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***J. Nat. Prod. Plant Resour*., 2016, 6 (4):24-29**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Role of plant growth promoting rhizobacteria for biocontrol of phytopathogens

Aarti Thakkar, Ruby Patel, Rinkal Mulani and Vibha Bhingradiya

President Science College, Gujarat University, Ahmedabad, Gujarat, India

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ABSTRACT

Plant diseases cause economical loss of billions of dollars by reducing crop yield, lower produce quality and contaminating food grain with toxic chemicals. The endless variety and complexity of the many diseases of plants caused by fungi have led to the development of a correspondingly large number of fungicides; unfortunately several plant pathogens have developed resistance to certain fungicides. Another approach is to apply genetically resistant cultivars, but this is not viable after a few years. The present review highlights the role of PGPR strains, specifically referring to allelochemicals produced and molecular mechanisms. Further research to fine tune combinations of allelochemicals, plant-microbe–pathogen interaction will ultimately lead to better disease control.

Key words: Biocontrol, Phytopathogens, Allelochemicals

INTRODUCTION

Biological control of plant pathogens is considered as a potential control strategy in recent years, because chemical control results in accumulation of harmful chemical residues, which may lead to serious ecological problems. At present, effective management of plant diseases and microbial contamination in several agricultural commodities is generally achieved by the use of synthetic pesticides. However, the continual and indiscriminate application of these chemical fungicides has caused health hazards in animals and humans due to residual toxicity.

In recent years, large numbers of synthetic fungicides have been banned in the western world because of their undesirable attributes such as high and acute toxicity. Many pathogenic microorganisms have developed resistance against chemical fungicides. This seriously hinders the management of diseases of crops and agricultural plants. Considering the deleterious effects of synthetic fungicides on life supporting systems, there is an urgent need for alternative agents for the management of pathogenic microorganisms. And also, there is a need to reduction or elimination of synthetic pesticide applications in agriculture is highly desirable. One of the most promising means to achieve this goal is by the use of new tools based on bio-control agents (BCAs) for pest and disease control alone or to integrate with reduced doses of chemicals in the control of plant pathogens resulting in minimal impact of the chemicals on the environment [1].

Biological control of plant diseases has been considered a viable alternative method to manage plant diseases [2]. Biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens or reproduction of one organism using another organism [3]. A variety of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people and the environment [3].

Weeds interfere crop growth and reduce yields, deteriorate crop quality, clog waterways and cause health problems; with eradication costs being massive [4]. About 240weeds have been reported to have allelopathic potential [5], although many of these species have been tested with unrealistic bioassays [6]. On the other hand, allelopathic crops that are able to chemically interfere with weed growth have also been identified, such as Secalecereale (rye)[7], Triticumaestivum (wheat) [8], Oryza sativa (rice) [9], Helianthusannus (sunflower) Nikneshan et al.[10] and Glycine max (Soyabean) [11]. In addition to beneficial chemical interference of crops with weed growth, there is potential for the advantageous use of allelopathy for practices such as crop rotation, cover and smothers crops and retention of crop residues [4].

Despite the tremendous growth in allelopathy research in recent years there are lots of areas that have yet not been studied. Isolation and identification of rice allelochemicals are important to toxicological and eco-toxicological studies before crossing between present traits and commercial germplasm. Agronomic managements of rice like date of sowing, seeding depth, standing water depth, amount and type of fertilizers, duration of dry period, density andspecies of weeds are to be investigated for rice based allelopathy. Using allelopathic potential, rice cultivars in crop rotation and as companioncrop need to be studied [12].

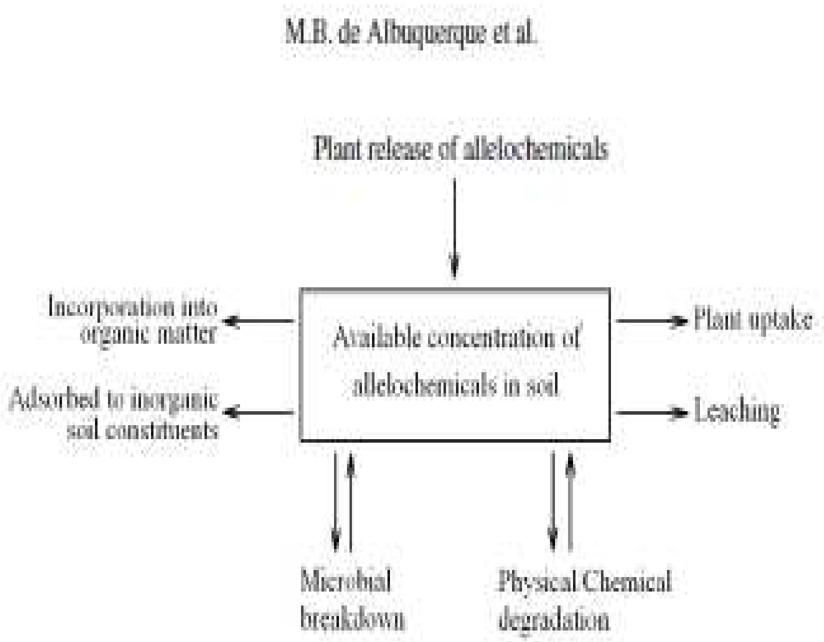
Antibiosis occurs during interactions involving low-molecular-weight diffusible compounds or antibiotics produced by Trichoderma strains that inhibit the growth of other microorganisms. Most Trichoderma strains produce volatile and nonvolatile toxic metabolites that impede colonization by antagonized microorganisms; among these metabolites, the production of harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-penthyl-α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others have been described [13]. In some cases, antibiotic production correlates with biocontrol ability, and purified antibiotics mimic the effect of the whole agent. However, there are also examples of antibiotic-overproducing strains, such as gliovirin over producing mutants of T. virens, which provide control similar to that of the wild-type, and of gliovirin-deficient mutants which failed to protect cotton seedlings from Phytiumultimum, whereas the parental strain did [14]. In general, strains of *T. virens* with the best efficiency as biocontrol agents are able to produce gliovirin[15]. Also, the most effective isolates of T. harzianum against *Gaeumannomycesgraminis* var. tritici produce pyrone antibiotics, and the success of the strains was clearly related to the pyrones they produced.

The combination of hydrolytic enzymes and antibiotics results in a higher level of antagonism than that obtained by either mechanism alone [16]. Synergetic effects between an endochitinase from T. harzianum and gliotoxin, and between hydrolytic enzymes and peptaibols on conidial germination of B. cinerea is well known [17].Research on the mechanisms responsible for the biocontrol exerted by Trichoderma spp. on phytopathogenic fungi have led to a better understanding of such mechanisms, as well as to the isolation of several genes encoding either enzymes and structural or regulatory proteins, or components of signaling pathways that are involved in processes such as the specific recognition of hosts by Trichoderma strains. These tools will allow the isolation of improved strains and thus of more efficient formulations to control fungal pathogens in pre- and post-harvest periods [18]**.**

In the soil environment there are many supposed allelochemicals. KIMBER [19] indicatedthat in nature, the concentrations range from inhibitory for some allelochemicals to stimulatory for other allelochemicals, and the resultant net effect in plants may be lower inhibition or stimulation or no effect atall. Some authors have argued that allelochemicals act synergistically, thus magnifying their phytotoxic capabilities [20].Few experiments were conducted to test this hypothesis. However, herbicide science indicates that synergism is a rare occurrence and usually antagonistic[21] or additive[22] effects are the norm. As expected, Duke et al. [23] reported antagonism between pcoumaric and ferulic acids on lettuce seed germination, and Blum et al. (1984) [24] observed antagonism between ferulic, caffeic, and vanillic acids on cucumber radicle growth.

Synergism among lytic enzymes and between enzymes and antibiotics suggests formulations to test mixtures of *Trichoderma* transformants that produce different enzymes, in order to improve the antagonistic effects of bio-control agents on phytopathogenic fungi. *T. harzianum* wild type inhibited the growth rate of *B. cinerea* by 30% and transformants expressing either a β-1,3 glucanase, a chitinase, or a β-1,6-glucanase inhibited the growth rate of *B. cinerea* by 60%. Transformants were differently combined in order to test synergism among the enzymes secreted against several phytopathogens. The combination that overproduced chitinase and β-1,3-glucanase was more effective than the individual transformants in inhibiting *Rhizoctonia meloni*, whereas using other combinations, the inhibition was not improved [25] .

AnalysisofthewholeFZB42genomerevealedanimpressive capability to produce adverse spectrum of different secondary metabolites aimed to suppress harmful microbes and nematodes Living with in the plant rhizosphere [26]. In total, 11 gene clusters representing more than 9%ofthe Genome are devoted to synthesizing anti microbial metabolites [27, 28]. By contrast, the genomes of the closely related non-plant associated members of the *B. subtilis* speciescomplexdevoteonlyaround5%oftheircapacityin synthesis of antimicrobials. According to numerous in vitro studies it is widely assumed that its antifungal activity is due to non-ribosomal synthesis of the cyclic LP bacillomycin D and fengycin [29], whilst its anti bacterial activity is mainly due to non-ribosomally synthesize dpolyketides [30],andbacilysin [31], and ribosomally synthesized bacteriocins [32].



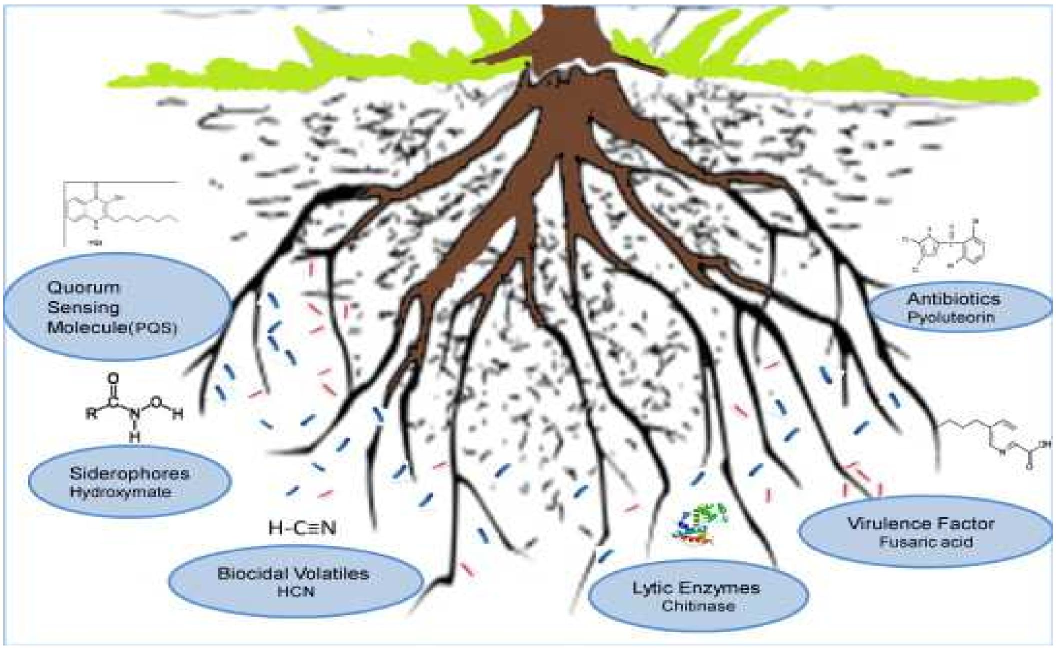
Input and output dynamics of allelochemicals in soil [33].

A review by Drobyet al[34] has well documented commercial antagonistic microorganisms available in the global market for postharvest control of decays in fruits and vegetable. These are Biosave (*Pseudomonas syringae* Van Hall), which are registered in the USA and used mostly for the control of sweet potato and potato diseases, and “Shemer”(*Metschnikowiafructicola* Kurtzman & Droby) registered in Israel and used commercially for the control of sweetpotato and carrot storage diseases. The two yeast-based products, Aspire TM (Ecogen, US) and Yield Plus (Anchor Yeast, South Africa) developed in the USA and South Africa is no longer available.

Currently, BioNext (Belgium) and Leasaffre International(France) have developed a commercial product, based onthe same yeast used in Aspire TM, *Candida oleophila*. A similar yeast-based product, *Candida saitoana* was developed by Neova Technologies (Abbotsford, British Columbia, Canada). Additionally, Spain has also developed a commercial formulation of *Candida sake* for use on pome fruit under the name “Candifruit”

Characteristic traits of an Ideal microbial antagonist
Several reviews have provided the good characteristic traits desired in microbial antagonist in the disease controlling process [35]. Wilson and Wisniewski [36] recommended a guideline to select an ideal antagonist, which are as follows:

1. Must be stable
2. Should be effective at low concentrations
3. Must not be demanding in terms of required nutrients
4. Must be able to survive under adverse environmental conditions
5. Should be effective against a wide spectrum of commodities and pathogens under different conditions
6. Should be amenable to production on inexpensive growth media
7. Should be amendable to formulations with a long shelf life
8. Should be easy to dispense without being hazardous to human health
9. Must be resistant to chemical used in the postharvest environment
10. Must be environmentally friendly
11. Must be compatible with commercial processing practices.
12. Should not be detrimental to the quality of the fruits and vegetables it preserves.



The rich microbial diversity provides a seemingly endless resource for this purpose. Rhizobacteria are most widely studied as plant growth-promoting bacteria (PGPB), associated with plant rhizosphere and are present in all agroecosystems[37]. Antagonistic bacteria are considered ideal biological control agents (BCA) because of the rapid growth, easy handling, and aggressive colonization of the rhizosphere[38]. The use of PGPR specifically as biocontrol agents of soil borne fungal plant pathogens as an alternative or complementary strategy to physical and chemical disease management have been investigated for over a century[39]. PGPR indirectly enhance plant growth via suppression of phytopathogens by producing chemicals that inhibit the growth of plant pathogens. Siderophores, antibiotics, biocidal volatiles, lytic enzymes and detoxification enzymes are all examples of allelochemicals produced by soil microbes [40].

Allelochemicals present in PGPR
Allelochemicals are associated with BCAs and are used in plant disease control that can be categorized based on various modes of action. Types of allelochemicals associated with PGPR strains and involved with disease management are shown in Table 1 [41-48].

Type and mode of action allelochemicals used in plant disease management

Sr. No	Type of Allelochemicals	Name of PGPR strain	Mode of action	Reference
.	Siderophore	*Alcaligenesfeacalis*	Growth inhibitions of *A. niger* NCIM 1025, *A. flavus* NCIM 650, *F.oxysporum* NCIM1008, and *A.alternata* IARI 715	Sayyed and Chincholkar (2009)
		Alcaligenes sp. STC1 and Acinetobacter sp. SH-94B	*A. niger* NCIM 1025, *A. flavus* NCIM 650,*F. oxysporum* NCIM 1281, *A. alternata* ARI 715, *C. arachichola, M. anisophilia* NCIM 1311, and *P. solanacerum* NCIM 5103.	Sayyed and Patel (2011)
2.	Antibiotics 2,4 DAPG	*Pseudomonas fluorescens*	effective against *S. rolfsii* (up to 75% inhibition)	Asadhi et al (2013)
	Pyrrolnitirin	*Pseudomonas chlororaphis* O6	*Rhizoctoniasolani* and *Fusarium graminearum*	Park et al (2011)
			Many fungal growth inhibition and confirmed antifungal gene by PCR	Grover et al (2010)
	Iturin A	*Bacillus subtilis* RP24	*S. sclerotiorum inhibition*	Kumar et al (2012)
	Bacillomycin D	*Bacillus* sp. A3F		

Alcaligenes sp. STC1 and A. niger NCIM 1025, A. flavus NCIM 650,F. oxysporum NCIM 1281, A. alternata ARI 715, C. arachichola, M. anisophilia NCIM 1311, and P. solanacerum NCIM 5103 Sayyed and Patel (2011) Acinetobacter sp. SH-94B 2. Antibiotics 2,4 DAPG P. fluorescens Effective against S. rolfsii (up to 75% inhibition) Asadhi et al. (2013) Pyrrolnitirin P. chlororaphis O6 Rhizoctonia solani and Fusarium graminearum Park et al. (2011) Iturin A Bacillus subtilis RP24 Many fungal growth inhibition and confirmed antifungal gene by PCR Grover et al. (2010) Bacillomycin D Bacillus sp. A3F S. sclerotiorum inhibition Kumar et al. (2012) 3. Lytic enzymes like chitinase, -1,3-glucanase, protease, etc. Pseudomonas PGC2 R. solani and P. capsici growth inhibition Arora et al. (2008) Bacillus alvei NRC 14 F. oxysporum inhibition in vitro and in vivo conditions Abdel-Aziz (2013) 4. Volatile metabolites Pseudomonas fluorescens, P. corrugate, P. chlororaphis, P. aurantiaca Inhibition of various pathogenic mycelium growth and spore germination Fernando et al. (2005) 5. Naturally produced allelochemicals (1) Phosphinothricin S. viridochromogenes allelochemicals as plant growth-regulating agents Barazani and Friedman (2001)

CONCLUSION

Biocontrol agents produce metabolites, chemicals and enzymes and rely on the emission for destruction of phytopathogens. Important discoveries pertaining to the genomics sequence of rhizospheric bacteria provide a variety of insights into the organisms lifestyle in plant microbes pathogens interaction. Further, developments and discovery of novel allelochemicals from PGPR would give greater insights into induction of increased disease resistance. In any case, the role of alleochemicals secreted by rhizospheric microbial community required for the studies, also because there is every reason to believe that going a greater understanding of these processes will facilitate in the long run efforts to mean off the dependence on agricultural chemicals.

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