



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

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## 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 devise 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

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

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**Keywords**

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

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## 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. Constraints 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. konongii*, 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*, *Cylindrocarpodestructans*, *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 *cry1* 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  $LD_{50} = 1.63 \text{ kJ/m}^2$  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 mycoparasitism 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,  $\beta$ -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.

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### 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 from 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.

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## 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.

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## 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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