (MacRae et al. 1989; Goldman 1995).

For sustaining the livelihoods of mountain people, we should emphasize an alternate technology that is nanotechnology to precisely improve the nutrient quantity in soil and promote crop productivity while ensuring environmental safety (Hansen 1996). The Indian Government is also looking towards nanotechnology to boost agricultural productivity in the Himalayan region. Planning Commission of India reported that nanotechnology could help in optimizing the water, nutrients and chemicals use efficiently. It can be implemented as environmental friendly technology that offers great promise for sustainable agriculture in harsh conditions. The productivity of crop yield is low in hills due to controlled climatic conditions (Kumar and Tripathi 1989; Sati 2005). Therefore, nanotechnology has potential to precisely improve the nutrient quantity in soil and promote crop productivity while ensuring environmental safety.

23.4 Nanotechnology

The term nano is originated from the Greek word "nanos" which means "dwarf" and it is one billionth of a meter (Committee on Technology 2014). The concept of nanotechnology was given by Richard P. Feynman who got Nobel Laureate in Physics in 1965. Nanotechnology has become the important tools in modern agricultural field for improving the productions through nutrient optimization (Batsmanova et al. 2013) in order to maintain environmental conditions. The foundation of nanotechnology is the nanomaterials or nanoparticles having different shapes, sizes (1–100nm), and structures. Li et al. (2006) reported that nanotechnology shows great potential in agricultural field due to the extremely small particle size and large surface area.

Nanoparticles are mainly composed of metals/metal oxides, and carbon/organic matter (Hasan 2015; Mukhopadhyay 2014) and generally classified into organic, inorganic, and carbon particles. Inorganic nanoparticles are composed of metals or metal oxides, metal-based nanoparticles are composed of metals including iron (Fe), aluminum (Al), silver (Ag), cobalt (Co), cadmium (Cd), copper (Cu), gold (Au), lead (Pb), and zinc (Zn), whereas metal oxide based nanoparticles are synthesized to modify the properties of adjacent metal-based nanoparticles (Salavati et al. 2008). These are magnetite (Fe_3O_4), iron oxide (Fe_2O_3), cerium oxide (CeO_2), aluminum oxide (Al_2O_3), zinc oxide (ZnO), silicon dioxide (SiO_2), and titanium oxide (TiO_2). Tai et al. (2007) reported that iron (Fe) nanoparticle in the presence of oxygen get oxidized into iron oxide (Fe_2O_3) that increases its reactivity. Kong et al. (2013) reported the iron-manganese (Fe-Mn) binary oxide nanoparticles which are more effective sorbent for arsenic (III) and arsenic (V) removal from groundwater. Organic-based nanoparticles such as micelles, dendrimers, liposomes, chitosan, and ferritin are biodegradable, nontoxic, and sensitive to thermal and electromagnetic radiation. The nanoparticles which are completely made of carbon are known as carbon-based nanoparticles (Bhaviripudi et al. 2007), such as fullerenes, carbon nano tubes (CNT), graphene, activated carbon, and carbon nanofibers (CNF).

23.4.1 Synthesis of Nanoparticles

Numerous synthetic methods have been developed to enhance the properties of nanoparticles in order to reduce the production cost (Cho et al. 2013). According to Royal Society and Royal Academy of Engineering, the two main processes are involved in nanoparticles synthesis, namely top-down approach and bottom-up approach. The top-down approach refers to the traditional micro-fabrication methods, which involve externally controlled tools to cut and shape nanomaterials. The other methods are mechanical milling (Yadav et al. 2012), laser ablation (Amendola and Meneghetti 2009), sputtering, and thermal decomposition (Salavati et al. 2008). The major drawback of this method is not dealing with the very tiny

Table 23.3 Metal/metal oxide and sulfide nanoparticles synthesized by microorganisms (Hasan 2015, Li et al. 2011)

Microorganism	Products	Location
Rhodococcus sp.	Au	Intracellular
Verticillium sp.	Ag	Extracellular
Fusarium oxysporum	Ag	Extracellular
Shewanella algae	Au	Intracellular
Candida utilis	Au	Intracellular
Yarrowia lipolytica	Au	Extracellular
Yeast cells	Fe_3O_4	Extracellular
Escherichia coli	Au	Intracellular
Lactobacillus	CdS	Intracellular
Shewanella oneidensis MR-1	Fe_2O_3	Intracellular
Rhodopseudomonas palustris	CdS	Intracellular
Brevibacterium casei	Au, Ag	Intracellular
Saccharomyces cerevisiae	Sb_2O_3	Intracellular
Coriolus versicolor	CdS	Extracellular
Lactobacillus sp.	$BaTiO_3$	Extracellular

objects. In contrast, bottom-up approaches play a crucial role in preparing nanoparticles having very small size. In bottom-up approaches, various methods are included such as sol-gel (Mann et al. 1997), spinning (Tai et al. 2007), chemical vapor deposition (CVD) (Bhaviripudi et al. 2007), pyrolysis (Kammler et al. 2001), and biosynthesis (Hasan 2015) (Table 23.3).

23.4.2 Biosynthesis of Nanoparticles

Nanoparticles produced by bio-enzymatic methods are more superior to chemical methods, still chemical methods are more reliable to produce large quantities of nanoparticles along with a proper shape and size, but this method is somehow complicated, costly and creates environmental toxicity (Awwad et al. 2013). Therefore, it must be needed to develop eco-friendly, cost-effective, profitable, and nontoxic nanoparticles. Biogenic synthetic method for nanoparticles is more adaptable, reliable, and environmental friendly. The particles synthesized by this process have specific surface area and higher catalytic reactivity (Mann 2001; Bhattacharya and Mukherjee 2008). The biogenic nanoparticles synthesis is classified based on the nanoparticles synthesis location into intracellular and extracellular synthesis (Mann et al. 1997). The intracellular method is based on transporting ions into the microbial cell and formation of nanoparticles with enzymes activity, whereas extracellular method consists of the nanoparticles trapped by specific metal ions on cell surface and reduces ion in the presence of enzymes (Zhang et al. 2011). Some typical metal, metal oxide and sulfide based nanoparticles produced by microorganisms are summarized in Table 23.4.

Table 23.4 Availability of groundwater resources and its utilization in IHR (CGWB 2006)

Region	Annual replenishable groundwater resource (bcm)[a]	Natural discharge during non-monsoon season (bcm)[a]	Net annual groundwater available (bcm)	Annual groundwater draft (bcm)	Stage of groundwater development[b] (percent)
Northern Himalayan states	5.44	0.48	5.0	1.84	37
North Eastern Hilly states	44	3.0	31	5.63	18
Whole India total	433.02	33.77	399.26	230.63	58

[a]Billion cubic meter

[b]Stage of groundwater development is calculated as the ratio of groundwater draft to total replenishable resource

23.4.3 Interaction of Nanoparticles with Plant Cell

The use of nanoparticles in agriculture is a very responsible task because it interacts with living organisms (Gajbhiye et al. 2009; Fulekar 2010). Therefore, the study of nanoparticles interaction with plant cell and tissue system is necessary to (Fig. 23.2). The size of nanoparticles is the key factor to determine the absorptivity rate in plant cell (Soni and Prakash 2012). It has reported that maximum dimensions (40–50 nm) of nanoparticle have higher chance to move and accumulate inside the plant cells (Salavati et al. 2008). In addition, other factors are also responsible such as nature and behavior of the nanoparticle, interaction with environment, and plant physiology (Rico et al. 2011). These factors are led to many morphological and physiological changes in plant cell (Siddiqui and Al-Whaibi 2013). Zhu et al. (2012) studied that crop species with different families were exposed with carbon-coated titanium dioxide nanoparticles. This particular nanoparticles exhibited diverse accumulation and absorption capacity inside the plant cell system (Sabo et al. 2012).

Once the nanoparticles are penetrated into a plant cell, there are mainly two ways for nanoparticles to pass through tissues, either apoplastic or symplastic pathway. The apoplastic pathway is crucial for radial movement within plant tissues which facilitate nanomaterials to accumulate in the root and aerial parts of the plant. In symplastic pathway, nanoparticles internalize by plant cell and go across the plasma

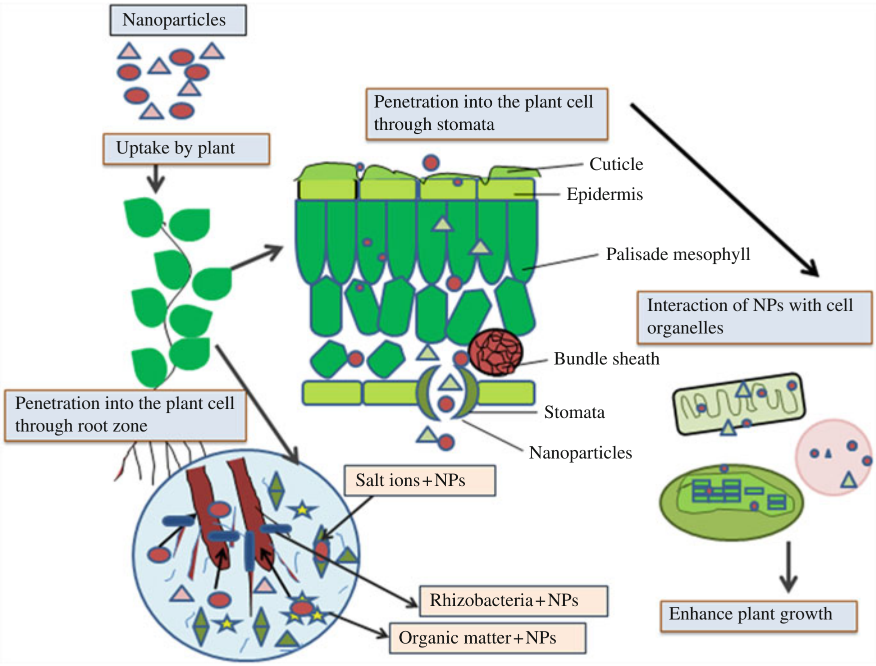

Fig. 23.2 Interaction of nanoparticles in plant cell

membrane through various channels, namely endocytosis (Etxeberria et al. 2006), carrier proteins, aquaporins, ion channels (Rico et al. 2011), and plasmodesmata (Roberts and Oparka 2003). Some of the channels are described below:

- Endocytosis—The nanoparticles are brought into the plant cell through plasma membrane (Etxeberria et al. 2006).
- Pore formation—Some nanoparticles can disrupt the plasma membrane, and enter into plant cell by inducing pore formation, and accumulate directly in the cytosol (Wong et al. 2016).
- Carrier proteins—Nanoparticles bind cell membrane proteins such as aquaporin involved in the movement of ions across the plasma membrane. Rico et al. (2011) has suggested the aquaporin (as transporters for nanomaterials inside the cell) but due to small pores it makes them inefficient channels for penetration of nanoparticles in plant cell (Schwab et al. 2015), unless pore size is increased.
- Plasmodesmata—It is the specialized structures for transporting the nanoparticles into cells through translocation process (Roberts and Oparka 2003).
- Ion channels—They have been observed as a probable transport channel for nanoparticles. However, the size of ion channels (around 1 nm) affects the nanoparticles transportation into plant cells without any effective modifications.

Out of these channels, endocytosis is the most suitable way to enter the nanoparticles into the plant cell. The movement of nanoparticles inside plants provides location and accumulation of nanoparticles about what parts of the plant they can accumulate and where they might be reached (Zhao et al. 2012). Zhu et al. (2012) reported that the accumulation of positively charged gold nanoparticles in radish and ryegrass roots are higher and faster than negatively charged gold nanoparticles in rice and pumpkin.

23.4.3.1 Factors Influencing Uptake and Transport of Nanoparticles in Plants

(a) Nanoparticles affect how they are taken up and translocated in the plant along application method in plant cell.
(b) In the soil, the presence of microorganisms and other compounds facilitates or hinders the absorption capacity of nanoparticles through interaction.
(c) Several plant tissues such as epidermis, hypodermis, and casparian strip must be crossed before reaching the vascular tissues either through roots or leaves.
(d) The mode of chosen pathways of nanomaterials follows either apoplastic or symplastic for radial and up down movement along the plant.

23.5 Nanoparticles and Sustainable Agriculture in IHR

As we know that, the Himalayan region contains fragile ecosystem zone with diversify natural resources. In IHR exploitation of natural resources at higher rates has created a wide gap between replenishment and exploitation of natural resources (Horowitz 1988). In addition, environmental deterioration in terms of deforestation, poor productivity, soil erosion, floods, and lower soil fertility on high altitudinal land are considered to be chief unsustainable trends in IHR (Eckholm 1979; Ives and Messerli 1989; Jodha 1990). Thus, mountain inhabitants are forced to abandon their agricultural land in order to secure their livelihood. Jhum cultivation in northeastern region is alternative way of conserving water and soil nutrients losses.

In IHR region, traditional crops have lost its entity because of low crop yields and low resistance towards harsh climate (Ramakrishnan et al. 1993; Bruijnzeel and Bremmer 1989). Thus, the mountain farmers have been using higher dose of chemicals including weedicides, pesticides, fungicides, and herbicides in order to increase crop yield. The use of chemicals is more reliant and cheapest mode to control pests and diseases in IHR crops. However, uncontrolled uses of pesticides and harsh climate conditions have impacted adversely Himalayan agroecosystem (Mousavi and Rezaei 2011). Therefore, modern innovative technologies should address productivity problem in IHR. There is a need of collaborative investigations and farmers-oriented intelligent use of chemicals at the nanoscale (Joseph and Morrison 2006). The advancement in the nanotechnology improved plant resistance against many biotic and abiotic stresses including resistance to pests and diseases, drought, and low temperature (Beyrouthya and El Azzi 2014; Srilatha 2011). Nanofertilizers help in increasing plant growth and yield, nano based sensors are useful in monitoring soil quality, and nanopesticides are highly useful to control pest and disease. Nano based agriculture can improve the plant efficiency via sustainable manner by generating less waste than conventional products and approaches. Some applications of nanotechnology in IHR are described in Fig. 23.3.

23.5.1 Nanotechnology and Water Management

In IHR, managing the water resource is a more complex and challenging task. This region is more dependent on water resources for hydropower, irrigation, food, and sanitation. Long rivers such as the Indus, Ganges, and Brahmaputra have a plentiful seasonal water supply. Despite this, people living in the mountain have limited access to water for drinking and irrigation purposes. The rapid climate changes in the Himalayas were observed by many scientific communities. The mean global temperatures have increased by an average of 1.5 °C. Monsoon and winter precipitation are observed to be fallen by 5 and 2%, respectively, but the pre-monsoon rain is increased by 4%, which represents a shift in rainfall patterns (Joshi and Kumar 2014). Therefore, significant variations in rainfall could be observed in IHR. The northern Himalayan region has steep slopes and high runoff. This region is covered by different rocks such as slate, sandstone, granites, and limestone. Although this area has little potential for groundwater storage, it still acts as a chief source for recharging plains around Indo-Gangetic and Brahmaputra region. British Geological Survey (2001) has reported that the groundwater is abundant in the aquifers of the Terai region. This groundwater is the main source of water for drinking and irrigation purposes. Despite abundant rainfall in this region, agricultural development is restricted by the limited development of irrigation. As per the assessment, salient

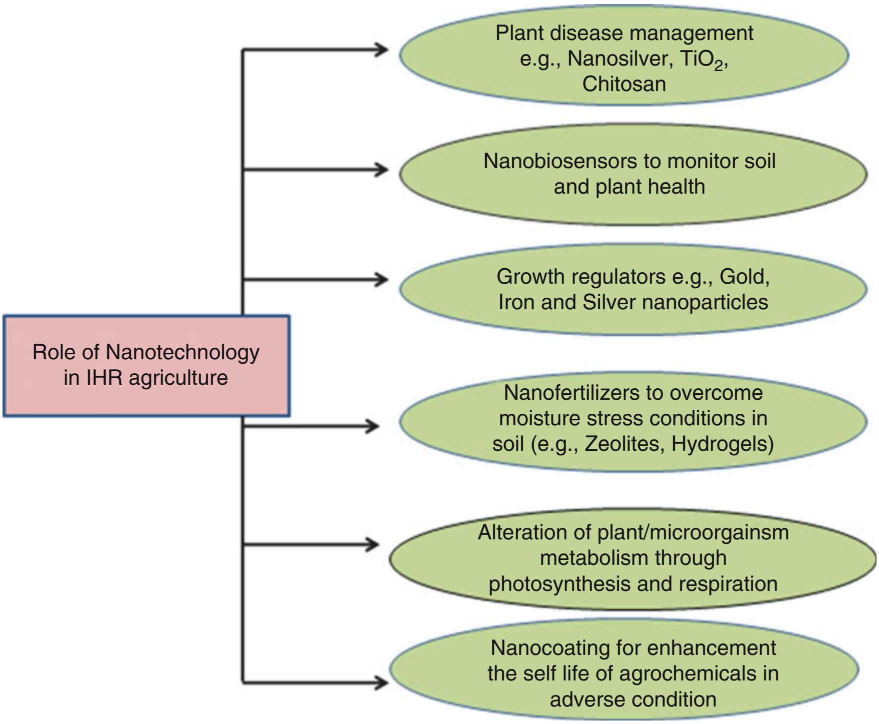

Fig. 23.3 Role of nanotechnology in IHR agriculture

details of groundwater resource availability and utilization, and stages of groundwater development are given in Table 23.5. A close examination of groundwater resources in the hilly region shows that out of the available (433 BCM) annual replenishable groundwater resources, 40 BCM are retained by Himalayan states (Central Ground Water Board 2006; Table 23.4).

The exploitation of this groundwater aquifer has been increasing rapidly in recent years. Shallow groundwater zone is at risk from contaminants such as pathogenic bacteria, pesticides, and nitrate, which is major problem in this region. Therefore, large masses of Himalayan people are facing acute water shortage for drinking purposes. The natural hazards such as faulting, folding, and fracture zones contribute to the loss of aquifer in the mountain belts. In recent times, both the quality and quantity of water availability from springs have been depleting at faster rate. The major cause of dependency on the spring water is due to climate change and variation in rainfall pattern.

The plant growth performance in agriculture is dependent on a suitable distribution of fertilizer and water. However, nitrogen (40–70%), phosphorus (80–90%), and potassium (50–70%) from inorganic fertilizers are released to the environment through leaching without being taken up by plants (Wang and Wang 2009). Therefore, nanoparticles-based formulation such as nanohydrogel can play an efficient

Table 23.5 Applications of variety of nanoparticles (NPs) for groundwater, water and soil remediation

Contaminated sources	Contaminants	Applied treatments	Reference
Groundwater	Chlorinated compounds (BHC, DDT, Lindane, Aldrin, Endrin)	Injection of nZVI in subsurface	Elliott and Zhang (2001)
	Heavy metals (Pd, Cr, Cu, As)	nZVI reduced graphite oxide modified composites	Wang et al. (2014)
Water	Hexavalent chromium Cr(VI)	Agarose stabilized nZVI	Jiao et al. (2014)
	SOx, NH_3, fungicides, heavy metals, insecticides, pesticides	Graphene oxide	Bao et al. (2011)
	Fluoride	Pristine graphene	Li et al. (2011)
	Heavy metals, chlorinated organic compounds (DDT, Lindane, Eldrin)	Iron-based NPs	Hooshyar et al. (2013)
	2-chlorophenol, *E. coli, Staphylococcus aureus*	TiO_2NPs	Park and Lee (2014)
Soil	Chlorinated and brominated compounds	Bimetallic NPs	
	Bisphenol A	Magnetic hybrid NPs modified with β-Cyclodextrin	Wang et al. (2014)

role in IHR agriculture activity to make agricultural production more sustainable (Thornton 2010). The nanohydrogel is biodegradable and more effective in decreasing the water stress in the environment (Senturk et al. 2013). Superabsorbent materials such as hydrogels are hydrophilic polymer which can absorb a large amount of aqueous fluids within a short period of time. Mann et al. (1997) studied the compatibility of silver-coated hydrogels in moisture stress region that indicates the higher water-holding capacity of soil than natural soil. A study on silver-coated hydrogel showed that hydrogel holds 7.5% more water in soil than natural soil. Zhang et al. (2006) reported a hydrogel that can store 130–190 times of its weight of rainwater/irrigation water. Pérez-de-Luque and Hermosín (2013) observed that seeds coated with nano-silver recorded higher water absorption, higher vitamin content, and increased crop yield. This shows that hydrogel is capable of absorbing water in soil and improves the water-holding capacity as well as water use efficiency in the soil.

23.5.2 Nanotechnology and Pest Management

In IHR, conventional methods are being used to control the pathogens and pests which leads to residual toxicity and environmental hazards. These conventional practices have shown adverse effects on the environment. Farmers of that region are adopting a number of traditional methods such as roughing (removal of infested plants) for managing the pest and diseases in the crop. On the other hand, the variability in climatic conditions also leads to pest or disease expansion in IHR. High summer temperatures have created a favorable condition for faster development of temperate zone insects. In the eastern Himalayan region, reduction in crop yield is due to a severe attack of various pests and diseases in crop plants. This region provides suitable climatic conditions like continuous heavy rainfall (159–671 mm), low temperature (7.5–28.2 °C), and high humidity (87.3–93.3%) for increasing the population of pests (Gopi et al. 2016). Chandola et al. (2011) investigated that in western Himalayan, white grub, a notorious pest, is found in the terraced slopes between 1400 and 2200 m elevations. The farmers of that region have adopted various kinds of measures such as setting fire in the field, using a higher dose of urea and table salt to control the growth of white bug in the field crop. Drought stress also made some plants to become more susceptible to pests and pathogens and suppress the plant defense system.

To overcome these constraints, the NPs applications are high in demand for sustainable agriculture. The efficient use of nanoparticles can be prepared as formulations of pesticides, insecticides, and insect repellants (Gajbhiye et al. 2009). Nanoparticles-based pesticides have the potential to increase the agriculture production in sustainable manner. Incorporation of metal-based nanoparticles (NPs) including AgNPs, AuNPs, CuNPs, FeNPs, FeS_2NPs, TiO_2NPs, and ZnNPs have developed (Dewan et al. 2012; Zhao et al. 2012). They can be introduced for crop management in hill region. Pérez-de-Luque and Hermosín (2013) observed that treatment with nanoparticles showed faster germination of seeds along with more resilience towards environmental stress. The advantages of nanotechnology can contribute to increasing the quality and quantity of crop yields and climate resilience at higher altitudinal agriculture (Forsberg and Lauwere 2013). Hafeez et al. (2015) studied the copper NPs and the appropriate concentration of copper (Cu) nanoparticles can enhance the growth of wheat. Taheri et al. (2015) studied ZnO nanoparticle that can improve the yield of corn in mineral poor soils. Rouhani et al. (2012) studied the effect of Ag and Ag-Zn nanoparticles on *Aphis nerii*. The result revealed that Ag nanoparticles can be used in controlling of pest, such as *Aphis nerii*. It has been studied the fungicidal effect of sulfur nanoparticles (SNPs) on phytopathogens, *Fusarium solani* and *Venturia inaequalis*. It has been found that small-sized SNPs are very effective and prevent fungal growth. Bhagat et al. (2013) have reported that nanogel which is prepared from a pheromone has a significant potential for crop protection and well suited for pest management in a variety of crops. Pallavi et al. (2016) investigated the impact of silver nanoparticles (AgNPs) on the rhizospheric bacterial zone and observed higher growth and root nodulation in different crop species with respect to concentration of AgNPs.

The crops in the Himalayan region are most susceptible to fungal diseases which causes major loss to the crop production. Several fungicides which have been used conventionally cause detrimental effects on plants. Nano fungicides have the potential to solve this problem. Shyla et al. (2014) reported antifungal activity of zinc oxide (35–45 nm), silver (20–80 nm), and titanium dioxide (85–100 nm) nanoparticles against *Macrophomina phaseolina*, a major soil-borne pathogen of oilseed and pulse crops. Similarly, Suriyaprabha et al. (2014) found that maize treated with nanosilica (20–40 nm) showed resistance against phytopathogens, *namely Fusarium oxysporum* and *Aspergillus niger*. This antifungal property of nanoparticles is due to the inactivation of thiol groups present in fungal cell wall which helps in disruption of transmembrane, followed by energy metabolism and electron transport chain.

23.5.3 Nanotechnology and Crop Nutrition

The fertilizers play an important role in plant growth and its development. At times fertilizers remain unavailable to plants due to leaching, insolubility, and decomposition, which lead to environmental problems worldwide. In IHR, uncontrolled use of pesticides, insecticides, and herbicide to get higher crop production has caused the adverse effect on Himalayan agroecosystem (Mousavi and Rezaei 2011). Thus, an effective approach need to be developed to minimize nutrient loss of fertilization and to increase the crop yield through nanotechnology. Application of nanotechnology in agriculture is an important approach to enhance crop production (Adrees et al. 2015). Nanofertilizer can tend to enhance the absorption capacity of plants to uptake soil nutrients (Huang et al. 2015). It has the ability to prevent nutrient losses and it also avoids the unwanted nutrients interaction with microbes, water, and air. The mechanism of absorption of nanofertilizer starts in plant cell through apoplastic or symplastic pathway. It has reported that nanofertilizers can be used as additives in fertilizers to increase water and minerals soil such as silicon and titanium-based nanoparticles. The higher use of nitrogen-based chemical fertilizer is more soluble in soil water and leached to the groundwater zone. Therefore, adsorbents like zeolite, montmorillonite, halloysite, and bentonite nanoclays can be used to develop nitrogen fertilizers with controlled-release characteristic (Sharmila 2010). It has been reported that the effects of NPs in crop/plants at certain concentrations are generally positive and increase the plant tolerance towards biotic and abiotic stresses such as less temperature, fewer nutrients availability in soil, and pathogens and pests. Huang et al. (2015) reported positive effect of zinc oxide nanoparticles (in colloidal solution form) when used as fertilizer in very small concentration. Therefore, nanofertilizer not only supplies flow of nutrients but also revives the soil to an organic state which maintains farm productivity.

Nanosized silica-silver has been reported to have wide range of antimicrobial activity and does not cause chemical damages to the human body and plants. It was also found that nanosized silica-silver also controls plant pathogens (Huang et al. 2015). The use of silver and gold nanoparticles with natural biofertilizers such as *Pseudomonas fluorescens*, *Bacillus subtilis* and *Paenibacillus elgii* has been observed to promote growth in in vitro conditions.

23.5.4 Seed Treatment and Preservation

In IHR, the major cause of low productivity is the seed susceptibility towards the biotic and abiotic conditions. The seed quality parameters, i.e., seed size and seed weight, affect the final yield in crop production. The market availability in IHR is far away. Therefore, suitable perseverant is needed for fruits and vegetables for a long time. Nanotechnology can enhance agriculture production and its applications. A very few nanotechnologies are available for the preservation of fruits, seeds, and vegetables, and many of those are in the initial stage of development, so further investigation is required. Seed treatment is required for higher crop growth. Nowadays, seed pretreatment with nanoparticles is more reliable towards the harsh environment. Treating seed with colloidal solution of metal nanoparticles is capable of storing genetic purity grade and improves plant immune status. Treating seeds with metal nanoparticles contributed significantly to increase crop only in combination with the use of fertilizers in a dose-dependent manner. Jia et al. (2008) identified a method of keeping nanoparticle complex latex fresh by coating loquat and cherry with sodium alginate. During preservation, he identified the water losses, decay indexes, respiratory intensity, total acid content, and soluble solids. These results were suggested that the complex latex can reduce water losses and decay indexes of loquat and control its respiratory intensity. Zhang et al. (2005) used the antimicrobial property of quasi nano-silver particle solution for antimicrobial preservation of tomato juice and a mixture of tomato-carrot juice.

23.5.5 Soil Micronutrient Management

Soil is a biogeochemical dynamic entity that plays an essential role in sustaining life forms by regulating different processes in terrestrial ecosystems (Adewopo and Bhomia 2012). Micronutrient deficiency in soil occurs due to the contribution of many natural factors. High erosion rates and leaching conditions in the Himalayan region lead to low concentrations of micronutrients. Pedagogically, most of the soils in the Himalayan regions consist of metamorphosed sedimentary rocks (Carson 1992). Nutrient-rich bedrocks and sediments such as basalts, limestone, and shales were deposited but are less widespread in this region. In IHR, conventional agriculture practices are being characterized by tillage and crop residue burning degraded the sustainability of crop. The conventional farming practices and continuous loss of soil fertility are threat to global agricultural production (Sati 1993). This type of conventional agricultural practices is capable of achieving the food production goals but simultaneously destroying the natural resources (Tao et al. 2012). In northeastern Himalayan region, most soils are rich in soil organic carbon (SOC), and nitrogen (N) content, except those under shifting cultivation. The soil in sub-tropical regions including Tripura, Assam, and other foothills is deficient in SOC and potassium (K). In this region, most soils is acidic in nature and addresses to the leaching of basic cations due to heavy rainfall.

Therefore, it has concluded that NPs play an important role in sustaining soil fertility, thus serving to achieve sustainable agricultural productivity. We should widen our vision to considering different potentials of the nanoparticles/materials for sustainable materials management. Different nanomaterials such as nanoclays, nanozeolites, and NPs-based hydrogels have been observed to enrich the water-holding ability of the soils (Sekhon 2014), hence these NPs act as a slow-release source of water and reduce the water shortage periods during the crop season. Applications of the NPs-based systems are advantageous for agricultural purposes and cultivation of degraded areas. Peteu et al. (2010) observed that nanoformulations of micronutrients can be used for enhancement of soil health and vigor by spraying on plants or direct supply to soil. Tao et al. (2012) have reported that organic such as carbon nanotubes, inorganic such as metals and metal oxide nanoparticles, and polymer are capable to absorb environmental contaminants to increase the soil remediation ability. There are so many micronutrients, namely iron, molybdenum, manganese, zinc, copper, boron, etc. which are essential for the growth and development of crop plants. In IHR, conventional agriculture practices have decreased the micronutrients (zinc, iron, and molybdenum) in the soil progressively (Huang et al. 2015).

23.5.6 Nanobiosensors

Recently, nanoparticles are being used in diagnostic tools for the detection of plant diseases. The nanoparticles-based biosensors can be used in farming to maximize output from crops, which is a long-desired goal. Nanobiosensor has the potential to minimize the input of herbicides and pesticides through monitoring localized environment condition. Ultimately, precision farming with the help of smart sensors allows enhanced agriculture productivity by providing careful information to make farmers take improved decisions (Cioffi et al. 2004). The nanosensors can also detect crop pathogens, plant viruses, and soil nutrients availability.

Fundamentally, a biosensor is made by coupling a ligand receptor to a signal transducer. It consists of a probe, a bioreceptor, and a transducer. Role of the transducer is to measure the produced electrical signal from the interaction of analytes and bioreceptor (Fig. 23.4). Lopez et al. (2014) reported that nucleotide changes in microbes and viruses are detected by nano-chips. Nano-chips contain fluorescent oligo capture probe to sense hybridization. Yao et al. (2009) studied that fluorescence silica NPs in combination with antibody are used for detection of disease in the plant. Mousavi and Rezaei (2011) reported that nano smart dust can be used in environmental pollutants evolution. Application of nanomaterials-based devices takes such as nanosensor in agriculture provides real-time monitoring of crops/plants, minimizing the use of pesticides and antibiotics, and detecting virus and many fungal or bacterial infections (Jayalakshmi 2014). Hence, nanosensors can replace conventional sensors via sensing number of fertilizers, pesticides, herbicides, and pathogens, and thus support sustainable agriculture by enhancing crop productivity.

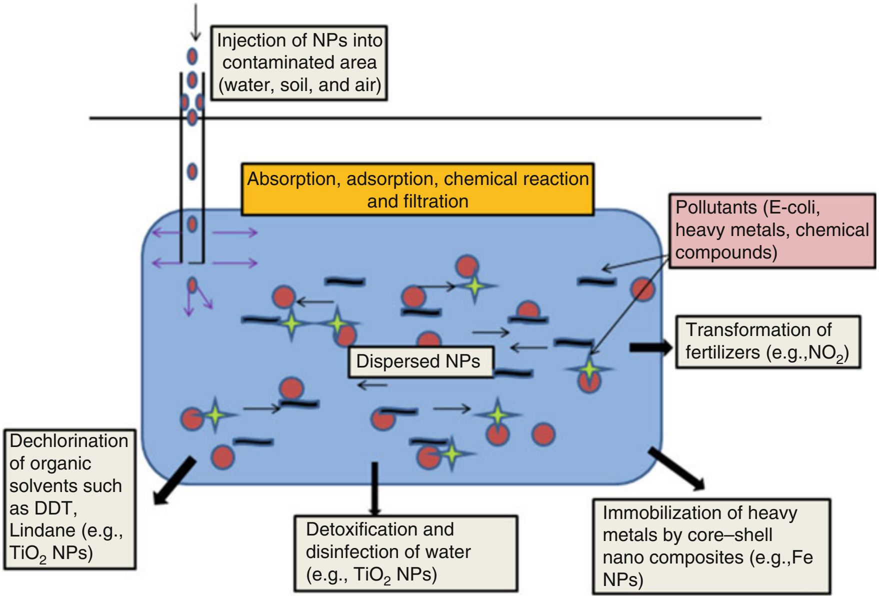

Fig. 23.4 Application of nanoparticles in environmental remediation

23.5.7 Environmental Remediation

The Himalayan agricultural farmers have limited resources for improving their crop verities and productivity. The mountains ecosystem contains fragility and susceptibility due to natural and anthropogenic factors. Mountain people are facing several challenging tasks such as higher physical demands, natural hazards, and agricultural production constraints. In IHR, only 3% land ranked as appropriate for rain-fed agriculture, which restricted the livelihood opportunities of mountain people (UNEP-WCMC 2002). The major restrictions are shortage of water, landslides, avalanches, foods, loss of genetic diversity, soil erosion, wildfires, and extremes temperature and radiation. Water quality of Himalayan rivers is continuously worsening due to anthropogenic activities, poorly constructed drainage and sewage systems, etc. The influence of such activities directly led to the loss of self-purification capability of water bodies. Farmers of the Himalayan region are using pesticides and herbicides for protecting their crop varieties. Those pesticides and herbicides are shown recalcitrant behavior towards environmental degradation and remain in the environment for a longer period.

Adrees et al. (2015) studied the effect of copper (Cu) pollution in the soils and found that it is due to overuse of pesticides, fungicides, and untreated industrial effluent. Higher dose of Cu exhibits adverse effects on plant growth and crop. Many studies have reported that Cu induced oxidative damage and antioxidant response in crops which led to growth inhibition in plants. Nano-based technologies can show a promising step in bioremediation. For example, the colloidal nanoparticle when mixed in contaminated soil resulted in decreased toxicity of resistant pesticides (Kong et al. 2013). Recent research has suggested that nanoscale iron particles based remediation techniques have several advantages such as nontoxic nature of NPs, effective for the transformation of a large variety of contaminants, and inexpensive (Navarro et al. 2008).

Al-Zawi et al. (2013) investigated that nano-based technologies are much more effective and less expensive and that could work to eliminate Cd and Pb from polluted soil. Recently, researchers observed in the laboratory that iron nanoparticles have worked effectively for reducing the large variety of chlorinated compounds and metal ions. These compounds have resulted from excessive use of chemical fertilizers. Chlorinated based pesticides are persistent in nature and readily degraded under reducing conditions (Chen and Yada 2011). Sabir et al. (2014) observed that zinc oxide nanoparticles have potential to enhance the production of crops and degradation materials of environmental contaminants. The nanoscale zero valent iron (nZVI) is widely used for detoxification of pesticides, and transformation of chlorinated compounds (Khin et al. 2012). They also played an efficient role towards transformation of a large range of environmental pollutants, rapid mobility, and inexpensive, fast mode of action, in-situ and ex-situ remediation flexibility (Zhang 2003) (Fig. 23.4) (Table 23.5).

23.5.8 Climate Change

The economy of the mountain farmers is completely dependent on agriculture and horticulture crops. These sectors are highly climate sensitive and in this regard climate change has emerged as serious environmental concerns. Reduction in snowfall and shrinking winters, increasing temperature during summer, uneven distribution of rainfall, and temperature rising above normal during the winter months were among the main changes in the climate observed. Climate change could be observed from the increasing air and ocean temperatures to widespread melting of snow and ice which leads to rising sea level. Rain-fed farming on slopes is the dominant features of hill farming and poses special changes in climate as compared to plain area agriculture (Vedwan and Rhoades 2001).

The biophysical and socio-economical nature of the mountains is characterized by extreme diversity and non-homogeneity. The effects of climate change in different mountain systems are more perceptible in high crop production areas due to an overall increase in the temperature and drying up of surface water resources including river water, springs, lakes, etc. Because of climate change, the mountain agriculture has shifted from traditional crops to the off-season crops like the area under basmati rice and maize was diverted towards local paddy (Vedwan and Rhoades 2001). To overcome from climate change constraints, we need to search new approaches to combat the climate change in Himalayan region. Therefore, nanotechnology may play an efficient role to combat the consequences of climate change.

23.6 Recommendations for Future Research in Nanotechnology

The use of NPs is shown promising applications in IHR region. Because of unfavorable biotic and abiotic stresses, that areas should be overlooked. Involvement of nanotechnology in IHR agricultural must be taken seriously for effective crop production. The cost for the synthesis of nanomaterials is the one factor which may obstruct their application in this field. Therefore, the production of nanomaterial for farmers field tests, nanomaterials should be produced at low cost. Natural NPs such as alginate (Silva et al. 2010), chitosan (Grillo et al. 2014; Maruyama et al. 2016), and lipids (Campos et al. 2015) are cost-effective and can be synthesized from natural existing compounds at low cost for field trials. The major drawback of implementing this technology in IHR is unawareness of mountain people. Therefore, it is necessary to explain them about nanotechnology and its application. In addition, we have to suggest them a clear cut overview of nanoparticles application in soil conditioning and soil physicochemical characteristics, before introducing the nanotechnological approaches.

23.7 Conclusion

The mountain ecosystem exhibits environmental stresses and climatic variations including frequent floods, droughts, hypoxia, low temperature, high solar intensity that possess unsustainable production of agricultural crops. Therefore, we must do proper planning and management which can provide the sustainable future for Himalayan region. We need an innovative technology that ensures that the development does not affect its bio-cultural diversity. Nanotechnology has potential to conserve the natural resources without harming any ecological systems. However, we must acknowledge the fact that whole IHR is facing natural and anthropogenic pressure leading to environmental degradation. Therefore, research on the working mechanisms of NPs in these region demands more attention.

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