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

16

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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

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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)

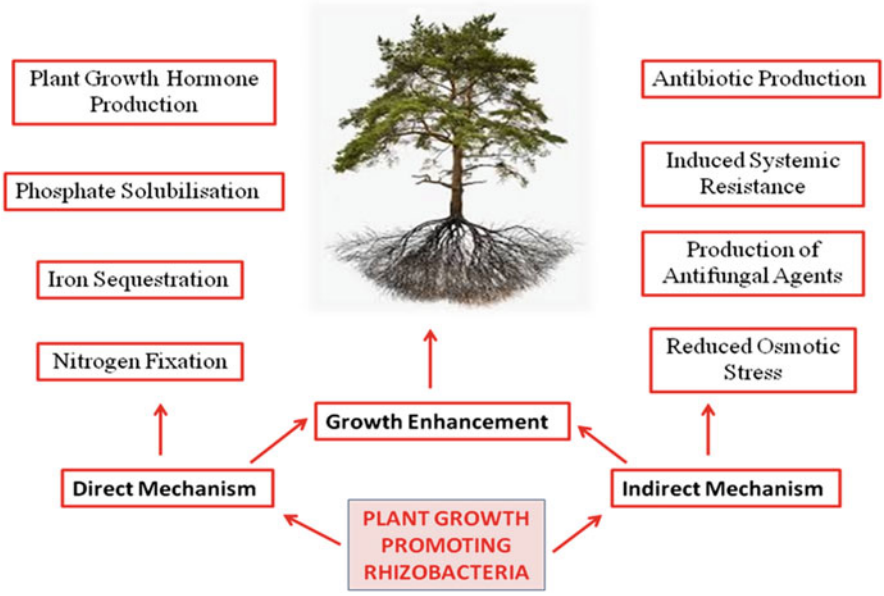

Fig. 16.1 Mechanism of plant growth promotion by plant growth promoting rhizobacteria

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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