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Review Article

Microbial consortia-mediated plant defense against phytopathogens and growth benefits

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Abstract

Microorganisms under natural habitats live in communities and some provide benefits to plants. The concept of development of microbial consortia for bio-control and crop sustainability relies on this fact. Microbes when introduced to soil as consortium interact with a host plant, partially mimic the natural soil conditions. To improve stability of the released microbes in different agricultural fields, use of microbial consortia is advocated. Further, microbes together can also offer multiple mechanisms of mycoparasitism, competition, antibiosis, induced systemic resistance etc., to fight pathogens. Microbes in communities also strengthen the capabilities of the partners in an additive or synergistic manner. Although development of microbial consortia is important for management of plant diseases, it is also equally important to understand how they influence plant metabolism when the consortium is introduced to soil. Various plant physiological parameters that are identified to aid biocontrol by pathogens include activation of phenyl propanoid pathway, activation of antioxidant pathways and various stress enzymes such as phenyl alanine ammonia lyase, peroxidase and polyphenol oxidase etc. The compatible microbial consortia trigger defence responses in an enhanced level in crop plants than the microbes alone, and provides better protection against pathogens. Evaluation of compatibility and synergism of microbial components is essential for the success of microbial consortia. Further rapid evaluation methodologies or kits need to be developed for quicker developments in the field of microbial consortia.

Keywords: Microbial consortia, systemic resistance, defense molecules, antibiosis, compatibility

1. Introduction

Rhizosphere is the region surrounding a root and is affected by the root itself. It is rich in microbial population and is a dynamic and complex environment. Generally, interactions between plants and microorganisms can be classified as pathogenic, saprophytic, and beneficial (Lynch, 1990). Beneficial interactions involve plant growth promoting rhizobacteria (PGPR), generally refers to a group of soil and rhizosphere bacteria colonizing roots in a competitive environment and exerting a beneficial effect on plant growth (Kloepper and Schroth,1978).Deleterious microorganisms living in the rhizosphere and interacting with the plant roots may cause development of plant diseases. In recent years, interest in the use of PGPR to promote plant growth has increased. Beneficial effect of PGPR on plant growth involves abilities to act as phytostimulators and biofertilizers. PGPR could enhance crop yield through nutrient uptake and plant growth regulators. PGPR could also act as biocontrol agents by production of antibiotics and siderophores and by triggering induced local or systemic resistance.

2. Advantages of biocontrol agents

1. Host specificity.
2. Ability to multiply in the target cells.
3. No problem of toxic residue.
4. No evidence or absence of resistance.
5. No problem of cross resistance.
6. Conventional technique or methods for applications.
7. Permanent control of pest or long persisting effect.
8. Ideally suited for integration with most other plant protection measures used in IPM programme.
9. No fear of environment pollution and hence ecofriendly.

3. Disadvantages of biocontrol agents

1. High selectivity or host specificity.
2. Requirement of additional control measures.
3. The correct time of application.
4. Delayed effect or mortality.
5. Storage problem.
6. Difficulty of culturing in large quantities.
7. Short residual effectiveness.

4. The concept of microbial consortium

A microbial consortium is two or more microbial groups living symbiotically.Microbial consortia have various advantages over single species, or "superbugs", such as efficiency, robustness, and modularity. Microorganisms under natural habitats live in communities and some provide benefits to plants. It is unveiled that microbes in small consortia enhance defense signaling cascades leading to enhanced transcriptional activation of several metabolic pathways. With progress in time a sizeable understanding on microbial consortium-induced plant defenses have been reached.

A better understanding of the major mechanism displayed in field soils will suggest what conditions are to be provided in order to optimize the antagonistic activities of inoculant strains. In this optic, controlled root exudation or nutritional amendment could lead to more successful disease management. Bacteria with more than one beneficial effect are of great interest in biocontrol. By combining strains with different disease-suppressive mechanisms, the impact of filed fluctuating biotic and abiotic conditions could be minimized, as some biocontrol mechanism could be effective even if others are unfunctional. In addition, such combinations could be effective against multiple phytopathogens.

5. Various applications of microbial consortia

- Bio fertilizers.
- Bio-control agents.
- Assisting mammals in food digestion.
- Reclamation of soils.
- Effective degradation of organic wastes.
- Rhizosphere bioremediation of pesticides.

6. Microbial consortium in disease suppression

Application of microbes in a consortium may improve efficacy, reliability and consistency of the microbes under diverse soil and environmental conditions (Stockwell et al., 2011).Use of different species of microbes occupy different niches in the root zone and thereby restrict competition among them. Diversity in biocontrol mechanisms offered by each microbial component may also help in enhancing disease suppressiveness. A number of microbial consortia have been developed by many scientists and were tested in different crops. These are abstracted in Table 1.

7. Microbial consortium-mediated plant defense

The microbial consortium activates the antioxidant enzyme activities and the phenylpropanoid pathway leading to accumulation of total phenolics, proline, and pathogenesis related (PR) proteins after the pathogen challenge (Fig. 1). Table 2 gives a summary of experimental studies on combined use of biological control agents (BCAs) to control plant diseases in comparison with the use of individual agents. Akanksha et al., 2012 studied the impact of triple microbial consortia consisting of fluorescent *Pseudomonas* (PHU094), *Trichoderma* (THU0816) and *Rhizobium* (RL091) for alleviation of biotic stress in chickpea through enhanced antioxidant and phenylpropanoid activities. Periodical studies revealed maximum activities of phenylalanine ammonia lyase and polyphenol oxidase and accumulation of total phenol content higher when challenged with the pathogen compared to the single microbe and dual microbial consortia. Jain et al. (2012) used *Pseudomonas aeruginosa* PJHU15, *Trichoderma harzianum* TNHU27 and *Bacillus subtilis* BHHU100 as a consortium to assess suppression of soft-rot pathogen *Sclerotinia sclerotiorum*. The triple-microbe consortium and single-microbe treatments showed 1.4–2.3 and 1.1–1.7-fold increment in defense parameters, respectively, when compared to untreated challenged control. The compatible microbial consortia triggered defense responses in an enhanced level in pea than when the microbes were alone, and provided better protection against *Sclerotinia* rot (Fig. 2). *Trichoderma* species and fluorescent *Pseudomonas* spp. have been reported to induce systemic resistance in plants. These biological control agents were tested as a single application and in combination for their abilities to elicit induced resistance in cucumber against *Fusarium oxysporum f. sp. radices cucumerinum* and in *A. thaliana* against *Botrytis cinerea*.The combination of Tr6 and Ps14 induced a significantly higher level of resistance in cucumber, which was associated with the primed expression of a set of defense-related genes upon challenge with *Fusarium*. In *Arabidopsis*, both Ps14 and Tr6 triggered ISR against *B. cinerea* but their combination did not show enhanced effects. In the induced systemic resistance-defective *Arabidopsis* mutant myb72, none of the treatments protected against *B. cinerea*, whereas in the SA-impaired mutant sid2, all treatments were effective. Taken together, these results indicate that in *Arabidopsis* Ps14 and Tr6 activate the same signaling pathway and thus have no enhanced effect in combination. The enhanced protection in cucumber by the combination is most likely due to activation of different signaling pathways by the two biocontrol agents(Hamidreza et al., 2013) (Fig.3).

Table 1. Microbial consortia effective against different plant pathogens, the rationale behind selection, and their mode of actions.

Sl. No.	**Microbial Consortium**	**Rationale behind selection of microbes and pathogens against whom tested**	**Plants**	**Mode of action**	**References**
1	*Trichodermaharzianum* Tr6 &*Pseudomonas sp.* Ps14	Ps14 Isolated from cucumber rhizosphere to test their combined effect against *Fusariumoxysporum*f. sp. *radiciscucumerinum*	*Cucumis sativus,* *A. thaliana*	Primed expression of a set of defense-related genes	Alizadeh et al. (2013)
2	*Azospirillum* sp. AZ204 &*Pseudomonas fluorescens* Pf1	Selected based on prior report of AZ204 as N fixer and P solubilizer, Pf1 as BCA and tested against *Rhizoctonia bataticola*	*Gossypium hirsutum*	Plant growth promotion	Marimuthu et al. (2013)
3	*P. fluorescens* EBC5 & *P. fluorescens* EBC6	Isolated from chilli root tissues and selected based on mycelial growth inhibition of *Pythiumaphanidermatum in vitro*	*Capsicum annum*	ISR	Muthukumar et al. (2010)
4	*Bacillus amyloliquefaciens*& IN937a *B. pumilus* IN937b	Prior use as mixtures provided broad spectrum of disease protection, tested against *Sclerotiumrolfsii*, *Ralstoniasolanacearum*, C. *gloeosporioides*	*Capsicum annum,* *Solanum lycopersicum*	Increased SOD and PO activities	Jetiyanon (2007)
5	Mixture of *Pseudomonads*	Selected based on antifungal activities of the strains, tested against *Gaeumannomycesgraminis var. tritici*	*Triticum aestivum*	Antibiosis	Duffy and Weller (1995)
6	*T. harzianum* , *P. fluorescens strain* 2-79RN10	Selected based on antifungal activities of the strains, tested against *Aphanomyceseuteiches f. sp. pisi*	*Pisumsativum*	Siderophore; ISR; Plant growth promotion	Dandurand and Knudsen (1993)
7	P. aeruginosa (PHU094) , *T. harzianum* (THU0816), *Mesorhizobium sp.* (RL091	Compatibility checked beforehand of the strains BCAs and antagonistic behavior against pathogen, tested against *Sclerotiumrolfsii*	*Cicerarietinum*	Activation of phenylpropanoid pathway and lignin deposition Antioxidant mechanisms	Singh et al. (2013)
8	*Rhizobia sp,* *B. cereus* strain BS03, *P. aeruginosa* RRLJ04	Randomly selected and mixed to see the efficacy of the consortium against *Fusariumudum*	*Cajanuscajan*	Increased PAL, PO, and PPO activities	Duttaet al. (2008)
9	*B. licheniformis* MML2501, Bacillus sp. MML2551, *P. aeruginosa* MML2212, *Streptomyces fradiae* MML1042	Randomly selected based on prior knowledge of effectivity against Sunflower necrosis virus disease (SNVD)	*Helianthus annuus*	Plant growth promotion	Srinivasan and Mathivanan (2009)

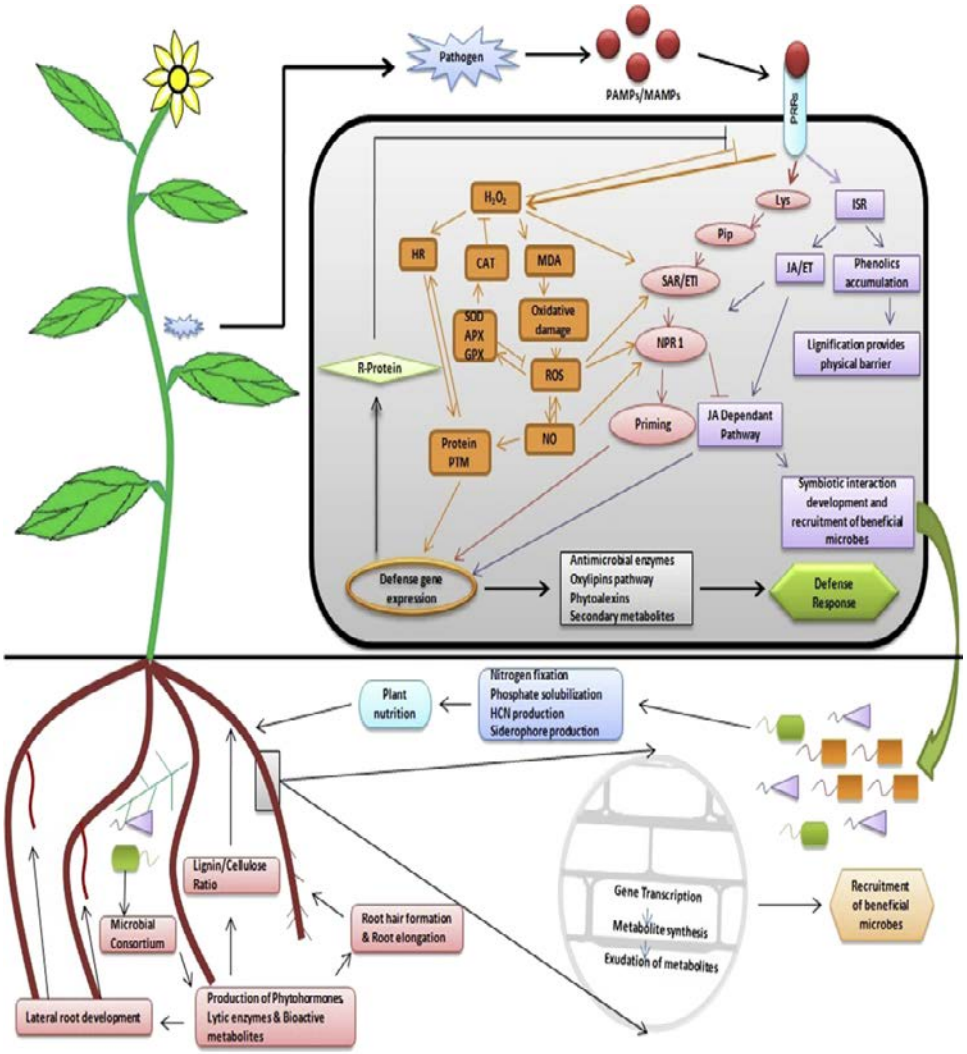

Fig.1: Mechanisms of rhizosphere microbe-mediated defense responses in plants against pathogenic stresses Ref**.** *Sarma et al.*, (2015).

Srinivasan and Mathivanan(2009) tested two plant growth promoting microbial consortia viz., PGPMC-1 consisting *of Bacillus licheniformis* strain MML2501 + *Bacillus sp. strain* MML2551 + *Pseudomonas aeruginosa* strain MML2212 + *Streptomyces fradiae* strain MML1042 and PGPMC-2 consisting of *B. licheniformis* MML2501 + *Bacillus sp*.MML2551 + *P. aeruginosa* MML2212 against Sunflower necrosis virus disease (SNVD).These formulations of the above plant growth promoting microorganisms (PGPMs) were evaluated along with farmers' practice (imidacloprid + mancozeb) and control in farmers' fields. Significant disease reduction, increase of seed germination, plant height and yield parameters with an additional seed yield of 840 kg/ha, an additional income of Rs. 10,920/ha and benefit cost ratio of 6.1 were recorded following treatment with a powder formulation of PGPMC-1 compared to control. The effects of *Glomus intraradices*, *Pseudomonas alcaligenes* and *Bacillus pumilus* on the root-rot disease complex caused by the root-knot nematode *Meloidogyne* incognita and the root-rot fungus *Macrophomina phaseolina* in chickpea was assessed by quantifying differences in the shoot dry mass, pod number, nodulation, and shoot content of chlorophyll, nitrogen, phosphorus and potassium. Application of microbial consortia showed better performance of these characters than their individual organism application (Akhtar and Siddiqui, 2008). The effect of a combined inoculation of *Rhizobium*, a phosphate solubilizing *Bacillus megaterium* sub sp. *phospaticum* strain-PB and a biocontrol fungus *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea were studied under glasshouse and field conditions. Combined inoculation of these three organisms showed increased germination, nutrient uptake, plant height, number of branches, nodulation, pea yield, and total biomass of chickpea compared to either individual inoculations or an uninoculated control. Increased growth and yield parameters were more pronounced when *T. harzianum*- PDBCTH 10 was inoculated along with the phosphate solubilizing bacterium and *Rhizobium*. Studies on population dynamics in the rhizosphere showed that there was no significant inhibition between the introduced organisms (Rudresh et al., 2004).

Table 2. Summary of experimental studies on combined use of biological control agents to control plant diseases in comparison with the use of individual agents.

Controlled organism	**BCA1**	**BCA2**	**Host type**	**Number of treatments**				
				Total	**>Pred**	**>Best**	**<Best**	**Ref**
Colletotrichum acutatum	*Cryptococcus laurentii*	*Metschnikowia pulcherrima*	Wounded apple	4	0	0	0	Conway et al.(2005)
Penicilliumexpansum	*Cryptococcus laurentii*	*Metschnikowia pulcherrima*	Wounded apple	4	0	0	0	Conway et al.(2005)
Botrytis cinerea	*Cryptococcus albidus,*	*C. laurentii,* *Rhodotorula glutinis*	Wounded apple	4	1	1	1	Calvoet al.(2003)
*Gaeumannomyces graminis*var. *tritici*	Several *Pseudomonas* spp. strains		Wheat seed	5	0	0	0	Pierson et al.(1994)
Rhizoctoniasolani..	*Trichodermas* pp	*Bacillus* sp	Cucumber seed	6	0	0	0	Yobo et al.(2010)
Pythiumultimum	*Trichodermavirens*	*Burkholderiaambifaria,* *B. cepacia,* *Serratiamarcescens*	Cucumber seed	22	0	0	0	Roberts et al.(2005)
Fusariumoxysporum, *R. solani*	*B. subtilis,* *B. amyloliquefaciens*	*B. licheniformis,* *Pseudomonas fluorescens,* *Chryseobacterium balustinum*	Tomato seed	3	0	1	0	Domenechet al.(2006).
Pyriculariaoryzae	*Pseudomonas fluorescens*	*Chryseobacterium balustinum*	Rice seed and leaves	1	0	0	0	Lucas et al.(2013).
Xanthomonas campestris	Five bacterial strains		Wounded anthurium leaves	9	0	0	0	Fukui et al.(1999).

Note: Total number of treatments with combined use of BCAs, number that resulted in greater control efficacies than predicted based on the Bliss assumption (>Pred), number that resulted in greater control efficacies than the more efficacious component BCA (>Best), and number that were less efficacious than the more efficacious component BCA (<Best). NA = not available.

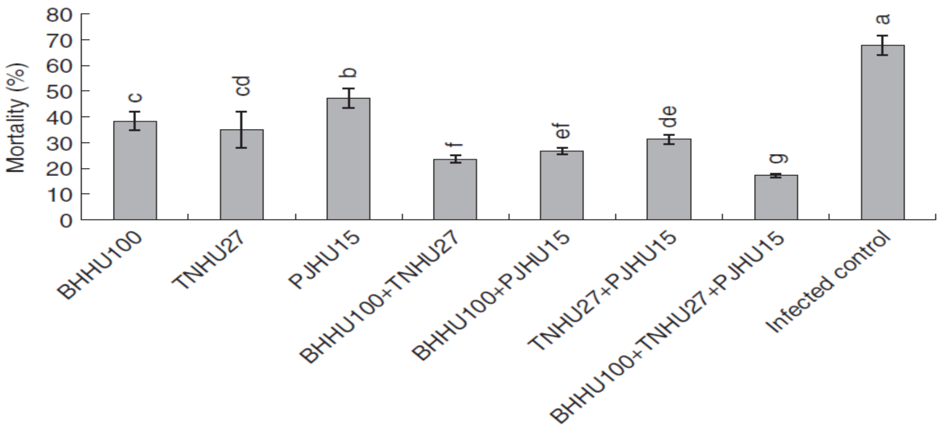

Fig.2: Mortality in pea treated with *Pseudomonas aeruginosa* PJHU15, *Trichodermaharzianum*TNHU27 and *Bacillus subtilis* BHHU100 when challenged with *Sclerotinia sclerotiorum*.

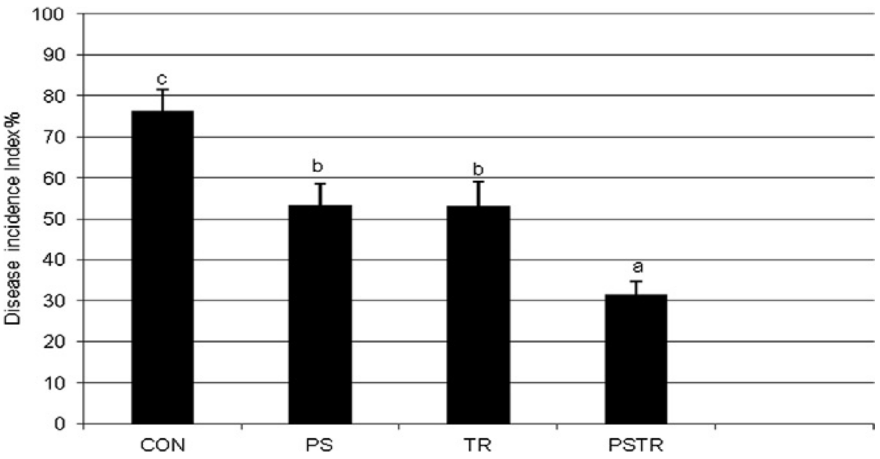

Fig.3: Control of *Fusarium* stem and root rot disease by individual and combined application of *T. harzianum* Tr6 (TR) and *Pseudomonas sp*. Ps14 (PS).

8. Limitations of microbial consortia

Several reports are available which demonstrated that certain microbial consortia were unable to show at least comparable effects on plants with respect to their individual applications. One of the major causes for such contrary results with microbial mixtures may be attributed to incompatibility of the microbes in the mixture with each other. The findings clearly advocate for screening of compatible microbes for development of microbial consortia. The basic objective of developing microbial consortium will fail if the microbes used in the consortium do not have any additive or synergistic effects on disease suppression.

9. Future perspectives and conclusions

Biological means of enhanced pathogen suppression in crop plants is clearly essential. To improve stability of the released microbes in different agricultural fields, use of microbial consortia is advocated. Consortia face the challenges of different soil and environmental conditions as they partially mimic the soil microbial communities. Microbes together can also offer multiple mechanisms (mycoparasitism, competition, antibiosis, induced systemic resistance, etc.) to fight pathogens. Study of microbial communities in natural rhizosphere also helps in the development of better microbial consortia.

Metabolic profiling of living microbial colonies facilitates studying spatiotemporal dynamics of metabolite production in microbial communities (Moree et al., 2012).Additional efforts are required to identify compatible strains of microbes that can positively influence the host physiological and transcriptional regulations for development of cost effective products for commercialization. Simple and rapid testing methodologies/kits need to be developed for evaluation of effective microbial consortia, which can predict probable impacts of the consortia on host plants through synergistic acts of the microbes.

Conflict of interest statement

We declare that we have no conflict of interest.

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