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Research Article

Selection of potential rhizobacterial isolates from ginger rhizosphere against bacterial wilt caused by *Ralstonia solanacearum*

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ABSTRACT

Eleven rhizobacterial isolates including the reference cultures of *Pseudomonas fluorescens* were evaluated for growth promotion and disease suppression *in vitro* based on various parameters *viz*., antagonism index, vigour index, hydrogen cyanide, ammonia and IAA production, 'P' solubilization and each was scored as per a modified standard score chart. Accordingly, the plant growth promoting index (PGPI) of 11 rhizobacteria were calculated based on the above six parameters and it was observed that four isolates out of 11 *viz*., RB-22, RB-144, RB-11 and RB-82 showed a PGPI of above 70 whereas the lowest index was recorded with RB-151. TLC analysis of growth regulators produced by the test isolates revealed that though all the cultures produced auxins and their related compounds, only two isolates *viz*., RB-141 and RB-11 produced both gibberellic acid as well as auxins. It was observed that all isolates produced considerable amounts of salicylic acid in varying amounts with the maximum by the two reference cultures followed by the isolates RB-11 and RB-22. The maximum number of antibiotics was produced by the isolate RB-22 comprising of pyoluteorin, pyrrolnitrin, pyocyanin and unidentified metabolite and this was closely followed by RB-144, RB-66, RB-11 and P.f2 which produced three antibiotics. The antibiotic 2, 4 DAPG was produced by RB-66, RB-11 and P.f2 apart from certain unidentified ones.

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INTRODUCTION

Ginger, one among the major highly valued spice crops grown in the country, is incited by the ubiquitous soil borne pathogen, *Ralstonia solanacearum*, causing bacterial wilt incidence. This pathogen inflicts heavy crop losses in ginger cultivation, thus affecting the economic well being of the cultivators. Currently, much importance is given to eco-friendly management strategies such as the use of bioagents due to the ecological hazards inflicted by the excessive use of plant protection chemicals. It is widely accepted that plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface (Rangajaran *et al*., 2003). These organisms can also be beneficial to the plant by stimulating growth (Moeinzadeh *et al.,* 2010). Among these organisms, fluorescent pseudomonads are considered to be the most promising group of plant growth promoting rhizobacteria (PGPR) involved in biocontrol of plant diseases (Moeinzadeh *et al*., 2010). They produce secondary metabolites such as antibiotics, phytohormones (Keel *et al.,* 1992) and volatile compounds like hydrogen cyanide (Defago and Haas, 1990). Plant growth-promoting ability of these bacteria is mainly because of the production of indole-3- acetic acid (IAA) (Patten and Glick, 2002). Production of antibiotics such as phenazine-1-carboxylic acid (PCA), pyocyanin, 2-acetamidophenol, pyrrolnitrin, pyoluteorin, Phenazine-1-carboxylic Acid, 2, 4-diacetylphloroglucinol, viscosinamide and tensin in different species of pseudomonads has been reported (Sunishkumar *et al*., 2005). The role of fluorescent pseudomonads in disease suppression caused by *R.solanacearum* has been established by Anith *et al*. (2000). In the present study, the possible growth-promoting and biocontrol potential of 11 efficient

strains has been examined by determining the metabolites, *viz*., indole acetic acid, salicylic acid, phosphorus solubilization, antibiotics and hydrogen cyanide production.

MATERIALS AND METHODS

In this study, 160 bacteria were isolated from ginger rhizosphere and on the basis of preliminary screening through dual culture assay and pot culture experiment, 11 potential antagonists against *Ralstonia solanacearum* including the two reference cultures of *P.fluorescens* from TNAU (P.f1) and KAU (P.f2) were selected. These antagonists were further subjected to various analysis for understanding the parameters that contribute to the growth promoting as well as disease suppressing effect against the bacterial wilt pathogen in ginger.

Antagonism index (AI) of potential antagonists:

The 11 potential isolates were screened again for their *in vitro* inhibitory effect against the pathogen and a modified antagonism index (AI) suggested by Kasinathan (1998) was calculated using the formula AI=PI x IZ where, AI – Antagonism index, PI – Per cent inhibition, IZ – Inhibition zone (mm). Then the per cent inhibition was calculated. The inhibition zone (IZ) produced by each isolate was further scored following the scale as: IZ zone of >1 <10 mm = 1; >10 <20 mm = 2; >20 <30 mm = 3 and > 30 mm = 4

Vigour index:

The isolates were bioassayed for the ability to promote or inhibit seedling growth using the method as described by Shende *et al.* (1977) and Elliot and Lynch (1984). Germination percentage, length of epicotyl and hypocotyl were measured after 72 h. The vigour index (VI) was calculated using the formula: VI = (Mean root length + Mean shoot length) x Germination percentage. The vigour index (VI) was scored as follows: VI of >1 <2 = 1; VI of >2 <3 = 2; VI of >3 <4 = 3 and VI >4 = 4

Production of hydrogen cyanide:

Production of hydrogen cyanide (HCN) by the potential isolates was detected by following the method of Wei *et al.* (1991). Change in colour of filter paper strips from yellow to brown and to red indicates the production of hydrogen cyanide. The reaction was scored on a 1-4 scale depending on the colour gradation.

Production of ammonia:

The qualitative estimation of production of ammonia was done following the method of Dye (1962). The presence of faint yellow to deep yellow or brown colour indicated production of ammonia. The reaction was scored as nil, low, medium and high in 1-4 scale based on the intensity of the colour.

Phosphorus (P) solubilization:

The phosphate solubilizing capacity of the potential rhizobacterial isolates was tested *in vitro* using Pikovskaya's agar as well as in its broth (Pikovskaya, 1948). Plates were observed for clearing zone around the colony and its diameter measured. For quantification, the isolates after inoculation into Pikovskaya's broth was scored following the scale based on the 'P' solubilization as: >1<3 mg 50ml^{-1} = 1; >3<6 mg50ml^{-1}= 2; >6<9 mg 50ml^{-1} =3 and > 9 mg 50ml^{-1} =4

Quantitative estimation of indole acetic acid (IAA):

A modified protocol by Bric *et al.* (1991) was used to estimate the IAA produced by the selected PGPR isolates. A standard curve was prepared with different concentrations of IAA and was used to quantify the IAA production and these were finally scored in a scale as: >0<15 µgml^{-1} =1; >16<30 µgml^{-1}=2; >31<45 µgml^{-1} = 3 and > 46µgml^{-1} = 4

Detection of auxins and gibberellins by TLC:

The growth promoting hormones produced by the potential rhizobacterial isolates was assayed by thin layer chromatography (TLC) as per the protocol of Hasan (2002). The TLC plates were observed under a UV transilluminator for yellowish green and orange-red fluorescence, which indicated the presence of GA and IAA, respectively. Plates were also observed in visible light for violet / red colour which indicated the presence of IAA.

Determination of PGPR index:

All the qualitative and quantitative data of plant growth promoting parameters *viz*., antagonism index, vigour index, HCN, ammonia, IAA production and phosphate solubilization were transformed into 1 to 4 scale and the PGPR index was calculated for each rhizobacterial isolate. The isolates which showed the higher index compared to that of control were considered as a potential antagonists. The PGPR index was calculated as suggested by Samanta and Dutta (2004) is as follows: PGPR index = (Net PGPR score / Gross PGPR score) x 100

Assay of secondary metabolites:

After assessing the various parameters which are known to contribute to plant growth, an effort was carried out to detect the production of secondary metabolites like salicylic acid, antibiotics and siderophores by the rhizobacterial isolates as these secondary metabolites play a significant role in imparting resistance and disease suppression.

Spectroscopy and TLC analysis of salicylic acid:

For both quantitative and qualitative analysis of salicylic

acid (SA) production, the isolates grown in casamino acid broth was estimated as per the protocol of DeMeyer and Hofte (1997). The absorbance of the purple iron - SA complex, which developed in the aqueous phase was measured at 527 nm in a spectrophotometer (Spectronic 20D+) and was compared with a standard curve of SA dissolved in ethyl acetate.

Detection of antibiotics by TLC:

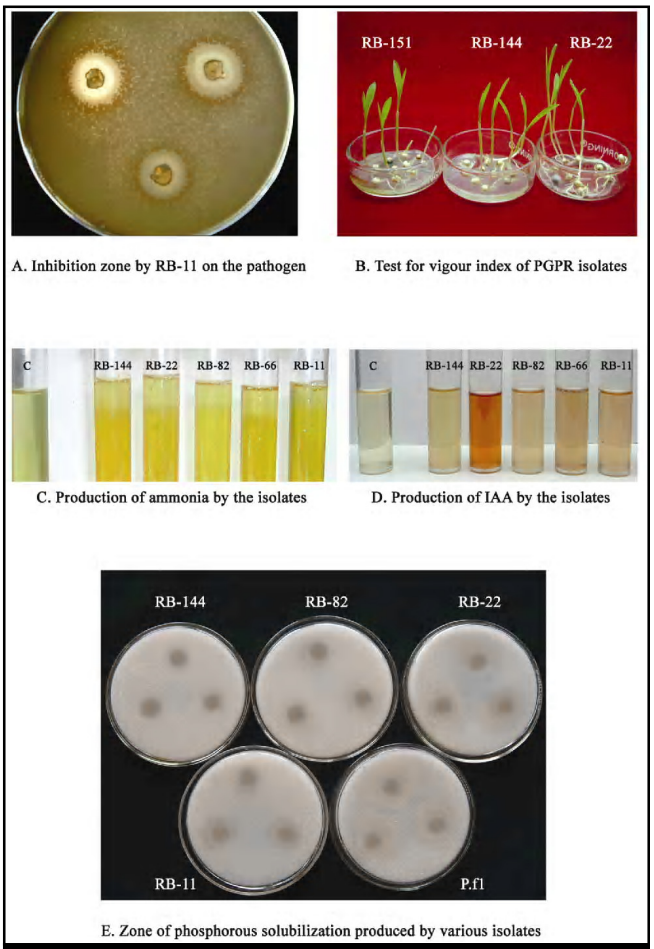
Antibiotic detection by TLC method was carried out as per the protocol of Howell and Stipanovic (1980) and Kraus and Loper (1992). The plates were observed under UV light (254 nm) and were also kept in iodine vapour chamber for 60 minutes for development of coloured bands. The Rf values were calculated and compared with the standard / reference values of the antibiotics.

RESULTS AND DISCUSSION

Over the last decades, many studies have been reported on natural activity of some fungi and bacteria against pathogens, and this is considered as a very appealing alternative to the use of chemical fungicides (Welbaum *et al*., 2004). The rhizosphere microorganisms, especially fluorescent pseudomonads, have exceptional ability to promote the growth of host plant by various mechanisms (O'Sullivan and O'Gara 1992). Also these bacteria have various mechanisms to suppress plant diseases including production of antibiotics, efficient root colonization and production of powerful siderophores (Haas and Defago, 2005). It is also necessary to prove the antibacterial mechanism of each isolate because the production and especially the quantity of secondary metabolites are often specific for each isolate. Consequently, they were tested for the production of antibacterial antibiotics, growth regulators, hydrogen cyanide, ammonia, salicylic acid and also their effect on phosphorus solubilization in comparison with the reference strains of *P. fluorescens*.

Antagonism index (AI) of potential rhizobacterial isolates:

As a first step to arrive at the PGPR index, the antagonism index of the potential rhizobacterial isolates was calculated and it was shown that six isolates showed the maximum antagonism index (AI) of above 500 with the maximum in P.f2 followed by RB-144 and RB-11 (Plate 1A). The least AI was noticed with RB-69 and RB-71. Similar results were reported for other pathosystems by various workers (Manimala, 2003) where they observed variation in the antagonistic reaction of different *Pseudomonas* spp. against *R. solanacearum.* According to Raupach and Kloepper (1998), the lytic activity by the rhizobacterial antagonists against the pathogen is mainly due to their production of lytic enzymes or by inhibitory metabolites.



A. Inhibition zone by RB-11 on the pathogen

B. Test for vigour index of PGPR isolates

C. Production of ammonia by the isolates

D. Production of IAA by the isolates

E. Zone of phosphorous solubilization produced by various isolates

Plate 1 : Various attributes of plant growth promoting index (PGPI)

Vigour index:

It was found that in general, the seeds bacterized with rhizobacteria improved the vigour index. Among the isolates, RB-22 was the most effective growth activator followed by RB-151, P.f1, RB-144 and RB-71 thereby, indicating that these rhizobacteria had potentiality to enhance the growth of the seedlings (Plate 1B). Such growth promoting effects of rhizosphere bacteria in various crops have been well documented (Dhoke and Kurundkar, 2005).

Production of hydrogen cyanide:

With regard to the production of HCN, it was seen that all the rhizobacterial isolates tested were non-cyanogenic in nature when tested with King's B media. Bano and Mussarat (2003) noticed low HCN production under iron limiting conditions. Lack of iron supplement in the media used in this study for HCN production may be the reason for failure in detection of HCN by the isolates. Such inability to produce HCN by rhizobacterial isolates have been noticed by Samanta

and Dutta (2004).

Production of ammonia :

The ability of the rhizobacterial isolates for the production of ammonia, a volatile compound having direct bearing on biocontrol activity were tested and it was found that all the isolates produced ammonia (Plate 1C). However, RB-151 and P.f2 produced less amount of ammonia while the others recorded higher production. Production of ammonia by rhizobacteria from mustard has been documented by Samanta and Dutta (2004) and concluded that ammonia production has a role in suppressing *S. sclerotiarum.* Further, Ryu *et al*. (2003) opined that volatiles produced by PGPR strains triggered growth promotion and ISR in *Arabidopsis thaliana.*

Phosphorus (P) solubilization:

It is well established that one of the important criteria for an efficient PGPR is their ability to transform unavailable 'P' to the available form (Plate 1E). Among the isolates, eight recorded 'P' solubilization in Pikovskya's solid media while the rest in liquid medium only (Fig. 1). The maximum 'P' solubilization was with RB-144, RB-82 and P.f2. Such capacity of rhizobacterial isolates in solubilizing 'P' were documented by Katiyar and Goel (2003) and Dey *et al.* (2004). Further, conferring resistance of plants to stress conditions by mobilizing 'P' for plant growth was also reported.

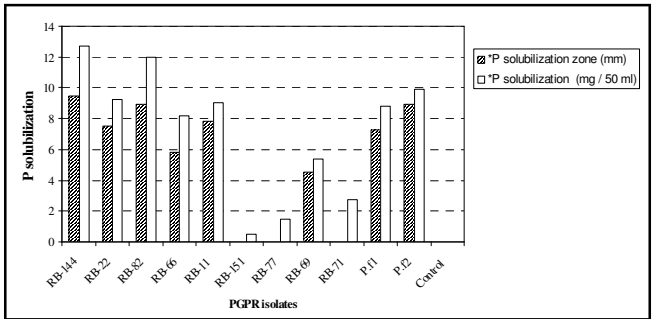


Fig. 1 : Phosphorus solubilization by PGPR isolates

Assay of growth promoting hormones :

It is very well established fact that many rhizobacteria including fluorescent pseudomonads produce plant growth promoting substances like gibberellins, cytokinins and IAA which can directly or indirectly modulate plant growth and development. Growth regulators like auxins and gibberellins are known to be produced by rhizobacteria in many phytosysytems which have a direct effect in growth and development of plants (Patten and Glick, 1996). Here, all the 11 isolates produced varying levels of IAA ranging from 9.02 to 56.89 μgml^{-1}. The maximum quantity was produced by RB-22 followed by RB-151 whereas RB-69 and RB-11 produced least quantity of IAA (Fig. 2 and Plate 1D). After quantifying the IAA production by spectral analysis, further TLC analysis of growth regulators produced by the 11 rhizobacterial isolates were carried out. Chromatographic separation of the metabolites revealed that isolates RB-144, RB-11 and P.f2 produced four metabolites. Of the four metabolites, two spots were noticed with RB-144 and RB-11 which are suggestive of gibberellins and auxins. The red and the yellowish green colour suggest the production of IAA and Gibberellins which were noticed with two isolates RB-144 and RB-11 while blue / violet coloured bands were developed with RB-144, RB-22, RB-82, RB-11 RB-151, P.f1 and P.f2 under U.V. which indicated production of derivatives of IAA with concomitant Rf values (Plate 2). The role of plant growth regulators has been well demonstrated by many researchers throughout the globe in many different agricultural crops with different genera of beneficial bacteria (Khalid *et al.,* 2004; Ebstam *et al.,* 2005). Production of IAA by different PGPR strains was quantified by many workers (Rubio *et al*., 2000; Bano and Mussarat, 2003; Bhatia *et al.*, 2005). They also noticed variation among the rhizobacterial strains in the production of IAA.

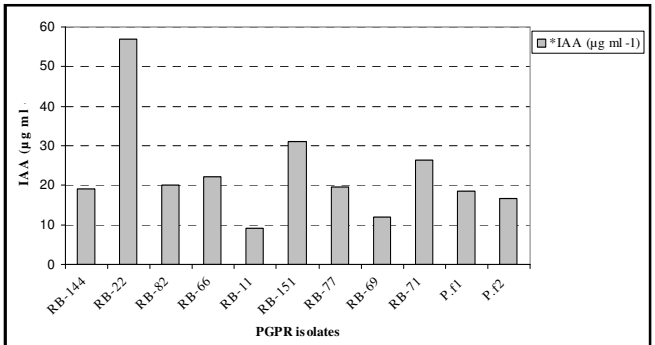


Fig. 2 : Production of indole acetic acid by PGPR isolates

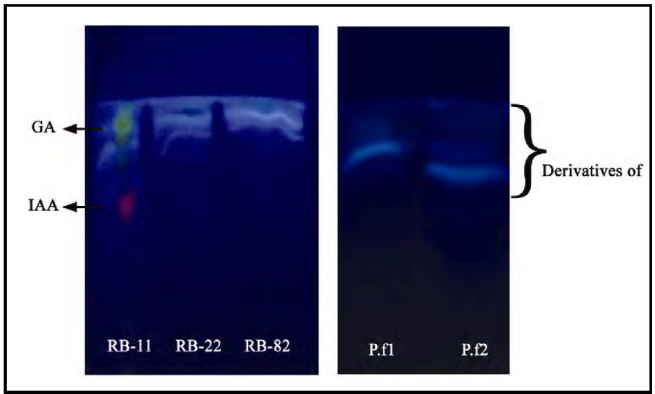


Plate 2 : TLC profile of growth regulators
GA-Gibberellic acid, IAA-Indole acetic acid

Determination of PGPR index:

PGPR index is a measure to detect the potentiality of the aforesaid bacteria. Thus, based on the data on vigour index, 'P' solubilization, IAA, NH_3 and HCN production, the rhizobacterial isolates showed different PGPR index (PGPI), among which, RB-22 recorded a higher PGPR index of 83.33 followed by RB-144, RB-11 and RB-82 (Table 1). The lowest index was with RB-151 (50). Similar line of work has been carried out by Samanta and Dutta (2004) and they observed differences in PGPI among the PGPR isolates tested. They concluded that among the PGPR parameters, 'P' solubilization and ammonia production had significant effect in attaining maximum PGPR index. Thus, the 11 isolates selected from the previous pot culture experiment possessed various mechanisms / attributes which might have played a role in growth promotion as well as their antagonistic activity against the pathogen. According to Chet (1993), production of various compounds of microbial origin is involved in growth promotion as well suppression of pathogen.

Assay of secondary metabolites:

Spectroscopic and TLC analysis of salicylic acid:

Quantitative estimation of the assay from cell free culture filtrates (CFCF) of the isolates revealed production of SA in varying quantities (Fig. 3 and Plate 3). The reference culture P.f2 produced the maximum quantity and the rest produced the same within a range of 9.42 to 75.68 μgml^{-1}. After the quantitative estimation, an attempt was made to separate SA and other related metabolites present in the CFCF by TLC. This chromatography study indicated the presence of SA as brown spot of Rf value 1.0 which was evident with six rhizobacterial isolates. It is interesting to note that in the TLC plate with RB-22, RB-66 and P.f2, two more spots were also visualized with yellow colour having different Rf values indicating the production of certain other metabolites in addition to SA by these isolates.

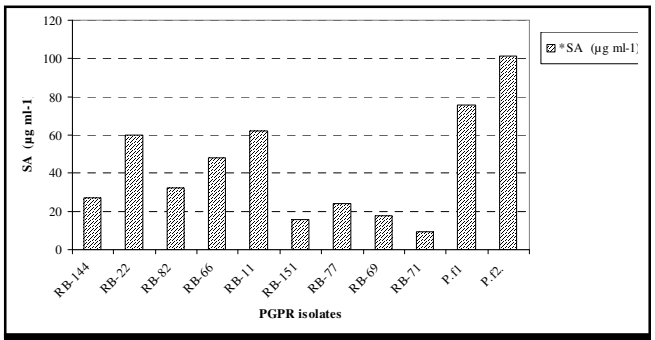


Fig. 3 : Production of salicylic acid by PGPR isolates

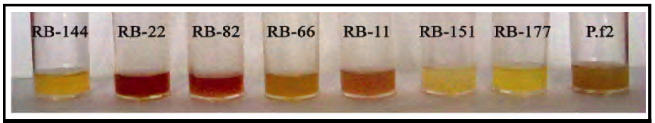


Plate 3 : Production of salicylic acid by PGPR isolates

Salicylic acid is known to play a central role as a signaling molecule in plant defense against microbial attack (Reimann and Haas, 2003). According to DeMayer and Hofte (1997), bacterially produced SA contributes to the induction of systemic resistance. However, van Loon *et al.* (1998) reported that the increase in SA in the bacterized plants was the result of induction by the bacteria in inducing SA synthesis in the plant or it is not clear that whether the plant takes up bacterially produced SA and translocates it to the leaves. Salicylic acid is a metabolite important in pathogen induced SAR and can induce systemic resistance to pathogens after root and soil

Table 1 : PGPR index of potential rhizobacterial isolates

Sr. No.	Isolate	Score for various parameters						*PGPR Index
		Seed germination	IAA production	NH_3 production	P solubilization	HCN production	Per cent inhibition	
1.	RB-144	4	2	4	4	1	3	75.00
2.	RB-22	4	4	4	4	1	3	83.33
3.	RB-82	4	2	3	4	1	3	70.83
4.	RB-66	4	2	4	3	1	2	66.67
5.	RB-11	4	1	4	4	1	3	70.83
6.	RB-151	4	2	2	1	1	2	50.0
7.	RB-77	4	2	4	1	1	3	62.50
8.	RB-69	4	1	3	2	1	2	54.17
9.	RB-71	4	2	4	1	1	2	58.33
10.	P.f1	4	2	4	3	1	2	66.67
11.	P.f2	3	2	2	4	1	3	62.50
12.	Control	3	1	1	1	1	1	33.33

treatment. Abeysinghe (2003) observed *P. fluorescens* strains WE16 and WE32 produced equal amount of SA *in vitro* unlike *Bacillus* strains and *P. putida* WE10 which did not produce any detectable amount of SA, indicating that SA may not play as a primary bacterial determinant for inducing systemic resistance. The role of SA in induction of systemic resistance in various crops by different rhizobacterial isolates was reported by Audenaert *et al.* (2002).

TLC profile of antibiotics:

It is well established that antibiosis plays an active role in the biocontrol of plant disease and often acts in concert with competition and parasitism. According to Ownley and Windham (2003), antagonists produce effective growth inhibitory substances that are active against a wide group of micro organisms and such compounds are referred to as antibiotics with broad spectrum activity. Hence, an effort was made to find out whether the antagonistic rhizobacteria used in the present investigation were able to produce any antibiotic. For this, the culture supernatants of various isolates were subjected to TLC analysis. In general, most of the selected isolates produced antibiotics comparable with the Rf value of that of pyrrolnitrin, pyoluteorin, 2,4-diacetyl phloroglucinol (2, 4 DAPG) and pyocyanin (Plate 4). However, the isolates RB-151, RB-69 and RB-71 did not produce any detectable antibiotic on the chromatogram. A blue coloured spot developed on the chromatogram of the isolates RB-144, RB-22, RB-82, RB-77 and P.f2 was identified as pyoluteorin while, the fluorescent one produced by RB-22, RB-66, RB-11 and P.f1 was recorded as pyrrolnitrin. The isolate RB-22 also produced another blue spot with different Rf value comparable with that of pyocyanin. RB-22 and RB-144 also produced two other unidentified metabolites. It is also interesting to note that the isolates RB-11, RB-66 and P.f2 showed a crimson yellow metabolite which was tentatively recorded as 2, 4-DAPG.

Here, it may be noted that detection of antibiotics in the chromatogram was made based on the Rf values of metabolites recorded and reported in the case of antagonistic fluorescent pseudomonads and other rhizobacterial strains reported by Duffy and Defago (1997). Hence, further confirmation need to be carried out in comparison with the standard antibiotics. However, this study indicated that the majority of the rhizobacterial isolates were able to produce many antibiotics which are capable of suppressing pathogenic organisms. Apart from these, isolates RB-144, RB-22, RB-82, RB-66, RB-11 and P.f2 are seen to produce certain other metabolites which did not match to the standard Rf values. It is to be noted that three isolates RB-66, RB-11 and P.f2 also produced 2, 4 DAPG, a phenolic metabolite known for its antibacterial activity (Ahmadzadeh *et al*., 2004). Production of this compound in the suppression of bacterial leaf blight (BLB) of rice was documented (Velusamy *et al.*, 2003).

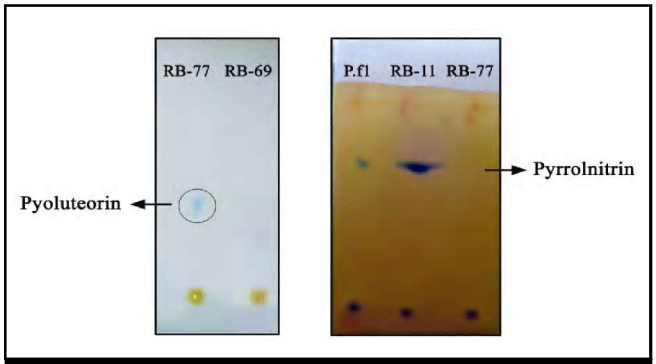


Plate 4 : TLC profile of antibiotics

The findings indicate that the PGPR isolates were able to substantially promote plant growth and suppress bacterial wilt incidence by way of production of metabolites like solubilization of phosphorus, production of auxins, gibberellins, salicylic acid, ammonia, hydrogen cyanide, antibiotics etc.

REFERENCES

Abeysinghe, S. (2003). Induction of systemic resistance in bean against *Uromyces appendiculatus* by different rhizobacteria, In: Proceedings of the 6th International PGPR Workshop, 5-10th Oct, 2003, Calicut, India, pp.570-573.

Ahmadzadeh, M., Tehrani, A.S. and Jahromi, K.T. (2004). Study on production of some antimicrobial metabolites by fluorescent pseudomonads. *Iranian J. agric. Sci.,* **35**: 731-739.

Anith, K.N., Manomohandas, T.P., Jayarajan, M., Vasanthakumar, K. and Aipe, K.C. (2000). Integration of soil solarization and biological control with a fluorescent *Pseudomonas* sp. for controlling bacterial wilt, *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al.* of ginger. *J. Biol. Control,* **14**: 25-29.

Audenaert, K., Damme, A., Cornelis, P., Cornelis, T. and Hofte, M. (2002). Induced resistance by *Pseudomonas aeruginosa* 7NSK2: bacterial determinants and reactions in the plant. *Bulletin OILB/ SROP*, **25**: 223-226.

Bano, N. and Musarrat, J. (2003). Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Cur. Microbiol.,* **46**: 324-328.

Bhatia, S., Dubey, R.C. and Maheshwari, D.K. (2005). Enhancement of plant growth and suppression of collar rot of sunflower caused by *Sclerotium rolfsii* through fluorescent *Pseudomonas. Indian Phytopath.,* **58**: 17-24.

Bric, J.M., Bostock, R.M. and Silversone, S.E. (1991). Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. *Appl. Environ. Microbiol*., **57**: 535-538.

Chet, I. (1993). *Biotechnology in Plant Disease Control*, Wiley-Liss, NEW YORK, 373p.

Defago, G. and Haas, D. (1990). *Pseudomonads* as antagonists of soil borne plant pathogens: mode of action and genetic analysis. *Soil Biochem*., **6**:249-291.

DeMayer, G. and Höfte, M. (1997). Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. *Phytopathology,* **87**: 588–593.

Dey, R., Pal, K.K., Bhatt, D.M. and Chauhan, S.M. (2004). Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiol. Res.,* **159**: 371-394.

Dhoke, P.K. and Kurundkar (2005). Efficacy of fluorescent *Pseudomonas* isolates on seed germination and seedling vigour of chickpea. *J. Pl. Dis. Sci.,* **1**: 85-87.

Duffy, B.K. and Defago, G. (1997). Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology,* **87**: 1250-1257.

Dye, D.W. (1962). The inadequacy of the usual determinative test for identification of *Xanthomonas* spp. *NZT Sci.,* **5**: 393-416.

Ebtsam, M. M., Selim, S. M., El-Sayed, S. A. and Zaki, M. M. (2005). Rhizobacteria producing growth promoting substances as a bio-control agent against *Rhizoctonia solani* and *Fusarium solani* causing root rot disease of tomato seedlings. *Ann. agric. Sci. Cairo,* **50**: 47-62.

Elliot, L.F. and Lynch, J.M. (1984). *Pseudomonas* as a factor in the growth of winter wheat (*Triticum aestivum* L.). *Soil Biochem*., **16**: 69-71.

Haas, D. and Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads *Nature Reviews Microbiology* | AOP, Available: published online doi: 10.1038/nrmicro1129, [10 March 2005].

Hasan, H.S.H. (2002). Gibberellin and auxin production by plant root fungi and their biosynthesis under salinity- calcium interaction. *Rostlina vyroba,* **48**: 101-106

Howell, C. R. and Stipanovic, R.D. (1980). Suppression of *Pythium ultimum* induced damping off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic pyoluteorin. *Phytopathology,* **70**: 712-715

Kasinathan, R. (1998). Studies on employing *Trichoderma* chitinases against plant pathogenic fungi. M. Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, COIMBATORE, T.N. (India).

Katiyar, V. and Goel, R. (2003). Solubilization of inorganic phosphate and plant growth promotion by cold tolerant mutants of *Pseudomonas fluorescens. Microbiol. Res.,* **158**: 163-168

Keel, C., Schnider, U., Maurhofer, M., Voisard, C., Laville, J., Burger, U., Wirthner, P., Haas, D. and Defago, G. (1992). Suppression of root diseases by *Pseudomonas fluorescens* CHAO: importance of bacterial secondary metabolite, 2,4-diacetylphoroglucinol. *Mol. Plant-Microbe Interact*., **5** : 4-13.

Khalid, A., Arshad, M. and Zahir, Z.A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. Applied Microbiology,* **96** : 473-480.

Kraus, J. and Loper, J.E. (1992). Lack of evidence for a role of antifungal metabolite production by *Pseudomonas fluorescens* Pf-5 in biological control of *Pythium* damping - off of cucumber. *Phytopathology,* **82**: 264-271.

Manimala, R. (2003). Management of bacterial wilt of solanaceous vegetable using Microbial antagonists, M.Sc (Ag) Thesis, Kerala Agricultural University, Vellanikkara, THRISSUR, KERALAK (India).

Moeinzadeh, A., Sharif-Zadeh, F., Ahmadzadeh, M. and Heidari Tajabadi, F. (2010). Biopriming of sunflower (*Helianthus annuus* L.) seed with *Pseudomonas fluorescens* for improvement of seed invigoration and seedling growth. *Australian J. Crop Sci.,* **4** :564-570.

O'Sullivan, D.J. and O'Gara, F. (1992). Traits of fluorescent Pseudomonas spp. involved in suppression of plant root pathogens. *Microbiol. Rev*., **56**:662-676.

Ownley, B.H. and Windham, M.T. (2003). Biological control of plant pathogens. In: *Plant Pathology: Concepts and Laboratory Exercises*, CRC Press, LLC, 10p.

Patten, C.L., and B.R. Glick (1996). Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol.,* **42**:207–220.

Patten, C. and Glick, B. (2002). Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Appl. Environ. Microbiol*ogy, **68**:3795-3801.

Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with activity of some microbial specie. *Microbiologiya,* **17**: 362-370.

Rangajaran, S., Saleena, L. M., Vasudevan, P. and Nair, S. (2003). Biological suppression of rice diseases by *Pseudomonas* spp. under saline soil conditions. *Plant Soil,* **251** : 73–82.

Raupach, G.S. and Kloepper, J.W. (1998). Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology,* **88**: 1158-1164.

Reimann, Gaille, C. and Haas, D. (2003). Evaluation of pyochelin and its biosynthetic precursors salicylate and dihydroaeruginoate as potential biocontrol factors in plant disease suppression. In : Proceedings of the 6th International Workshop on PGPR, 5-10th Oct, 2003, Calicut, India, p.563.

Rubio, T.M.G., Plata, V.S.A., Castillo, B.J. and Nieto, M.P. (2000). Isolation of *Enterobacteria, Azotobacter* sp. and *Pseudomonas* sp., producers of indole-3-acetic acid and siderophores, from Colombian rice rhizosphere. *Revista Latinoamericana de Microbiologia,* **42**: 171-176.

Ryu, C.M., Hu, C.H., Reddy, M.S. and Kloepper, J.W. (2003). Different signaling pathways of induced resistance by rhizobacteria in *Arabidopsis thaliana* against two pathovars of *Pseudomonas syringae. New Phytologist*, **160**: 413-420.

Samanta, S.K. and Dutta, S. (2004). Potential of native plant growth promoting rhizobacteria in the management of *Sclerotinia* stem rot of mustard. *J. Mycol. Pl. Pathol.,* **34**: 761-768

Shende, S.T., Apte, G. and Singh, T. (1977). Influence of *Azotobacter* on germination of rice and cotton seeds. *Curr. Sci.,* **46**: 675-676

Sunishkumar, R., Ayyadurai, N., Pandiaraja, P., Reddy, A.V., Venkateswarlu, Y., Prakash, O. and Sakthivel, N. (2005). Characterization of antifungal metabolite produced by a new strain *Pseudomonas aeruginosa* PUPa3 that exhibits broadspectrum antifungal activity andvbiofertilizing traits. *J. Applied Microbiol.,* **98**:145-154

Van Loon, L.C., Bakker P.A.H.M. and Pieterse, C.M.J. (1998). Systemic resistance induced by rhizosphere bacteria. *Ann. Rev. Phytopath.,* **36**:453-483

Velusamy, P., Kavitha, S., Vasudevan, P. and Gananmanickam, S.S. (2003). Role of plant associated bacteria and their antimicrobial products in the suppression of major rice disease in India. In : Proceedings of the 6th International PGPR Workshop, 5-10th Oct, 2003, Calicut, India, pp. 256-259.

Wei, G., Kloepper, J.W. and Tuzun, S. (1991). Induction to systemic resistance of cucumber to *Colletotrichum orbiculare* by selected strains of plant growth promoting rhizobacteria. *Phytopathology,* **81**: 1508-1512.

Welbaum, G., Sturz, A.V., Dong, Z. and Nowak, J. (2004). Fertilizing soil microorganisms to improve productivity of agroecosystems. *Critical Reviews in Plant Sci.,* **23**:175- 193.

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