Original Research Article

Biocontrol activity of PGPR species isolated from Lonar-lake

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Abstract

Biological control of plant diseases is gaining attention due to increased pollution caused by pesticides application for crop protection and development of resistance in plant pathogens resistance. The use of environment friendly microorganisms has proved useful in plant growth and disease control in modern agriculture. PGPR inoculum is a promising agricultural approach, which plays a vital role in crop protection, growth promotion or biological control. In the present investigation invitro inhibition of mycelial growth of three soil-borne fungal phytopathogens by candidate bacterial species was evaluated by dual culture method.

Key Words: PGPR, Biocontrol agent, Phytopathogens, Antifungal metabolites.

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INTRODUCTION

The recent update in agriculture field has strongly proposed strategies like integrated plant nutrient management (IPNM) and integrated plant disease management (IPDM) to improve overall growth and productivity of crop. The first clear indication of improved plant growth and biological control of root pathogens due to seed bacterization with rhizobacteria came from the works of Burr *et al.*, (1978) and Kloepper *et al.*, (1980) who reported the plant growth promoting effects of *Pseudomonas* strains which were antagonistic to a wide range of plant pathogens *in vitro*. These studies also provided the first evidence that the rhizosphere microbiota could be modified significantly with microorganisms introduced with the planting material. Kloepper *et al.*, (1989) coined the term plant growth promoting rhizobacteria (PGPR) to include bacteria inhabiting the root and rhizosphere soil which have the ability to increase plant growth. Bacteria that can improve plant growth through various mechanisms have been known for decade and have been introduced in to soil, on seeds or roots to improve plant growth and health. *Rhizobium* spp. which fix nitrogen from the atmosphere and form root nodules on legumes, were the first biofertilizers identified and have been used commercially as inoculants for legumes for over 100 years (Kannaiyan, 2002). Bacterial inoculants are able to increase plant growth, speed up seed germination, improve seedling emergence, responses to external stress factors, protect plants from disease and root growth pattern (Lugtenberg *et al.*, 2002). The mechanisms of plant growth stimulation by associative bacteria are mobilization of nutrients, stimulation of root growth by production of phytohormones and antagonism against soil borne plant pathogens (Hoflich *et al.*, 1994). Successful examples of inoculation of maize, canola, wheat and other crops with PGPR species *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Enterobacter* have been achieved both in laboratory and field trials (Sharma and Johri, 2003). Wu *et al.*, (2005) reported in their studies that microbial inoculum *Bacillus megaterium* and *Bacillus mucilaginous* not only increased the plant growth, but also improved nutritional assimilation of plant (total N, P and K). Plant growth promoting bacteria have an ability to convert nutritionally

important elements from unavailable to available form through biological processes (Vessey, 2003). Several mechanisms have been suggested by which PGPR can promote plant growth Viz direct growth promoting mechanisms as nitrogen fixation, solubilization of phosphorus, sequestering of iron by production of siderophores, production of phytohormones such as auxins, cytokinins, gibberellins while indirect mechanisms of plant growth promotion by PGPR include antibiotic production, depletion of iron from the rhizosphere, synthesis of antifungal metabolites, production of fungal cell wall lysing enzymes, competition for sites on roots and induced systemic resistance (Glick *et al*. 1999). In the present investigation bacterial species isolated from lonar lake, Dist-Buldhana (MS) have been evaluated for their PGPR and biocontrol activities against some common phytopathogens.

MATERIAL AND METHODS

Invitro antifungal activity: Bacterial isolates were evaluated for their antifungal activity against *Sclerotium rolfsii*, *Aspergillus niger*, *Aspergillus flavus* and *fusarium oxisporum* by dual culture technique (Rangeshwaran and Prasad, 2000). For testing antagonistic activity of isolates against fungal cultures PDA plates were prepared and used separately for each isolate. For which fungal disc of 6 mm diameter was placed on one edge of the plate (1 cm from the corner) and bacterial isolate was streaked or spot inoculated on the other edge of the plate (1 cm from the corner) followed by incubation at 28°C for 3-5 days (96 h) or till the pathogen covered the entire plate in the control. Inhibition of fungal mycelium (halo- zone) around the bacterial colony was scored positive and inhibition zone was measured. Each experiment was carried out in triplicates. The percent growth inhibition was calculated using

% Inhibition = [(R-r)/Rx100]

Where, r is the radius of the fungal colony opposite the bacterial colony and R is the maximum radius of the fungal colony away from the bacterial colony.

Protease production: Production of extracellular protease was tested as described by Maurhofer *et al*, (1995). Bacterial isolates were spotted on plates of Skim Milk Agar (SMA) plates and incubated at 28°C for 48 h. Semi quantification of protease was carried out by measuring a halo-zone around bacterial colonies. The experiment was performed in triplicate.

RESULT AND DISCUSSION

During isolation representative samples from Lonar lake were subjected for further processing in the laboratory and from total 32 isolates two isolates named *Enterobacter cloacae* R10-1A and *Lysinibacillus fusiformis* NBB1were evaluated for their biocontrol activity.

In vitro antifungal activity of isolated strains: In vitro inhibition of mycelial growth of three soil-borne fungal phytopathogens by siderophorogenic *Enterobacter cloacae* R10-1A and *Lysinibacillus fusiformis* NBB1 was tested by dual culture method on iron deficient King's B (KB) medium and iron sufficient potato dextrose agar (PDA). It showed the ability of *Enterobacter cloacae* R10-1A and *Lysinibacillus fusiformis* NBB1 to inhibit fungal phytopathogens. The degree of inhibition varied with the test organism and the media used. The zone of inhibition was wider in most cases on King's B medium, indicating the involvement of siderophores in the antagonistic activity. Furthermore the zone of inhibition was found to be reduced many fold on PDA which clearly demonstrates that other antifungal compounds like cell wall degrading enzymes may be involved in the inhibition. The reason behind such a difference in the degree of inhibition may be due to (i) iron limitation or competition for iron. (ii) Degree of diffusion level of siderophores in the media. From the results it is evident that the antagonistic action exerted by the isolates dominantly is due to siderophores and supported by certain secondary metabolites seems to have a good cumulative effect. These results are in accordance with the results obtained by few other laboratories (Johri *et al*., 1997). In the present investigation dual culture assay revealed that both siderophorogenic isolates are positive and can be used as biocontrol agents. *E. cloacae* R10-1A inhibited the growth of *Sclerotium* and *Fusarium*. This inhibition could be presumably due to siderophore production and cell wall degrading enzymes or production of hydrolytic enzymes like protease by the bacterial isolates in the culture medium. *Enterobacter cloacae* R10-1A was able to inhibit *Fusarium oxysporium* (58%). Similarly when *Enterobacter cloacae* R10-1A and *Lysinibacillus fusiformis* NBB1 were tested against *Sclerotium rolfsii*, it was observed that % inhibition were 69% and 67% respectively. Production of extracellular protease was tested according to Maurhofer *et al*. (1995) on Skim Milk agar plates. *Enterobacter cloacae* R10-1A and *Lysinibacillus fusiformis* NBB1 showed strong proteolytic activity by protease showing zone of clearance of 15 mm and 14 mm respectively around the colony. Biological control agents (BCA) synthesizes extracellular cell wall degrading enzymes these lytic enzymes are mostly inducible enzymes that hydrolyze fungal cell wall components (e.g. chitinase, glucanase, laminarinase, cellulase, and protease) (Compant *et al*., 2005). Some examples of biological control of fungal pathogen by bacterial antagonists have been reported. Biocontrol of *Fusarium oxysporium* with combinations of bacterial

strains of *Paneibacillus* sp. and *Streptomyces* sp. that produces chitinase and β-1, 3 glucinase (Singh *et al*., 1999). Finally from the investigation and observed facts it is concluded that both the isolates used in the study of growth promotion as well biocontrol activity are effective PGPB and BCA and are alternate to chemical input for sustainable agriculture.

REFERENCES

1. Burr, T. J., Schroth, M. N. and Suslow, T. V. (1978) Increased potato yields by treatment of seed pieces with specific strains of Pseudomonas fluorescens and P. putida. Phytopathol. 68: 1377-1383.
2. Compant, S., Duffy, B., Newak, J., Clement, C., and Bark, E. A. (2005) Use of plant growth promoting bacteria for biocontrol of plant diseases: principle, mechanism of action, and future prospects. Applied and Environmental Microbiology. 71: 4951-4959.
3. Glick, B.R., Patten, C.L., Holguin, G. and Penrose, D.M. 1999. Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press,London, United Kingdom, p 267.
4. Höflich, G., Wiehe, W., Ku¨hn, G. (1994) Plant growth stimulation with symbiotic and associative rhizosphere microorganisms. Experientia 50, 897–905.
5. Johri, B. N., Rao, C. N. S., and Goel, R. (1997) Fluorescent Pseudomonads in plant disease management, in Biotechnological approaches in soil microorganisms for sustainable crop production (Eds. K. K. Dadarwal), Scientific Publishers, Jodhpur, India. 193-223.
6. Kannaiyan, S. (2002) Biofertilizers for sustainable crop production. In: Kannaiyan, S. (Eds.) Biotechnology and biofertilizers, Kluwer Academic Publishers, Dordrecht, The Netherlands. 9-49.
7. Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N. (1980) Pseudomonas siderophores: a mechanism explaining disease-suppressive soils. Current. Microbiol. 4: 317–320.
8. Kloepper, J. W., Lifshits, R. and Zablotowicz, R. W. (1989) Free living bacterial inocula for enhancing crop productivity. Trends Biotechnol. 7: 39-43.
9. Lugtenberg, B., Chin-A-Woeng, T., Bloemberg, G. (2002) Microbe– plant interactons: principles and mechanisms. Antonie van Leeuwenhoek 81: 373–383.
10. Maurhofer, M., Keel, C., Haas, D., Defago, G. (1995) Influence of plant species on disease suppression by Pseudomonas fluorescens strain CHAO with enhanced production. Plant Pathol. 44: 40-50.
11. Rangeshwaran, R. and Prasad, R. D. (2000) Isolation and evaluation of rhizospheric bacteria for biological control of chick pea wilt pathogens. Journal of Biological Control. 14(1): 9-15.
12. Sharma, A., Johri, B. N. (2003) Growth promoting influence of siderophore producing Pseudomonas strain GRP3A and PRS9 in maize (Zea mays L.) under iron limiting conditions. Microbiol. Res. 158: 243–248.
13. Singh, P. P., Shin, Y. C., Park, C. S., and Chung, Y. R. (1999) Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. Phytopathology. 89: 92-99.
14. Vessey, J. K. (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 255(2): 571-86
15. Wu, S. C., Caob, Z. H., Lib, Z. G., Cheunga, K. C., Wonga, M. H. (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma. 125: 155–166.

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