Crop production and disease suppression of fluorescent siderophore of KS1 and PS2 ,on *Fusarium* infested agriculture land.

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Abstract

From the aspects of environmental pollution ,an un controlled use of pesticides and other agrochemicals decrease the crop production and plant growth it may also induce the mutagenic or carcinogenic effects on non-targeted soil microorganisms migrate from the soil .These organism migrate from the soil to human ,animals and plants in different ways. PGPRs are great boon for agriculture. These bacterial siderophore are biodegradable, which can improve plant growth also. The siderophore have multi specialties in their uses .The foot rot is an important emerged diseases of paddy plants, agricultural practices and climatic factors imparts a major role to wide spreading of this disease . Thus two strains were selected from a series of isolated from paddy roots at different locations of Champakkulam village Alappuzha District. KS1 and PS2 were screened *in vitro* for their bio chemical growth promoting abilities and assays. Both strains were potential to enhance the plant growth. *Fusarium moniliforme* a phyto pathogen of Bakanae disease. Which occurs fairly dry soil as well as wet soil. Cereal crops faced these problem and decrease it production rate. Indigenous association of PGPR with Paddy plant influence the suppression of disease symptoms and thus increase the yield. Summer climate condition is also an influencing factor to reduce spreading of disease through the action of siderophore production. This study aimed on the bio control properties of PGPR through siderophores and enhancement of yield without any toxic agents.

Introduction

These Fluorescent *Pseudomonads* potential as bio pesticides , growth enhancer, and disease suppresser in various ways. Microrganisms works naturally not toxic to soil and others (Singh 2008).PGPRs widely used as bio control agents agaist soil-borne fungal pathogens in agriculture (O Sullivan and Gara 1992; Ganeshan and Kumar 2006; Maurya .,2014).

The PGPR potentiality in agriculture increased the use of these siderophore instead chemical contained pesticides, herbicides, fertilizers, and another supplements to the plants . *Fusarium* rot disease usually starts on seedlings stage at the junction of root and stem of the germinated seedlings. Later it break near the place of seed attachment , plant attempt to overcome disease through the new production of roots ,but eventually dies and decay the plant due to the fungal attack. Seed treatment using these PGPR strains reduce the severity of this disease and make it more productive than test control plants. Recent studies and research progresses on the biodiversity of PGPRs point out the possibilities of new bio medicines to the crop plants without any damage to the soil and living things.

Materials and methods

Isolation, purification of bacterial strains : From the 120 isolates best two KS1 and PS2 were collected from the rhizoplane of Paddy (*Oryza sativa L)* the field of different agro-ecological zones of Kuttanad. These strains were purified multiple slanting. These were maintained by periodical transfer of KB slants.

***Fusarium moniliforme* culture:**

The cultural characteristics were studied on PDA (Potato Dextrose Agar) and NA (Nutrient Agar).About 20 ml. of sterile medium poured on each petri dishes. Actively growing mycelial disc (5mm) inoculated and incubate at 28±2C.Observations continued till it recorded. *Fusarium moniliforme* (IMTECH Chandigarth ,India).

***In vitro* antibiosis studies**

KB and PDA contained petri dishes are used to inoculate fungus .*Pseudomonas* (24 h old) strain streaked 2 cm periphery of petridish and incubate at 36C ±2C. After 48 h old an actively growing mycelial 4mm disc placed at the centre of petri dishes. Then kept it under 36C in a BOD. Inhibition zone measured after 3 days of growth. Untreated petri dishes considered as control. Average of length and width of these strains measured, and recorded it as inhibition zone.

Spectral analysis details

Succinate medium used to measure OD. 24 h old culture centrifuged at 10,000 rpm and cell free supernatant were screened by absorbance using UV spectrophotometer. Peak at 404±5nm indicated the presence of siderophore. Standard peak is between 420-450 nm is indicating the presence of ferric hydroxamate type (Ali and Vidhale, 2011).

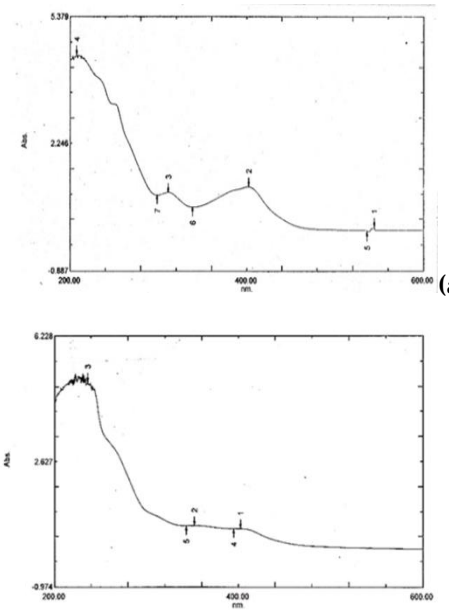
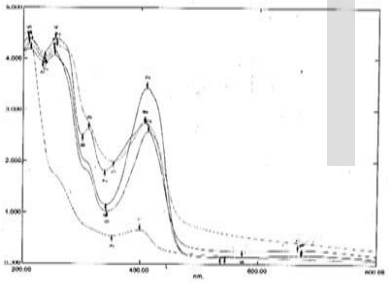


Fig.1 (a) spectral graph of strain KS1,(b) PS2 and co-relation graph of all strains showing absorption peak at or near 409 nm (peak between 300-450 nm indicates the presence of hydroxamate type siderophores.)



Seed bacterisation , growth promotion and yielding :
Special seed treatment performed with *Pseudomonas* strains (Dileep *et al*). Healthy certified seeds (Onam Rice variety) were collected for the study. These were washed carefully with sterile distilled water for 3 or 4times.Then it surface sterilized with 0.5% sodium hypochlorite for 5 minutes. Washed thoroughly & repeatedly about 4 minutes using sterile distilled water. Allow it into dry in sterile air for 12 hours. After that collected healthy seeds were dipped bacterial solution for 5 minutes in already prepared 48 hour old culture solution with 1% CMC (Carboxy Methyl Cellulose as base medium).Bacterized seeds were sowed on pots in nursery conditioned experiments and sowed widely in selected paddy field for large scale experiment studies. Seeds with 1%CMC served as control for this study. Plant growth ,yield and disease suppression are observed at regular intervals of time. Five replicates were taken from 25 plants, different plant growth measures observed and tabulated. Non-bacterized control plants also observed and noted it growth details to compared with test plants.

Crop production of treated paddy plants from pot study:

Strains	N	Mean	Std.Dev.	Minimum	Maximum	F-Value (P-Value)
PS2	5	91.70	3.21	87.9	95.3	0.029
PS3	5	91.70	3.76	87.7	96.2	(<.993)
KNP	5	91.19	3.39	86.0	94.4	
KS1	5	91.36	3.14	87.0	94.3	
Total	20	91.49	3.11	86.0	96.2	

Table:1 1. % of healthy grains in ear of paddy :
ANOVA shows there is no significant difference in 1% of healthy grains among different strains.

Strains	N	Mean	Std.Dev	Minimum	Maximum	F-Value (P-Value)
PS2	5	3.88 a	0.73	2.86	4.89	6.02
PS3	5	3.67 a	1.06	2.32	4.86	(<.01)
KNP	5	3.48 a	0.62	2.81	4.20	
KS1	5	5.22 b	0.13	5.00	5.32	
Total	20	4.06	0.96	2.32	5.32	

Table 2 . Total weight of grain.
a,b are homogenous subsets formed by DMRT .
ANOVA shows there is no significant difference in **total wt. of grains** among different strains at 1% level of significance (P-Value <.01).The DMRT shows KS1 is significantly different from all other strains.

Strains	N	Mean	Std.Dev	Minimum	Minimum	F-Value (P-Value)
PS2	5	15.40	7.02	7	24	0.536
PS3	5	14.00	6.96	6	24	(.664)
KNP	5	14.80	6.61	8	25	
KS1	5	19.20	7.53	12	30	

Total	20	15.85	6.78	6	30	

Table .3. Total no: of chaff in each ear of paddy

ANOVA shows there is no significance difference in total no:of chaff among different strains.

Siderophore production of strains at different level of temperature:

Temperature	*Pseudomonas aeruginosa* strains Mean and SD	
	KS1	PS2
25C	2.33^{b} ±0.25	1.91^{b} ±0.46
30C	3.64^{d} ±0.24	2.83^{c}±0.26
35C	2.81^{c}±0.09	$2.46^{b,c}$±0.71
40C	2.23^{b} ±0.16	2.06^{b}±0.06
45C	1.44^{a} ±0.15	0.54^{a}±0.17
Total	**2.49±0.76**	**1.96±0.88**
F-Value	91.74	23.17
(P-Value)	(<.01)	(<.01)

Table 4.Relation between temperature and siderophore production .

a,b,c,d are homogenous subsets formed by DMRT.

ANOVA shows there is significant difference in Production of siderophore at different temperature levels in both the strains KS1 and PS2 at 1% level of significance (<.01).The DMRT shows the value at 30C is highest and significantly different from others in KS1 .In PS2 ,the value is highest at 30 C but is not significantly different from at 35C.

Paired Sample Test (Comparison between KS1 and PS2)

Strains	Mean	SD	t-Value (P-Value)
KS1	2.49	0.76	5.4
PS2	1.96	0.88	(<.01)

Table 5.The value is high in KS1.The pair- wise t-test shows that the production of siderophores is significantly different in between the strains KS1 and PS2 at 1% level of significane.

Disease suppression

This was done under controlled condition (Kumar 1999). Pot study and field study were carried under natural condition. These all are conducted in 5 separate sets under nursery condition. 5 identified *Pseudomonas* strains were used to this experiment. Small plastic pots infested *Fusarium moniliforme* mycelium taken .Then bacterized seeds (1.2×10^{6}cfu/seed) sown on this fungal infested soil. Non-bacterized and bacterized with pathogen free soil contained pots were considered as control pots. Then this was repeated in small field beds. All growth parameters recorded after 28d. *Pseudomonas* culture applied in each week at regular intervals of time.

Strains	N	Mean	Std.Dev	Minimum	Maximun	F-value (P-Value)
KS1	25	31.6^{a}	5.9	25.0	40.0	40.75
PS2	25	56.4^{b}	9.7	45.0	69.0	(<.01)
Control	25	76.2^{c}	7.4	65.0	85.0	
Total	75	54.7	20.2	25.0	85.0	

Table. 6: Treated plant's shown less symptoms on nursery bed.

a,b,c are homogenous subsets formed by DMRT.

Root colonization studies

To evaluate the efficacy of potential bacterial strain root colonization study (Dileep C,2010)was essential. Use Pesticide resistant (Chlorpyriphos) bacterial strain KSI is used for it.14 day old uprooted paddy plants were dug out from the soil carefully. Gently remove the soil, shaking and weighed.1g root segments placed in 100 ml distilled water. Then shake it well to release rhizosphere bacteria. Serial dilution of these root washings were placed on PDA contained petri dishes amended chlorpyriphos pesticide. *Pseudomnas aeruginosa* strain were resistant in 650 ppm. Count *Pseudomonas aeruginosa* colonies and other aerobic bacteria present in the petri dishes. The cfu/g of fresh root weight was enumerated after 48 h of incubation at 28±2C.

Strains	N	*cfu* after 28th week of cultivation		TARB (Total Agro Rheanizobacterium)		F-Value (P-Value)
		Mean	SD	Mean	SD	
PS2	5	21.2^{b}	0.8	25.4^{c}	1.1	0.197 (.659)
PS3	5	16.4^{a}	1.1	16.8^{a}	1.6	
KS1	5	41.6^{c}	2.9	34.0^{d}	1.0	
KNP	5	16.6^{a}	1.1	21.8^{b}	1.5	
FPO4	5	22.0^{b}	1.9	24.8^{c}	1.3	
Total	5	23.6	9.6	24.6	5.9	
F-Value P-Value	179.29 (<.01)	110.75 (<.01)				

Table .6..a,b,c,d are homogenous subsets formed by DMRT (among strains).

ANOVA shows there is significant difference in population destiny among different strains in *cfu* after 28th day of cultivation and TARB at 1% level of significance (P-Value <.01).The DMRT shows that highest observed in KS1 in both cases and which is significant .

Statistical analysis

Treatments were only compared with it control plants .The study repeated with 5 replicates of each other from the 25 test plants. ANOVA, t-test ,DMRT were used as statistical tools.

Results and Discussion

The rhizosphere is an ecological niche, in which beneficial bacteria compete with other micro biota for organic carbon compounds and interact with flora through root colonization activity to the soil. These root-colonizing beneficial rhizobacteria can colonize endophytically and multiply inside the root. Rhizosphere, these components contribute to growth processes, like cell growth, cell differentiation, and suppression of plant pathogenic microbes. However the use of these synthetic chemicals during the last three decades creates a number of ecological problems. Recently many scientists diverted their keen attention to solve the problem. As a result beneficial rhizobacteria were exploring for plant protection .*Pseudomonas aeruginosa* ,*P.putida*, *P.fluorescene*, and *Bacillus substilis* are important rhizosphere bacteria for the pest and disease management.(S.Nakkeeran *et al*.,2005).PGPR promote the plant growth,yield , and suppress the common fungal diseases (Pleban et al.,1995,Uthkede et al.,1992).

In vitro antagonism of these bacterial strain and *Fusarium moniliforme* were checked under lab condition. Inhibition zones were produced in PDA and NA medium petri dishes.KS1 strain were more effective than other PS2 and non -infested control plate. Soil is selected for the study was common garden soil. soil analysis were done and noted its TARB(Total Agro Rhizo Bacterium) and present population density of *Pseudomonas* colonies .Soil pH is 6.2 slightly acidic nature.

Seedlings treated with selected PGPR strain enhanced its root growth and shoot growth compared with control plants. The order of growth enhancement mentioned in KS1 > PS2 > KNP > PS3 > this manner. Length of root, no :of shoot leaves , fresh and dry weights, yield of plant are the growth parameters .Plant KS1 treated samples more resistant than other bacterized control and non-infested control. Infected seedlinsgs shows nematode attack partially. They try to live the junction of root and shoot. Very rarely appeared the symptoms on the seedlings .Bacterial suspension sprayed at regular intervals in a week. After 1 month it can reduce gradually.

Conclusion

The presents results clearly shows the presence of these iron chelating agent can reduced the pathogenic effect of fungal conidia. The root exudates are essential constituents ,which promote plant growth, to influence beneficial secondary metabolite production , disease suppression ,and crop yield. There is no doubt these PGPRs are potential tool for sustain the agriculture not only in our country ,all over the world. Low cost of production make PGPR favourite to below average people and its also eco friendly.Hence we can expect more from new emerging strains of PGPR mixes.

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