IOSR Journal of Pharmacy and Biological Sciences (IOSRJPBS)
ISSN : 2278-3008 Volume 1, Issue 4 (July-August 2012), PP 24-32
www.iosrjournals.org

Biological Control of Fusarium Oxysporum and Aspergillus Sp. By Pseudomonas Fluorescens Isolated From Wheat Rhizosphere Soil Of Kashmir.

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Abstract: *As Pseudomonas fluorescens possess a variety of promising properties which make it a better biocontrol agent. In this current study wheat rhizosphere soil collected from different wheat growing regions of Kashmir valley were evaluated for presence of Pseudomonas fluorescens using King's B medium. Based on colony morphology, siderophore production and biochemical tests, out of 136 rhizosphere soil samples, only 52 isolates were identified as Pseudomonas fluorescens. Out of 52, only 7 isolates viz., SKWI, BG6, BG27, Bandi6, Bandi 11, Bandi24 and Bandi63 belonging to different regions showed remarkable antifungal activity against Fusarium oxysporum and Aspergillus species with different levels of inhibition pattern. Interestingly, Bandi6 and Bandi11 collected from Bandipora region of Kashmir valley demonstrated highest antifungal activity against Fusarium species with 29mm and 28mm zone of inhibition respectively. Similarly, BG6 isolate collected from Budgam region of Kashmir valley showed highest zone of inhibition (20mm) against Aspergillus species. In this study, our investigations clearly indicate that isolates collected from this region of India can be better exploited to be used as potential biocontrol agents in agriculture system.*

Keywords: *Aspergillus species, Fusarium oxysporum, King's-B, Pseudomonas fluorescens and wheat rhizosphere*

I. Introduction

The use of chemical fertilizers and pesticides has caused an incredible harm to the environment. These agents are both hazardous to animals and humans and may Persist and accumulate in natural ecosystems (Musa *et al.*, 1976). An answer to this problem is replacing chemicals with biological approaches, which are considered more environment friendly in the long term. One of the emerging research area for the control of different phytopathogenic agents is the use of biocontrol plant growth promoting rhizobacteria (PGPR), which are capable of suppressing or preventing the phytopathogen damage (Venant Nihorembere *et al*., 2011).

Fluorescent pseudomonads representing group of PGPR can promote growth and suppress plant pathogens by multiple mechanisms. Their applicability as biocontrol agents has drawn wide attention because of production of secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes and phytohormones (Gupta *et al*., 2001). *Pseudomonas fluorescens* is considered as biological biocontrol agent against various plant related diseases including root diseases (Ursula *et al*., 2000). They can be utilized in low-input sustainable agricultural applications, such as biocontrol, on account of their ability to synthesize secondary metabolites with antibiotic properties (Franks *et al.*, 2006). Many of such antibiotics produced have a broad-spectrum activity but strain to strain variations do exist (Raaijimakers *et al*., 2002). These secondary metabolites include 2, 4-diacetylphloroglucinol (DAPG), phenazine (Phz), pyrrolnitrin, oomycin A, viscosinamide, pyoluteorin and hydrogen cyanide (HCN). Among these secondary metabolites, DAPG has received the particular attention because of its production by a wide range of pseudomonads used for the biological control of root diseases (Dowling and O'Gara., 1994; Keel *et al*., 1996; Sharifi-Tehrani *et al*., 1998).

DAPG antibiotic producing *Pseudomonas fluorescens* have been recovered from soil and rhizosphere samples of many crop species as well as from marine environments (Thomashow &Weller., 1995; Keel *et al*., 1996; McSpadden Gardener *et al*., 2000; Lee & Kim, 2001; Weller *et al*., 2002; De La Fuente *et al*., 2004; Isnansetyo *et al*., 2003). These bacteria have been studied intensively because they are responsible for the suppressiveness of some soils against take-all disease of wheat caused by *Gaeumannomyces graminis* var. tritici (Weller *et al*., 2002; De Souza *et al*., 2003b; fusarium wilt of tomato Tamietti *et al*., 1993), black root of tobacco caused by *Thielaviopsis basicola* (Stutz *et al.*, 1986) and damping-off of sugar beet caused by *Pythium ultimum* (Fenton *et al.*, 1992). In addition to their antifungal activity, such bacteria have been found to possess some antiviral properties (Tada *et al.*, 1990) and also inhibit the growth of soft-rotting bacteria and cyst nematodes of potato (Cronin *et al.*, 1997) due to presence of DAPG. Thus the study of genotypic and phenotypic diversity of *Pseudomonas* spp. Along with their plant growth-promoting potential is important not only for understanding

their ecological role in the rhizosphere and the interaction with plants, but also for any biotechnological applications as plant growth-promoting rhizobacteria.

II. Materials and Methods

2.1 Sampling and Geographic Location

In order to collect samples of wild type of *Pseudomonas fluorescens* the Wheat Rhizosphere soil samples were collected from two different fields of SKAUST-K (Sheri Kashmir Agriculture University of Science & Technology) Shalimar, growing two different wheat varieties viz, SKW-196 and MSV (Mansarovar). Soil samples were also collected from Budgam and Bandipora fields growing wild variety of wheat. They are new districts in the state of Jammu & Kashmir, India. Budgam (Budgam District latitude 34.06077^0 N, longitude 74.79934^0 E) and Bandipora (Bandipora District latitude 34.4167^0 N, longitude 74.6500^0E) .The samples were collected at vegetative stages of growth during the year 2007 – 2008. The soils were collected from the upper 30 cm of the soil profile, stored in plastic bags on ice prior to use. The top of each plastic bag was closed with a rubber band to prevent drying of soil and immediately rushed to Department of soil science, SKAUST-K and subjected to analysis.

2.2Processing of soil samples

2.2.1Isolation of *Pseudomonas fluorescens* from wheat Rhizosphere soil

A total of 136 rhizosphere soil samples collected from wheat crop were processed after 22 hours of collection. The intact root systems were collected. Loosely adhering soils were shaken and detached from the roots and was discarded. 4.5 gms of root portions by weight with a layer of closely adhering rhizosphere soil was then transferred to 45.5 ml sterilized d.dH_2O and vigorously shaken for 10 minutes. The suspensions from all soil samples were serially diluted upto 10^{-7} with three replications for each sample. 100 µl of 10^{-1},10^{-3},10^{-5} and 10^{-7}diluted samples were spread on Pseudomonas selective medium based on King's medium B(KMB) (King et al., 1954) that was made selective for isolation of *Pseudomonas fluorescens* by adding chloramphenicol (13µg/ml), cyclohexamide (100µg/ml) and Ampicillin (50µg/ml) in them (Simon& Ridge,1974). Three replicate plates were incubated at 27°C for 48 hours. After 48 hours of incubation, all the isolates were checked for fluorescence under UV light (Sharifi-Tehrani et al., 1998) and representative types of colonies were selected and further purified on KMB agar medium. Pure isolates were preserved at -80°C after an addition of glycerol to a final concentration of 40% (v/v).

2.3Morphological and biochemical characterization of isolates

Morphological features including colony type, bacterial shape and growth characteristics of the isolates were determined using King's B agar medium. Catalase test, fluorescent pigment on KB media and siderophore detection were obtained according to previously reported manual (Goszczynska *et al.* 2000, Arnow, 1937 and Pickett *et al*. 1991). HI Assorted Biochemical kit (HI Media, Mumbai) containing sterile media for Citrate, ornithine, lysine decarboxylase, urease`, phenylalanine deaminase, Nitrate reduction, H2S production test and 5 different carbohydrates for fermentation test-Glucose, Adonitol, Lactose, Arabinose, and Sorbitol was used for further screening of isolates.

2.4 Preparation of inoculum

Single well isolated colonies were picked up with sterile loop and inoculated in 5 ml nutrient broth and incubated at 37°C for 4-6 hours until the inoculums turbidity reached 0.1OD at 620 nm. The biochemical test kit was opened and used as per vendor's instructions. Each well was inoculated with 50 µl of the overnight grown cultures by surface inoculation method and kept for incubation at 35-37°C for 18-24 hours. At the end of the incubation period, a series of reagents were added to carry out biochemical tests.

2.*5 In vitro* fungal inhibition assay

The fungal test cultures used in the current study were obtained from Division of Plant Pathology, SKAUST, Srinagar (Kashmir), India and grown on Potato dextrose agar (PDA) (peeled potato 250 g, dextrose 20g, agar 15 g, distilled water 1L). Further, purification was done by single spore isolation technique and cultures maintained at 27°C on PDA slants. Seven to ten days old cultures of *Fusarium oxysporum* and *Aspergillus* species were used in the experiment. Fungal bioassay was performed by using the paper disc method (Bahraminejad *et al.,* 2008). Fungus suspension was spread over the PDA plates and an overnight grown *Pseudomonas fluorescens* used as a source of antifungal agent. A fresh colony of *Pseudomonas fluorescens* isolated from rhizosphere soil was inoculated in nutrient broth and incubated at 27 °C for 24 hrs. Paper discs were soaked in 5 ml of this bacterial culture for 30 seconds and later placed on agar plates. The discs were dried between each application and were applied on agar plates within 15 mints after fungus inoculation, and plates incubated at 27^0 C for 3-7 days. At the end of incubation period, the plates were checked for clear zones of

inhibition formed around the discs. The experiment was done in triplicate for each antifungal *Pseudomonas fluorescens* isolates.

2.6 Production of antifungal metabolite

2.6.1 Siderophore

Siderophore production was analysed using Chrome Azurol S (CAS) medium (Schwyn and Neilands., 1987). Formation of yellow to orange halo zone around the colonies after incubation at 27^0C for 48h to 72hrs was considered to be positive.

2.7 PCR amplification of *PhlD* Gene:

PCR amplification of *PhlD* gene was performed using self designed SGF (5'-CCAAGGGCATAGCTCATCAT) forward and SGR (3'-GCTCAAGGAGCAATCGTTTC) reverse primer. PCR amplification was carried out in 25µl reaction mixtures containing 2µl DNA, 1 µl of each forward and reverse primers, 2.5 µl of 1x Taq polymerase buffer with 1.5Mm of $MgCl_2$, 2µl of 25 mM dNTP mix (Fermentas,USA), 1 U of Taq DNA polymerase (Fermentas,USA) and rest 14 µl of sterile water with following cycling conditions: Initial denaturation at 94°C for 5min followed by 35 cycles of 94°C for 30sec, 56°C for 30sec, 72°C for 30 secs with a final extension of 72°C for 10 min. Amplification was carried out in Techne cycler (TC-2295, UK).

The amplification products were electrophorised on 1% (w/v) agarose gel with 0.5x TBE buffer. Before loading, reaction mixtures (8 µl) were properly mixed with 2 µl of gel loading dye and loaded onto the wells of the gel along with DNA ladder. After electrophoresis, the gel stained with ethidium bromide (1.0 µg/ml) was visualized using a UV Trans-illuminator and photographed using Gel Documentation system (BIO-RAD, India).

III. Results

3.1Isolation of Pseudomonas fluorescens

In the present study, from one hundred and thirty five rhizosphere soil samples, only 52 isolates were able to fluoresce under UV light (Fig 5.4. a &b). At biochemical level all the 52 isolates showed Catalase and Oxidase activity and also produced yellowish green fluorescein pigment on King's B medium peculiar to *Pseudomonas fluorescens* (Table5.1). Results were further authenticated by HI Assorted Biochemical Kit (Table5. 2).

3.2 Production of Siderophore

Interestingly, all of the 52 isolates produced Siderophore on CAS (Chrome Azurol S) blue agar except SKW2 and MSV5 by changing the colour of the CAS medium from blue to light yellow or orange (Fig 5.5)

3.3 Antifungal assay *in vitro*

It was noteworthy that among the 52 *Pseudomonas fluorescens* isolates, only 7 isolates were found to inhibit the growth of *Fusarium oxysporum* and *Aspergillus* species (Fig 5.6 a & b& Fig 5.7 .a& b) with variable antifungal activities. (Table 5.3 & Fig 5.9). As shown in figure 3 and 4 the highest zone of inhibition against *Fusarium sp.* was shown by Bandi 6 (29mm), followed by Bandi 11(28mm). Whereas highest inhibition against *Aspergillus sp.* was shown by BG6 (20mm). Bandi 6 and Bandi 11 showed best result against *Fusarium sp.* While as, BG6 showed best result against *Aspergillus species.* These results gave us a clue that all the 7 isolates posing antifungal activity may be involved in the production of *PhlD* antifungal metabolites (Antibiotic) and could be better exploited as biocontrol agents. Plates inoculated with fungus only served as control. Three replications were maintained for each isolate.

3.4 PCR-based screening method to detect DAPG production

In order to check the mode of action, all the seven *Pseudomonas fluorescens* isolates posing antifungal activity were subjected to PCR with self designed SGF and SGR forward and reverse primers respectively. Out of seven isolates only 4 isolates were able to produce DNA fragments corresponding to approximately 745-bp size that corroborated with the predicted known *PhlD* sequence of Pf-5(NRRL B-23932) used as positive control in the current study (Fig.5.8).

3.5 Sequencing of PCR products

Sequencing of PCR products for *PhlD* gene was performed using both forward and reverse primers at Bioaxis labs, Hyderabad (India). Partial *PhlD* gene sequences of *Pseudomonas fluorescens* SKW1 (Sagik2r), BG6 (Sagik1f) and Bandi6 (Sagik4r) retrieved in this study were deposited in the GenBank database under accession numbers **JQ765867, JQ765868 and JQ765869.**

IV. Discussion

Fluorescent pseudomonads, normally present in soil are effective colonizers of the rhizosphere of many crop plants possessing potential to inhibit growth of number of phytopathogenic fungi (Weller D.M., 1988). To our best knowledge, we are reporting first time from Kashmir valley of India, *Pseudomonas fluorescens* with potential antifungal activity. Such type of bacteria have been studied mainly because of their widespread distribution in soil, their ability to colonize the rhizosphere of host plants and ability to produce a wide range of compounds inhibitory to a number of serious plant pathogens (Anjaiah *et al* 1998; Weller 1988; Copper and Higgins 1993; Vidhyasekaran and Muthamilan 1995). They aggressively colonize rhizosphere of various crop plants, and have a broad spectrum antagonistic activity against plant pathogens, such as antibiosis (the production of inhibitory compounds) siderophores production and nutrition or site competition (Cartwright *et al.,* 1995; Rosales *et al*., 1995, Bull *et al,* 1991).

Keeping such plant health promoting activities of the particular bacteria in view in our current study we were able to isolate 52 *Pseudomonas fluorescens* through biochemical screening of 136 wheat rhizosphere soil samples. As nowadays, biological control agents are highly appreciated as alternative to the use of fungicides, for suppression of fungal pathogens in agricultural production (Sigler *et al*., 2001). Reports have shown that among the many organisms suited for biocontrol of soil borne diseases, the root-colonizing bacterium *Pseudomonas fluorescens* CHA0 has become a model for studying the behavior of biocontrol inoculants in the soil ecosystem (Rezzonico *et al.,* 2003). Our results also indicated good presence of such bacteria in this part of India. *Pseudomonas fluorescence* have been reported to show known biological control activity against certain soil-borne phytopathogenic fungi including *Macrophomina phaseolina*, *Rhizoctonia solani*, *Phytophthora nicotianae* var. *parasitica*, *Pythium* sp. and *Fusarium* sp. by producing known secondary metabolites such as siderophore, HCN and protease (Ahmadzadeh *et al.*, 2006; Fuchs and De'fago.,1991) and biosynthesis of these metabolites is modulated by a number of biotic and abiotic factors (Duffy & Défago, 1999; Raaijmakers *et al*., 2002).

It is well known that geographical locations play a big role in maintenance of different ecological niches. In our study also the different regions of Kashmir (SKAUST-K, Bandipora and Budgam) from where isolates were obtained varied in their agro climatic conditions. Some of the soil factors that have been identified to have a significant influence on variable biocontrol activity of *Pseudomonas fluorescens* isolates have been examined in many earlier studies. Reports have shown that production of siderophores by *Pseudomonas* spp. is greatest at 25–27°C (Barton *et al*., 1996) and is repressed at 37°C (Marugg *et al*., 1985). Likewise, it is well known that ammonium forms of nitrogen can reduce the severity of take-all disease (Colbach., 1997; Huber., 1968; Mc Nish., 1988). Absorption of ammonium-nitrogen by wheat roots leads to excretion of corresponding H^+ ions (Thomashow & Weller., 1996) and a corresponding reduction in rhizosphere pH (Weller., 1988). Taking all these things into consideration, the soil type sample sites was analyzed and SKAUST-K soil was found to be of clay loam type having pH 7.3, whereas soil type of Bandipora and Budgam was silty clay loam (pH 7.16) and sandy loam (pH 7.4) respectively. Secondly temperature that has been reported to be a key factor influencing both colonization by rhizobacteria and expression of biocontrol mechanisms (Burpee., 1990; Beauchamp *et al*., 1991) was found to vary in the range of 25-30^o C in these selected regions of Kashmir. Our results clearly indicated that due to variable soil chemistry there is a wide variety of *Pseudomonas fluorescens* present in Kashmir valley.

As *Pseudomonas fluorescens* has been reported to provide 79-82 % control of rice blast and sheath blight that in turn increase grain yield in rice (Gnanamanickam *et al.,* 1998). The quantity and quality of nutrients available and the ability to compete successfully for them are also major determinants of microbial population size and metabolic activity, both of which are integrally linked to the regulation of antibiotic synthesis (Weller., 1990; Weller., 1993).

In the current study out of 52 isolates, only 7 isolates of *Pseudomonas fluorescens* showed antifungal activity against *Fusarium oxysporum* and *Aspergillus* species. One isolate from SKAUST-K (SKW1), two isolates from Budgam (BG6 & BG27), four isolates from Bandipora due to different soil chemistry (Bandi6, Bandi11, Bandi24 & Bandipora63) demonstrated varied antifungal activity against both the fungus. The proportions of antifungal isolates obtained were higher in Bandipora soil followed by Budgam. Literature cited indicates that biotic and abiotic factors associated with the crop and environment affect the performance of fluorescent pseudomonads (Thomashow and Weller., 1995; Duffy and Défago, 1997; Notz *et al*., 2002) and due to varied soil chemistry, the isolates with highest inhibitory activity against *Fusarium oxysporum* were retrieved from Bandipora soils compared to the soils of SKAUST-K and Budgam. While as, isolates with highest inhibitory activity against *Aspergillus* species were found from Budgam soil.

The 745-bp internal fragment from the *phlD* gene of *Pseudomonas fluorescens* has been extensively used to enumerate producers of 2, 4-DAPG among fluorescent pseudomonads from the rhizosphere of wheat (Duffy & Défago., 1999; Notz., 2002) and maize (Bangera & Thomashow., 1999). Therefore, in order to check the mode of resistance offered by 7 isolates towards two different fungi, in our study PCR based analysis of 2,

4-DAPG was carried out. Detection of *PhlD* gene fragment corresponding to 745bp DNA fragment in four of the seven isolates i.e. SKW1, BG6, Bandi6 and Bandi24 corroborated well with previously reported *PhlD* gene (Wang and co-workers., 2001). Interestingly, the results showed that there is no significant link between inhibition of fungal pathogens and existence of *PhlD* gene in Bandi11, Bandi63 and BG27. The important factor that may account for the lack of this relationship may be due to the other factors imparting antifungal activity to these isolates.

It is suggested that difference in biocontrol capacities may be due to one or more of the following factors. First, different genotypes produce different amounts of 2, 4-DAPG and other antifungal metabolites *in vitro* (Keel *et al.*, 1996; Sharif-Tehrani *et al.*, 1998) and such differences also may occur in situ. Second, the degree to which strains can colonize the rhizosphere may impact their ability to suppress invading pathogens. Thus, in this study it was concluded that variable antifungal activity of 7 different isolates could be attributed to production of antibiotic as well as to different locations and abiotic and biotic factors of soil from where they were collected.

Work related to *in situ* detection of 2, 4-DAPG is being carried out in our laboratory for its better exploitation as biocontrol agent.

V. Tables and Figures

Table5.1 Morphological Features Of *Pseudomonas Fluorescens*.

Microbial isolate	Variable	Characteristics
Colony & cell morphology	Colony size Surface Opacity colour	Large Round Opaque Yellow green
Biochemical characteristics	Oxidase Catalase Siderophore production	Positive Positive Positive
Fluorescent diffusible pigment on King's B	Blue green Yellow green	Not produced Produced
Growth characteristics	Growth at 4^0 C Growth at 28^0 C Growth at 41^0C	Positive Posi tive Negative

Table 5.2 Based On The Reactions That Appear As Change In Coloration, Following Symbols (+ve, -ve and V) Were Assigned *+ve = Positive (more than 90% -ve = Negative (more than 90%) V = 11-89% Positive.*

Well No.	Test	Pseudomonas fluorescens (ATCC 13525)	SKW1	BG 6	BG2 7	Bandi 6	Bandi 11	Bandi 24	Bandi63
1	Citrate utilization test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
2	Lysine decarboxylase	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
3	Ornithine decarboxylase	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
4	Urease	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5	Phenylalanine deamination	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6	Nitrate reduction	v	+	-ve	+ve	-ve	-ve	-ve	-ve
7	H_2 s production	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
8	Glucose fermentation	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9	Adonitol	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
10	Lactose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11	Arabinose	v	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12	Sorbitol	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 5.3 Variable Zone Of Inhibition

	Fungal pathogens			
SL NO	Place of collection	Isolates	Fusarium sp. Inhibition zone in mm(6th day of inoculation)	Aspergillus sp. Inhibition zone in mm(5th and 7th day of inoculation)
1	SKAUST-K	SKW1	22	10
2	Budgam	BG6	13	20
3	Budgam	BG27	12	11
4	Budgam	Bandi6	29	10
5	Bandipora	Bandi 11	28	11
6	Bandipora	Bandi 24	21	9
7	Bandipora	Bandi 63	11	9

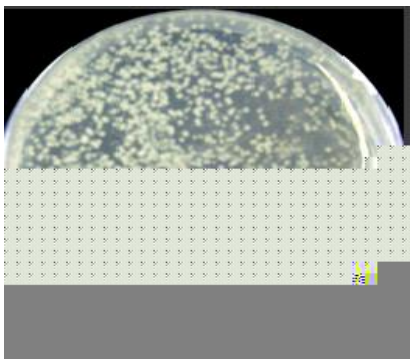

Fig.5.4 (a) culture of *Pseudomonas fluorescens* on King's B under normal light. (b) culture of *Pseudomonas fluorescens* on King's B under uv.

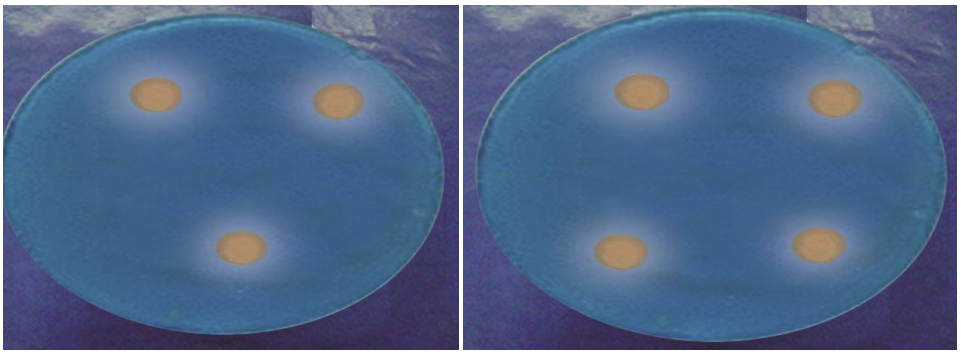

Fig. 5.5 siderophore producing *Pseudomonas fluorescens* isolates using CAS agar plates.

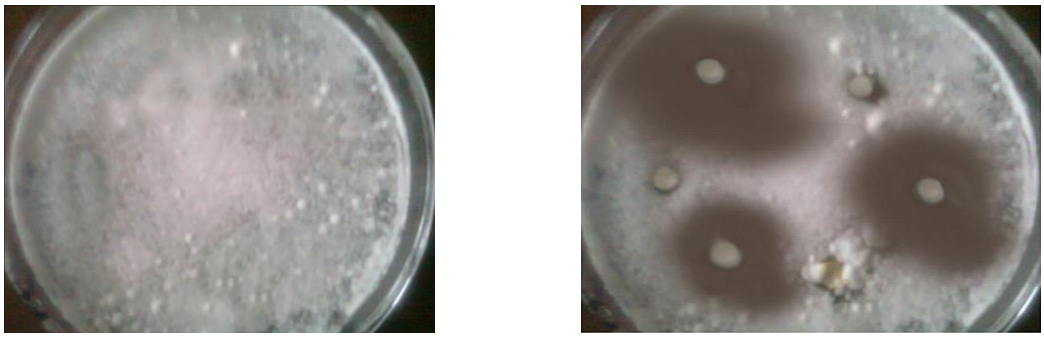

Fig. 5.6 (a) Fusarium *oxysporum* as a positive control (b) growth inhibition of *Fusarium oxysporum* by *Pseudomonas fluorescence* isolates

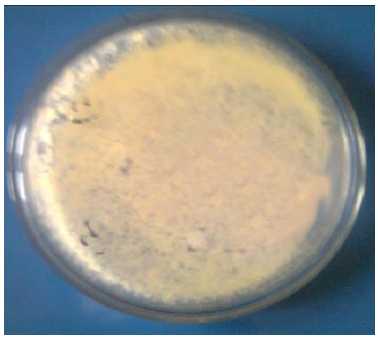

Fig 5.7 (a) *Aspergillus* species as a positive control

(b) growth inhibition of *Aspergillus* Species by *Pseudomo* *fluorescens* isolate.

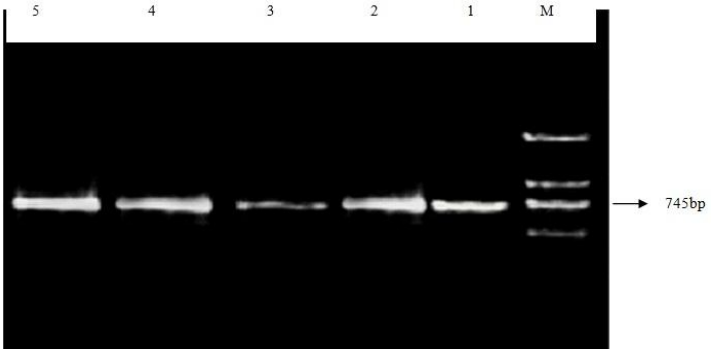

Fig 5.8 photograph showing amplified product of *PhlD* gene electrophoresed in 1.0 % Agarose gel. Lane 2-5: 745bp fragment of *PhlD* gene of *Pseudomonas fluorescens* isolates. Lane 1: Pf-5 (Reference strain) Lane- M: 1 Kb Marker (Bioline).

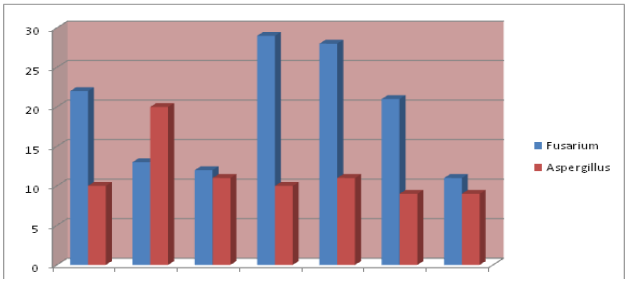

Fig 5.9 variable antifungal activities of *Fusarium oxysporum* and *Aspergillus* species.

VI. Conclusion

The metabolite 2, 4- DAPG produced by *Pseudomonas fluorescens* is a major factor in controlling a range of plant pathogens. The experiment performed under *in vitro* conditions demonstrated that wild isolates of *Pseudomonas fluorescens* can inhibit the growth of *Fusarium oxysporum* and *Aspergillus* species that infect wheat crop of Kashmir. The isolates restricted the growth of tested fungi by the production of Siderophore and 2, 4-diacetylphloroglucinol.

Further *in situ* analysis of the antifungal metabolite produced by wild isolates of *Pseudomonas fluorescens* needs to be done.

VII. Acknowledgement

We are highly thankful to all the faculty and non-faculty members of Department of Biosciences, JMI, New-Delhi and Honorable Vice Chancellor, SKAUST-K for their support and help.

References

[1] Musa S, Horrible Food Pollution. Kodansha, Tokyo, 220 pp. mutants. *Plant Growth Promoting Rhizobacteria: Progress and Prospects. Bulletin OILB 14*, 1976, 332–5.

[2] Venant Nihorembere, Marc Ongena, Maite Smargiass, *Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnol.Agron. Soc. Environ.15 (2)*, 2011, 327-337.

[3] Gupta C.P., Dubey R.C., Kang S.C., Maheh Wari D.K. *Antibiosis-mediated necrotrophic effect of Pseudomonas GRC2 against two fungal plant pathogens. Current Science 81*, 2001, 91-94.

[4] Ursula, S.K., Arnaud, S., Monika, M., Caroline, B., Brion, D., Cecile, G.B., Cornelia, R., Regina, N., Genevie, V.D.F., Dieter, H and Christoph, K.L. *Autoinduction of 2, 4 Diacetylphloroglucinol biosynthesis in the biocontrol agent* Pseudomonas fluorescens *CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. Journal of Bacteriology. 182(5)*, 2000, 1215-1225.

[5] Franks, A., Ryan, R. P., Abbas, A., Mark, G. L., and O'Gara, F. *Molecular tools for studying plant growth-promoting rhizobacteria (PGPR)*, 2006, 116-131.

[6] Raaijmakers JM, Vlami M, de Souza JT. *Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81,* 2002, 537–47.

[7] Dowling, D.N. and O'Gara, F. *Metabolites of Pseudomonas involved in the biocontrol of plant disease. Trends Biotechnol. 12*, 1994, 133-141.

[8] Keel, C., Weller, D.M., Natsch, A., Défago, G., Cook, R.J. and Thomashow, L.S. *Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent Pseudomonas strains from diverse geographic locations. Applied and Environmental Microbiology 62*, 1996, 552–563.

[9] Sharifi-Tehrani A, Zala M, Natsch A, Moënne-Loccoz Y, Defago G. *Biocontrol of soil-borne fungal plant diseases by 2,4-diacetylphloroglucinol-producing fluorescent pseudomonads with different restriction profiles of amplified 16S rDNA.Eur J Plant Pathol.104*, 1998, 631-643.

[10] Thomashow LS & Weller DM. *Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. Plant–Microbe Interactions, Vol. 1* (Stacey G & Keen N, eds), Chapman & Hall, New York, NY, 1995, 187-235.

[11] McSpadden Gardener BB, Schroeder KL, Kalloger SE, Raaijmakers JM, Thomashow LS, Weller DM. *Genotypic and phenotypic diversity of phlD-containing Pseudomonas strains isolated from rhizosphere of wheat. Appl Environ Microbiol. 66,* 2000, 1939-1946.

[12] Lee E-T & Kim S-D. *An antifungal substance, 2, 4- diacetylphloroglucinol, produced from antagonistic bacterium Pseudomonas fluorescens 2112 against Phytophthora capsici. Kor Appl Microbiol Biotechnol 29*, 2001, 37–42.

[13] Weller DM, Raaijmakers JM, McSpadden Gardner BB, Thomashow LS. *Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology 40*, 2002, 308–348.

[14] De La Fuente L, Thomashow LS, Weller DM, Bajsa N, Quagliotto L, Chernin L & Arias. *Pseudomonas fluorescens UP61 isolated from birds foot trefoil rhizosphere produces multiple antibiotics and exerts a broad spectrum of biocontrol activity. Eur J Plant Pathol 110*, 2004, 671–681.

[15] Isnansetyo A, Cui L, Hiramatsu K and Kamei Y. *Antibacterial activity of 2,4 diacetylphloroglucinol (DAPG) produced by Pseudomonas sp. AMSN isolated from a marine alga, against vancomycin-resistant Staphylococcus aureus (VRSA). Int J Antimicrob Agents 22*, 2003, 545-7.

[16] De Souza, J.T., Weller, D.M. and Raaijmakers, J.M. *Frequency, diversity, and activity of 2, 4-diacetylphloroglucinol-producing fluorescent Pseudomonas species in Dutch take-all decline soils. Phytopathology 93*,2003b, 54–63.

[17] Tamietti, G., L. Ferraris, A. Matta and I.A. Gentile. *Physiological responses of tomato plants grown in fusarium suppressive soil. J. Phytopathol 138*, 1993, 66-76.

[18] Stutz, E., Defago, G., and Kern, H. *Naturally occurring fluorescent pseudomonads involved in suppression of black root rot of tobacco. Phytopathology 76*, 1986, 181-185.

[19] Fenton AM, Stephens PM, Crowley J, O' Callaghan M, O'Gara F**.** *Exploitation of gene(s) involved in 2,4- diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a Pseudomonas strain. Appl Environ Microbiol 41*, 1992, 109-117.

[20] Tada, Y., Sakamoto, M. and Fujimura, T. Theor. *Appl. Genet 80*, 1990, 475-480.

[21] Cronin, D., Y. Moënne-Loccoz., A. Fenton, C. Dunne, D.N. Dowling, and F. O'Gara**.** *Role of 2, 4-diacetylphloroglucinol in the interaction of the biocontrol pseudomonad strain F113 with the potato cyst nematode Globodera rostochiensis. Appl. Environ. Microbial.63*, 1997b**,** 1357-1361.

[22] King, et al. *Media for the demonstration of pyocyanin and fluorescein, J. Lab. Clin. Med. 44*,1954, 301.

[23] Simon, A. and Ridge, E.H. *The use of ampicillin in a simplified selective medium for the isolation of fluorescent pseudomonads. Journal of Applied Bacteriology 37*, 1974, 459- 460.

[24] Goszczynska, T./ Serfontein, J.J./ Serfontein, S. *Introduction to Practical Phytobacteriology. Bacterial disease unit. ARC-Plant Protection Research Institute Pretoria, South Africa*. 2000.

[25] Arnow, L.E. *Colorimetric determination of the component of 3, 4 hydroxyphenyl alaninetyrosine mixtures. Ann.Rev. Biochem.50,* 1937, 715–731.

[26] Pickett, M.J. / Goodneer, J.R./ Harvey, S.M. *Test for detecting degradation of gelatin: Comparison of five methods. J. Clinical Microbiol. 29*, 1991, 2322–2325.

[27] Bahraminejad S, Asenntorfer R E, Riley IT, Schultz CJ. *Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots oats (Avena sativa L.). J. Phytopathol. 156,* 2008, 1-7.

[28] Schwyn, B. and Neilands, J.B. *Universal chemical assay for the detection and determination of siderophores. Anal. Biochem., 160*, 1987, 47–56.

[29] Weller DM. *Biological control of soil borne plant pathogens in the rhizosphere with bacteria. Annul Rev Phytopathol 26,* 1988, 379-407.

[30] Anjaiah V, Koedam N, Thompson NB, Loper JE, Hofte M, Tambong JT and Cornelis P. *Involvement of phenazines and anthranilate in the antagonism of P. auregenosa PNA1 and Tn5 derivatives towards Fusarium sp. Pythium sp. Mol Pl Microb Interact 11*,1998, 847-854.

[31] Copper AL and Higgins KP. *Application of Pseudomonas fluorescens isolates to wheat as potential biological control agents against take-all. Plant Pathol 42*, 1993, 560-567.

[32] Vidhysekaran P and Muthamilan M. *Development of formulations of Pseudomonas fluorescens for control of chickpea wilt. Plant Disea 79*, 1995, 782-786.

[33] Cartwright, D.K., W.S. Chilton, D.M. Benson. *Pyrrolnitrin and phenazine production by* Pseudomonas cepacia, *strain 5.5 B, a biological agent of Rhizoctonia solani*, Appl. Microbiol. Biotechnol. *43*, 1995, 211-121.

[34] Rosales A.M., L. Thomashow, R..J. Cook, T.W. Mew. *Isolation and identification of antifungal metabolites produced by rice-associated antagonistic Pseudomonas spp., Phytopathology 85,* 1995, 1028-1032.

[35] Bull, C. T., D. M. Weller, and L. S. Thomashow. *Relationship between root colonization and suppression of Gaeumannomyces graminis var. tritici by Pseudomonas fluorescens strain 2-79. Phytopathology 81,* 1991, 954-959.

[36] Sigler, W., Nakatsu, C.H., Reicher, J., Turco, R. *Fate of the biological control agent Pseudomonas aureofaciens TX-1 after application to turfgrass. Applied and Environmental Microbiology 67,* 2001, 3542–3548.

[37] Rezzonico, F., Moenne-Loccoz, Y., Défago, G. *Effect of stress on the ability of a phlA-based quantitative competitive PCR assay to monitor biocontrol strain Pseudomonas fluorescens CHAO. Applied and Environmental Microbiology 69,* 2003, 686-690.

[38] Ahmadzadeh, M., Afsharmanesh, H., Javan-Nikkhah, M.A. & Sharifi-Tehrani. *Identification of some molecular traits in fluorescent pseudomonads with antifungal activity. Iranian Journal of Biotechnology. 4,* 2006, 245-253.

[39] Fuchs, J., De´fago, G. *Protection of cucumber plants against black root rot caused by Phomopsis sclerotioides with rhizobacteria. IOBC/ WPRS Bulletin 14,* 1991, 57–62.

[40] Duffy BK, Défago G. *Environmental factors modulating antibiotic and siderophore biosynthesis by* Pseudomonas fluorescens *biocontrol strains. Appl. Environ. Microbiol. 65,* 1999**,** 2429-2438.

[41] Barton HA, Johnson Z, Cox CD, Vasil AI, Vasil ML. *Ferric uptake regulator mutants of Pseudomonas aeruginosa with distinct alterations in the iron-dependent repression exotoxin A and siderophores in aerobic and micro aerobic environments. Molecular Microbiology 21,* 1996, 1001–17.

[42] Marugg JD, van Spanje M, Hoekstra WPM, Schippers B, Weisbeek PJ. *Isolation and analysis of genes involved in siderophore biosynthesis in plant-growth-stimulating Pseudomonas putida WCS358. Journal of Bacteriology 164,* 1985, 563–70.

[43] Colbach, N., P. Lucas, and J.-M. Meynard. Influence of crop management on take-all development and disease cycles on winter wheat. Phytopathology 87, 1997**,** 26-32.

[44] Huber, D. M., C. G. Painter, H. C. McKay, and D. L. Peterson. *Effect of nitrogen fertilization and take-all of spring wheat. Phytopathology 62,* 1968, 434-436.

[45] McNish, G. C. *Changes in take-all (*Gaeumannomyces graminis *var. tritici), rhizoctonia root rot (*Rhizoctonia solani*) and soil pH in continuous wheat with annual applications of nitrogenous fertiliser in western Australia. Aust. J. Exp. Agric. 28,* 1998**,** 333-341.

[46] Thomashow, L. S., and D. M. Weller. Molecular basis of pathogen suppression by antibiosis in the rhizosphere, *In Hall, R. (ed). Principles and Practice of Managing Soil borne Plant Pathogens. American Phytopathological Society, St. Paul, MN* 1996 80-103.

[47] Burpee LL. *The influence of abiotic factors on biological control of soilborne plant pathogenic fungi. Canadian Journal of Plant Pathology 12,* 1990, 308–17.

[48] Beauchamp CJ, Kloepper JW, Chalifour FP, Antoun H. *Effects of temperature on growth promotion and root colonization of potato by bioluminescent rhizobacteria mutants. Plant Growth Promoting Rhizobacteria: Progress and Prospects. Bulletin OILB 14,* 1991, 332–5.

[49] Gnanamaickam SS, Valasubramanian K, Thara V and Chaterjee AK. *Microbial antagonists for rice diseases control: molecular approaches in microbes: for health, wealth and sustainable environment. Ed Ajit varma, 1998,* 371-388.

[50] Weller, D. M., and L. S. Thomashow. *Antibiotics: evidence for their production and sites where they are produced, In R. R. Baker and P. E. Dunn (ed.), New Directions in Biological Control: Alternatives for Suppression of Agricultural Pests and Diseases. Alan R. Liss, New York* 1990, 703-711.

[51] Weller, D. M. and L. S. Thomashow. *Microbial metabolites with biological activity against plant pathogens, In R. D. Lumsden and J. L. Vaughn (ed.), Pest Management: Biologically Based Strategies. American Chemical Society, Washington, DC* 1993, 172-180.

[52] Duffy and Défago, B.K. Duffy, G. Défago**.** *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,* 1997, 1250–1257.

[53] Notz, R., Maurhofer, M., Dubach, H., Haas, D., and Défago, G. *Fusaric acid producing strains of Fusarium oxysporum alter 2, 4-diacetylphloroglucinol biosynthesis gene expression in Pseudomonas fluorescens CHA0 in vitro and in the rhizosphere of the wheat. Appl. Environ. Microbiol. 68,* 2002, 2229–2235.

[54] Bangera, M.G. and Thomashow, L.S. *Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2, 4-diacetylphloroglucinol from Pseudomonas fluorescens Q2-87. J. Bacteriol. 181,* 1999, 3155-3163.

[55] Wang C, Ramette A, Punjasamarnwong P, Zala M, Natsch A, Moënne-Loccoz Y, Défago G. *Cosmopolitan distribution of phlD-containing dicotyledonous crop associated biocontrol pseudomonads of worldwide origin. FEMS Microbiol Ecol. 37,* 2001, 105 -116.