Chapter 16
Biotic Stress Management in Agricultural Crops Using Microbial Consortium

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16.1 Introduction

The rhizosphere provides home to a large number of active microbial populations capable of exerting beneficial, neutral or detrimental effects on plant growth. Microbes in general live in a heterogeneous community bound to the root surfaces and the plant–microbe signaling permits them to live and work as a community. The majority of interactions studied so far were normally concerned with single pathogen and a single biocontrol agent in the rhizosphere (Wilson and Backman 1999). But, this may sometimes account for the inconsistent performance as a single agent is not active in all soil conditions or against all pathogens that attack the host plant. Failure of the introduction of antagonistic microorganisms seems to be due to environmental factors resulting in inadequate distribution, insufficient establishment of rhizobacterial strains, poor expression of their antagonistic activity (Schippers et al. 1987), or difficulty in attaining threshold population. Indeed, variability is likely to be increased by numerous biotic and abiotic factors, and among them fluctuations in antagonistic activity is the most important one. Combining microbes as a control strategy may prove to be more relevant in the long term to give better yield and quick results (Duffy and Weller 1995; Bashan 1998). On the other hand, mixtures of biocontrol agents (BCAs) may be useful for biocontrol of different plant pathogens via different mechanisms of disease

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suppression. The recent focus on the use of multiple microorganisms with diverse activities as consortium is thus gaining momentum. By combining microorganisms, multiple antagonistic and stress tolerant traits can be combined and one may assume that at least one biocontrol mechanism will be functional under the circumstances faced by the released BCAs. Combinations of BCAs are, therefore, expected to result in a higher level of protection (Dunne et al. 1998), through reduced variability of biological control (Guetsky et al. 2001, 2002), and potentiality to suppress multiple plant diseases (Jetiyanon and Kloepper 2002).

Enhanced disease suppression may indirectly result from the multiplicity of interactions between the introduced strains which may positively influence growth, root colonization, better substrate colonization, population, and activity of each other. Combining microbial strains is not only advantageous because it can lead to enhanced levels of disease suppression, but also beneficial when a single strain fails to suppress a disease, the others in combination still may contribute to disease suppression (de Boer et al. 2003; Jain et al., 2012 a, b; Singh et al., 2012). Mixing of BCAs belonging to taxonomically different groups requires optimization of temperature, moisture, and pH for imparting benefits to the host by aggressive root colonization. Moreover, BCAs like fungi and bacteria may provide greater benefits to the host plants by being operative under varied conditions at different times and by occupying different or complementary niches.

16.2 Mechanisms of Biocontrol

Beneficial microorganisms may contribute either directly to the growth of plants or indirectly by reducing plant disease incidence (Jetiyanon et al. 2003; Gray and Smith 2005; Hass and Defago 2005). The antagonistic microorganisms exercise various mechanisms to accomplish disease control viz., inhibition of the pathogen by antimicrobial compounds (antibiosis); competition for iron through production of siderophores; competition for colonization sites and nutrients supplied by seeds and roots; induction of plant resistance mechanisms; inactivation of pathogen germination factors present in seed or root exudates; or degradation of pathogenicity factors of the pathogen such as toxins and parasitism that may involve production of extracellular cell wall-degrading enzymes like chitinase and β-1,3 glucanase that degrades pathogen cell walls (Keel and Défago 1997; Whipps 1997). Several modes of action can be exhibited by a single BCA, whereas microbial consortium can guarantee different mechanisms or combinations of mechanisms in the suppression of different plant diseases (Fig. 16.1). Most of the effects of the individual microorganisms in mixture are additive, although synergistic effects have also been reported in some cases (Ravnskov et al. 2006; Kohler et al. 2007).

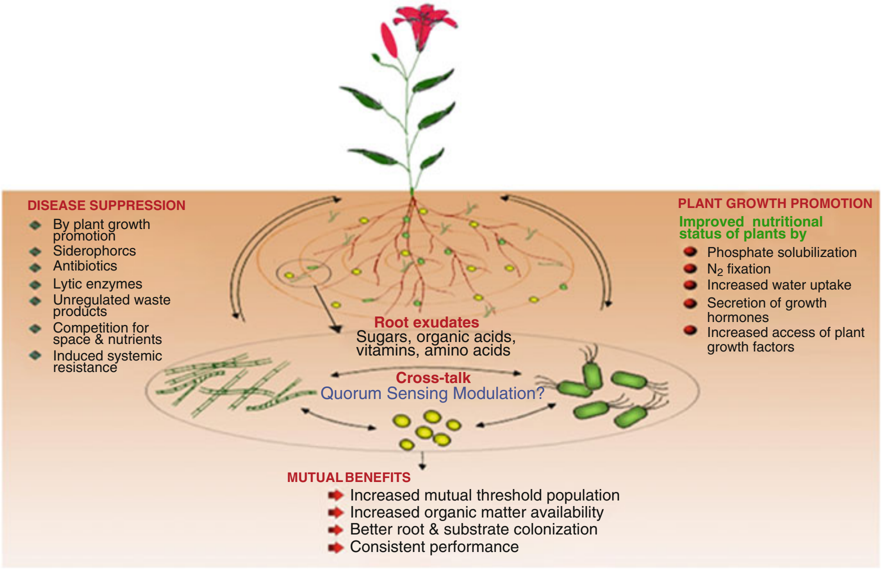

Fig. 16.1 Schematic representation of multiple interactions taking place in the rhizosphere and multifacet benefits imparted to the plants

16.2.1 Antibiosis

Antibiotics are microbial toxins which at low concentrations poison or kill other microorganisms. One of the important mode of disease suppression by BCAs include production of one or more antibiotics (e.g., 2,4-diacetylphloroglucinol (DAPG), viscosinamide, tensin, pyoluteorin (Plt), zwittermicin A, kanosamine, phenazine-1-carboxylic acid (PCA), butyrolactones, oligomycin A, oomycin A, pyrrolnitrin (Pln), xanthobaccin, or toxic substances like cyanide) (Milner et al. 1996; Keel and Défago 1997; Whipps 1997; Nielsen et al. 1998; Kang et al. 1998; Kim et al. 1999; Nakayama et al. 1999; Thrane et al. 2000). Antibiotic synthesis is very closely linked to the overall metabolic profile of the cell, which in turn is governed by nutrient availability and other environmental factors like major and minor minerals, type of carbon source and supply, pH, temperature, and other parameters (Thomashaw and Weller 1996).

Antibiotic production by bacteria, particularly by pseudomonads, seems to be closely regulated by a two-component system involving an environmental sensor and a cytoplasmic response factor (Keel and Défago 1997). By using *P. aeruginosa* PAO1 mutant unable to produce HCN, it was confirmed that cyanide poisoning is responsible for killing the nematode *Caenorhabditis elegans* (Gallagher and Manoil 2001). *Pseudomonas* species can synthesize enzymes which may also modulate the plant hormone levels and limit the available iron by production of siderophores apart from killing the pathogen by producing antibiotics (Siddiqui 2006).

The extent of mechanisms for biocontrol and the effectiveness of many BCAs are reported to depend on metabolites with antimicrobial activity in combination with other mechanisms (Raaijmakers et al. 2002). For example, *Bacillus megaterium* KL39, a BCA of red-pepper Phytophthora blight disease, produces an antifungal antibiotic active against a broad range of plant pathogenic fungi (Jung and Kim 2003). Similarly, *B. subtilis* also synthesizes an antifungal antibiotic that inhibits *Fusarium oxysporum* f. sp. *ciceri*, the causal agent of wilt of chickpea (Kumar 1999), and a strain of *B. subtilis* RB14 produces the cyclic lipopeptides antibiotics iturin A and surfactin active against several phytopathogens. *Rhizobium* spp. have also been reported to produce extracellular compounds (such as trifolitoxin) with direct antimicrobial activities (Breil et al. 1996). Raaijmakers et al. (2002) reviewed antibiotics produced by bacterial BCAs and their role in microbial interaction. In the *Phytophthora oligandrum*/*Trichoderma harzianum* interactions disintegration of cytoplasm and cell components and loss of turgidity resulted when fungal cells were exposed to antibiotics (Floch et al. 2009). Enhanced suppression of take-all of wheat by the consortium of a nonpathogenic isolate of *Gaeumannomyces graminis* var. *graminis* and a mixture of pseudomonads was reported to be the result of direct competition for substrates at favored sites in combination with antibiotic production by the pseudomonads (Duffy and Weller 1995). The wide array of mode of action of these antibiotics obviously calls for designing consortium with different sites of action.

16.2.2 Siderophore Production

Almost all the microorganisms require iron as an essential element in a variety of metabolic and cellular pathways, and in most microbial habitats, Fe (II) is oxidized to Fe (III) which forms stable complexes. When concentration of iron is too low (10^{-6} M) to support the growth of the microorganisms, some organisms secrete iron-binding ligands called siderophores which have high affinity to sequester iron from the micro-environment. Antoun et al. (1998) determined that out of 196 *Rhizobium* spp. tested, 181 produced siderophores. Several evidences also indicate that siderophore production under iron limiting condition is responsible for the antagonism by some strains of *P. aeruginosa* against *Pythium* sp., the causal agent of damping-off and root rot of many crops (Buyens et al. 1996; Charest et al. 2005). The pseudobactin siderophore of *P. putida* WCS358 was found to increase the intensity of antagonism of the *F. oxysporum* strain Fo47 against the pathogenic *F. oxysporum* by making the pathogen more sensitive to competition for carbohydrates by Fo47 (Lemanceau et al. 1993). The involvement of siderophores in disease suppression by *P. putida* RE8 and WCS358 was investigated by de Boer et al. (2003). The pseudobactin siderophore of strain WCS358 was found to inhibit in vitro growth of RE8, whereas RE8 does not affect growth of WCS358. The improved control of Fusarium wilt of carnation by the combination of a nonpathogenic *F. oxysporum* strain Fo47 with *P. putida* WCS358 had indirect effect on competition for iron that made the pathogenic *F. oxysporum* more sensitive to competition for substrate with the nonpathogenic strain (Lemanceau et al. 1992, 1993).

16.2.3 Root Colonization

Root colonization is important being the first step in both infection by soil-borne pathogens and beneficial associations with microorganisms. Rhizosphere competence by BCAs is acquired by effective root colonization along with the ability to survive, outcompete, and proliferate on growing plant roots over a considerable time period, in the presence of indigenous microflora (Whipps 1997; Lugtenberg and Dekkers 1999). The competitive exclusion of deleterious rhizosphere organisms is also directly linked to the ability of BCAs to successfully colonize a root surface. Moreover, the O-antigen chain of bacterial lipopolysaccharides contributes to root colonization, but in a strain-dependent fashion. The O-antigenic side chain of *P. fluorescens* WCS374 does not contribute to potato root adhesion (de Weger et al. 1989), whereas the O-antigen chain of *P. fluorescens* PCL1205 is involved in tomato root colonization (Dekkers et al. 1998). Similarly, *Enterobacter cloacae* suppresses the infection of *Pythium ultimum* by effective catabolism of the available nutrients in the spermosphere (van Dijk and Nelson 2000; Kageyama and Nelson 2003).

The population dynamics of *P. putida* strains RE8 and WCS358 in the rhizosphere were investigated by applying them either singly or in combination to assess their ability to colonize roots. The population density of RE8 when combined with WCS358r was significantly higher compared to its single inoculation (de Boer et al. 2003). These findings are of considerable significance and give proper insights into the complementary/synergistic effect of the microbes in consortium for better colonization of roots.

16.2.4 Influence of Organic and Inorganic Matter

Root exudates offer a carbon-rich food to the rhizospheric microorganisms and provide them large amount of organic acids and sugars as well as variable amounts of amino acids, nucleobases and vitamins. Sometimes exudates have a major share of antimicrobial agents which give ecological niche advantage to organisms that have ability and adequate enzymatic machinery to detoxify them (Bais et al. 2004). Endophytic bacteria are shown to be selectively attracted more towards the rice exudates compared to other microbes (Bacilio-Jiméne et al. 2003). Another recent report says that root-secreted malic acid attracts beneficial soil bacteria like *B. subtilis* FB17 towards the root (Rudrappa et al. 2008).

Similarly, studies on the influence of organic matter on AM fungi and soil microbiota interactions are very limited (Green et al. 1999; Albertsen et al. 2006). AM fungi are obligate biotrophic and they receive carbon from their host plant only, whereas *Clonostachys rosea* relies on organic matter for carbon supply. The content of organic matter in the environment may therefore influence the interaction between these fungi. Thus, different types of organic matter affect the interactions between AM fungi and other soil microorganisms differently (Ravnskov et al. 1999), and the benefits derived by the host plants are highly dependent on these factors.

16.2.5 Lytic Enzymes

Diverse microorganisms having biocontrol potential secrete and excrete various metabolites that can interfere with pathogen growth or activities. Individual enzyme activities involved in the antagonism can be exploited as indicators in microbial screening to assess the antagonistic potential of strains for their precise use. Chitinase produced by *Serratia plymuthica* C48 inhibited spore germination and germ-tube elongation in *Botrytis cinerea* (Frankowski et al. 2001). Similarly, extracellular chitinases are considered crucial for *Serratia marcescens* to act as antagonist against *Sclerotium rolfsii* (Ordentlich et al. 1988), and for *Paenibacillus* sp. strain 300 and *Streptomyces* sp. strain 385 against *Fusarium oxysporum* f. sp. *cucumerinum* (Lim et al. 1991). An endophytic strain of *P. fluorescens* transformed with the chiA gene encoding the major chitinase of the *S. marcescens* provided effective control of *Rhizoctonia solani* on bean seedlings under growth chamber conditions (Downing and Thomson 2000). Similarly, β-1,3-glucanase contributes significantly to the biocontrol abilities of *Lysobacter enzymogenes* strain C3 (Palumbo et al. 2005). Bacterial chitinases and β-glucanases are involved in biological control of various plant pathogenic or wood deteriorating fungi (Pleban et al. 1995; Podile and Prakash 1996; Arora et al. 2007). Efforts are being made to identify cell wall-degrading enzymes produced by bacterial biocontrol strains, even though relatively little direct evidence for their presence and activity in the rhizosphere has been recorded. *Micromonospora carbonacea*, a cellulose producing isolate, was found to control root rot caused by *Phytophthora cinnamomi* (El-Tarabily et al. 1996) and actinomycete isolates that suppressed *Phytophthora fragariae* were shown to produce β-1,3-, β-1,4- and β-1,6-glucanases (Valois et al. 1996). Lytic enzyme regulation, especially proteases and chitinases particularly, involves the GacA/GacS (Gaffney et al. 1994; Natsch et al. 1994; Sacherer et al. 1994; Corbell and Loper 1995) or GrrA/GrrS regulatory systems (Ovadis et al. 2004) and colony phase variation (Lugtenberg et al. 2001).

16.2.6 Plant Growth Promotion

The plant–microbe interactions can significantly influence plant growth and crop yields. Plant growth-promoting rhizobacteria (PGPR) competitively colonize plant roots, and stimulate plant growth and/or reduce the incidence of plant diseases (Kloepper and Schroth 1978). Mechanisms of plant growth promotion include the production of volatile compounds and phytohormones, lowering of the ethylene level in plant, improvement of the plant mineral uptake, reduction of diseases, encouragement of other beneficial symbiosis, protection against degrading xenobiotics, and stimulation of disease-resistance mechanisms (ISR) (Jacobsen 1997). These PGPRs mostly belong to *Pseudomonas* and *Bacillus* spp., and are antagonists of recognized root pathogens.

Plant growth promoting microorganisms (PGPMs) such as *Rhizobium* and *Glomus* spp. can promote plant growth and productivity as their primary effect, but have also been shown to play a role in reducing disease as a secondary effect. Conversely, BCAs, such as *Trichoderma* and *Pseudomonas* spp., can control disease as their primary effect but have recently demonstrated to be a stimulator to plant growth additionally (Avis et al. 2008). Several microbes that have been studied extensively as BCAs against various phytopathogens also showed plant growth promotion activities (Singh et al. 2003; Srinivasan et al. 2009). The increase in plant growth is mostly attributed to synthesis of phytohormones such as IAA, cytokinins, and GA_3 (Shanmugaiah et al. 2006; Srinivasan et al. 2009). Many strains of fluorescent pseudomonads have shown to possess the ability to stimulate germination of seeds as well as development of shoot and root in different crops (Kloepper et al. 1988). The improvement in nutritional status of the plant has also been recognized as a possible mode of resistance to various pathogens (Karagiannidis et al. 2002; Sahni et al. 2008). Similarly, the combination of *P. fluorescens* strains EBC 5 and EBC 6 was found to increase the germination percentage, shoot length, and root length of chilli plants significantly (Muthukumar et al. 2010).

In a study on peas, three strains of *T. harzianum* increased fresh shoot weight, root weight, and/or root length (Naseby et al. 2000). Tripartite interactions among *Paenibacillus lentimorbus* NRRL B-30488 (B-30488), *Piriformospora indica* DSM 11827 (DSM 11827), and their consortia with native rhizobial population in the rhizosphere of *Cicer arietinum* L. was found to enhance nodulation and increase plant growth (Nautiyal et al. 2010). In a separate study, Seneviratne (2003) demonstrated that co-inoculation and coculture of microbes performed the tasks better than the individual microbes. When microbes are mixed into an inoculum consortium, each member of the consortium not only out-competed others for root colonization, but also complemented functionally for plant growth promotion (Shenoy and Kalagudi 2003).

16.2.7 Induced Systemic Resistance

PGPM and BCAs primarily affect plant productivity and health, but in addition to this more recently discovered effects like ISR has sparked an interest among the plant growers to use these beneficial microbes in the field (Vassilev et al. 2006). Plants have evolved a number of inducible defense mechanisms against pathogen attack (Durrant and Dong 2004). Use of microbial consortium would indirectly increase the stimulation in the plant to activate its defense mechanisms when challenged by a pathogen through strengthening of cell walls; deposition of callose and lignin (Singh et al., 2012); and the production of plant defense compounds such as phenolics, phytoalexins, and flavonoids, with simultaneous enhancement of enzyme activities such as chitinase, peroxidase, polyphenol oxidase, ascorbate peroxidase and phenylalanine ammonia lyase (Jain et al., 2012 a, b).

Bacillus sp. strain mixtures IN937b + SE49 and T4 + INR7 suppressed mosaic and anthracnose diseases in both winter and rainy seasons when compared with the nonbacterized control (Jetiyanon et al. 2003). Plants treated with the bacterial mixture of *B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b had superoxide dismutase (SOD) and peroxidase (PO) activity levels 25–30 % greater than the nonbacterized pathogen control. Additionally, significant disease protection in each plant pathosystem was observed with the bacterial mixture (Jetiyanon 2007). An increased level of defense-related enzymes viz., L-phenylalanine ammonia lyase (PAL), peroxidases, and polyphenol oxidase (PPO), was recorded on co-inoculation of rhizobia with *Bacillus cereus* strain BS 03 and a *P. aeruginosa* strain RRLJ 04 under the stress generated by Fusarium wilt of pigeon pea (Dutta et al. 2008). The expression of pathogenesis-related proteins (PR-proteins) can be used as a marker of ISR (van Loon 1997). Whipps (2004) also indicated that the plant defense responses occurring during *Glomus* spp. mycorrhization include phenolic and phytoalexin production, formation of structural barriers, and production of (PR) proteins and enzymes associated with plant defense mechanisms, showing the ability of some useful microbes in inducing ISR (van Loon et al. 1998).

16.3 Development of a Microbial Consortium

A preliminary step before development of a consortium requires gaining insight into the compatibility of the microorganisms used in vitro and to be used in the rhizosphere of the concerned host plant. The combination of antagonists used should be evaluated for their capacity to coexist in the rhizosphere. A successful and consistent biocontrol requires compatibility among co-inoculated microorganisms, their co-establishment in the rhizosphere, and the lack of competition among them. Evaluation is arguably, therefore, the most important phase during development of microbial consortium because it provides an understanding of its contribution in decreasing disease severity and increasing plant growth. Attempts are being made to develop microbial consortium for disease suppression and plant growth promotion (Nautiyal et al. 2005, 2006; Singh et al. 2006).

16.4 Microbial Consortium Comprising only Bacterial Strains

The use of combinations of antagonistic organisms may provide improved disease control over the use of single bioinoculant (Srivastava et al. 2010). Biocontrol by bacteria is mainly achieved through antibiosis, competition for space or nutrients in the rhizosphere, and ISR. Successful application of *Bacillus*, *Pseudomonas*, and *Streptomyces* spp. has already been reported for the control of various plant diseases in different crops (Emmert and Handelsman 1999; Anjaiah et al. 2003; Chung et al. 2005; Hass and Defago 2005). Chilli seeds treated with endophytic strains of

P. fluorescens in combination (EBC 5 and EBC 6) showed the lowest incidence of pre- and postemergence damping-off caused by *Pythium aphanidermatum* compared to individual treatment (Muthukumar et al. 2010). The combination of *P. putida* strains WCS358 and RE8 also enhanced suppression of Fusarium wilt of radish (de Boer et al. 2003). Combination of *Paenibacillus* sp. and a *Streptomyces* sp. suppressed Fusarium wilt of cucumber effectively than when used alone (Singh et al. 1999), and a combination of *P. fluorescens* and *Stenotrophomonas maltophila* improved protection against *Pythium*-mediated damping-off in sugar beet compared to when they were applied singly (Dunne et al. 1998). *P. putida* strains WCS358 and RE8 have different disease-suppressive mechanisms: pseudobactin-mediated competition for iron and/or another yet unknown disease suppressive trait for WCS358, and ISR for RE8. Combining these mechanisms by applying a mixture of the biocontrol strains leads to more effective, or at least more reliable, biocontrol of Fusarium wilt of radish (de Boer et al. 2003). Another possible reason of enhanced disease suppression may be induction in mutual population of the microbes. The population densities of *P. putida* WCS358 that developed in the presence of *P. putida* RE8 were above the threshold level required for disease suppression (Raaijmakers et al. 1995a). Similarly, *P. fluorescens* NBRI-N6 and *P. fluorescens* NBRI-N in a consortium controlled collar rot in betelvine caused by *S. rolfsii* more than either of the strains did individually (Singh et al. 2003). *Rhizobium* and *P. striata* when inoculated together improved growth and reduced nematode multiplication more than each inoculated alone. This may be due to increased availability of nitrogen (N) and phosphorus (P), as these nutrients have been reported to have adverse effect on nematode multiplication (Pant et al. 1983). Use of *Rhizobium* with *P. striata* has also been reported to reduce the multiplication of *Meloidogyne incognita* on pea (Siddiqui and Singh 2005; Kumar et al. 2005).

Under severe disease pressure of dry bean root rot caused by *Fusarium solani* f. sp. *phaseoli*, only co-inoculation with *B. subtilis* MBI600 (Epic) and *Rhizobium tropici* significantly reduced disease severity and enhanced yield compared to control and standard seed treatment (de Jensen et al. 2002). Some combinations of fluorescent pseudomonad strains increased wheat yield compared to the same strains used singly, and interestingly the best combinations did not always produce the same results in different crops (Pierson and Weller 1994). In contrast *Bacillus* strain mixture of IN937a and IN937b improved yield of all plants compared with that of plants treated with individual strain IN937a, suggesting that the combination could be useful on tomato, long cayenne pepper, and cucumber (Jetiyanon et al. 2003). Two species microbial consortium of *Burkholderia* sp. MSSP and *Sinorhizobium meliloti* PP3 were found to promote growth of pigeon pea because of increased IAA production and ability to solubilize phosphate (Pandey and Maheshwari 2007). Similarly, Dutta et al. (2008) observed promising results on combined use of *B. cereus* strain BS 03 and a *P. aeruginosa* strain RRLJ 04 with rhizobia for induction of systemic resistance against fusarial wilt in pigeon pea.

A mixture of *B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b has previously shown to consistently provide a broad spectrum of disease protection against both soil- and air-borne pathogens, like *Colletotrichum gloeosporioides*, *Ralstonia solanacearum*, *R. solani*, *S. rolfsii*, and cucumber mosaic virus (Jetiyanon and Kloepper 2002; Jetiyanon et al. 2003). The mixture was found to induce SOD and PO activities and significant disease protection in four plant/pathosystems, viz., tomato with *S. rolfsii* and *Ralstonia solanacearum* and pepper with *S. rolfsii* and *C. gloeosporioides* (Jetiyanon 2007). Similarly, combining proteolytic and phloroglucinol-producing bacteria can improve biocontrol of *Pythium*-mediated damping-off of sugar beet (Dunne et al. 1998). A mixture of bacteria producing chitinases and antibiotics was found to effectively suppress rice sheath blight caused by *R. solani* (Sung and Chung 1997). Thus to enhance biocontrol efficacy and consistency in performance, use of several strains from the same antagonistic microorganism, or combination of different biocontrol species should be emphasized (Alabouvette and Lemanceau 1998; Guetsky et al. 2002).

16.5 Microbial Consortium Comprising Fungal and Bacterial Strains

The use of bacterial and fungal strain mixtures is a promising way to improve efficacy of biocontrol strains. *Pseudomonas* and *Trichoderma* strains are the most commonly studied BCAs for developing consortium and they have been reported to improve overall plant growth and suppress disease incidence in different crops (Mathivanan et al. 2000; Thirup et al. 2003). Also, a positive synergistic combination of *Trichoderma* spp. and bacterial antagonists *P. syringae* has been reported to control plant pathogens (Whipps 1997). Similarly, certain reports have shown that carbon from Arbuscular Mycorrhiza mycelium is rapidly incorporated into microbial biomass (Paterson et al. 2008) and therefore, these fungi have the potential to be important conduits of energy into rhizosphere bacteria like *P. fluorescens* for biocontrol. Brulé et al. (2001) selected a mycorrhiza helper bacterial strain *P. fluorescens* BBc6 to improve the efficiency of *Laccaria bicolor* S238N inoculation in French nurseries.

Similarly, synergistic effect has been obtained in controlling *F. oxysporum* f. sp. *radicis-lycopersici* by combining a fluorescent *Pseudomonas* sp. with a nonpathogenic *F. oxysporum* (Alabouvette et al. 1996) where the nonpathogenic *F. oxysporum* competes for carbon sources and the bacterial antagonist produces a siderophore to fulfill its nutritional requirement of iron (Lemanceau et al. 1993). Effective control of *F. oxysporum* f. sp. *cucumerinum* was achieved by the interactive effect of the bacterium *P. putida* with saprophytic strains of *F. oxysporum* (Park et al. 1988). Application of *P. fluorescens* and *T. viride* also significantly reduced sheath blight disease incidence compared to control (Mathivanan et al. 2005). In a similar way, root rot of pea caused by *Aphanomyces euteiches* f. sp. *pisi* was significantly reduced by the combined application of *T. harzianum* and phenazine antibiotic producing *P. fluorescens* strain 2-79RN10, compared to *T. harzianum* treatment alone (Dandurand and Knudsen 1993). The ability of *Bacillus mycoides* and *Pichia guilermondii* has also been found successful in suppressing *Botrytis cinerea* infection on strawberry (Guetsky et al. 2001). In this case, the yeast effectively competed with *B. cinerea* for nutrients, whereas the *Bacillus* secreted inhibitory compounds and activated the defense systems of the host (Guetsky et al. 2002). Combined use of *Rhizobium* and *Glomus intraradices* had adverse effect on the pathogens by increasing the availability of N and P to plants and thus limiting it for pathogens (Akhtar and Siddiqui 2008). By combining the nonpathogenic *F. oxysporum* strain Fo47 with the bacterial strain *P. putida* WCS358, two different disease-suppressive mechanisms were noted to act together to enhance suppression of Fusarium wilt of carnation and flax (Lemanceau et al. 1992, 1993; Duijff et al. 1999). Overlap between fungi and bacteria in utilization of root exudates can result in selective competitive pressure and therefore have an additional impact in tackling different pathogens at the same time.

16.6 Consortium Having more than Two Microbial Components

For better and consistent performance of BCAs, it is a prerequisite to identify strains of BCAs with diverse activities. A consortium of four different PGPMs, namely, *Bacillus licheniformis* strain MML2501, *Bacillus* sp. strain MML2551, *P. aeruginosa* strain MML2212, and *Streptomyces fradiae* strain MML1042, was highly effective in reducing the sunflower necrosis virus disease (SNVD) and increased the plant growth (Srinivasan et al. 2009). Use of the consortium comprising of bioagents *T. harzianum*, fluorescent *Pseudomonas*, and *G. intraradices* against Fusarium wilt not only suppressed disease incidence but also helped in sustenance and growth promotion of crop through mechanisms like enhanced plant growth promotion and nutrient uptake (Srivastava et al. 2010). Similarly, reduced disease intensity in combined application of *G. intraradices* with *Rhizobium* and *P. striata* was observed in a study conducted on root disease complex of chickpea (Akhtar and Siddiqui 2008). The synergism between various bacterial genera such as *Bacillus*, *Pseudomonas*, and *Rhizobium* has also been demonstrated to promote plant growth and development (Halverson and Handelsman 1991; Vessey and Buss 2002). Kandan et al. (2005) observed increased leaf area and shoot length, in tomato plants treated with a consortium of three different *P. fluorescens* strains, CHA0, CoT1, and CPO1. In a similar way combination of three PGPRs, *B. pumilus*, *B. subtilis*, and *Curtobacterium flaccumfaciens* provided greater control of several pathogens on cucumber than they were inoculated individually (Raupach and Kloepper 1998). *Pythium oligandrum* inoculum containing three strains with different biological traits was proved to be greatly strain-dependent (Vallance et al. 2008). Floch et al. (2009) observed that close contact between the hyphae of *Fusarium oxysporum* Schltdl. strain Fo47 with *Trichoderma harzianum*, and *P. oligandrum* cells, caused the destruction to the latter. However, in the rhizosphere, hyphae are frequently separated by a certain distance and this allows the coexistence and persistence of the three microorganisms on the root systems.

Tomato and leek plants grown in the presence of *Glomus mosseae* along with genetically modified strains of *P. fluorescens* CHA96 and CHA0 pME3424 produced enhanced levels of antifungal compounds and had a significantly higher shoot dry weight than those grown in the absence of *G. mosseae*. Colonization and activity of *G. mosseae* was found to be unaltered in the presence of *P. fluorescens* isolates and presence of *G. mosseae* increased the population of *P. fluorescens* in the rhizosphere (Edwards et al. 1998). Inoculation of *G. intraradices*, *P. striata*, and *Rhizobium* caused a significant increase in plant growth, number of pods, and chlorophyll, nitrogen, phosphorus, and potassium contents of pathogen-inoculated chickpea plants (Akhtar and Siddiqui 2008).

Combined inoculation of *Rhizobium*, a phosphate-solubilizing *B. megaterium* sub sp., Phospaticum strain-PB, and a biocontrol fungus *Trichoderma* sp. showed increased germination, nutrient uptake, plant height, number of branches, nodulation, pod yield, and total biomass of chickpea compared to either individual inoculations or an uninoculated control (Rudresh et al. 2005). *B. licheniformis* MML2501, *Bacillus* spp. strain MML2551, *P. aeruginosa* MML2212, and *Streptomyces fradiae* MML1042 were evaluated against SNVD as consortia in all possible combinations under greenhouse conditions (Srinivasan 2007) and found that they effectively improved plant growth and reduced SNVD incidence under greenhouse conditions (Srinivasan et al. 2009). Apart from the positive results, there are reports on adverse effects of some isolates of *Trichoderma* and *Streptomyces griseoviridis* on arbuscular mycorrhiza formation (Wyss et al. 1992; McAllister et al. 1994) limiting the possibility of using microbes for developing consortia without thorough screening.

16.7 Shortcomings

Various reports indicate that use of beneficial microbial mixtures generally increase plant growth and/or decrease plant disease relative to inoculation with a single beneficial organism (Chandanie et al. 2006; Raimam et al. 2007). However, some authors have pointed out that this beneficial effect was not always observed. For example, a combination of *Bacillus subtilis* and nonpathogenic *Fusarium oxysporum* did not provide control over Fusarium wilt of chickpea (*F. oxysporum* f. sp. *ciceri*), whereas either applied singly did (Hervas et al. 1997). It indicates that the results of co-inoculation of these microorganisms on plant health and productivity should be determined on the basis of the case under study (Siddiqui and Shaukat 2002; Whipps 2004).

de Boer and coworkers (2003) documented that at the most, only limited competition for iron occurred between the strains of *P. putida* strains WCS358 and RE8. A possible explanation may be that WCS358 and RE8 colonize different niches, and therefore, no competition recorded for iron. In a mixture of microbes, one may negatively influence root colonization of others. Sometime interactions between an introduced microbial mixture can also negatively influence disease control. For instance, siderophore-mediated competition for iron or competition for substrate may limit the colonization or activity of introduced biocontrol strains (Raaijmakers et al. 1995b; Kragelund and Nybroe 1996). Even strains of the same species can exhibit significant differences. Natural microbial communities are more closely mimicked through application of a microbial mixture comprising of several species, and therefore this control strategy may prove to be more relevant in the long term. A prerequisite for successful and consistent biocontrol is to identify different BCAs with diverse activities, which can collectively increase the crop performance. The compatibility of microbes used in microbial mixture, their co-establishment in the rhizosphere of plants, and the lack of competition among them are compulsory requirements for a microbial mixture to qualify the eligibility criteria. The complexity of the interactions taking place in the rhizosphere between BCA used in the mixture among themselves and the indigenous microbiota needs to be considered and studied deeply during development of a successful microbial consortium.

16.8 Future Prospects

Our increasing knowledge on the beneficial effects of microbial consortium would most likely enhance the usefulness of these microorganisms regardless of their sole function in agricultural management practices. Mixtures of microorganisms may increase the genetic diversity of biocontrol systems which may persist longer in the rhizosphere and utilize a wider array of mechanisms for disease control (Pierson and Weller 1994). Further studies require focus on their additive and synergistic mode of actions. Additional information would assist us in appropriate application of these organisms in improving agricultural management practices. More focus should be paid on assessing the added value of the microbial consortium in comparison to a single bioinoculant. Moreover, crop-based microbial consortium may be developed to meet the specific requirements. With the advent of functional genomics and proteomics studies of these microorganisms (Marra et al. 2006; Chacón et al. 2007), work on genes and gene products would provide more precise information on modes of action. Statistical procedures have been devised using which separation of direct growth promotion effects of a biocontrol agent from the effect obtained by disease control is possible, using data from factorial experiments in which BCA were applied in the presence or absence of pathogens (Larkin and Fravel 1999; Ryder et al. 1999). Mathematical modeling can also help us predict the results of interactions among consortium microbes and pathogen in the rhizosphere. Future relies on the application of modern molecular techniques and along with conventional experimental procedures to understand and utilize plant–microbe interactions occurring in soil. Its application would increase our knowledge about their combined mode of action, particularly with induced resistance in plants. Formulation also plays a significant role in determining the final efficacy of the mixture. A large number of microbial mixtures have been reported till date (Table 16.1), but further studies are needed to explore interactions between microbial agents to get maximum benefits out of them.

Table 16.1 Microbial consortium reported for the management of various plant diseases

Microbial mixture	Disease/pathogen	Mechanisms involved	Reference
Rhiozobim and *P. straita*	Nematode multiplication	Increased N and P availability	Pant et al. (1983)
P. putida, *Fusarium oxysporum* strain F047	*Fusarium oxysporum*	Siderophore-mediated competition for carbohydrate	Lemanceau et al. (1993)
T. harzianum and P. fluorescens strain 2-79RN10	*Aphanomyces euteiches* f. sp. *pisi*	Siderophore, ISR, and plant growth promotion	Dandurand and Knudsen (1993)
Gaemannomyces graminis var. *graminis* + mixture of pseudomonads	Take-all disease of wheat (*Gaemannomyces graminis* var. *tritici*)	Antibiosis	Duffy and Weller (1995)
P. fluorescens and *Stentrophomonas maltophila*	*Pythium*-mediated damping-off in sugarbeet	ISR	Dunne et al. (1998)
P. putida RE8 and WCS358	Fusarium wilt of radish	Siderophore and ISR, Mutual increase in population	de Boer et al. (2003)
Pichia guilermondii and *B. mycoies*	*B. cinera*	Competition for nutrients and secretion of inhibitory compound	Guetsky et al. (2001, 2002)
P. fluorescens NBRI-N6 and *P. fluorescens* NRI-N	*Sclerotium rolfsii*	ISR	Singh et al. (2003)
Rhizobium and *P. straita*	*M. incognita*	Plant growth promotion	Siddiqui and Singh (2005)
Bacillus sp. strain mixture IN937b + SE49 and T4 + INRN	Cucumber mosaic virus and *Colletotrichum gloeosporioides*	ISR	Jetiyanon et al. (2003)
P. fluorescens and *T. viride*	Sheath blight	ISR and plant growth promotion	Mathivanan et al. (2005)
B. amyloliquefaciens IN937a and *B. plumilus* IN937b	*S. rolfsii*, *Ralstonia solanacearum* and *Colletotrichum gloeosporioides*	Increased SOD and PO activity	Jetiyanon (2007)
Rhizobia with *B. cerus* strain BS03 and *P. aeruginosa* RRLJ04	*Fusarium udum*	Increased PAL, PO, and PPO activity	Dutta et al. (2008)
Bacillus licheniformis strain MML2501, *Bacillus* sp. strain MML2551, *P. aeruginosa* strain MML2212 and *Streptomyces fradiae* strain MML1042	SNVD	ISR and plant growth promotion	Srinivasan and Mathivanan (2009)

(continued)

Table 16.1 (continued)

Microbial mixture	Disease/pathogen	Mechanisms involved	Reference
P. fluorescens EBC5 and EBC6	*Pythium aphanidermatum*	ISR	Muthukumar et al. (2010)
T. harzianum, fluorescent *Pseudomonas* and *G. intraradices*	Fusarium wilt	Plant growth promotion and increased nutrient uptake	Srivastava et al. (2010)

16.9 Conclusions

The increased growth and reduced intensity of disease by using microbial consortium may be attributed to a combination of mechanisms acting in an additive manner. Microorganisms in a cocktail may enhance the level and consistency of performance of biocontrol by increasing effectiveness over a wide array of biotic and abiotic stresses and proving to be more stable by mimicking natural communities. In particular, combinations may provide protection at different times and under different conditions, by occupying different or complementary niches, supplementing each other's requirements. The present chapter on microbial consortium emphasizes their potential role in plant growth promotion and disease control. By using microbial cocktails, we can make sure that atleast one organism used in the mixture is functional under a particular stress and in a particular environmental niche. Additive and synergistic effects of mode of actions in combination would increase their potential as a BCA and would serve us with better disease control, higher yield, and improve soil quality results. However, precise knowledge of their mode of action and plant–microbe interactions would help us in their appropriate release and multi-faceted uses in managing plant health. The potential increase in use of these cocktail microorganisms along with their ability to impart multiple benefits may further help in reducing problems associated with the use of synthetic chemicals in agriculture and managing biotic stress in crop plant in an ecologically acceptable way.

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