International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.3, pp 1370-1375, April-June 2013

IPACT-2013[14th – 15th March 2013]

National Conference on Industrial Pollution And Control Technology-2013

Phytological Analysis For Designinig A Microbial Consortium To Enhance Plant Growth.

Jelin J[1]*, Selvakumar TA[2], Dhanarajan MS[3]

[1]Faculty of Biotechnology, Sathyabama University, Chennai,India.
[2]Department of Biotechnology, Rajalakshmi Engineering College, handalam, Chennai, India..
[3]Jaya College of Arts and Science, Chennai, India.

*Corres.author: jelinilay@yahoo.co.in
PH:9445489844

Abstract: During the past couple of decades the use of plant growth promoting rhizobacteria(PGPR) for sustainable agriculture has increased tremendously in various parts of world. Significant increases in response to inoculation with PGPR have been repeatedly reported. Biofertilizers are also available for increasing crop nutrient uptake of nitrogen from nitrogen fixing bacteria associated with roots, sulfur uptake from phosphate mineral solubilizing bacteria. In the present study consortium of microorganisms were designed to cater different needs of growing plants and to understand interaction between consortium of microbial inoculants and plant systems will pave way to harness more benefits for improving plant growth and yield. The germination percentage of the Paddy seeds control is 66.7% on fourth day but varying results was obtained with Azotobacter, Pseudomonas, Bacillus, sth1 and sth2 as 83.3%, 83.3%, 50%, 66.7% and 66.7% respectively. The vigor index value for control is 113.39. Bacillus is 40 and Azotobacter, Pseudomonas, sth1 and sth2 are 149.99, 141.6, 73.37 and 66.7 respectively. The germination index is 2 for control, sth1 and sth2 1.75 for Bacillus and 2.25 for Azotobacter and Pseudomonas. The germination percentage of green gram seeds is 100% on 4th day and germination index was calculated as 2.5 for all the organisms. The vigor index is less for control (160) and 210 for Bacillus and sth1, 200for sth2, 180 for Azotobacter and 170 for Pseudomonas. The Field culture study gives a clear view of practical applications and explains the synergistic or antagonistic effect with the seeds. The results of the present study suggest that pressuring bacterial treatments have a significant effect on the plant. The field culture analysis revealed that seed inoculation with all bacteria resulted in an increased plant height and weight. Thus Azotobacter, sth1, Bacillus and Pseudomonas together as a consortium can be used as a biofertilizer for the growth of paddy, green gram and Maize. Future study can be done on the increase of seed number and yield. The designed consortium can be further formulated based on concentrations and designed for large scale production and commercialization. This when applied would increase the quality and fertility of the land.
Keywords: PGPR, Biofertilizers, Microbial Consortium.

Introduction

Biotechnology has opened up new possibilities concerning the application of beneficial bacteria to the soil for the promotion of plant growth and the biological control of soil-borne pathogens. Since the large scale release of genetically engineered bacteria to the environment faces a number of regulatory hurdles, the need to isolate and select superior, naturally occurring rhizosphere bacteria continues to be of interest. Apart from rhizobia symbionts, the rhizosphere-associated beneficial bacteria consist of the following genera: [1] *Pseudomonas* and *Bacillus,* which antagonize pathogenic or deleterious microorganisms (biological control) and [2] bacteria that enhance plant growth directly such as *Azospirillum*, *Acetobacter*, *Azotobacter*, and *Pseudomonas*, as well as many unidentified rhizosphere isolates.

The nutritional and environmental requirements of these bacteria are very diverse, and hence there is no general method that can be used to isolate all species of Plant Growth- Promoting Rhizobacteria (PGPR). Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Accordingly, a variety of methods have been developed, primarily within the last two decades.

The use of microorganism with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture. Some of the mechanisms, which can probably be active simultaneously or sequentially at different stages of plant growth, are increased mineral nutrient solubilization and nitrogen fixation, making nutrients available for the plants[3] .The bacteria presenting one or more of these characteristics are known as plant growth promoting rhizobacteria –PGPR[4] According to Kleoppar, numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in on or around plant tissues and stimulate plant growth by plethora of mechanisms are collective known as *PGPR.*[5] Gray and Smith (2005) have a recently shown the PGPR associations. This can be separated into extracellular(PGPR), existing in the rhizosphere, on the rhizoplane, and intracellular (iPGPR), which exist inside root cells generally in specialized nodular structures. PGPR inoculants currently commercialized that seem to promote growth by improved nutrients acquisition (biofertilizers)[5] .

During the past couple of decades the use of plant growth promoting rhizobacteria(PGPR) for sustainable agriculture has increased tremendously in various parts of world. Significant increases in response to inoculation with PGPR have been repeatedly reported.[5]

In the present study consortium of microorganisms were designed to cater different needs of growing plants and to understand interaction between consortium of microbial inoculants and plant systems will pave way to harness more benefits for improving plant growth and yield. The present study was under taken to screen to PGPR strains on seed germination and growth of rice, green gram and Maize seedling as well. Maize is one of the most important cereal crops after wheat and rice in the world.[6]

In the west African of Benin republic, maize is mainly produced by small scale farmers with little or no financial means to buy fertilizers. Implementing the use of PGPR in farming systems represents therefore, one of the most promising alternatives to improve maize yield improvement under field study has hindered the ability at national scientists in Benin to efficiency use these natural Biofertilizers. the objective of the work to (i) characterize the maize specific PGPR: and (ii) establish the efficient maize specific PGPR inoculation for an improved Maize Growth And Productivity.[7]

Methods

Isolation Of Bacteria From Soil

The screening should be done according to morphological, physiological, nutritional, and

biochemical characteristics in pure culture, with the guiding principle that more tests are better than fewer. Many such tables, lists, and tests have been published. For example, Ten grams of sample soil were taken into a 250ml of conical flask , and 90 ml of sterile distilled water was added into it. The flask was shaken for 10 min on a rotary shaker. One milliliter suspension was added to ml of via and shaken for 2 min. serial dilution technique was performed up to 10^{-7} an aliquot (0.1ml) if this suspension was spread on the plates of nutrient agar YEMA medium, Cetrimide agar, Azotobacter isolation agar medium. Plates

were incubated for 24 hrs at 37˚c and 60˚c observe the colonies of bacteria, after incubation, different characteristics of colonies such as shape, size, elevation, surface, margin, color, odor, pigmentation, etc staining and biochemical reactions were done although outdated for several species, the *Bergey's Manual for Systematic Bacteriology[* should be consulted, at least for the genus description. The sources for species description data are the following: For *Azospirillum* [8,9,] and for *Acetobacter*.[10] As for Pseudomonas, many species of *Pseudomonas* isolated from the field are very heterogeneous, vaguely defined, and often fail to fit precisely into established taxonomic subdivisions[11] Bacterial members can be classified into different groups based on (1)phenotypic characteristics (2) their cultural and biochemical characters,[12,13] (3) rRNA-DNA homology[14] .

Seed Germination Test

Seeds of 3 plants oryza sativa, zea Mays, vigna radiate were collected from the agricultural office in Thiruvallur. After sun drying for three days the seeds were kept in glass bottles at temperature 45˚c for 11 days. Thirty healthy seeds of uniform size were selected for each treatment. Seeds were allowed to germinate on two sheets of filter paper was moistened with 10 ml of water was then placed in seed incubator temperature in the germinator was maintained at 25+_ 1˚c with 12 hr light. The petridishes were arranged in completely randomized design(CRD) with 4 replications of each treatment. The number of germinated seeds were counted daily and root length of seedlings were measured at 12 DAS(days after seeding). After final count, final germination%(FGP) and germination index (GI), which indicates the seeds of germination, were calculated as described in the association of official seed analysis (AOSA,1983). Mean germination time(MGT) was calculated as per Ellis and Robert(1981)[15,16]. The time to 50% germination (T50) was calculated according to the method of abdul-baki and Anderson,1973[17]. The relative growth value rate of different weed species was calculated by following the formula proposed by Asraf and Waheed (1990)[18]

This experiment was repeated two times. In the preliminary analysis two times. In the preliminary analysis two experiment data were considered together taking time as a factor but there was no significant differences between the means of two time data (F value=0.1857) so, the pooled values are presented. Data were analyzed using analysis of variance(ANOVA) and means were separated by least significant difference (LSD) using statistical analysis system (SAS, version 9.0)

Formula

$$GI = \frac{\text{No of germinated seeds}}{\text{Days of first count}} + \frac{\text{No of germinated seeds}}{\text{Days of final count}}$$

Vigor index = mean root length + mean shoot length * germination

Field Culture Study

Fire bacterial isolates Azotobacter, bacillus, Pseudomonas and two thermophiles named sth1 and sth2 (soil thermophilic) were maintained in broth. The field soil was collected and given to agriculture office, Thiruvallur for soil test analysis. Seeds of oryza sativa, zea Mays and vigna radiate were washed with distilled water then seed inoculation was performed by a suspension of an bacteria(10^8 cfu ml^{-1}) with perlit mixture. Treatments were arranged as randomized complete block design with three rows for each plant totally ranging a durscors. Seeds were placed at 5cm depth. Five mature oryza sativa, zea Mays, vigna radiate plants were sampled from each treatment for final measures for every 7 days until 28 days from sowing. Sample were separated into components and oven derived at 60˚ c until it reaches a constant weight.

Harvesting Of The Plants And Analysis

Rice and Maize plants were harvested after 28 days of seed sowing through separating of plants from soil. The plants were washed through dipping into vessel. Plant height (cm) and root length (cm) of each plant were recorded. Dry weights of shoot and root were recorded after drying in an oven for 1 day at 60°C. Data was

analyzed statistically by F-test. The significance of differences between mean Values was evaluated by DMRT (Duncan's New Multiple Range Test).

Result And Discussion

The soil sample was collected and three isolates of mesophilic bacteria and two isolates of thermophilic bacteria were isolated on agar. This isolates were identified as Azotobacter, Pseudomonas, Bacillus and two new thermophilic isolates named as sth1 and sth2, which implies soil thermophilic (Table 1 and 2). Pure cultures were obtained and there colony morphology observed to be distinct.

Sth1 has large colonies with irregular edges having central depression and translucent. Sth2 was mucoidal smooth large colony with central raise and opaque. The colonies are subjected to staining. Bacillus, Sth1 and Sth2 were gram positive rods actively motile, capsulated with terminal spores. Pseudomonas and Azotobacter are gram negative non motile, non-capsulated and non sporing rods. Enzyme utilization test were performed and tabulated.

The germination percentage of the Paddy seeds control is 66.7% on fourth day but varying results was obtained with Azotobacter, Pseudomonas, Bacillus, sth1 and sth2 as 83.3%, 83.3%, 50%, 66.7% and 66.7% respectively. (Table 3). The vigor index value for control is 113.39. Bacillus is 40 and Azotobacter, Pseudomonas, sth1 and sth2 are 149.99, 141.6, 73.37 and 66.7 respectively. The germination index is 2 for control, sth1 and sth2 1.75 for Bacillus and 2.25 for Azotobacter and Pseudomonas. The germination percentage of green gram seeds is 100% on 4^{th} day and germination index was calculated as 2.5 for all the organisms. The vigor index is less for control (160) and 210 for Bacillus and sth1, 200for sth2, 180 for Azotobacter and 170 for Pseudomonas. The germination percentage of maize without inoculates is66.7% and is common for Azotobacter, Bacillus and sth2. (Table 3)

The fresh weight of the shoot of plants were measured sth1 shows maximum fresh weight of 191.33 mg (Table 2) for Paddy and Rs sth2 shows the least weight 52.64 mg. In case of Green gram maximum fresh weight is obtained for control (233 mg) by Azotobacter. In case of maize fresh weight is high for sth1 inoculants 1075 mg. The dry weight of plants shoot were measured sth1 shows maximum dry weight of 53mg for paddy and Pseudomonas shows the least weight of 24.66mg. In case of green gram maximum dry weight is obtained for Azotobacter 0.03 mg. In case of maize dry weight is high for sth1 inoculants 570 mg.

In the dry weight of shoot of plants were measured Azotobacter shows maximum fresh weight of 150.33 mg for paddy and shows the least weight of 22.66 mg for sth1. In case of green gram maximum fresh weight is obtained for 283 mg. In case of maize fresh weight is high for sth1 inoculants 290 mg.

In the dry weight of root of plants were measured sth1 shows maximum dry weight of 56mg for *paddy* and Pseudomonas shows the least weight of 15.66 mg. In case of green gram maximum least weight is obtained for sth2 70mg. In case of maize fresh weight is high for sth1 inoculants 290mg.

Paddy grows best in combination of Azotobacter and sth1. Maize grows best at combination of sth2, Bacillus and green gram grows best with rhizospheric micro flora itself and enhanced by Pseudomonas and Bacillus. In the present study a consortium of bacteria were designed to improve plant growth. The consortium includes Geobacillus Sub terrareans Bacillus, Pseudomonas and Azotobacter which can be commercialized as Biofertilizers.

Table 1 Effects Of Pgpr On Germination And Seedling Growth Of Maize

Isolates	Days Of Germinatiom			Seed Germination (4^{th} Day)%	Germination Index	Root Length	Shoot Length	Vigor Index
	2	4	6					
Control	-	3	6	66.67	1.75	0.6	0.8	40.82
Azotobacter	-	4	6	66.7	2	0.8	0.6	40.82
Pseudomonas	-	5	6	**83.33**	2.25	**0.9**	**0.9**	**133.32**
Bacillus	-	4	6	66.7	2	0.8	0.3	113.39
Sth 1	-	5	6	**83.33**	2.25	**0.9**	**0.9**	**133.32**
Sth 2	-	4	6	66.7	2	**0.9**	0.6	120.05

Table 2 Mean Height And Weight Of Maize

Isolates	Maize		Paddy		Green Gram	
	Height	Weight	Height	Weight	Height	Weight
Control	33.13	36.9	26.4	243.33	34.36	1283
Azotobacter	14.56	1063.1	37.13	270.66	32.4	1343
Bacillus	37.7	6.36	39.93	321.32	45.66	2116
Pseudomonas	38.86	5.73	27.26	223	28.69	243.33
Sth 1	40.46	6.78	32.83	311.83	36.99	1714
Sth 2	32.19	1006.22	22.66	122.66	30.46	1300

Table 3 Effects Of Pgpr On Germination And Seedling Growth

Isolates	Paddy			Green Gram			Maize		
	Seed Germination	Vigor Index	Germination Index	Seed Germination	Vigor Index	Germination Index	Seed Germination	Vigor Index	Germination Index
Control	50	85	1.75	100	160	2.25	66.67	40.82	1.75
Azotobacter	**83.33**	**149.99**	**2.25**	100	180	2.5	66.67	40.82	2
Pseudomonas	**83.33**	141.66	**2.25**	100	170	2.5	**83.33**	99.99	**2.25**
Bacillus	50	40	1.75	100	210	2.5	66.7	113.39	2
Sth 1	66.7	73.37	2	100	210	2.5	**83.33**	133.32	2.25
Sth 2	66.7	66.7	2	100	**220**	2.5	66.7	**120.05**	2

References

1. Kole, M.M., Page, W.J., and Altosaar, I., Distribution of *Azotobacter* in Eastern Canadian soils and in association with plant rhizospheres, *Can. J. Microbiol.,* 1988, 34, 815
2. Juhnke, M.E., Mathre, D.E., and Sands, D.C., Identification and characterization of rhizospherecompetent bacteria of wheat, *Appl. Environ. Microbiol.,* 1987, 53, 2793.
3. Reinhold, B., Hurek, T., Fendrik, I., Pot, B., Gillis, M., Kersters, K., Thielemans, S., and de Ley, J., *Azospirillum halopraeferens* sp. nov., a nitrogen fixing organism associated with roots of Kallar grass (*Leptochloa fusca* (L.) Kunth), *lnt. J. Syst. Bacteriol.,* 37, 43, 1987.
4. Matthew, J. Gray1, Loren M. Smith, Debra. Miller, and Charles R. Bursey Influences of agricultural land use on *clinostomum attenuatum* metacercariae prevalence in southern great plains amphibians, U.S.A.*4* Herpetological Conservation and Biology 2(1):23-28
5. Kloeppar J.W. Scoth MN, Effect of rhizoshphere cownization by plant development and yield. Phytopstool 70:1078-1082.
6. Bashan, Y. and Levanony, H., Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture, *Can. J. Microbiol.,* 1990, 36, 591,
7. Bergey's *Manual for Systematic Bacteriology,* Williams & Wilkins, U.S.A.
8. Quesada, E., Ventosa, A., Rodriguez-Valera, F., and Ramos-Cormenzana, A., Types and properties of some bacteria isolated from hypersaline soils, *J. Appl. Bacterial.,* 1982, 53, 155.
9. Page, W. J., Sodium-dependent growth of *Azotobacter chroococcum, Appl. Environ. Microbiol.,* 1986, 51, 510.
10. Kloepper, J. W., Lifshitz, R., and Schroth, M. N., *Pseudomonas* inoculants to benefit plant production,in ISI *Atlas of Science: Animal and Plant Sciences,* 1988, 60.

11. Bashan, Y., Singh, M., and Levanony, Y., Contribution of *Azospirillum brasilense* Cd to growth of tomato seedlings is not through nitrogen fixation, *Can. J. Bot.*, 1989, 67, 2429.
12. Magalhães, F. M. M. and Döbereiner, J**.,** [Occurrence of *Azospirillum amazonense* in some Amazonian ecosystems], *Rev. Microbiol., São Paulo*, 1984, 15, 246, (in Portugese).
13. Tarrand, J. J., Krieg, N. R., and Döbereiner, J., A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense sp.* nov., *Can. J. Microbiol.*, 1978, 24, 967.
14. Bashan, Y., Mitiku, G., Whitmoyer, R. E., and Levanony, H., Evidence that fibrillar anchoring is essential for *Azospirillum brasilense* Cd attachment to sand, *Plant Soil*, 1991, 132, 73.
15. Ejaz, H. S, Muhammad A, and Nudrat A. A, Variation in seed germination and seedling growth in some diverse lines of safflower (*carthamus tinctorius*l.)under salt stress*Pak. J. Bot*., 2007, 39: 1937-1944**.**
16. Döbereiner, J., Marriel, I. E., and Nery, M., Ecological distribution of *Spirillum lipoferum* Beijerinck, 344, *Methods in Plant Molecular Biology and Biotechnology.Can. J. Microbiol.*, 1976, 22, 1464.
17. Abdual-baki, A.A., J.D. Anderson, Relationship between decarboxilation of glutamic acid and vigour in soybean seed, *Crop Sci*., 1973, 13, 222–226.
18. Glick BR. The enhacement of hands plant growth promotion by freeliving bacteria. Can *J.Microbiol.*, 1995, 41, 109-117.

* * * * *