CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Online International Journal Available at http://www.cibtech.org/cjm.htm
2012 Vol. 1 (2-3) Jul.-Sept. & Oct.-Dec., pp.57-66/Amutharaj et al.
Research Article

INTERGENERIC MICROBIAL COAGGREGATES: BIOINOCULATION EFFECT OF ACC DEAMINASE POSITIVE WILD STRAINS OF *PSEUDOMONAS* AND *PAENIBACILLUS*, AS COAGGREGATES, ON THE MAXIMIZATION OF ISR AGAINST *PYRICULARIA ORYZAE* IN UPLAND RICE CV. ASD-19

***P. Amutharaj, C. Sekar and S. Esath Natheer**
Department of Microbiology, Faculty of Science, Annamalai University,
Annamalai Nagar, Tamil Nadu, India
**Author for Correspondence*

ABSTRACT
The bioinoculation effect of different formulations of *Pseudomonas* and *Paenibacillus viz*., control, single strains inoculation (wild), single strains inoculation (*acd* mutant), coinoculation of wild strains, coinoculation of *acd* deficient mutant strains, coaggregates of *acd* positive strains, coaggregates of *acd* deficient mutant strains and challenge inoculation of *Pyricularia oryzae* on the enhancement of ISR mediated bio control in upland rice cv.ASD-19 under *in-vitro* condition. It was observed that the application effect of PGPR cells, as coaggregates containing *acd* positive, wild strains of *Pseudomonas* and *Paenibacillus*, together with challenge inoculation of *Pyricularia oryzae* positively altered the biochemical constituents *viz*., increasing total phenol content, reduction in reducing sugar and non reducing sugar level and increased starch content of the host plant. Moreover, the application of the same also augmented the defense enzyme activities *viz*., peroxidase (PO) and polyphenol oxidase (PPO) of the host plant to a maximum level when compared to the application other formulations of PGPR cells and suggesting the induction of systemic resistance in the host plant against the phytopathogen of *Pyricularia oryzae*.
The results of the present study clearly envisaged the augmenting role of *acd* positive wild application of PGPR application, as coaggregates on the induction of systemic resistance against *Pyricularia oryzae* when compared to other formulation tested.

Key Words: *Upland Rice, Pgpr, Coaggregates, Isr, Acc Deaminase, Pyricularia Oryzae*

INTRODUCTION
Rice (*Oryza sativa* .L), as a cereal grain, is widely consumed as staple food for large part of world's population, especially, in Asia as well as in Africa and Latin America. To feed the ever increasing population of these regions, the world's annual rice production must be increased from present 560 to 750 million tonnes by 2020 (IRRI, 1993). The future increase in rice production has to come from the same or even reduced land area. Hence, the productivity yield of rice (per ha) must be greatly enhanced by providing additional nutrient inputs and through effective control of phytopathogens. Blast disease of rice caused by *Pyricularia oryzae* Cav. is one of the most destructive fungal disease of rice, causing an yield loss upto 90 per cent and has an ubiquitous occurrence in almost all the rice growing countries (Mehrotra, 1980).
Now-a-days, upland rice production management strategies mainly focus on the enormous use of synthetic chemical fertilizers and pesticides at high rates to enhance the per hectare yield of the same. The persistent, injudicious use of these chemicals has toxic effects on non-target microorganisms of the soil and can cause undesirable changes in the environment, also. In this context, the use of plant growth promoting rhizo bacteria (PGPR), as a biological approach, might be an alternative strategy to overcome the biological and environmental hazards posed by the persistent use of synthetic chemicals (Hussain and Sabari, 1980).

Rhizosphere bacteria that favourably affect the plant growth and yield of commercially important crops are denominated as "plant growth promoting rhizo bacteria" (PGPR) (Kloepper *et al*., 1980). Several mechanisms of plant-microbe interaction may participate in the association and affect plant growth, including, N-fixation, hormonal interaction, improvement in root growth, solubilisation of nutrients, alleviation of salinity and bio control against phytopathogens. Thus, the PGPR affect the plant growth directly by producing and secreting plant growth promoting substances or eliciting root metabolic activities by supplying biologically fixed nitrogen and indirectly by acting against phytopathogenic microorganisms (Kloepper, 1993). The well known PGPR include genera, namely, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Bacillus*, *Azoarcus*, *Klebsiella*, *Arthrobacter*, *Enterobacter*, *Serratia* and *Rhizobium* on non-legumes (Burdman *et al*., 2000).

Pseudomonas and *Paenibacillus* are the two important PGPR genera that are frequently encountered from the rhizosphere of upland rice (Guemouri-Athamani, 2000; Reddy *et al*., 2007; Prasanna Reddy *et al*., 2010; Vonderwied *et al*., 2000). The PGPR characteristics of the genus *Pseudomonas* (Brandl *et al*., 2001; Martinoz-viveros *et al*., 2010) and *Paenibacillus* (Gjing Kahug, 2001) have been frequently reported. The use of the same, as bioinoculant, is also known to induce systemic resistance (ISR) in the host plant against different phytopathogens and ultimately result in lesser disease incidence with higher crop productivity.

ISR can be defined as a phenomenon by which plants exhibit increased level of resistance to broad spectrum of phytopathogens by the prior activation of genetically programmed defense pathways (van Loon *et al*., 2003). The host plant which is exposed to different biotic and abiotic agents exhibit an induced defense activities against phytopathogens when the same is spatially separated from the inducer agent (Pieterse and Van Loon, 1999). The potential role of PGPR, as biotic agent of ISR, has already been reported (Mariano and Kloepper, 2000). The positive role of *Pseudomonas* and *Paenibacillus*, as biotic agents, in augmenting the ISR of host plant has been reported by many authors (Pieterse *et al*., 1998; Halfeld-Vieira *et al*., 2006; Saravanakumar and Samiyappan, 2007; Sziderics *et al*., 2007).

Interestingly, different bacterial strains of PGPR exhibited different signaling pathways for the elicitation of ISR in host plants. Certain rhizobacteria possess an enzyme *viz*., ACC-deaminase (*acd*) that hydrolyses ACC into ammonia and α-ketobutyrate (Mayak *et al*., 1999). PGPR strains containing ACC-deaminase activity could suppress the accelerated endogenous ethylene synthesis and thus facilitate the root elongation and nutrient absorption of host plant which resulted in improved growth and yield of crop plants (Zafar-ul-Hye 2008). Moreover, Pieterse *et al*., (2000) suggested the positive role of bacterial ACC (1-amimocyclopropane-1-carboxylate) deaminase enzyme in modulating the ethylene level at crop rhizosphere and proposed the same as an elicitor for ISR in host plants. However, there were no earlier reports available on the comparative performance of *acd* positive (wild) and *acd* deficient mutants of *Pseudomonas* and *Paenibacillus* on the enhancement of ISR mediated biocontrol against *Pyricularia oryzae* in upland rice.

Hence, the present study has been undertaken with an aim to exploit the comparative performance of *acd* positive (wild) and *acd* deficient mutant strains of *Pseudomonas* and *Paenibacillus* on the enhancement of ISR mediated biocontrol in Rice - *Pyricularia oryzae* pathosystem under upland condition.

MATERIALS AND METHODS

Pseudomonas fluorescens (PF-5) and *Paenibacillus polymyxa* (PP-5), obtained from the rhizosphere of upland rice cv. ASD-19, grown at Periyapattu, Cuddalore district, Tamil Nadu state, India, were used for the present study. The isolates were positive for the ACC deaminase (*acd*) activity, maintained in King's B and nutrient glucose agar slants, respectively, at 30 ± 2ºC with monthly transfer. The ACC deaminase (*acd*) deficient mutants of the isolates were constructed according to Miller (1972).

Preparation of Pseudomonas and Paenibacillus Coaggregates

The coaggregation of *Pseudomonas* and *Paenibacillus* isolates were prepared in Co-Ag buffer as described by Grimaudo and Nesbitt (1997). One ml aliquot of each PGPR isolates *viz*., PF-5 and PP-5

were mixed together in 10 ml Co-Ag buffer. The mixtures were vortexed for 10 s, shaken on a rotary platform shaker for 3 min and left undisturbed at room temperature for 24 h. All Co-Ag reactions were performed in triplicate and uninoculated buffer served as control.

Preparation of Different Bioformulations

One ml culture of each PGPR isolate (1×10^7 CFU/mL) under different formulations were prepared and used to evaluate their effect on the alteration of physiological and biochemical constituents of rice cv. ASD-19 with following treatments *viz*., control, *Pseudomonas fluorescens* wild (PF-5W), *Pseudomonas fluorescens* (*acd*) mutant (PF-5M), *Paenibacillus polymyxa* wild (PP-5W), *Paenibacillus polymyxa* (*acd*) mutant (PP-5M), co-inoculation of *Pseudomonas fluorescens* and *Paenibacillus polymyxa* wild (Co-I PF-5W + PP-5W), co-inoculation of *Pseudomonas fluorescens* and *Paenibacillus polymyxa* (*acd*) mutant (Co-I PF-5M + PP-5M), coaggregates of *Pseudomonas fluorescens* and *Paenibacillus polymyxa* wild (Co-A PF-5W + PP-5W) and coaggregates of *Pseudomonas* fluorescens and *Paenibacillus polymyxa* (*acd*) mutant (Co-A PF-5M + PP-5M)

Preparation of Growth Chamber

The growth chamber used for the study was a dessicator (12 x 10 cm) consisting of two parts. The lower part was filled with Weaver's medium (Weaver *et al*., 1975) whereas the upper part contained stainless steel wire mesh (mesh size 3 mm) supports. The lid was placed over the cotton and the chamber was closed before sterilization.

ASD-19 rice seeds were surface sterilized by immersion in 95 percent ethanol for 1 minute, followed by 20 min in 1 per cent NaOCl. After rinsing three times with sterile distilled water, the sterile seeds were placed on the surface of one percent water agar in petriplates (9cm dia, at the rate of 5 seeds per plate). Then, they were incubated in an inverted position for 3 days at room temperature to allow germination. The plates were sealed with wax to avoid agar dryness during germination. Fifty germinated rice seeds with coleoptiles (2 cm high) were transferred aseptically onto the stainless steel wire mesh, incubated for 10 d in the growth chamber with 14 h day and 10 h night cycle and at a temperature ranging from 24°C at night to 30°C around noon. By this time, the rice roots yielded many lateral roots, well spread in the Weaver's medium maintained at the lower part of the growth chamber.

Challenge Inoculation of Rice Plant with Pyricularia Oryzae

Pyricularia oryzae AU-1 (provided by Department of Plant Pathology, Annamalai University) was maintained in Potato Dextrose Agar (PDA) medium and used for the challenge inoculation purpose. Rice plants cv.ASD-19 were challenge inoculated by spraying the *Pyricularia oryzae* spore suspension (50,000 spore/mL inoculum level) on 10^{th} DAS with an atomizer and the control plant was sprayed with sterile Weaver's medium. The spraying of spore suspension was done under proper humid condition.

After one week of challenge inoculation, three plants from each treatment were carefully removed and rinsed with sterile distilled water. The blast disease incidence was enumerated with a score chart of 0 to 9 grades devised on Standard Evaluated System (SES) proposed by International Rice Research Institute (1980).

Estimation of Bio-Chemical Constituents

Methods of sampling

Plant samples from each treatment were taken on 0, 7, 14 and 21 days after challenge inoculation of *Pyricularia oryzae* for estimating the changes in the biochemical constituents *viz*., reducing sugars, non-reducing sugars, starch, ortho-dihydroxy phenol (OD Phenol) and total phenol and defense enzymes activities *viz*., peroxidase (PO) and polyphenol oxidase (PPO)

Preparation of Ethanol Extract

Plant samples were collected, pooled and 4 g of pooled samples were taken for extraction. They were chopped and then extracted in boiling 80 per cent ethanol (Mahadevan and Sridhar, 1986) and the same was used for the estimation of sugars and phenol and for defense enzyme activities.

Quantitative Estimation of Sugars

Reducing sugar present in the extract was estimated according to Nelson (1944) method while the estimation of non-reducing sugars was carried out according to InMan (1965).

Quantitative Estimation of Phenols

Total phenols present in the extract were estimated by employing Folin-ciocalteau reagent (Bray and Thorpe, 1954). The OD phenol content was assayed according to Arnow's reagent (Johnson and Schaal, 1957).

Enzyme Assay

One g of the leaf material was cut into small bits, crushed in chilled 0.1 M sodium phosphate buffer at pH 7.1 and the volume was made upto 5 ml with the same buffer, centrifuged at 2,100 rpm for 30 min and the supernatant was used as the enzyme source for all the enzyme assays *viz*., polyphenol oxidase and peroxidase. The activity of polyphenol oxidase was estimated by the method as described by Matta and Dimond (1963) whereas the activity of Peroxidase was assayed according to Hampton (1968). The enzyme activities in the sample were expressed in terms of unit/minute/mg of protein.

RESULTS AND DISCUSSION

The bioinoculation effect of different formulations of wild and *acd* deficient mutants of *Pseudomonas* and *Paenibacillus* cells, namely, single strain inoculation of either wild or *acd* mutant of *Pseudomonas* or *Paenibacillus*, co-inoculation of either wild or *acd* mutant of *Pseudomonas* and *Paenibacillus* and coaggregates application of either wild or *acd* mutant of *Pseudomonas* and *Paenibacillus*, on ISR mediated biocontrol against *Pyricularia oryzae* in upland rice cv. ASD-19 was studied under *in-vitro* condition.

Table 1: Response of different formulations of *Pseudomonas* and *Paenibacillus* cells on blast disease incidence in rice cv.ASD-19 under *in vitro* condition

Treatment [a]	Percentage of disease incidence[b]
Control	90.8 ± 1.60
Pseudomonas fluorescens (Wild)	44.6 ± 0.85
Pseudomonas fluorescens (Mutant)	52.8 ±0.62
Paenibacillus Polymyxa(Wild)	49.4 ± 0.37
Paenibacillus Polymyxa (Mutant)	59.8 ± 0.24
Co-I- *Pseudomonas fluorescens* + *Paenibacillus Polymyxa* (Wild)	28.6 ± 0.56
Co-I- *Pseudomonas fluorescens* + *Paenibacillus Polymyxa* (Mutant)	38.8 ± 0.11
Co-A- *Pseudomonas fluorescens* + *Paenibacillus Polymyxa* (Wild)	10.5 ± 0.10
Co-A- *Pseudomonas fluorescens* + *Paenibacillus Polymyxa* (Mutant)	19.9 ± 0.43

a - at 1x10^7 CFU/mL inoculum level; b - Values are mean of three replications ± SD
Co-I - Coinoculation; Co-A - Coaggregates

Among the different formulations of *Pseudomonas* and *Paenibacillus* cells, the application of *Pseudomonas* and *Paenibacillus* wild cells, as coaggregates, reduced the blast disease incidence to the highest level followed by the coaggregates containing *acd* deficient mutants of *Pseudomonas* and *Paenibacillus*, coinoculation of wild strains, coinoculation of *acd* deficient mutant strains, single wild strain inoculation, single *acd* deficient mutant strain inoculation and control (Table 1).

Moreover, the application of *Pseudomonas* and *Paenibacillus* wild strains, as coaggregates, positively altered the biochemical constituent's *viz*., reduction in reducing and non-reducing sugar level, increased the starch content, total and OD phenol contents and also the defense enzyme activities *viz*., PO and PPO in upland rice plant to a maximum level than other treatments (Fig. 1 to Fig. 7).

In the present study, the application of PGPR coaggregates containing ACC-deaminase positive wild strains of *Pseudomonas* and *Paenibacillus* augmented the total and OD phenol content of rice plant to a higher level when compared to control (without any bioinoculation) and the individual application of wild

or *acd* deficient mutants of *Pseudomonas* or *Paenibacillus* during the challenge inoculation of *Pyricularia oryzae* in rice plant. A positive correlation between the increased levels of total phenol and OD phenol with host plant resistance has been proposed by Farkas and Kiraly (1962).

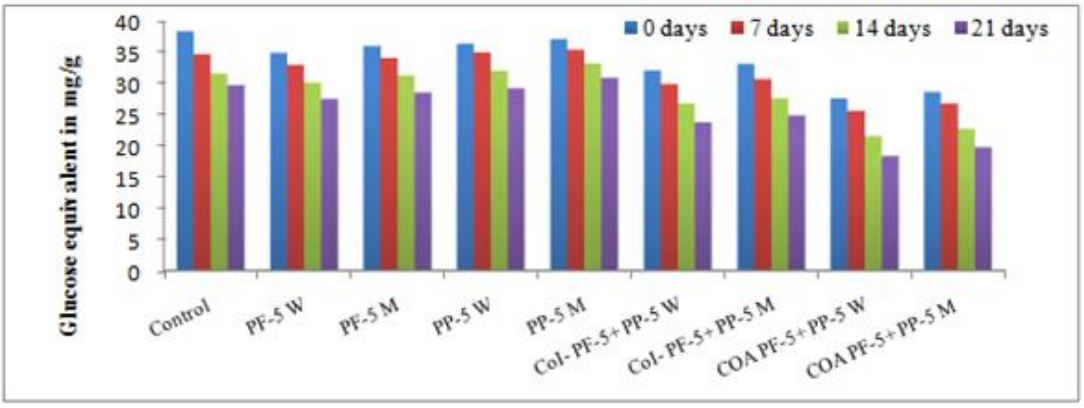

Figure 1: Changes in reducing sugar content of ASD-19 rice as influenced by different formulations of *acd* positive wild and *acd* deficient mutant strains of *Pseudomonas fluorescens* and *Paenibacillus Polymyxa* during *Pyricularia oryzae* incitation

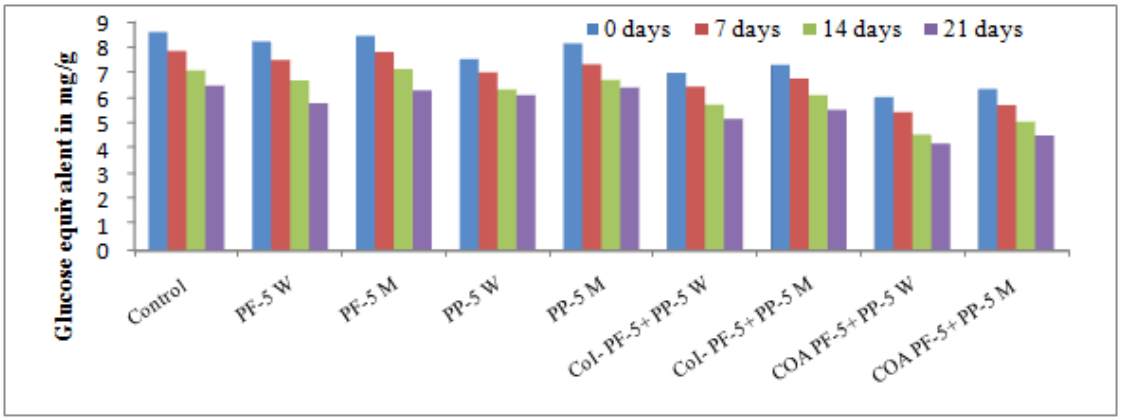

Figure 2: Changes in non-reducing sugar content of ASD-19 rice as influenced by different formulations of *acd* positive wild and *acd* deficient mutant strains of *Pseudomonas fluorescens* and *Paenibacillus Polymyxa* during *Pyricularia oryzae* incitation

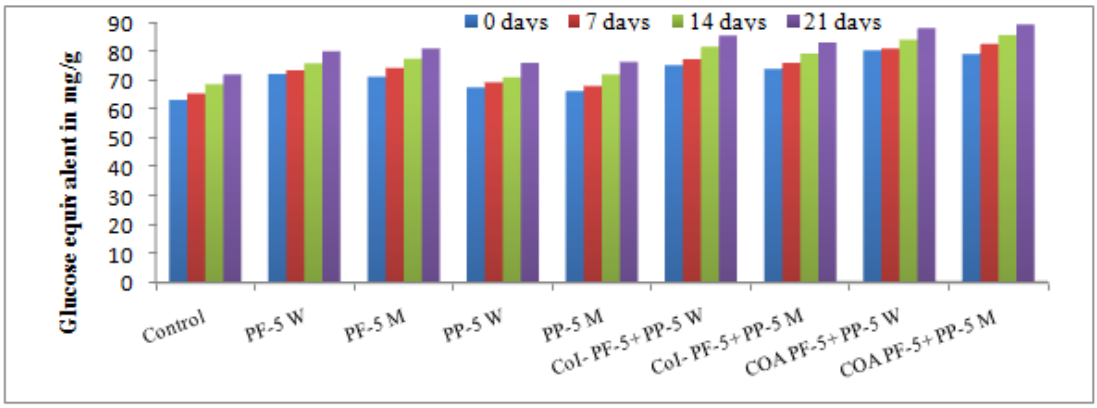

Figure 3: Changes in starch content of ASD-19 rice as influenced by different formulations of *acd* positive wild and *acd* deficient mutant strains of *Pseudomonas fluorescens* and *Paenibacillus Polymyxa* during *Pyricularia oryzae* incitation

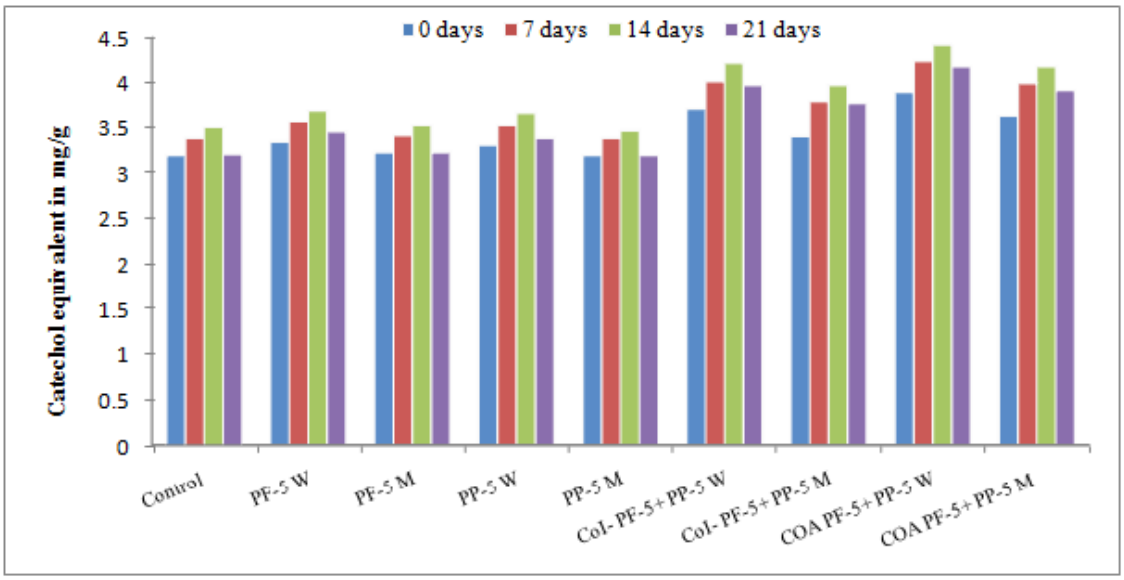

Figure 4: Changes in total phenol content of ASD-19 rice as influenced by different formulations of *acd* positive wild and *acd* deficient mutant strains of *Pseudomonas fluorescens* and *Paenibacillus Polymyxa* during *Pyricularia oryzae* incitation

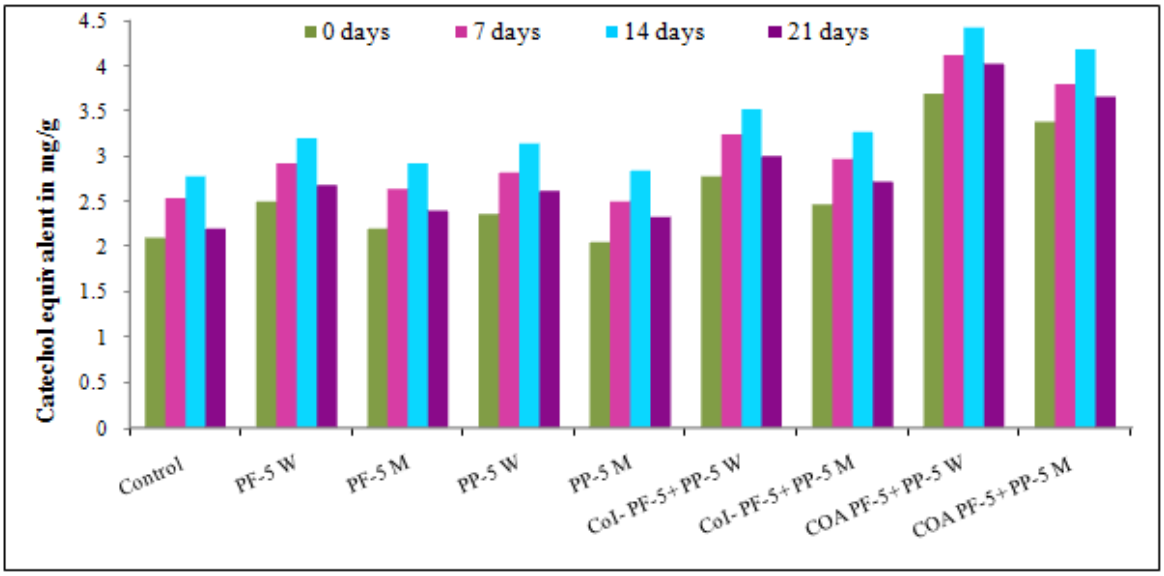

Figure 5: Changes in OD phenol content of ASD-19 rice as influenced by different formulations of acd positive wild and acd deficient mutant strains of Pseudomonas fluorescens and Paenibacillus Polymyxa during Pyricularia oryzae incitation

The oxidation of phenols is mediated by the enzyme PPO and PO and the resulting quinines are effective inhibitors of SH group of enzymes which may be inhibiting to the pathogen (Goodman *et al*., 1967). Oxidation of phenols to oxidized products (quinine) play an important role in induced systemic resistance (ISR) which limits the activities of fungal phytopathogens (Hassan *et al*., 2007; Shalaby *et al*., 2001). The reduction in the soluble sugars may be due to higher polymerization of sugars into starch (Chaboussou, 1972). It may be presumed that the reducing sugar levels may be inadequate for the

colonization of the pathogen and the Post-infectional decrease in the sugar reserve of the host plant rice has been recorded by several workers (Rao and Nayudu, 1979; Sridhar, 1970).

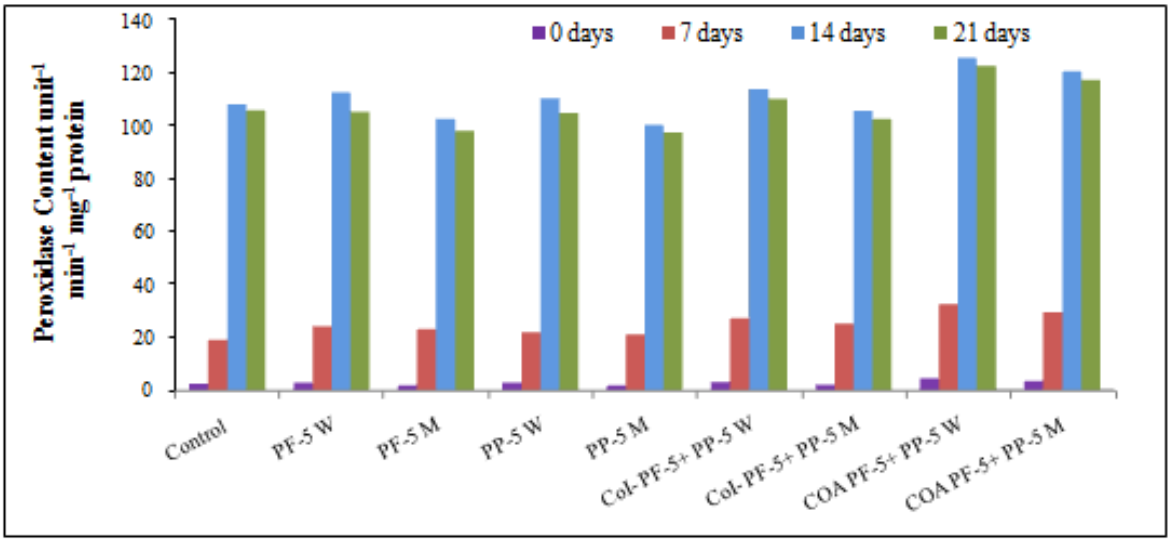

Figure 6: Changes in peroxidase content of ASD-19 rice as influenced by different formulations of acd positive wild and acd deficient mutant strains of Pseudomonas fluorescens and Paenibacillus Polymyxa during Pyricularia oryzae incitation

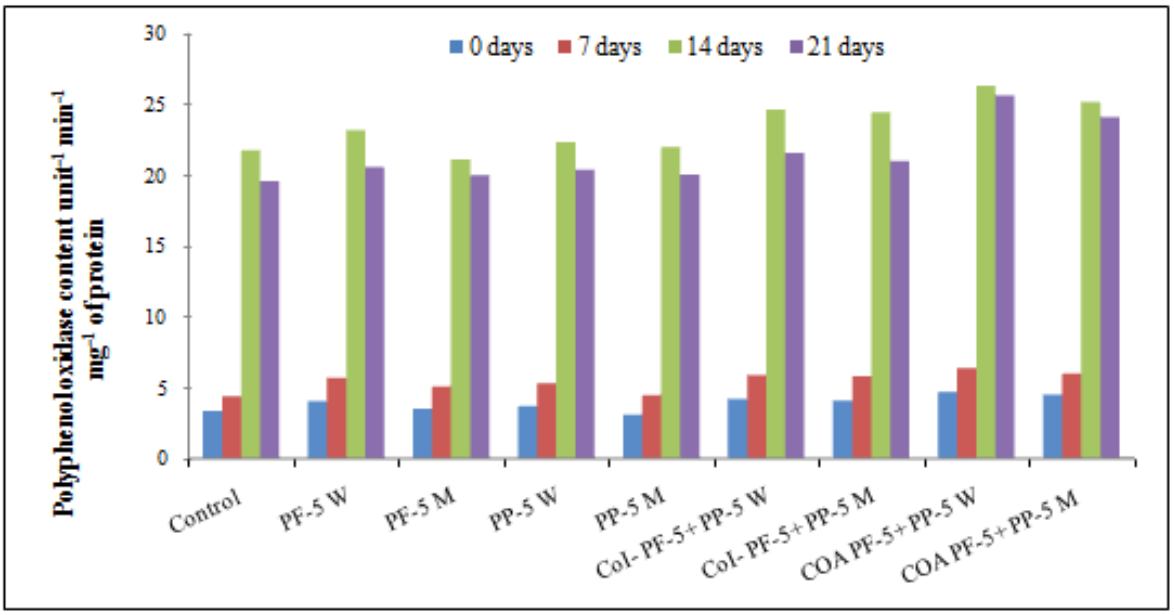

Figure 7: Changes in poly phenol oxidase content of ASD-19 rice as influenced by different formulations of *acd* positive wild and *acd* deficient mutant strains of *Pseudomonas fluorescens* and *Paenibacillus Polymyxa* during *Pyricularia oryzae* incitation

The accumulation of starch in ACC-deaminase positive PGPR coaggregates applied plants may also be due to stimulated activity of starch synthetase (Penrose and Glick, 2001). Pieterse *et al*. (2000) reported the positive role of ACC-deaminase containing rhizobacterial strains on the modulation of ethylene in crop rhizosphere which leads to the induction of systemic resistance (ISR) in *Arabidopsis thaliana*.

Similarly, the synergistic effect of PGPR strain mixtures on the bio control of phytopathogens has been reported frequently (Pierson and Weller, 1994).

The results of present study clearly envisaged the positive effect of PGPR strains, containing ACC deaminase positive wild strains of *Pseudomonas* and *Paenibacillus*, in augmenting the ISR mediated biocontrol against *Pyricularia oryzae* in upland rice plant when compared to the PGPR strains, containing *acd* deficient mutant strains of *Pseudomonas* and *Paenibacillus*.

Interestingly, the same effect was more pronounced during the application of *Pseudomonas* and *Paenibacillus* strains, as coaggregates. However, the mechanism of *acd* positive wild strains of *Pseudomonas* and *Paenibacillus* in augmenting ISR mediated biocontrol against *Pyricularia oryzae* is unknown and needs further exploitation. This is the first comprehensive report regarding the bioinoculation effect of *acd* positive PGPR strains *viz*., *Pseudomonas* and *Paenibacillus*, as coaggregates, on the maximization of ISR mediated bio control against *Pyricularia oryzae* in upland rice.

REFERENCES

Bray HG and WV Thorpe (1954). Analysis of phenolic compounds of interst in metabolism. *Methods of Biochemical Analysis* **1** 27-52.

Burdman S, Okon Y and E Jurkevitch (2000). Surface characteristics of *Azospirillum* brasilense in relation to cell aggregation and attachment to plant roots. *Critical Reviews in Microbiology* **26** 91-110.

Chaboussou F (1972). The role of potassium and cation equilibrium in the resistance of plants towards disease. *Potash Review Submission* **23(**39) 10-15.

Farkas GL and Kiraly Z (1962). Role of Phenolic compounds in the physiology of plant disease and disease resistance. *Phytopathology* **44** 105-150.

Gjung Kahng, Seo-Hyung Lim, Han-Dae Yan and Weon-Taek Seo (2001). Production of extracellular polysaccharide, EPS WNG, from *Paenibacillus sp*. WN9 KCTC 8951P and its usefulness as a cement mortar admixture. *Biotechnology and Bioprocess Engineering* **6** 112-116.

Goodman RN, Kiraly Z and Zaitlin M (1967). The Biochemistry and Physiology of Infectious plant Diseases, (D. Van, No strand Co. Inc., Princeton), New Jersey.

Grimaudo NJ and Nesbitt WE (1997). Coaggregation of *Candida albicans* with Oral *Fusobacterium sp*. Oral Microbiol. *Immunology* **12** 168-73.

Guemouri – Athmani S, Berge O, Bourrain M, Mavingui P, Thiery JM, Bhatnagar T and Heulin T (2000). Diversity of *Paenibacillus polymyxa* in the rhizosphere of Wheat (*Triticum durum*) in Algerian Soils. *European Journal of Soil Biology* **36** 149-159.

Halfeld-Vieira BA, Vieira JR Jr, Romeiro RS, Silva HAS and Baract-Pereira MC (2006). Induction of systemic resistance in tomato by autochthonus phylloplane residente *Bacillus cereus*. *Pesquisa Agropecuária Brasileira* **41** 1247-1252.

Hampton RE (1968). Activity of some soluble oxidases in carrot slices infected with Thielaviopsis. *Phytopathology* **53** 497-499.

Hassan Maggie EM, Abd El-Rahman,. Saieda S, El-Abbasi IH and Mikhail MS (2007). Changes in peroxidase activity due to induced resistance against fababean chocolate spot disease. *Egyptian Journal of Phytopathology* **35** 35-48.

Hussain S, Siddique T, Arshad, M and Saleem M (2009). Bioremediation and Phytoremediation of Pesticides: Recent Advances, *Critical Review in Environmental Science and Technology* **39** 843-907.

Inman RE (1965). Quantative sugar changes in barley nfected with a facultative parasite. *Phytopathology* **55** 341-345.

IRRI (1980). International Rice Research Institute. Annual Report for 1979. Los Banos, Philippines 171-182.

IRRI (1993). Rice research in a time of change. International Rice Research Institute, Medium term plan for 1994-1998.

Johnson and Schaal (1957). Chlorogenic acid and other ortho-dihydroxy phenols in scap susceptible Triump potato tubers of different maturities. *Phytopathology* **47** 253.

Kloepper JW (1993). Effect of seed piece inoculation with plant growth promoting rhizobacteria on population of *Erwinia carotovora* on potato roots and daughter tubers. *Phytopathology* **73** 217-219.

Kloepper JW, Schroth MN and Miller TD (1980). Effect of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. *Phytopathology* **70** 1020-1082.

Lugtenberg BJJ, Dekkers L and Bloemberg GV (2001). Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annual Review of Phytopathology* **38** 461-490.

Mahadevan and R Sridhar (1986). Methods in physiological plant pathology, III. Edn. Sivakami publication Madras 82.

Mariano RLR and Kloepper JW (2000). Me´todo alternativo de biocontrole: Resisteˆncia sisteˆmica induzida por rizobacte´rias. *Revisa˜o Anual de Patologia de Plantas* **8**121–137

Martínez-Viveros O,Jorquera MA, Crowley DE, Gajardo G and Mora ML(2010). Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of Soil Science and Plant Nutrition* **10**(3) 293-319.

Matta A and Domond AE (1963). Symptoms of *Fusarium* wilt in relation to quantity of fungus and enzyme activity in tomato stems. *Phytopathology* **53** 574-575.

Mayak S, Tirosh T and Glick BR (1999). Effect of wild type and mutant plant growth promoting rhizobacteria on the rooting of mung bean cuttings. *Journal of Plant Growth Regulation* **18** 49-53.

Mehrotra RS (1980). Plant pathology, Tata McGraw Hill Publication Co. Ltd. New Delhi.

Miller JF (1972). Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Nelson M (1944). A photometric adoptation on the somogyi methods for the determination of glucose. *The Journal of Biological Chemistry* **153** 375-380.

Penrose DM and Glick BR (2001). Levels *of* 1-aminocyclopropane-l-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth-promoting bacteria. *Canadian Journal of Microbiology* **47** 368-372.

Pierson EA and Weller GM (1994). Use of mixtures of *fluorescent pseudomonads* to suppress take – all and improve the growth of wheat. *Phytopathology* **84** 940-947.

Pieterse CMJ, Johan A, Van pelt I, Jurriaan T, Stefanie P, Martin J Mueller, Antony JB, Jean-pierre MT and van Loon LC (2000). Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. *Physiological and Molecular Plant Pathology* **57** 123-134.

Pieterse CM and Van Loon LC (1999). Salicylic acid – independent plant defense pathways. *Trends in Plant Science* **4** 52-58.

Pieterse CMJ, vanWees SCM, Van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ and van Loon LC (1998). A novel signaling pathway controlling induced systemic resistance in Arabidopsis. *The Plant Cell* **10** 1571-1580.

Prasanna Eddy B and Rao KS *(*2009). Biochemical and PCR-RAPD characterization of *pseudomonas fluorescens* produced antifungal compounds inhibit the rice fungal pathogens *in vitro*. *Electronic Journal of Environmental Agricultural and Food Chemistry* **8** 1062-1067.

Rao NS and Nayudu MV (1979). Changes in the organic constituents of rice leaves infected by *Xanthomonas oryzae*. *Phytopathol* 94 357-366.

Reddy KRN, Choudary Anitha K and Reddy MS (2007). Antifungal metabolites of *Pseudomonas fluorescens* isolated from Rhizosphere of rice crop. *Journal of Mycology and Plant Pathology* **37** 431-437.

Saravanakumar D and Samiyappan R (2007). ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance In groundnut (*Arachis hypogea*) plants. *Journal of Applied Microbiology* **102** 1283-1292.

Shalaby IMS, El-G anaieny RMA, Botros SAD and El Gebally MM (2001). Efficacy of some natural and synthetic compounds against charcoal rot caused by *Machrophomina phaseaolina* of sesame and sunflower plants. Assiut *Journal of Agricultural Science* **32** 47-56.

Sridhar R (1970). Nitrogen fertilization and the rice blast disease. *Plant Disease Reporter* **54** 632.

Sziderics Rasche AH, Trognitz F, Sessitsch AF and Wilhelm E (2007). Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.). *Canadian Journal of Microbiology* **53** 1195-1202.

Van Loon LC(2000). Systemic induced resistance. Slusarenko AJ, Fraser RSS, 2000. Eds, Mechanism of resistance to plant diseases. *Dordrecht Kluwer* 521-574.

Von der weid IA, Paiva E, Nobrega A, Van Elsas JD and Seldin L (2000). Diversity of *Paenibacillus polymyxa* strains isolated from the rhizosphere of maize planted in Cerrado soil. *Research in Microbiol*ogy **151** 369-381.

Weaver PK, Wall JD and Gest H (1975). Characterization of Rhodopseudomonas capsulate, *Archive of Microbiology* **105** 207-216.

Zafar-ul-Hye M (2008). Improving nodulation in lentil through co-inoculation with rhizobia and ACC-deaminase containing plant growth promoting Rhizobacteria. PhD Thesis. University of Agriculture, Faisalabad, Pakistan 198.