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A Study on Isolation, Partial Characterisation and antifungal activity of Pseudomonas *fluorescens* from soil

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Abstract:* *Pseudomonas fluorescens* are organisms which are abundant in soil and influence plant by growth promotion and disease control. Of 50 samples, thirty isolated samples obtained from soil was partially characterized as Pseudomonas *fluorescens*. They were classified into 5 biovars BV1,II,III,IV and V . Among the Biovars BV II is the most abundant (26.6%)followed by BV IV(23.3%),BV I(20%),BV V(16.6%) and BV III (13.3%)All of them produced siderophores in CAS medium.Minimal Inhibitory Concentrations(MIC) of the two heavy metals) and two antibiotics (Penicillin and Streptomycin) were observed as shown in table 3.All biovars showed resistance to 2 heavy metals(Lead and mercury and 2 antibiotics(Penicillin and Streptomycin). So they can be used in soil contaminated with heavy metals and also in the presence of antibiotics. Strain BV V was found to be the most resistant strain and was used for further studies. Four basal media supplemented with different concentration of iron, were employed to study the effect of iron and different organic carbon sources on siderophore production in *Pseudomonas fluorescens*. The highest siderophore production was obtained in KB medium(24.3 µM) and the lowest production was in glycerol medium(2.45 µM) with no Iron added. The standard KB medium without added iron permitted the synthesis of greater amount of siderophores. Fusarium. All the isolates of *Pseudomonas fluorescens* inhibited the pathogenic fungi Fusarium isolated from soil. Both the culture containing cells and cell free extract shown inhibition of Fusarium. Among broth cultures *Pseudomonas fluorescens* BV III showed more inhibition (63.3%) on third day of inoculation.Cell free extract of *Pseudomonas fluorescens* BV V on third day of incubation showed more inhibition (67.7%)than culture containing cells(46.6%). Special analysis of crude extract of culture filtrate, revealed the production of siderophores by fluorescent Pseudomonas. The maximum absorption was found it to be at 373nm. Further studies are needed to confirm the specific molecule which causes inhibition in *Pseudomonas fluorescens.*

***Keywords:* Antibiotics, Biovars, CAS medium, Cell free extract Fusarium, Heavy metals, MIC, *Pseudomonas fluorescens* ,Siderophore.**

1. INTRODUCTION

The members of the genus *Pseudomonas* may be described as gram negative, non spore forming, straight or slightly curved rods. They are typically motile by means of one or more flagella. Generally common to all constituent species of the genus *Pseudomonas* have certain physiological properties such as chemotropic nutrition, aerobic metabolism, absence of fermentation, absence of photosynthesis, inability to fix nitrogen and capacity for growth at the expense of a large variety of organic substrates.(Palleroni;1984)*Pseudomonas* species have very simple nutritional requirements. In the laboratory they grow well in media with some organic matter in solution, at neutral pH and at temperatures in the mesophilic range. One of the handiest media for culturing *Pseudomonas* in the laboratory is King's B medium. (Duffy etal; 1996)

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A common characteristic of the fluorescent Pseudomonas is the production of pigments that fluoresce under short wavelength (254 nm) ultraviolet light, particularly under conditions of iron limitation. Some of these pigments and/or their derivatives are to play a role as siderophores in the iron uptake systems of the bacteria, and hence, their production is markedly enhanced under conditions of iron deficiency. The ability of Pseudomonas to grow and to produce siderophore is dependent on the iron content and type of carbon sources in the medium. Under conditions of low iron concentration, the Pseudomonas isolates studied produced yellow-green fluorescent iron-binding peptide siderophores and the biosynthesis of this siderophores was affected by several different parameters. Interest in the Pseudomonas has increased recently because of the possible use of siderophores as biopesticides and the possible use of Pseudomonas in detoxifying chemical wastes through a wide range of enzymatic metabolic activities. Iron limitation has also been studied because of the potential role of microbial siderophores in facilitating uptake of heavy metals and their mobilization under certain growth conditions.(Rachid etal; 2005).

Fusarium is a large genus of filamentous fungi widely distributed in soil and in association with plants. The genus *Fusarium* currently contains 20 species. The most common of these are *Fusarium solani*, *Fusarium oxysporium* and *Fusarium chlamydosporum*.*Fusarium oxysporium* is a fungus that causes Fusarium wilt diseases in more than a 100 species of plants. It does so by colonizing the water-conducting vessels (xylem) of the plant. As a result of this blockage and breakdown of xylem, symptoms appear in plants such as leaf wilting, yellowing and eventually plant death.

Chemical fungicides not only may pollute the atmosphere but also can be environmentally harmful as chemical spread out in the air and accumulated in the soil. Furthermore the repeated use such chemicals has encouraged the development of the target organism. Despite the undesirable problems caused by the synthetic fungicides, fungicides will be increasingly applied in agriculture in the near future, provided that safer and ecologically friendly fungicides become available. The requirements for the fungicides to be practically used in fields are excellent potency against a variety of plant pathogens and safety, not only for humans, animals, and host plants but also for the ecosystems. These concerns highlight the need for selective fungicides with higher degradability in nature. Fungicides of microbial origin, which are synthesized biologically, have been demonstrated to be not only specifically effective on the target organism but also inherently biodegradable. (Carmi etal; 1994).

Some *Pseudomonas fluorescens* strain has biocontrol properties, protecting the roots of some plant species against parasitic fungi such as *Fusarium* or *Phythium*, as well as some phytophagous nematodes. It is not clear exactly how the plant promoting properties of *Pseudomonas fluorescens* are achieved.Theories include:That the bacteria might induce systemic resistance in the host plant, so it can better resist attack by a true pathogen.(Brion.K.Duffy,1997)The bacteria might out compete other (pathogenic) soil microbes, e.g.: by siderophores giving a competitive advantage at scavenging for iron. The bacteria might produce compounds antagonistic to other soil microbes, such as phenanzine type antibiotics or hydrogen cyanide.

The biological control of soil-borne pathogens with antagonistic bacteria, Pseudomonas species belonging to plant growth promoting Rhizobacteria has received prominent attension because of the dual role of these bacteria in plant growth promotion and disease control. Main objective of present study is the isolation and characterization and Classification of biovars of *Pseudomonas fluorescens*.Also Detection of antifungal activity of culture containing cells and cell free extract.

2. MATERIALS & METHODS

Isolation of *Pseudomonas fluorescens* from soil

1. Sample collection

Soil samples were collected from different rhizosphere mainly rose rhizosphere and also from workshop soil.

2. Isolation of organisms

Enrichment culture 1 gm of soil sample were transferred to 9ml king's broth in a screw cap vial. The bottles were shaken well and incubated for 48-72 hours for enrichment. After incubation 1 loopful of culture was streaked on F agar plates containing antibiotics, incubated for 48-72 hours at room temperature. Pigment producing colonies from F agar was isolated and streaked on KB agar slants.

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3. Characterization of Isolates

a. Pigment production : Colonies on King's B agar and F agar were checked for pigment production.

b. Macroscopic characterization: size, shape, appearance and other colony characteristics were observed.

c. Microscopic characterization: Gram staining, Metachromatic granule staining by Albert – Laybourn Method and Hanging drop Method

4. Characterization based on Biochemical reactions

Oxidase test, Catalase test,Gelatin liquefaction,Starch hydrolysis,Lactose fermentation,Glucose fermentation,Indole production test,Citrate utilization test,Nitrate reduction test,Denitrification,Esculin decomposition,Lysine decarboxylase and arginine dihydrolase and Litmus milk

5. Detection of siderophore production

The four solutions of CAS medium were mixed after autoclaving. The medium was poured into petriplates and allowed to solidify. Active bacterial colonies were spotted on the surface of CAS medium. The plates were incubated at 28^0C for 48 hours and examined for the development of orange halo against a dark blue background suggests siderophore production. (Jagadeesh et al 2001)

6. Effect of iron and growth inhibitions on siderophore production

The liquid king's B medium was modified by inclusion of inhibition at different concentration and was inoculated with 100 ml of cell suspensions from preculture of 24 hrs. The following concentration of inhibition was tested. Lead (200, 500,700,1000,1200,1600,2000,4000,6000, 8000µM) Mercury (6.25, 12.5, 25, 50,100µM), penicillin (2.5, 50,100 Units/10ml) and streptomycin (0.10, 0.20, 0.40, 0.60, 0.80mg/l). Only the most resistant strain was used in subsequent studies.

7. Effect of iron concentration and various carbon sources on siderophore production

Cultures were grown for 40 hrs at 25^0C with shaking (200rpm) in 500ml Erlenmeyer flasks containing 125ml medium, with the pH adjusted to 7. Four basal media were employed with $FeCl_3$ added in increasing amounts (5, 10, 50,100, 200, 250 and 300 mg/ml). The media were Asparagine medium, Glycerol medium and succinate medium.

8. Measurement of growth and siderophore assay

Bacterial growth was estimated turbidimetrically at 600nm. Amount of siderophore secreted into the culture medium was determined by removing bacteria by centrifugation and measuring the absorbance of the supernatant at 400nm. Concentration was calculated using absorption maximum (d = 400nm) & molar extinction coefficient ε = 20,000.

9. Effect of the bacterial growth inhibitors on siderophore production

The influence of bacterial growth inhibitors on siderophore production under different concentration of Fe^{3+} in KB medium. The strains were grown in KB medium with different concentrations of the following inhibitors: Lead (2000µM), Mercury (100), Penicillin and streptomycin (50). (Djibauirachid etal, 2005)

10. Isolation of pathogenic fungus from soil

Rhizosphere soil samples were collected from the field and it is serial diluted and plated on PDA medium

11. Detection of antifungal activity of culture containing cells

PDA plates were prepared and wells were cut on the plates. The bacterial cultures from Ist, IInd, IIIrd, IVth day were taken and the organisms were added the appropriate wells on PDA plates and pathogenic fungus – Fusarium were inoculated at the centre of the plates. The development of fungal growth was observed up to 6 days to find out fungal growth inhibition.

12. Detection of antifungal activity of cell free extract:

PDA plates were prepared and wells were cut on the plates. The cell free extracts were taken from 1st, 2nd, 3rd and 4th day bacterial cultures by centrifugation followed by filter sterilisation. The filtrates were added to appropriate wells on PDA

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plates and pathogenic fungus - Fusarium were inoculated at the centre of the plates. The development of fungal growth was observed up to 6 days.

3. RESULTS

1. Isolation of *Pseudomonas Fluorescens*

Of the fifty samples tested thirty isolates were obtained on King's B agar medium.

2. Characterization

a. Macroscopic Examination

Pigment Production:The isolates produced green-yellow fluorescent pigment on Pseudomonas F agar plates and KB agar plates.

b. Microscopic Examination

All 30 isolates were found to be gram –ve, short, stout motile rods.

c. Biochemical Reactions

All the 30 isolates were identified as *Pseudomonas fluorescens* based on biochemical reactions.

Table 1: Classification of *Pseudomonas Fluorescens* into biovars

Samples	Non fluorescent pigment				Substrate used for growth						Biovars
	Green	Orange	Blue (non diffusable)	Denitrification	L-arabinose	Sucrose	Propionate	Butyrate	Sorbitol	Ethanol	
Pf1	_	_	_	+	+	+	_	d	+	+	BV II
Pf2	_	_	_	_	+	+	+	_	+	_	BV I
Pf3	_	_	_	+	+	+	_	d	+	+	BV II
Pf4	_	_	_	+	+	+	+	_	+	_	BV I
Pf5	_	_	_	_	+	+	+	_	+	_	BV I
Pf6	_	_	_	+	d	_	d	d	d	d	B V III
Pf7	_	_	_	_	d	d	+	d	d	d	BV V
Pf8	_	_	_	+	+	+	_	d	+	+	BV II
Pf9	_	_	_	+	d	_	d	d	d	d	BV III
Pf10	_	_	+	+	+	+	+	+	+	_	BV IV
Pf 11	+	+	+	+	+	+	+	+	+	_	BV IV
Pf12	_	_	_	_	d	d	+	d	d	d	BV V
Pf 13	_	_	_	_	d	d	+	d	d	d	BV V
Pf 14	_	_	+	+	+	+	+	+	+	_	BV IV
Pf15	_	_	_	+	+	+	-	d	+	+	BV II
Pf16	_	_	_	_	d	d	+	d	d	d	BV V
Pf17	_	_	+	+	+	+	+	+	+	_	BV IV
Pf18	_	_	_	+	+	+	_	d	d	+	BV II
Pf19	_	_	_	+	+	+	_	d	+	+	BV II
Pf20	_	_	_	+	d	_	d	d	d	d	BV III
Pf21	_	_	_	+	+	+	_	d	+	+	BV II
Pf22	_	_	_	_	+	+	+	_	+	_	BV I
Pf23	_	_	_	+	d	_	d	d	d	d	BV III
Pf 24	_	_	_	_	d	d	+	d	d	d	BV V
Pf 25	_	_	+	+	+	+	+	+	+	_	BV IV
Pf26	_	_	_	_	+	+	+	_	+	_	BV I
Pf 27	_	_	+	+	+	+	+	+	_	_	BV IV
Pf28	_	_	_	_	+	+	+	_	+	_	BV I
Pf29	_	_	_	+	+	+	_	d	+	+	BV II
Pf30	_	_	+	+	+	+	+	+	_	_	BV IV

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Table 2: Percentage Occurrence of Biovars

	Number of Isolates	Percentage Occurrence
BV I	6	20%
BV II	8	26.6%
BV III	4	13.3%
BV IV	7	23.3%
BV V	5	16.6%

Detection of Siderophore Production in CAS medium the isolates showed orange halo against a dark blue background indicates production of siderophore.

Table 3: Minimal Inhibitory concentration of some growth inhibitors

Inhibitor	MIC (µM) for the following strains of *Pseudomonas fluorescens*				
	BV I	BV II	BV III	BV IV	BV V
Pb^{2+}	1000	1500	1500	1000	2000
Hg^{2+}	50	50	50	100	100
Penicillin	25	50	50	25	50
Streptomycin (mg/l)	0.1	0.1	0.1	0.1	0.2

The most resistant strain BV V was used in further studies.

Table 4: Influence of iron and medium content on growth and siderophore production .

Fe^{3+} added µg/l	King's B		Glycerol		Asparagine		Succinate	
	Growth	Siderophore concentration (µM)	Growth	Siderophore concentration (µM)	Growth	Siderophore concentration (µM)	Growth	Siderophore concentration (µM)
0	0.682	24.3	0.118	2.45	0.126	4.05	0.136	5.6
5	0.561	18.15	0.110	2.1	0.122	3.55	0.125	5.5
10	0.487	15.8	0.078	1.8	0.108	3.40	0.112	3.9
50	0.462	15.3	0.054	1.6	0.086	3.25	0.095	4.3
100	0.462	15.25	0.084	1.8	0.078	2.6	0.086	3.8
150	0.326	14.95	0.082	1.75	0.081	2.8	0.075	3.55
250	0.312	14.9	0.114	1.70	0.069	2.45	0.074	3.1
300	0.302	10.2	0.110	1.55	0.061	2.05	0.069	2.6

Table 5: Effect of heavy metals and bacterial growth inhibitors on siderophore production in KB medium.

Medium Fe^{3+} added Mg/l	King's B(control)		Mercury(100)		Lead(2000)		Penicillin(50)		Streptomycin(0.2)	
	Growth	Siderophore (µM)	Growth	Siderophore	Growth	Siderophore	Growth	Siderophore	Growth	Siderophore
0	0.682	24.3	0.123	3.7	0.220	5.9	0.105	4.5	0.112	2.6
5	0.561	18.15	0.165	4.0	0.256	6.4	0.115	4.9	0.106	3.0
10	0.487	15.8	0.214	4.2	0.288	6.4	0.121	5.4	0.144	3.6
50	0.462	15.3	0.220	4.3	0.360	6.7	0.147	6.0	0.191	4.0
100	0.462	15.25	0.266	4.3	0.398	7.2	0.149	6.2	0.194	3.0
150	0.326	14.95	0.310	4.5	0.415	8.2	0.155	6.8	0.225	2.7
250	0.312	14.9	0.415	7.1	0.498	9.5	0.161	7.2	0.266	2.8
300	0.302	10.2	0.419	7.3	0.515	9.9	0.160	7.1	0.277	3.0

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The highest siderophore production was obtained with a standard king's medium. The lowest production was in glycerol medium.

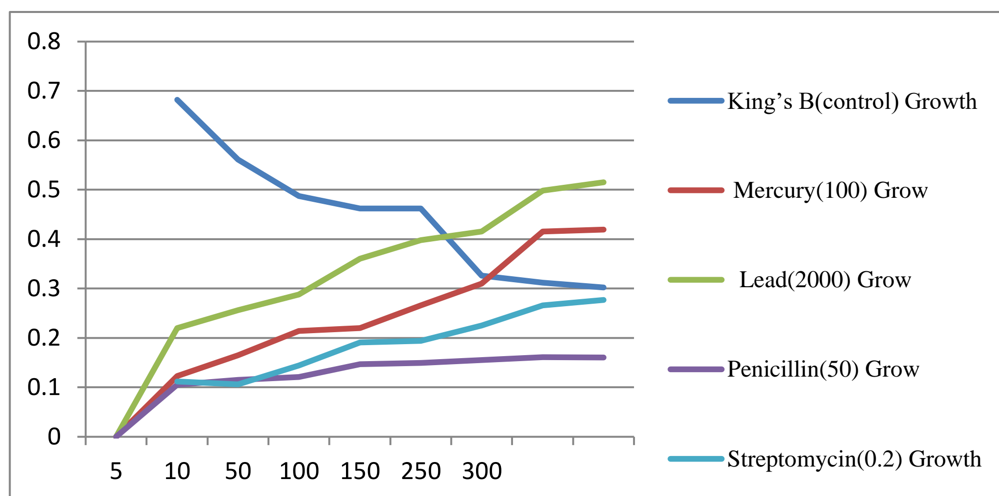

Fig.1.Effect of heavy metals and antibiotics on growth of *Pseudomonas fluorescens*

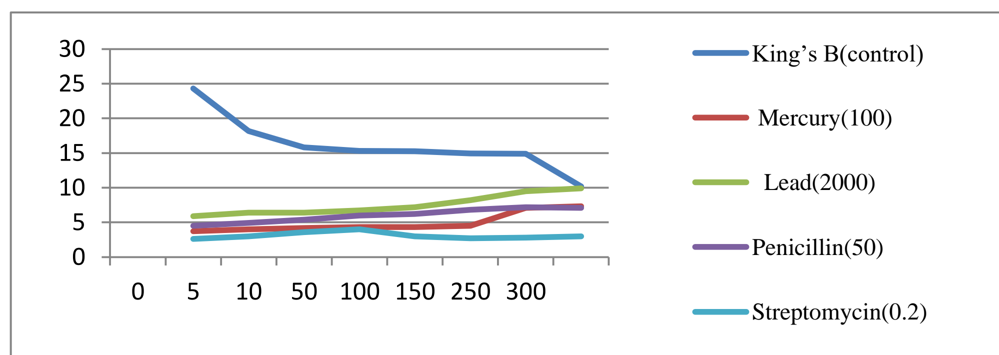

Fig.2.Effect of heavy metals and antibiotics on siderophore production of *Pseudomonas fluorescens*

Table 6: Antifungal activity of both culture

	Zone diameter in mm				Percentage of inhibition			
Broth Culture	1st day	IInd day	IIIrd day	IVth day	Ist day	IInd day	IIIrd day	IVth day
Control	9	9	9	9	_	_	_	_
BV I	3.6	3.8	4	4	60%	57.7%	55.5%	55.5%
BV II	4.0	4.1	4.2	4.1	55.5%	54.4%	53.3%	54.4%
BV III	3.2	3.6	3.3	3.8	64.4%	60%	63.3%	57.7%
BV IV	4.3	4.5	4.0	4.3	52.2%	50%	55.5%	52.2%
BV V	4.9	5.5	4.8	5.6	45.5%	38.8%	46.6%	37.7%

Table 7: Antifungal activity of cell free extract

	Zone diameter in mm				Percentage of inhibition			
Cell free extract	Ist	IInd	IIIrd	IVth	Ist	IInd	IIIrd	IV th
Control	9	9	9	9	_	_	_	_
BV I	4.9	4.6	4.4	4.5	45.5%	48.8%	51.1%	50%
BV II	4.8	4.4	4.1	4.2	46.6%	51.1%	54.4%	53.3%
BV III	4.6	4.0	3.6	4.1	48.8%	55.5%	60%	54.4%
BV IV	4.8	4.3	3.0	4.3	46.6%	52.2%	66.6%	52.2%
BV V	4.4	4.0	2.9	3.8	51.1%	55.5%	67.7%	57.7%

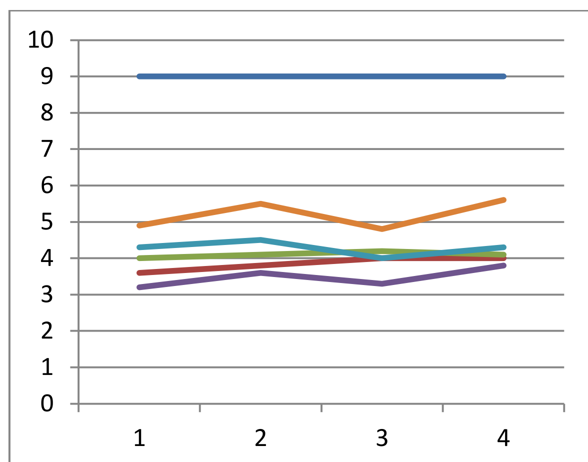

Fig .3. Antifungal activity of both culture

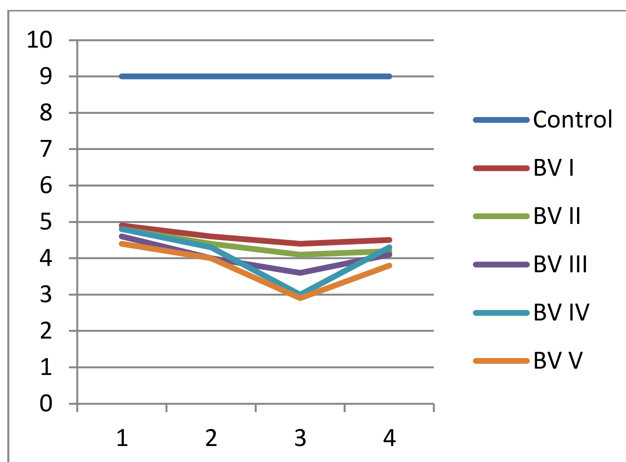

Fig.4. Antifungal activity of cell free extract

Table 8: Spectral Analysis of cell free extract

Extracts	Maximum absorption (in nm)
Pseudomonas fluorescens BV I	373
BV II	373
BV III	373
BV IV	373
BV V	373

4. DISCUSSION

Pseudomonas fluorescens are organisms which are abundant in soil and influence plant by growth promotion and disease control. All the thirty isolated samples obtained from soil was identified and characterized based on the biochemical reactions as *Pseudomonas fluorescens*. All isolates grown on F agar medium containing antibiotics and it also showed fluorescence on King's B agar. Isolates of Pseudomonas fluorescens were positive for catalase,oxidase,arginine dihydrolase, gelatinase,litmus milk and it not hydrolase starch,and they were classified into 5 biovars BV1,II,III,IV and V designated by Stainer et al in 1966.Among the Biovars BV II is the most abundant (26.6%)followed by BV IV(23.3%),BV I(20%),BV V(16.6%) and BV III (13.3%)All of them produced siderophores in CAS medium.

Minimal Inhibitory Concentrations(MIC) of the two heavy metals (Lead and mercury) and two antibiotics (Penicillin and Streptomycin) were observed as shown in table 3.All biovars showed resistance to 2 heavy metals and 2 antibiotics. So they can be used in soil contaminated with heavy metals and also in the presence of antibiotics. Strain BV V was found to be the most resistant strain and was used for further studies.

Four basal media supplemented with different concentration of iron, were employed to study the effect of iron and different organic carbon sources on siderophore production in *Pseudomonas fluorescens*. The highest siderophore production was obtained in KB medium(24.3 µM) and the lowest production was in glycerol medium(2.45 µM) with no Iron added. The standard KB medium without added iron permitted the synthesis of greater amount of siderophores. As the concentration of Iron increased in the medium production of siderophore get reduced. The ability of Pseudomonas to grow and to produce siderophore is dependent on the iron content and the type of carbon sources in the medium (Budzikkiewicz, 1993). In King's medium even though growth and siderophore production decreases with increase in Iron concentration,control medium with added Mercury,Lead,penicillin and Streptomycin(at their MIC)showed an increase in growth and siderophore production as concentration of Iron increased(Table 5 & fig .1 and 2) .The bacteria *Pseudomonas fluorescens* has the ability to protect and stimulate plant growth in soil which are polluted with pesticides and agrochemicals.(Vivas et al,2003)

Plant pathogen isolated on PDA medium was identified as Fusarium. On PDA medium all the isolates of *Pseudomonas fluorescens* inhibited the pathogenic fungi Fusarium. Both the culture containing cells and cell free extract shown

inhibition of Fusarium. Among broth cultures *Pseudomonas fluorescens* BV III showed more inhibition (63.3%) on third day of inoculation.Cell free extract of *Pseudomonas fluorescens* BV V on third day of incubation showed more inhibition (67.7%)than culture containing cells(46.6%).Therefore cell free extract of *Pseudomonas fluorescens* BV V after third day of inoculation can be effectively used for treatment of Fusarium,but on fourth day activity decreases(Table6&7 and Fig 3&4).

Use of synthetic agrochemicals to enhance crop productivity and control of fungal and insect pest is a serious concern in modern day agriculture. The sky rocketing prices of these compounds and the increasing risk of residue toxicity and pathogen resistance urge agriculturalists to look for viable alternative methods. Use of naturally occurring, root colonizing, beneficial rhizobacteria may be a safe alternative approach. Invitro antagonism showed by Pseudomonas determined that fungal inhibition is a complex phenomenon and a number of mechanisms operated on currently which make it difficult to assign antagonistic influence to specific inhibitory molecule.

Special analysis of crude extract of culture filtrate, revealed the production of siderophores by fluorescent Pseudomonas. The maximum absorption was found it to be at 373nm.

5. CONCLUSION

All the thirty isolated samples obtained from soil was identified and characterized based on the biochemical reactions as *Pseudomonas fluorescens*. All isolates grown on F agar medium containing antibiotics and it also showed fluorescence on King's B agar. Among the Biovars BV II is the most abundant.All of them produced siderophores in CAS medium. All biovars showed resistance to 2 heavy metals and 2 antibiotics. So they can be used in soil contaminated with heavy metals and also in the presence of antibiotics. Strain BV V was found to be the most resistant strain and was used for further studies.Standard KB medium without added iron permitted the synthesis of greater amount of siderophores. In King's medium even though growth and siderophore production decreases with increase in Iron concentration,control medium with added Mercury,Lead,penicillin and Streptomycin(at their MIC)showed an increase in growth and siderophore production as concentration of Iron increased. *Pseudomonas fluorescens* inhibited the pathogenic fungi Fusarium isolated from soil. Both the culture containing cells and cell free extract shown inhibition of Fusarium. Cell free extract of *Pseudomonas fluorescens* BV V after third day of inoculation can be effectively used for treatment of Fusarium,but on fourth day activity decreases.Use of synthetic agrochemicals to enhance crop productivity and control of fungal and insect pest is a serious concern in modern day agriculture. The sky rocketing prices of these compounds and the increasing risk of residue toxicity and pathogen resistance urge agriculturalists to look for viable alternative methods. Use of naturally occurring, root colonizing, beneficial rhizobacteria may be a safe alternative approach. Invitro antagonism showed by Pseudomonas determined that fungal inhibition is a complex phenomenon and a number of mechanisms operated on currently which make it difficult to assign antagonistic influence to specific inhibitory molecule. Special analysis of crude extract of culture filtrate, revealed the production of siderophores by fluorescent Pseudomonas. Further studies are needed to confirm the specific molecule which causes inhibition in *Pseudomonas fluorescens*

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REFERENCES

[1] Bergey's Manual of Systems Bacteriology – 1st and 2nd edition -1996, 2001.

[2] Budzikkiewicz, H 1993. Secondary metabolites from fluorescent. Pseudomonas. FEMS Microbiol. Rev.104: 209-228

[3] Brion K Duffy and Geneviere Defugo 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.

[4] Duffy , B.K. , Simons A and Weller D.M (1996) – Combination of *Trichoderma koningii* with *fluorescent Pseudomonas* for control of take-all on wheat phytopathology 86:188-194

ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 3, Issue 3, pp: (6-14), Month: July - September 2016, Available at: www.paperpublications.org

[5] K.S.Jagadeesh ,J.H Kulkarni ,P.O.Krishnaraj (2001) – Evaluation of the role fluorescent siderophore in the biological control of bacterial wilt in tomato using tn^5 mutants of fluorescent Pseudomonas sp.Current Science ,vol.81,No:8,25

[6] N Kannan Ph.D. 2003, Head of department of Microbiology PSG College of Arts and Science, Coimbatore - Hand book of laboratory culture media, reagent stains and buffers .Panima Publishing Corporation, New Delhi Pg: 179-180; 117-118

[7] Rachana Srirastara and Shalini(2005) – Antifugal activity of *Pseudomonas fluorescens* against different plant pathogenic fungi .Electronic journal of environmental, agricultural and food chemistry. Pg: 2881-2889. 11) Rachid Lahlali, Y Brostaux, MH Jijakli (2005) Control of apple blue mold by the antagonistic yeast Pichia anomala strain K: screening of UV protectants for preharvest application.Plant disease 95 (3), 311-316.

[8] Schneider U, Keel C, Blumer C, Troxler J, Defugo G and Haas D (1995) – Amplification of the house keeping sigma factor in *Pseudomonas fluorescens* CHAO enhances antibiotic production and improves biocontrol abilities. J Bacteriol 177:5387-5392.

[9] Vivas A, Azcón R, Biró B, Barea JM, Ruiz-Lozano JM.2003a. Influence of bacterial strains isolated from lead-polluted soil and their interactions with arbuscular mycorrhizae on the growth of Trifolium pratense L. under lead toxicity. Canadian Journal of Microbiology 49, 577–588.