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SHORT COMMUNICATION

Antimicrobial activities of rhizobacterial strains of *Pseudomonas* and *Bacillus strains* isolated from rhizosphere soil of carnation (*Dianthus caryophyllus* cv. Sunrise)

Sapna Sharma · Mohinder Kaur

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Abstract Under the present study, an attempt was made to characterize rhizobacteria i.e. *Pseudomonas* and *Bacillus* species isolated from rhizosphere of carnation to evaluate their growth promoting effect on carnation so as to select and develop more efficient indigenous plant growth promoting and disease suppressing bioagents of specific soil type and specific plant type. Maximum strains of *Pseudomonas* and *Bacillus* sp. showed significant antimicrobial activities against most of the microorganisms tested. On the basis of in vitro antagonistic activities ,the best strains were selected and used in field trial to study the influence of these strains on the growth of carnation. Results have shown marked effect on growth parameters and disease incidence has also been reduced significantly.

Keywords Rhizobacteria · Antagonistic activity · Antifungal activity · PGPR

S. Sharma (✉) · M. Kaur
Department of Basic Sciences,
Dr. Y.S. Parmar University of Horticulture and Forestry,
Nauni-Solan - 173 230, H.P.,
India

E-mail: sapna04_82@rediff.com

Introduction

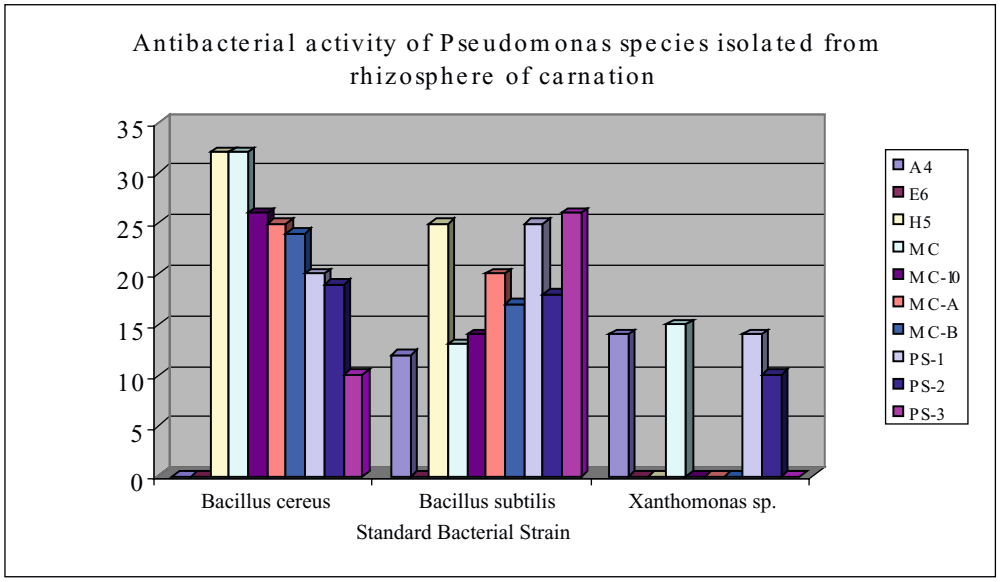
Carnation is an important cash crop. The most devastating disease of carnation is wilt caused by *Fusarium* sp. and *Rhizoctonia* sp. In recent years biological control has emerged as an important alternative method in managing soil borne plant diseases. Several rhizobacteria have been used extensively as biological agent to control many soil borne plant pathogens (Amico et al. 2005;Raj kumar et al. 2005). Rhizobacteria exert their beneficial effect on plants by various mechanisms viz. siderophore production, HCN, antibiotics, lytic enzymes, competition and by inducing systemic resistance (Pieterse et al. 2001; Tag et al. 2003).

Rhizosphere organisms exhibit strong antagonistic activities against bacterial and fungal pathogens which can cause enormous loss to crop. This loss can be minimized by chemical or biological means. Hence the present study is aimed to isolate and characterize *Pseudomonas* and *Bacillus* sp. for biological control and also to promote plant growth activities.

Samples were collected from rhizosphere of carnation. Isolation was done using standard serial dilution method and media employed were nutrient agar, *Bacillus* agar and King's B (for *Pseudomonas*) and identified on the basis of morphological and biochemical tests as per their genera laid down in Bergey's Manual of Systematic Bacteriology and were named as per the name of site from where samples were collected .

All the bacterial isolates were screened out for antagonism against the plant pathogens *Bacillus subtilis, B. cereus, and Xanthomonas* sp. and human pathogens. viz*., E. coli, Klebsiella* sp*., Salmonella typhi, S. paratyphi, Shigella* sp. *Staphyllococcus* sp. Plant pathogenic indicator test fungi

viz. *Fusarium* sp., *Alternaria* sp., *Aspergillus* sp. *Penicillium* sp. *Rhizoctonia* sp. *Sclerotium* sp. *Phytophthora* sp. *Trichoderma* sp. and *Trichothecium* sp. were procured from Department of Mycology and Plant Pathology and Department of Basic Science, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan .Antibacterial and antifungal activity of each strain of *Pseudomonas* sp. and *Bacillus* sp. was checked by bit/well plate assay methods (Fleming et al. 1975). Culture bit of each indicator bacteria bored with the help of sterile cork borer and was placed on the side of prepoured plate of nutrient agar in duplicates already having a lawn of indicator bacteria. In this method, wells were cut on nutrient agar plates and 100 µl of 72 h old cell free culture supernatants of test strains were added to each well on nutrient agar plates having lawn of indicator bacteria . Plates incubated at 37°C and 28 ± 2°C for *Bacillus* sp. and *Pseudomonas* sp. respectively for 24–48 h and observed for formation of clear zone of inhibition around well and bit against control. For testing of antifungal activity 72 h old bit of indicator fungi were placed on one side of prepoured potato dextrose agar plate in duplicates and on the other side of plates well was cut with the help of sterile cork borer. 100 µl of 72 h old cell free culture supernatant of each bacterial strain was added to each well. Plates were incubated at 28 ± 2°C for 3–5 days and observed for clear zone formation around well against control.



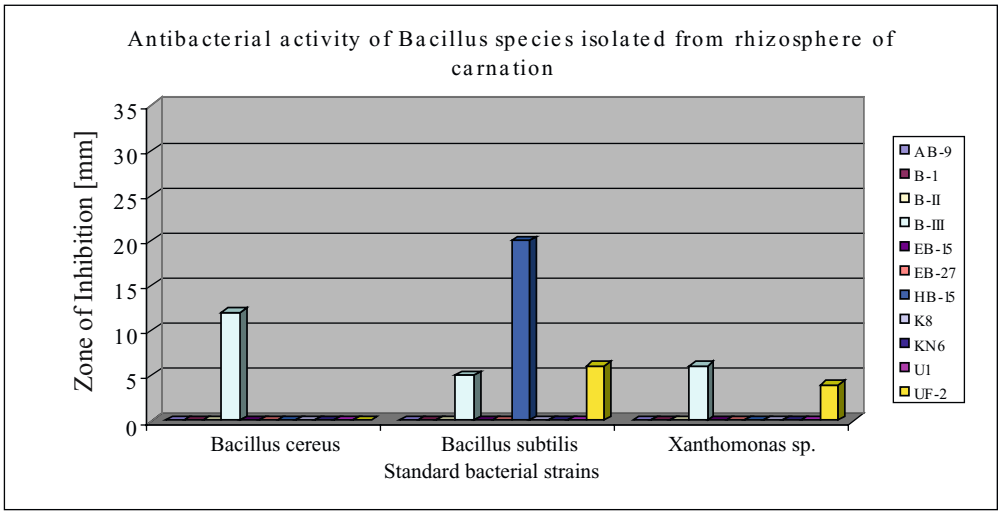


Fig. 1 Antibacterial activity of pseudomonas species and Bacillus species isoloated from rhizosphere of carnation.

On the basis of antagonistic activities four strains were used in field to study the effect of isolates on the growth and disease incidence in carnation in comparison to control at the concentration of 8×10^8 CFU/ml.

The most effective mechanisms that a PGPR (plant growth promoting rhizobacteria) can employ to prevent growth of phytopathogen is the synthesis of antibiotics. The *Pseudomonas* and *Bacillus* strains isolated from carnation were screened out for the in vitro production of antibacterial and antifungal activities (Figs. 1, 2) against various plant as well as human pathogens. Present isolates from the rhizosphere of carnation were found to be effective against various bacteria and fungi, indicating that antagonistic metabolites may be broad spectrum in nature like antibiotics.Where the *Pseudomonas* sp. and *Bacillus* sp. contain one or more than one type of secondary metabolites has not been ascertained. It may be due to that the microorganisms selected from rhizosphere enrichment environment may be so diversified and may be able to produce bioactive substances and biocatalysts due to environmental stress conditions (Steele and Stowers, 1991). In the present study *Pseudomonas* isolates had shown the better antagonistic activities than *Ba-*

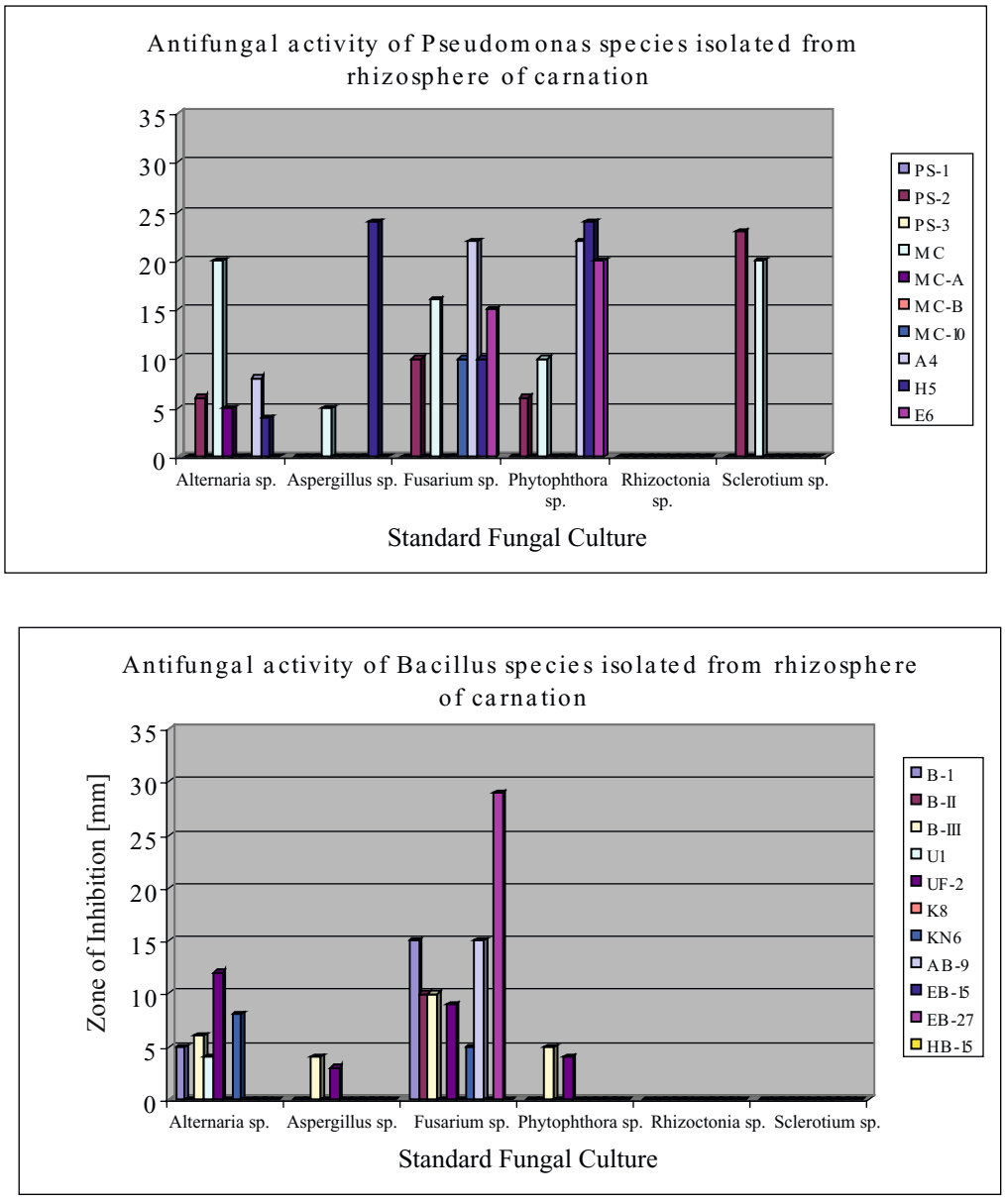


Fig. 2 Antifungal activity of pseudomonas species and Bacillus species isoloated from rhizosphere of carnation.

cillus sp. Most of the *Pseudomonas* isolates have inhibited plant pathogens, as *B. subtilis* inhibited by ~90% isolates, *B. cereus* by ~80% isolates and *Xanthomonas* by ~40% isolates. Among *Bacillus* isolates only two isolates were able to inhibit the above plant pathogens. Among the various isolates of *Pseudomonas* four strains were found to give best antagonistic activities against all tested pathogens.

Antibiotics are secondary metabolites produced by microorganisms, plants and made synthetically. They play a role in disease suppression of bacteria and fungi. Microbial interactions in the rhizosphere of plants are of considerable importance to agriculture (Harrison et al. 1993; Pal and Jalali, 1998). Bacteriocins are the simple protienaceous metabolites that are antimicrobial to other closely related organisms and inhibit the growth. Bacteriocins from *Pseudomonas* sp. can be used against *Staphyllococus* sp. *Xanthomonas* sp. *Erwinia* sp. (Padilla et al. 1996). Similar activity may also be produced by our *Pseudomonas* and *Bacillus* sp. (Figs. 1, 2) isolated from rhizosphere of carnation. Biological control of plant diseases using antagonistic bacteria may be alternative approach to the use of hazardous chemical fungicides (Meena et al. 2000).

Result of field experiment revealed that effect of organisms were statistically significant over control. Disease incidence was reduced upto 40 % as compared to control. This may be due to the secretion of antibiotics or secondary metabolites by the bacteria in root system which inhibited the phytopathogens. Despite of reduction of disease incidence an increase in growth parameters was also obtained, like shoot length was found to increase by 10%, yield was increased by 40%and flower diameter was also increased. This study has shown that these rhizosphere organisms have plant growth promoting potential and biocontrol activities. This growth promotion may be due to other mechanisms as production of siderophores, growth regulators, lytic enzymes etc.

Root colonization is another important factor in biological control of soil borne plant pathogens. Cook (1993) showed that bacteria isolated from rhizosphere of a specific crop showed a better control of diseases than organisms isolated from other crops. Therefore in present study the antagonists were isolated from rhizosphere of carnation and applied in carnation to study the biocontrol effect.

These indigenous strains isolated from specific plant and specific soil type may prove to be novel PGPR strains for carnation. They can reduce the disease incidence and will improve the fertility status of soil and may also protect the environment and will be cost effective. These strains can also be used as plant growth promoting bioagents for growth promotion and good health of plants.

References

1. Amico ED, Cavalca L, Andreoni V (2005) Analysis of rhizobacterial communities in perennial Graminaceae from polluted wate meadow soil,and screening of metal resistant potentiality plant growth promoting bacteria. FEMS Microbial Ecology 52:153–162
2. Cook RJ (1993) Making greater use of introduced microorganisms for biological control of plant pathogens.Annual Review of Phytopathology 31:53–80
3. Fleming HP, Etchells JL and Costilow RH (1975) Microbial inhibition by an isolate of *Pedicoccus* from cucumber brines. Applied Microbiology 30:1040–1042
4. Harrison, LA, Letrendre L, Kovacerich P, Pierson E a and Weller D M (1993) Purification of an abtibiotic effective against *Gaeumawaomyces glaminis* var. Tritici produced by a biocontrol agent *Pseudomonas ameofaciens*. Soil Biology and Biotechemistry 25:215
5. Meena B, Ramamoorthy V A, Marimuthu T and Velazhehen. (2000) *Pseudomonas fluorescens* mediated systemic resistance against late leaf spot in groundnut. Journal of Mycology and Plant Pathology 30 (2):151–158
6. Pal V and Jalali I (1998) Rhizosphere bacteria for biocontrol of plant diseases. Indian Journal of Microbiology 38: 187–204
7. Padilla C, Brevis P, Lobbos O and Hubort E (1996) Bacteriocin activity of *Pseudomonas* sp. on enteropathogenic bacteria in an artificial aquatic system. Letter in applied Microbiology 23:371–374
8. Pieterse CMJ, Van Pelt JA, Van Wees SCM, Ton J, Leonkloosteriziel KM, Keurentjes JJB, Verhagen BWM, Knoester M, Van der Sluis I, Bakker PAHM, Van Loon LC (2001) Rhizobacteria mediated induced systemic resistance: triggering,signaling and expression. Eur J Plant Pathology 107:51–61
9. Rajkumar M, Lee KJ, Lee WH and Banu JR (2005) Growth of Brassica juncea under chromium stress :influence of siderophores and indole 3 aceic acid producing rhizosphere bacteria. Journal of Environmental Biology 26 (4):693–699
10. Steele DB and Stowers MD (1991) Techniques for selection of industrially important microorganisms. Annual Review of Microbiology 45:89–106
11. Tag EL, Lim SK, Nam DH, Khang YH, Kim SD (2003) Pyoverdin 2112 of *Pseudomonas fluorescence* 2112 inhibits *Phytophthora capsici,*a red peppe blight causing fungus. J Microbiol Biotechnol 13:415–421