PLANT GROWTH-PROMOTING RHIZOBACTERIA IN ENHANCING PLANT GROWTH AND SUPPRESSING PLANT PATHOGENS

N. SAKTHIVEL, E. SIVAMANI, N. UNNAMALAI and S. S. GNANAMANICKAM
Centre for Advanced Studies in Botany, University of Madras, Madras 600 025, India.

ABSTRACT

Several strains of *Pseudomonas fluorescens* were isolated from rhizosphere of plants and were identified as biotypes C and G. These siderophore (fluorescent pigment) producing strains showed antagonism, in *in vitro* tests, to plant pathogenic fungi, *Fusarium oxysporum*, f. sp. *cubense*, *F. oxysporum*, f. sp. *vasinfectum*, *Rhizoctonia solani* and *Acrocylindrium oryzae* and bacteria, *Xanthomonas campestris* pv. *oryzae* and *Pseudomonas syringae* pv. *phaseolicola*. Further, selected strains of *P. fluorescens* used for bacterization of rice and cotton seeds enhanced the plant growth by 12 to 27% in rice and 8 to 40% in cotton. The potential that these plant growth-promoting rhizobacteria have for Indian agriculture for biological fertilizing and biological control of plant diseases is discussed.

INTRODUCTION

A NUMBER of bacterial strains of the *Pseudomonas fluorescens–putida* group isolated from plant rhizospheres have recently been used as seed inoculants on crop plants to promote growth and increase yields[1-3]. These bacteria, termed plant growth-promoting rhizobacteria (PGPR) have also demonstrated their potential role in the reduction of plant diseases[2, 4-8]. Kloepper *et al*[2] presented evidence to explain the mechanism by which PGPR, showing antagonism to potentially deleterious rhizoplane fungal and bacterial pathogens, enhanced plant growth. These workers and others found that the plant growth-promoting activity of PGPR was due to the production of extracellular siderophores (microbial iron-transport agents) which complex environmental iron, making it less available to certain endemic microorganisms thus inhibiting their growth[2,5,8,9,14].

Interest in PGPR-like organisms and in bacterization of plants arose because of their potential applications to Indian agriculture. The preliminary report of Unnamalai and Gnanamanickam[10] on the identification of such PGPR-like bacteria antagonistic to citrus canker pathogen, *Xanthomonas campestris* pv *citri* prompted further work. In this report, we describe the identification of several strains of *P. fluorescens* with *in vitro* antibiosis to important plant pathogenic fungi and bacteria and causing, enhanced growth of rice and cotton plants.

MATERIALS AND METHODS

Fluorescent bacteria were isolated from root and leaf samples of a number of crop plants like rice, citrus, groundnut, cowpea, black gram, cotton, grapevine and potato. For routine isolations, plates of King's medium B (KB)[11] were used. A number of strains which were fluorescent on KB but not on $FeCl_3$ (1 μM)–amended KB were isolated. These strains tentatively identified as *P. fluorescens*, were further characterized by biochemical tests into biotypes as outlined in Bergey's manual[12] and by Unnamalai and Gnanamanickam[10].

In vitro antagonism between strains of *P. fluorescens* and fungal and bacterial plant pathogens was examined. The pathogens used were, *Fusarium oxysporum* f sp *cubense* (Panama wilt of banana), *F. oxysporum* f sp *vasinfectum* (wilt of cotton), *Rhizoctonia solani* (seedling disease of cotton), *Acrocylindrium oryzae* (sheath rot of rice) and bacteria, *Xanthomonas campestris* pv. *oryzae* (bacterial leaf blight of rice) and *Pseudomonas syringae* pv. *phaseolicola* (halo-blight of French bean). Antibiosis against fungi were detected using the method of Howell and Stipanovic[6] on potato-dextrose agar (PDA) and against bacteria by the method of Kloepper *et al*[2] on KB. In addition, the following siderophore-plug assay was also employed: Selected strains of *P. fluorescens* were grown on cellophane overlaid KB plates for 2 weeks. Cellophane along with bacterial

growth was removed from the agar which contained the siderophore (fluorescent pigment). One or two siderophore plugs (6 mm dia) were transferred to PDA or KB plates and were spray-inoculated with cell suspensions (*ca* 10^6 conidia/ml) of the test organisms. After incubation at 23°C for 2–7 days, inhibition zones observed around the plugs indicated antibiosis.

To check the growth-promoting activity of PGPR, selected strains of *P. fluorescens* were used as inoculants of rice (cv. TKM9, IR50 and IR 20) and cotton (cv. DCH 32) seeds. For such bacterization of seeds, the technique described by Suslow and Schroth[3] was used. *P. fluorescens* strains grown on KB for 48 hr were scrapped into a suspension of 1% carboxy methyl cellulose (CMC) and mixed with surface-sterilized seeds. The bacterized seeds were dried overnight. Seeds coated likewise with CMC alone served as control.

Coated seeds were sampled for the number of colony forming units (CFU) of bacteria by macerating 10 seeds in a pestle and mortar with 100 ml of phosphate buffer (pH 7.2) and plating 0.1 ml of the appropriate dilutions onto plates of KB. The bacterized seeds (with 10^8 cells/seed) were sown in pots containing non-sterile field soil. Seedlings were maintained in greenhouse. The growth was measured when seedlings were 4 weeks old.

RESULTS AND DISSCUSSION

All the strains of *P. fluorescens* produced a fluorescent pigment (siderophore) on KB but not on $FeCl_3$-amended KB medium. These were negative for levan production and positive for arginine dihydrolase, oxidase, denitrification and gelatin liquefaction. Based on their ability to utilize carbon sources,[12] eight *P. fluorescens* strains were further grouped into biotypes C(3 strains) and G(5 strains). The biotype C strains utilized sorbital as the sole carbon source whereas biotype G strains did not. Other characteristics were common.

Strains of *P. fluorescens* showed *in vitro* antagonism to all the fungal and bacterial pathogens tested. Growth of *F. oxysporum* f sp *cubense* (12 strains, collected from wilted banana plants from different locations of Tamilnadu) were inhibited by *P. fluorescens* (figure 1) in both methods of *in vitro* bioassays. In these tests, *P. fluorescens* also inhibited *F. oxysporum* f sp *vasinfectum* (one strain), (figure 1), *R. solani* (one strain), *A. oryzae* (one strain), *pseudomonas syringae* pv *phaseolicola* 0456 (one strain) and *Xanthomonas campestris* pv. *oryzae*.

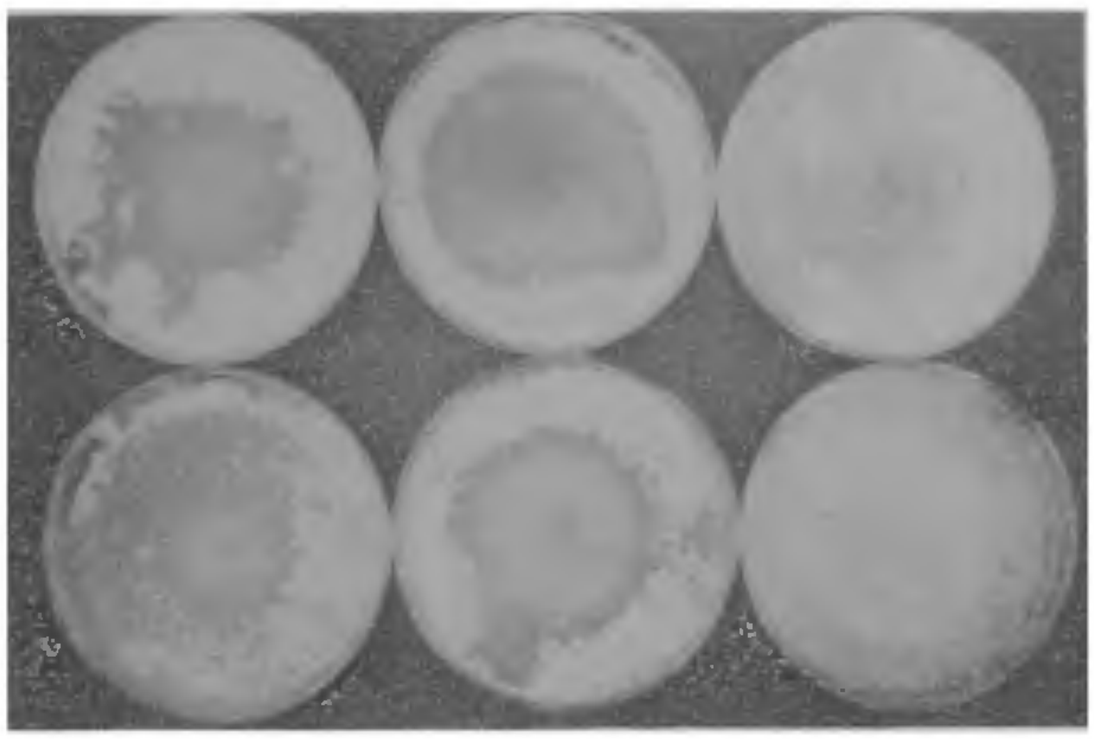

Figure 1. Inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *cubense* (Fc) and *F. oxysporum* f. sp. *vasinfectum* (Fv) by PGPR strains.

Plate	*Fusarium oxysporum (Fc or Fv)*	*PGPR Strain*
Top–left	Fc-E3 (from Erode)	Pfco (cotton)
middle	Fc-E3	Pfct (citrus)
right	Fc-K3 (from Kolli Hills, Salem)	Pfct
Bottom–Left	Fc-M12 (from Madurai)	Pfbg (black gram)
middle	Fc-M12	Pfct
right	Fv	Pfct

The results on the effect of bacterization of seeds with *P. fluorescens* strains (table 1) show that plant growth is enhanced. Seedlings of all the 3 rice cultivars, TKM9, IR 20 and IR 50 showed such enhanced growth due to bacterization with different strains. Except in 2 experiments (Pfp + TKM 9 and Pfco + IR 50) in all the other treatment combinations, enhanced growth of (12.4 to 27.0%) was observed. Enhancement of growth was maximum (27%) when rice seedlings of cv. IR 50 were bacterized with a potato strain (Pfp) of *P. fluorescens* (table 1), (figure 2). In 2 experiments, bacterized cotton plants of cv DCH 32 showed 8% and 40% growth enhancement with PGPR strains Pfco (cotton) and Pfgn (groundnut) respectively (table 1).

The results presented here reveal that the PGPR-like bacteria isolated from plant rhizospheres show *in vitro* antibiosis to some of the important plant pathogens. The list of plant pathogens include the Panama wilt pathogen, *F. oxysporum* f sp *cubense*, perhaps the most important fungal pathogen of *Musa* spp. Others like the sheath rot pathogen of rice, *A. oryzae* is also a pathogen which is becoming increasingly important to Indian rice ecology. If these experiments can be extended to field conditions, it may be possible to

Table 1 *Effect of bacterization with strains of Pseudomonas fluorescens on plant growth.*

Name of crop and cultivar	Plant height (cm)[a] Nontreated	bacterizeed		Enhanced growth[b] (per cent)
Rice cv TKM9	15.43	15.13	(Pfp)[c]	—
		17.35	(Pfgv)	12.44
		17.94	(Pfgn)	16.27
Rice cv IR 20	18.70	21.14	(Pfp)	13.05
	16.34	19.12	(Pfp)	17.01
Rice cv IR 50	22.44	22.01	(Pfco)	—
	15.61	18.72	(Pfct)	19.92
		19.83	(Pfp)	27.03
Cotton cv DCH 32	19.44	27.27	(Pfgn)	40.28
	23.70	25.65	(Pfco)	08.23

[a] Rice seedlings heights (total shoot length) were from measurements of 30 to 60 seedlings for each treatment. In cotton plants measurements were made from the cotyledonary node to shoot tips of seedlings.

[b] Percent increase in height of bacterized plants over those of controls.

[c] PGPR strains in parentheses: Pfp is *P. fluorescens* strain isolated from potato, Pfgv is from grapevine, Pfgn is from groundnut, Pfco is from cotton and Pfct is from citrus.

Figure 2. Enhanced growth of rice plants (cv. IR 50) due to bacterization with PGPR (strain Pfp). Left: bacterized; Right: control

obtain biological control against these pathogens. Recently, Mew and Rosales[13], in the Philippines, obtained substantial reduction of sheath blight of rice by bacterization of rice seeds. Our results also confirm earlier findings that PGPR show antagonism to *X. campestris*. pv *oryzae*[10] and *P. syringae* pv. *phaseolicola*[3]. Antagonism between rhizobacteria and *F. oxysporum* f sp *cubense* has not been reported thus far.

The results on bacterization of seeds with PGPR strains are encouraging. Enhancement of growth naturally leads to increased yields. Pending confirmation, the pot culture experiments detailed here reveal that the different PGPR strains are capable of causing enhanced plant growth. For example, rice plants of cv TKM 9 respond differently to different PGPR strains. This suggests the importance of finding the most effective PGPR strain by exhaustive screening. Likewise, rice cv IR 50 showed 19% growth increase due to Pfct strain and 27% due to Pfp strain. While a 27% growth increase in rice and 40% growth increase in cotton has to be recognized as significant, these results offer much more scope for improvement in plant growth and yield if more effective strains of PGPR are used. The search for such strains and translation of enhanced growth in terms of increased yield in rice (grain) and cotton (lint) are in progress.

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ANNOUNCEMENTS

MAHENDRALAL SIRCAR AWARD IN PHYSICS FOR 1984

The above award instituted by the Indian Association for the Cultivation of Science, Calcutta, has been awarded to Prof. S. Chandrasekhar, FRS, of Raman Research Institute, Bangalore, at a function to mark the 150th birth day celebration of Dr Sircar, the founder secretary of the organisation.

The award includes a medal, a certificate and Rs.10,000 in cash.

JAWAHARLAL NEHRU SCIENCE AWARD FOR 1983

The Jawaharlal Nehru Science Award, instituted by the Madhya Pradesh Government, has been awarded to Prof. M. G. K. Menon, FRS, Member of the Planning Commission and a member of the working committee of Current Science Association. The corresponding award for Engineering and Technology for the year 1983, has been awarded jointly to Prof. Satish Dhawan, former Chairman of the Indian Space Commission, Bangalore and a former member of the working committee of Current Science Association and Dr Raja Ramanna, Chairman of the Atomic Energy Commission. The Social Sciences award has been given to Prof. K. N. Raj, Honorary Emeritus Fellow of the Centre for Development Science Studies, Trivandrum.

RAMESHWARDAS BIRLA SMARAK KOSH AWARD FOR 1985–86

Dr M. S. Valiathan, FRCS, Director, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum and a pioneer in the area of biomaterials and bioimplants has been awarded the 'Rameshwardas Birla Smarak Kosh Award for 1985–86. The award is made for the outstanding research in medical or related fields to an Indian National and carries a cash prize of Rupees one lakh.

Dr M. S. Valiathan is a member of the Editorial Board of *Current Science* and we wish him many more such honours to follow.