Vol III Issue IX March 2014

Impact Factor : 2.2052(UIF)

ISSN No :2231-5063

# International Multidisciplinary Research Journal

## Golden Research Thoughts

**IMPACT FACTOR : 2.2052**(UIF)

**Welcome to GRT**

**RNI MAHMUL/2011/38595** **ISSN No.2231-5063**

Golden Research Thoughts Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial board.Readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.



**Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India**
**Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.aygrt.isrj.net**

Golden Research Thoughts
ISSN 2231-5063
Impact Factor : 2.2052(UIF)
Volume-3 | Issue-9 | March-2014
Available online at www.aygrt.isrj.net

# EFFECT OF CO-FLOCS OF PGPR EXOPOLYSACCHARIDES (EPS) ON PLANT GROWTH PROMOTING AND BIOCONTROL CHARACTERISTIC AGAINST RICE (*PYRICULARIA ORYZAE*) DISEASE (IR-50) IN LOWLAND CONDITION.

**S. Kalaiarasu and S. Vivekanandhan**

Department of Microbiology, Annamalai University, Annamalainagar , India.

**Abstract:**-The effect of exopolysaccharides of *Azospirillum* isolates viz., Azo-16, Azo-19, PS-24 and PS- 28 and certain ISR inducing chemicals viz., Salicylic acid, Jasmonic acid and Azibenzolar on the enhancement of plant growth and bio-control against blast disease in lowland rice crop was studied under in-vitro condition. It was observed that the application of EPS, collected from *Azospirillum* isolates and Pseudomonas isolates, augmented the height of rice plant and reduced the blast disease incidence in lowland rice to a higher level when compared to the application of ISR inducing chemicals alone. Eventhough, the application of ISR inducing chemicals was found to reduce the blast disease incidence, as in the case of purified EPS application of *Azospirillum* isolates and Pseudomonas isolates, but did not augment the growth of rice plant and clearly revealed the absence of phytostimulatory activities of ISR inducing chemicals.The study on the optimization of different concentrations of purified EPS viz., 100, 200, 300 ppm on the blast disease incidence of rice revealed that the application of the same at 200 ppm concentration could effectively controlled the disease incidence to a higher level when compared to other concentration.

The results of the present study clearly revealed the effect of *Azospirillum* and Pseudomonas EPS on the enhancement of host plant growth as well as the bio-control against *Pyricularia oryzae* whereas the application of ISR inducing chemicals confined with reduction in blast disease incidence alone. Moreover, the *Azospirillum* and Pseudomonas EPS at a concentration of 200 ppm level could be optimized as effective one for the control of blast disease in lowland rice.

**Keywords:**Pgpr Exopolysaccharides , Biocontrol Characteristic , Promoting , Azospirillum .

## INTRODUCTION:

In India, rice is grown under both lowland and upland conditions and out of 44 million hectare of rice cultivated area, 12 per cent of the same grown under rainfed lowland condition. However, the production of lowland rice has been volatile and the yield of the same is affected by a lot of biotic and abiotic factors including nutrition, temperature, water stress and disease incidence. Generally, large quantities of synthetic chemical fertilizers and pesticides are used to replenish the same. Recently, a biological approach of using plant growth promoting rhizobacteria (PGPR) was attempted to reduce the drastic effects caused by consistent use of synthetic chemical fertilizers and pesticides and to improve the productivity of lowland rice crop. Moreover, the biological approach has a great potential in supplying 'N' nutrition and biocontrol of phytopathogens which eventually leads to sustainable production of rice grown under lowland rice ecosystem. The blast disease, caused by *Pyricularia oryzae*, is one of the most destructive fungal disease of lowland rice crop, causing an yield loss up to 90 percent. PGPR mediated Induced systemic resistance (ISR) against blast pathogen seems to be a promising approach in the reduction of biological and environmental hazards posed by the application of synthetic chemical pesticides.

The occurrence of *Azospirillum*, as PGPR, in the rhizosphere of rice has been reported by many authors (Lakshmi *et al.*, 1977; App *et al*., 1980; Baldani and Doberenier 1980; Thomas-Bauzon *et al*., 1982; Rao *et al*., 1983; Rao and Rajarammohan rao 1983; Nayak *et al*., 1986; Dung et al., 1988). Azospirillum exerted PGPR characteristics viz., Nfixation, hormonal interaction, improvement in root growth, solubilisation of nutrients, alleviation of salinity and biocontrol against phytopathogens in the host rhizosphere (Nadeem *et al*., 2006; Gholami *et al*., 2009). Hence, the development and deployment of this organism, as an agricultural bioinoculant, will be the suitable biological approach for the maximization of growth and

yield in lowland rice crop. Neyra *et al*., (1995) proposed the use of "EPS mediated *Azospirillum* bioflocs", as a delivery system, for the enhancement of growth and yield of crop plants under stress conditions, including, moisture and temperature. The effect of rhizobacterial EPS, as an elicitor of ISR, against phytopathogens of crop plants has already been reported Kyungseok et al., (2008). Eventhough, many reports suggested the positive role of *Azospirillum* and *Pseudomonas* inoculation in rice crop, the role of EPS-rich flocculated culture of *Azospirillum* and *Pseudomonas* application on the induction of systemic resistance (ISR) against P.oryzae in lowland rice has not been studied, so far.

The present investigation has been undertaken with an aim to elucidate the role of *Azospirillum* and *Pseudomonas* EPS, as an elicitor, on the induction of systemic resistance (ISR) against Pyricularia oryzae in lowland rice crop.

## MATERIALS AND METHODS

### Preparation of inoculum

Four efficient *Azospirillum* and *Pseudomonas* isolates viz., Azo-16, Azo-19, PS-24, PS-28, isolates from the rhizosphere of lowland rice var.IR-50, were used in the present study. All the *Azospirillum* and *Pseudomonas* isolates were grown separately in synthetic malate broth and Melted King's agar medium (Day and Dobereiner, 1976). Then, the medium was centrifuged at 5000 x g for 10 min to harvest the log phase cells and the pellets washed three times with 0.1 M phosphate buffer (pH 6.8), finally, the cells were resuspended in the same buffer to a cell concentration of 1 x 109 cfu/ml by measuring the OD at 420 nm and used as inoculum.

### EPS production

Minimal salts medium (Neyra and Van Berkum, 1977) was used for the present study together with addition of 8mM fructose and 0.5mM KNO3, as sole carbon and nitrogen source (Sadasivan and Neyra 1985). Each one ml culture of *Azospirillum* and *Pseudomonas* isolates (1x10-7 cfu/ml) was added to 100ml of fructose medium dispensed in 250ml Erlenmeyer flask and incubated at 30 ± 20°C for 5 days under shaking condition (250rpm) in a rotary shaker. After the incubation period, the EPS produced by individual *Azospirillum* and *Pseudomonas* isolates were extracted and purified according to Kyungseok *et al*., (2008) and used at different concentrations viz., 100, 200, and 300 ppm.

### Preparation of ISR inducing chemicals

ISR inducing chemicals viz., salicylic acid, jasmonic acid and Azibenzolar (Himedia, India) at a level of 0.01 percent concentration were used.

### Treatments

The following treatments viz., ISR inducing chemicals at 0.01 percentage concentration and *Azospirillum* and *Pseudomonas* EPS at 200 ppm concentration were used for assessing the biocontrol ability against *Pyricularia oryzae* whereas the optimization of different concentration of *Azospirillum* and *Pseudomonas* EPS on the blast disease incidence was tested at 100, 200 and 300 ppm concentration levels.

### Preparation of growth chamber

The growth chamber was a desiccator (12 x 10 cm) consisting of two parts. The lower part was filled with Weaver's medium and 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. The growth chamber was sterilized by autoclaving. After the sterilization of growth chamber, fifty germinated rice seeds with coleoptile (2 cm high) were transferred aseptically onto the stainless steel wire mesh and incubated for 10 days. The growth chamber was maintained under 14 h/day and 10 h/night cycle and the temperature ranging from 24°C at night to 32°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*

*P. oryzae* AU-1 (provided by Dept.of plant pathology Annamalai University) was maintained in oat meal agar (OMA) medium and used for the challenge inoculation purpose. Thick spore suspension of the same was prepared with sterile distilled water from 10 day old culture maintained in OMA medium and strained through double layer muslin cloth so as to get a free suspension of conidia. The population was adjusted with the help of Haemocytometer and a spore suspension with optimum spore concentration (50,000 spore's ml-1) was prepared. Then, the spore suspension was added with few drops of Tween-80 which increased the adherence capacity of the spores and acts as a sticker. The spraying of spore suspension was done under proper humid condition. Control plants were also sprayed with sterile distilled water.

After one week of challenge inoculation the blast disease incidence was enumerated with a score chart of 0-9 grades devised by International Rice Research Institute (1980). The statistical analysis was carried out according to Gomez and Gomez, (1984).

## RESULT AND DISSCUSSION

The dual effect of the Purified EPS of *Azospirillum* and *Pseudomonas* isolates viz., Azo-16, Azo-19, Ps-24 and Ps-28 and ISR inducing chemicals, namely, salicylic acid, jasmonic acid and azibenzolar on the growth and *Pyricularia oryzae* disease incidence in rice was studied under in vitro condition (Table 1).

It was observed that the EPS application of each *Azospirillum* and *Pseudomonas* isolates was found to enhance the plant height and reduced the disease incidence in lowland rice var.IR-50. Interestingly, the application of EPS, collected from the *Azospirillum* and *Pseudomonas* isolates, augmented the height of rice plant and reduced the disease incidence to a higher level when compared to the application of ISR inducing chemicals.

Eventhough, the application of ISR inducing chemicals was also found to reduce blast disease incidence as in the case of purified EPS application of *Azospirillum* and *Pseudomonas* isolates but did not augment the growth of the rice plant. The results of the present study clearly revealed the absence of phytostimulatory activities of these chemicals. The results of the present study also suggested the effect of *Azospirillum* and *Pseudomonas* EPS on the augmentation of growth of the host plant as well as the reduction in disease incidence whereas, the ISR inducing chemicals confined with reduction in blast disease incidence alone. Usharani, (2005) reported the phytostimulatory and biocontrol effect of Pseudomonas fluorescens against Pyricularia oryzae in lowland rice. Bahat-Samet et al., (2004) reported the phytostimulatory effect of *Azospirillum* and *Pseudomonas* EPS on wheat. The results of the present study clearly revealed the effect (Phytostimulatory and biocontrol) of Azospirillum and Pseudomonas EPS and in conformity with the earlier findings of Bahat-Samet et al., (2004) and Usharani, (2005).

The effect of Purifed EPS of *Azospirillum* and *Pseudomonas* isolates viz., Azo- 16, Azo-19, PS-24 and PS-28 at different concentrations, viz., 100, 200 and 300 ppm on the blast disease incidence of rice was studied under in vitro condition (Table- 2).

**Table - 1**
**Response of *Azospirillium* and *Pseudomonas* exopolysaccharides (EPS) and ISR inducing chemicals on the enhancement of growth and blast disease incidence (Pyricularia oryzae) in rice.**

| Treatment | Plant Height(cm) *** | Disease incidence (%) a,b |
|---|---|---|
| Control | 15.22 ±1.00 | 83.51 ±1.21 |
| ISR inducing chemicals** | | |
| Salicylic acid | 16.00 ± 0.31 | 21.13 ± 0.45 |
| Jasmonic acid | 15.13 ± 045 | 21.44 ±0.11 |
| Azibenzolar | 15.00 ±0.16 | 21.13 ± 0.33 |
| Purified EPS (Azo -16) * | 24.13 ± 0.11 | 20.81 ± 0.14 |
| Purified EPS (Azo -19) * | 21.07 ± 0.31 | 19.81 ± 0.39 |
| Purified EPS (Ps -24) * | 22.17 ± 0.42 | 21.00 ± 0.18 |
| Purified EPS (Ps -28)* | 25.13 ±0.36 | 20.101±0.14 |

* EPS collected from minimal medium of Neyra and Van Berkum (1977) supplemented with 0.1% pectic acid and 0.005 % after KNO3 after 48 hr of incubation. Purified EPS was prepared according to Kyungseok et al.(2008).
** at 0.01 per cent
*** 20th DAS
a.Disease incidence estimated 7 days after challenge inoculation with *Pyricularia oryzae*

b.Values are mean of three replications ± SD

**Table – 2**
**Effect of *Azospirillium* and *Pseudomonas* EPS at different concentrations on blast disease incidence in rice (IR-50)**

| Treatment | Concentration of EPS (ppm) | Disease incidence | Statistics [b,c] |
|---|---|---|---|
| Control | - | 79.9 ±1.04 | - |
| Purified EPS from (Azo-16) | 100 | 16.2 + 0.42 | e |
| | 200 | 17.4 ±0.31 | f |
| | 300 | 15.0 ±0.11 | f |
| Purified EPS from (Azo -19) | 100 | 21.4 ±0.18 | a |
| | 200 | 18.1 ±0.12 | b |
| | 300 | 20.0 ±0.16 | b |
| Purified EPS from (PS -24) | 100 | 17.7 ±0.39 | c |
| | 200 | 18.9 ±0.22 | d |
| | 300 | 16.5 ±0.45 | d |
| Purified EPS from (PS -28) | 100 | 16.6 ±0.13 | g |
| | 200 | 13.4 ±0.21 | h |
| | 300 | 15.4 ±0.44 | h |

*EPS collected from minimal medium of Neyra and Van Berkum (1977) supplemented with 0.1% pectic acid and 0.005 % after $KNO_3$ after 48 hr of incubation and Purified EPS was prepared according to Kyungseok et al.(2008).

a.Disease incidence estimated 7 days after challenge inoculation with *Pyricularia oryzae*
b.Values followed by different letters are significantly differed at 5 % level according to student't' test
c.Values are mean of three replications ± SD

It was found that the application of purified EPS, collected from each *Azospirillum* and *pseudomonas* isolates, was found to reduce the blast disease incidence in rice to a higher level when compared to control plants. Among the different concentrations of EPS, the application of purified EPS at 200 ppm level reduced the blast disease incidence to a level on par with 300 ppm level of EPS application. However, a marked variation was observed between 100 and 200 ppm level of EPS application regarding the blast disease resistance in rice. The study clearly revealed the importance of *Azospirillum* and *Pseudomonas* EPS application at 200 ppm level on the effective reduction of blast disease incidence in rice.

However, there were no earlier reports regarding the biocontrol effect of *Azospirillum* and *Pseudomonas* EPS at different concentrations, available against *P.oryzae*. The results of the present study clearly revealed the optimization of *Azospirillum* and *Pseudomonas* EPS (200 ppm) for the effective biocontrol of *Pyricularia oryzae* incidence in rice and the subject needs further elaborate reserach.

**ACKNOWLEDGEMENT**

The Authors would like to thank the university grants commission (UGC) for providing financial assistance under Major Research project.

**REFERENCES**

1.App, A.A.I. Watanabe, M.Alexander, W.Ventura, C.Daez, T.Santiago and S.K.DeDatta. 1980. Non-symbiotic nitrogen fixation associated with the rice plant in flooded soils. Soil Sci., 130: 283-289.
2.Bahat – Samet, E., S. Castro – Sowinski, and Y. Okon, 2004. Arabinose content of extra cellular polysaccharide plays a role in cell aggregation of Azospirillum brasilense FEMS Microbiol. Lett., 237 : 195-203.
3.Baldani, V.L.D. and J. Dobereiner, 1980. Host-plant specificity in the infection of cereals with Azospirillum spp. Soil Biol. Biochem., 12: 433-439.
4.Day, J.M. and J.Dobereiner. 1976. Physiological aspects of N2 fixation by a Spirillum from Digitaria roots. Soil Biol. Biochem., 8: 45-50.
5.Dung, N.N.,N.P.Chi and L.K.Bang.1988. Azospirilla from rice fields near Hanoi. In: Azospirillum IV: Genetics, Physiology and Ecology (Ed.) Klingmuller, W. Sringerverlag, Berlin. pp. 242 – 246.
6.Gholami, A., S. Shahsavani and S. Nezarat. 2009. The effect of plant growth promoting Rhizobacteria (PGPR) on germination, seedling growth and yield of maize. World Academy of Science, Engineering and Technology, 49:19-24.
7.Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for agricultural research, John Wiley and Sons, New York, USA.IRRI. 1980. International Rice Research Institute. Annual Report for 1979. Los Banos, Philippines, pp. 171-182.

8.Kyungseok, P., J.W. Kloepper, and C.M. Ryu, 2008. Rhizobacterial exopolysaccharides elicit induced resistance on cucumber. J. Microbiol. Biotechnol. 18 : 1095-1100.
9.Lakshmi, V., A. Satyanarayana Rao, K. Vijayalakshmi, M. Lakshmi Kumari, K.V.B.R.Tilak and N.S.Subba Rao. 1977. Establishment and survival of Spirillum lipoferum. Proc. Indian. Acad. Sci. Sec. B, 86: 397 – 404.
10.Nadeem, S.M., Z.A. Zahir, M. Naveed, M. Arshad, and S.M. Shahzad, 2006. Variation in growtha nd ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress, Soil & Environ, 25 : 78-84.
11.Nayak, D.N., J.K. Ladha and I. Watanabe. 1986. The fate of marker Azospirillum lipoferum inoculated into rice and its effect on growth, yield and 15N2 feeding and 15N dilution techniques. Biol. Fertil. Soils, 2: 7 -14.
12.Neyra, C.A. and P. Van Berkum, 1977. Nitrogen fixation grasses. Advan. Agron., 29: 37-38
13.Neyra, C.A., A.L. Atkinson and O. Olubayi. 1995. Coaggregation of Azospirillum with other bacteria basis for functional diversity NATO ASI Ser. G. Ecol., 37: 429- 439.
14.Rao J.L.N., and V.R.Rajamamohan Rao. 1983. Nitrogenase activity in the rice rhizosphere soil as affected by Azospirillum inoculation and fertilizer nitrogen under lowland conditions. Curr. Sci., 52: 686 - 688.
15.Rao.V.R., D.N.Nayak, P.B.D. Charyulu and T.K. Adhya, 1983. Yield response of rice to root inoculation with Azospirillum J. Agric. Sci., 100: 689-691.
16.Sadasivam, L. and C.A. Neyra 1985 Flocculation in Azospirillum brasillense, Azospirillum lipoferum: exopolysaccharides and cyst formation. J. Bacteriol., 163: 716-723.
17.Thomas-Bauzon, D., P.Weinhard, P.Ville Court and J.Balandreau. 1982. The Spermosphere model. 1. Its use in growing, counting and isolating nitrogen fixing bacteria from the rhizosphere of rice. Can. J. Microbiol., 28: 922- 928.
18.Ushrani, G. 2005. Studies on the development of Pseudomonas fluorescens, as plant growth promoting rhizobacteria (PGPR) and biocontrol agent, of rice (Oryza sativa L.) in Cuddalore district, Tamilnadu, Ph.D. Thesis, Annamalai University, India.