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PREPARATION AND STANDARDIZATION OF BIOCOMPATIBLE BACTERIAL CONSORTIUM FOR THE ENHANCED GROWTH OF CROP PLANTS

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ABSTRACT

Intensive application of agrochemicals leads to several agricultural problems and poor cropping systems. The extensive research program on beneficial bacteria and fungi has resulted in the development of a wide range of bio-fertilizers, which satisfied the nutrient requirements of crops and increased the crop yield as well.In the present study;variousPlant Growth Promoting Rhizobial Bacteria from the 6 soil samples were isolated, characterised and stored. PGPR- Biofertilizer was prepared by mixing isolated PGPR-BACTERIA with various compounds. Seeds of Bengal gram,Green gram and Chicken peas were collected and treated with biofertilizer; controls (without biofertilizers) also were maintained for comparison. Biofertilizers (*Azotobacter, Pseudomonas, Enterobacter,Bacillus, Phosphobacteria* and *Rhizobium)* were used alone as well as in combination of all inoculates. Seeds were sown in cups and grown in three batches. The growth and yield parameters of those plants such as length of root and stem, leaves count, total chlorophyll, carotenoid and protein content were observed.The total chlorophyll and carotenoid content in plants were estimated. High chlorophyll, carotenoid and protein content were observed in Rhizobium inoculants when compared with other inoculants. High chlorophyll content was observed in Chicken peas and high carotenoid content in Green gram and Chicken peas. High chlorophyll was observed in Chicken peas. High protein content was observed in T5 and low protein content was observed in T2 when compared with other inoculants, Chicken pea showed high protein content and Bengal gram showed low protein content.

Key words: Biofertilizer, Bacterial Consortium, *Rhizobium.*

1. INTRODUCTION

A biofertilizeris a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Singh *et al*., 2008).Bio-fertilizers provide eco-friendly organic agro-input and are more cost-effective than chemical fertilizers. Bio-fertilizers such as *Rhizobium, Azotobacter, Phosphobacterium, Pseudomonas* and *Bacillus*, have been in use a long time. *Rhizobium* inoculants are used for leguminous crops. *Azotobacter* can be used with crops like wheat, maize, mustard, cotton, potato and other vegetable crops. *Pseudomonas* inoculations are recommended mainly for sorghum, millets, maize, sugarcane and wheat. *Rhizobium* fixes atmospheric nitrogen and are used as inoculations for paddy crop grown both under upland and low-land conditions.Other types of bacteria, so-called phosphate-solubilising bacteria, or *Pseudomonas putida* P13 (Vijila*et al*., 2008) are able to solubilise the insoluble phosphate from organic and inorganic phosphate sources (Abbas*et al*.,2013).

Bengal gram is the third important pulse crop in India. It is annual pulse crop and native to central Asia. It is also extensively grown in West Indies, Japan and other tropics/sub-tropical countries. Bengal gram seeds are highly nutritious containing higher amount of protein (24 to 26%) and are reported to be rich in potassium, phosphorous and calcium with good amount of sodium. Green gram is one of important pulse crop in India. Commonly called as mung beans are occasionally used in Indian cuisine; beans without skins are boiled to make dry preparation often served with rice. Chicken pea is one of the earliest cultivated legumes: 7,500- year old remains have been found in the Middle East. Chicken peas are known for their medical uses such as increasing sperm and milk, provoking menstruation and urine and helping to treat kidney stones.

2. MATERIALS AND METHODS

2.1. Isolation and Identification of Microorganisms

Soil samples were collected andsubjected to serial dilution with dilution factor 1:10 and bacteria's were grown on nutrient agar using spread plate method. After that, different bacteria's were grown on their selective media's and then streaked on nutrient agar plates to obtain pure cultures.

2.2. Preparation of Biofertilizer

The compounds (given in table) were mixed with all isolated cultures (5ml each) in a beaker

under sterile conditions to prepare PGPR biofertilizer.

Table1. Composition of biofertilizer

S.No	Compounds	Weight (g/30ml)
1.	Dolomite Talc	100g
2.	Carboxy Methyl Cellulose	1g
3.	Calcium Carbonate	1g
4.	Crab Shell Powder	0.05g
5.	Barley Powder	0.05g

2.3. Determination of Plant Growth Using MICR the Plants

Seeds of Bengal gram, Green gram and Chicken peas were collected. Seeds were treated with biofertilizers and controls (without biofertilizers) also were used as reference. The microbial consortium consists of *Azotobacter, Pseudomonas, Enterobacter, Bacillus, Phosphobacterium* and *Rhizobium* were used alone as well as in combination of all inoculates. Seeds were sown in cups. The plants were grown in three batches. The batch –I includes inoculants such as T0-control (without any microbial inoculation), T1-*Rhizobium*,T2- *Pseudomonas*, T3- *Phosphobacteria*, T4-*Bacillus*,T5- *Enterobacter*, T6- *Azotobacter* and T7- *Rhizobium*+*Pseudomonas* + *Phosphobacteria* + *Bacillus* + *Entero* + *Azotobacter.* The batch –II includes biofertilizer powder mixed with seeds. The batch –III includes soil mixed with bio fertilizer powder. From each entry, 10 plants were randomly selectedfor recording observations on important yield attributing characters, plant height,and length of the root, leaves count, estimation of total chlorophyll, carotenoid and protein content during the plant growth period.

2.4. Estimation of Plant Chlorophyll

About 0.5 mg of fresh leaf was ground in a mortar and pestle with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was saved. The pellet was restricted with 5 ml of 80 per cent acetone each time, until it become colourless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was measured at 645 and 663nm in spectrophotometer. The chlorophyll content was determined by using the following formulae.

Chlorophyll 'a' (mg/g fr. wt.) = $(0.0127) \times (OD_{663}) - (0.00269) \times (OD_{645})$

Chlorophyll 'b' (mg/g fr. wt.)

$= (0.229) \times (OD_{645}) - (0.00488) \times (OD_{663})$

Total chlorophyll(mg/g fr. wt.)

$= (0.0202) \times (OD_{645}) - (0.00802) \times (OD_{663})$

2.5. Estimation of Plant Carotenoid

The same chlorophyll extract was measured at 480nm, in spectronic-20 to estimate the carotenoid content.

Carotenoid (mg/g fr. wt.) = D×F×V×10/wt. ×2500

$= (OD_{480}+OD_{114})$ '(OD_{663})-$(OD_{638}$ 'OD $_{645})$

2.6. Estimation of Plant Protein (Lowry et al)

About 0.5 mg of plant materials was macerated with a pestle and mortar with 10 ml of 20 per cent trichloroacetic acid. The homogenate was centrifuged for 15 min at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 ml of 0.1 N NaOH. This extract was used for the estimation of protein. From this extract, 1 mL of sample was taken in a 10 mL test tube and 5 mL of reagent was added. The solution was mixed well and kept in dark for 10min. Later 0.5 mL folinphenol was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in the Spectronic-20. Blank prepared without protein sample was used for zerosetting.

3. RESULTS AND DISCUSSION

In the present investigation, the rhizosphere soil samples were collected from crop plants, and it was subjected to isolate the rhizospheric bacteria by standard methods, all the rhizospheric bacteria were used to prepare the effective microbial consortia as plant growth promoting agent for crop plants.

3.1. Isolation and Identification of Microorganisms

The various PGPR-BACTERIA'S were isolated from the soil and their characteristics were studied using selective medium and pure cultures were stored. There was significant number of different bacterial isolates were obtained and enriched for making biomass, which is listed below (Table 2). The preliminary tests and growth characteristics on respective selective medium supports to precede the identification of PGPR. Further, the biochemical profiles evidenced for naming the isolated bacteria at the genus level with reference to the Bergey's manual for bacterial identification.

Table 2. Growth characteristics of rhizosphere bacteria

S.No.	Bacteria	Selective Media	Characteristics
1.	*Pseudomonas* sp.	King's-B Agar	Fluorescent colonies were observed.
2.	*Enterobacter* sp.	Violet Red Bile Glucose Agar	Red coloured colonies were observed.
3.	*Rhizobium* sp.	Yeast Mannitol Agar	White colonies along with air bubbles (gas production).
4.	*Phosphobacterium* sp.	Nutrient Agar	White colonies were observed.
5.	*Bacillus* sp.	Nutrient Agar	Zone of hydrolysis(iodine test) was observed
6.	*Azotobacter* sp.	*Azotobacter* medium	White colonies were observed

3.2. Determination of Plant Growth by the Activity of PGPR

The growth and yield parameters of Bengal gram, Green gram and Chicken peas such as plant height, root length, leaves count, total chlorophyll, carotenoid and protein content were significantly increased by PGPR application in all concentrations when compared to control plants.

Utilization of biological fertilizer increased fresh and dry weight; no. of pods per plant that it could be due to increasing other nutrient absorption, also biological phosphate fertilizer can be used as a solution for increasing phosphate and micronutrient absorption in the alkaline soil. Both qualitative and quantitative characteristics were significantly increased by phosphate-solubilising microorganisms and also increased the growth and resistance of plants in water deficit conditions (Selvakumar *et al.,* 2012).

The utilization of phosphate-solubilising microorganisms, account for about 45% of the total biofertiliser production and use. This bacterium helps in increasing crop productivity by way of helping in solubilisation of insoluble phosphorus, stimulating growth by providing hormones, vitamins and other growth factors. The availability of phosphorus to legume crop is a key constraint to its production (Laemmli *et al*., 1970).

Several genera of rhizobacteria belonging to *Pseudomonas* spp. And *Bacillus* spp. are reported to solubilise zinc. Microbes solubilise the metal forms by protons, chelated ligands, and oxido reductive systems present on the cell surface and membranes (Crane *et al.,* 1985; Hughes and Poole, 1991; Wakatsuki, 1995). These bacteria also exhibit other traits beneficial to plants, such as production of phytohormones, antibiotics, siderophores, vitamins, antifungal substances, and other cellular components (Rodr´ıguez and Fraga, 1995) Microbes are potential alternate that could cater plant nutrient requirement by up taking the energy materials from the soil.

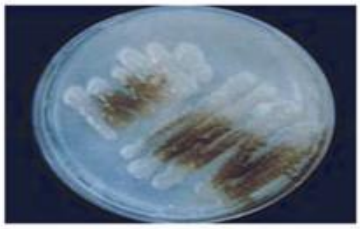
The soil microorganisms are responsible for transfer of the immobilized soil phosphorus into available form through which phosphorus becomes easily available to these plants. Co-inoculation can benefit plant growth by different mechanisms. However one of the most commonly reported plant growth promotion mechanism by bacteria is the changing of morphological and physiological changes in root system. An increase in the number of lateral roots and root hairs cause addition of root surface available for nutrients and water uptake. Higher water and nutrient uptake by inoculated roots caused an improved water status of plant, which in turn could be the main factor enhancing plant growth (Parvatham *et al*., 1989).

Table 3. Measurement of length of stem, root and No. of leaves Batch- I

Sample		T0	T1	T2	T3	T4	T5	T6	T7
Green Gram	Stem Length (cm)	18.4	20.2	-	23.0	25.3	28.1	18.2	-
	Root Length(Cm)	3.9	6.3	-	4.8	3.9	6.7	6.9	-
	Leaves Count	2	5	-	6	8	12	10	-
Chicken Peas	Stem Length (cm)	16.1	26.3	28.4	28.8	31.7	38.5	21.3	19.2
	Root Length (cm)	3.9	4.3	5.5	5.9	7.9	9.0	5.8	4.4
	Leaves Count	4	7	6	8	8	16	10	6
Bengal Gram	Stem Length (cm)	21.2	18.3	12.7	17.2	19.5	-	16.2	-
	Root Length(cm)	5.2	6.4	5.8	6.2	6.5	-	7.0	-
	Leaves Count	3	10	4	7	5	-	8	-

T0 – Control (without bacterial strain), T1- *Rhizobium*, T2- *Pseudomonas*, T3- *Phosphobacteria*, T4-*Bacillus*, T5- *Enterobacter*, T6-*Azotobacter* and T7- *Rhizobium*+ *Pseudomonas* + *Phosphobacteria* + *Bacillus* + *Enterobacter* + *Azotobacter*

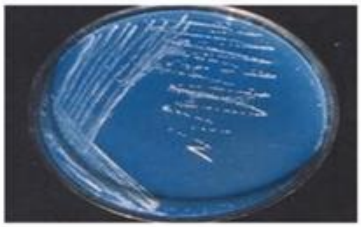
Rhizobium



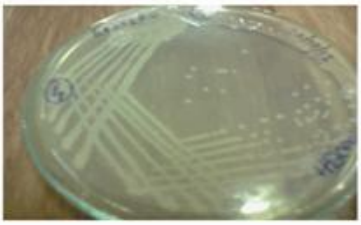
Azotobacter

Bacillus

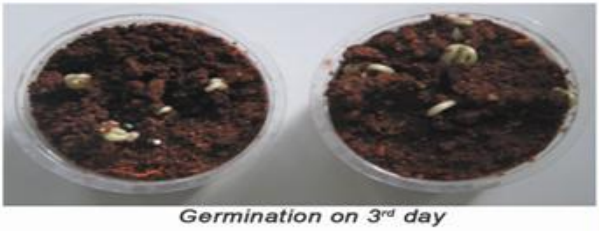
Phosphobacterium



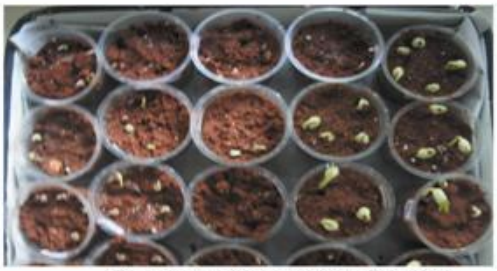
Azospirillum



Enterobacter

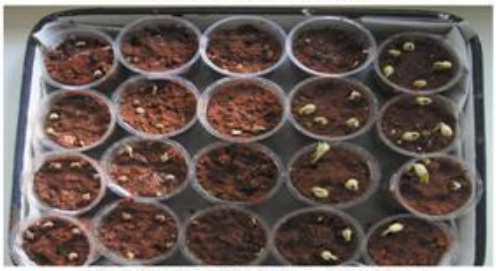


Germination on 3rd day



Plants on pot system-Batch-I

Germination on 5th day



Plants on pot system-Batch-II

Germination on 15th day

GERMINATION IN BATCH-I ON 9th day

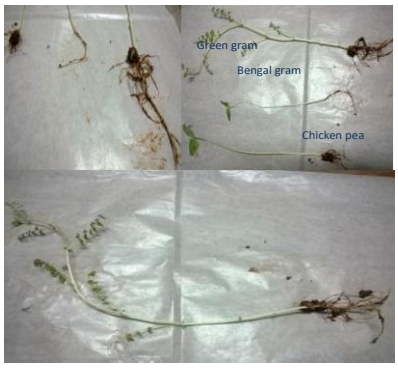
GERMINATION IN BATCH-II ON 9th day

GERMINATION IN BATCH-III ON 9th day

Table 4. Plant growth on Batch-II & III

Content	Bengal Gram		Chicken Peas		Green Gram	
	Batch-II	Batch-III	Batch-II	Batch-III	Batch-II	Batch-III
Stem length (cm)	23.4	21.3	32.4	38.6	26.1	28.6
Root length (cm)	12.3	12.2	17.3	19.4	15,3	16.2
Leaves count (No.)	17	19	29	38	24	29

Various root and stem length in three plants



Significant Leaves Count In Chicken Peas

3.3. Measurement of Length of Stem, Root and No. of Leaves

Ten plants were selected from those grown in a randomised block design in three replications and important yield attributing characters like number of leaves, length of the stem and length of root were observed and recorded.

3.4. Estimation of Chlorophyll and Carotenoid

The total chlorophyll and carotenoid content in Batch 1, Batch 2, and Batch 3 was estimated (Table 5, 6, 7). In Batch 1, high chlorophyll content was observed in T1 Chicken peas and high carotenoid content in T5 Green gram, T5 Chicken peas. In Batch 2, high chlorophyll and carotenoid content was observed in Chicken peas. In Batch 3, high carotenoid content was observed in Bengal gram and high carotenoid content in Green gram. The isolate T7 did not showed significant growth of green gram and Bengal gram. All the experiments were done with triplicates.

3.5. Existence of plant protein due to bacteria consortia

The protein content in all batches was estimated. In Batch 1, high protein content was observed in T5 when compared with others and low protein content was observed in T2. In Batch 2 and Batch 3, Chicken pea showed high protein content and Bengal gram showed low protein content.

Table 5 Estimation of Chlorophyll and Carotenoid on Batch - I

Sample (mg/g)		T0	T1	T2	T3	T4	T5	T6	T7
Green Gram	Chlorophyll	1.08	3.4	-	2.67	2.4	2.1	2.78	-
	Carotenoid	0.02	0.02	-	0.04	0.01	0.09	0.03	-
Chicken Peas	Chlorophyll	1.98	3.45	3.23	3.11	2.98	2.94	2.67	1.01
	Carotenoid	0.09	0.03	0.05	0.03	0.07	0.09	0.02	0.01
Bengal Gram	Chlorophyll	0.90	2.56	2.3	2.90	2.08	-	2.67	-
	Carotenoid	0.03	0.02	0.06	0.03	0.45	-	0.89	-

T0 – Control (without bacterial strain), T1- *Rhizobium*, T2- *Pseudomonas*, T3- *Phosphobacteria*, T4-*Bacillus*, T5- *Enterobacter*, T6- *Azotobacter* and T7- *Rhizobium*+ *Pseudomonas* + *Phosphobacteria* + *Bacillus* + *Enterobacter* + *Azotobacter*

Table 6. Estimation of Chlorophyll and Carotenoid on Batch – II & III

Sample (mg/g)	Bengal Gram	Chicken Peas	Green Gram
Chlorophyll	2.02	2.98	1.92
Carotenoid	0.56	0.89	0.71

Table 7. Estimation of Chlorophyll and Carotenoid on Batch - III

Sample (mg/g)	Bengal Gram	Chicken Peas	Green Gram
Chlorophyll	2.95	2.91	2.04
Carotenoid	0.45	0.55	0.78

Table 8. Estimation of protein on Batch-I.

Protein	T0	T1	T2	T3	T4	T5	T6	T7
Green gram	3.09	3.23	-	3.31	3.22	3.98	3.20	-
Chicken peas	3.45	3.67	3.34	3.12	3.76	3.44	3.71	3.33
Bengal gram	3.00	3.21	3.09	3.45	3.81	-	3.27	-

T0 – Control (without bacterial strain); T1- *Rhizobium*, T2- *Pseudomonas*, T3- *Phosphobacteria*, T4-*Bacillus*, T5- *Enterobacter*, T6- *Azotobacter* and T7- *Rhizobium+ Pseudomonas + Phosphobacteria + Bacillus + Enterobacter + Azotobacter*

Table 9. Estimation of protein on Batch-II & III

Protein	Bengal Gram	Chicken Peas	Green Gram
Batch –II	3.11	3.56	3.28
Batch –III	3.49	3.89	3.71

The present study clearly demonstrated that inoculation with plant growth promoting Rhizobacteria significantly enhanced the growth of Green gram in all dimensions. In this study application of microbial consortia in the preparation of biofertilizer has increased the total biomass of plants include root, stem and leaf count. Also it is showed that PGPR inoculation effectively increases surface area of roots and root weight. The earlier study also showed the similar findings (Cakmakc *et al.,* 2007). The variation in enhancement of plant growth by these strains may be due to the difference in the quantity of Chlorophyll and Carotenoid induced by each strain on the respective crop plant batches.

REFERENCES

Abbas, M and Rahim, (2013). Effect of Integrated Application of Bio-fertilizer on Grain Yield, Yield Components management and Associated Traits of Maize Cultivars.*American-Euraisan Journal of Agriculture and Environmental Science,* 271-277.

Cakmakc, R., M. Erat, U. Erdogan and M. F. Donmez, (2007). The influence of plant growth-promoting Rhizobacteria on growth and enzyme activities in wheat and spinach plants.*Journal of Plant Nutrition and Soil Science*, **170** (2): 288–295.

Crane, F.L., I. L. Sun, and M. G. Clark (1985).Transplasma-membrane redox systems in growth and development. *Biochimica et Biophysica Acta*, 81(3): 233–264.

Hughes, M.N and R. K. Poole, (1991).Metal speciation and microbial growth—the hard (and soft) facts.*Journal of General Microbiology*,**137**(4): 725–734.

Laemmli U.K., (1970). Cleavage of structural proteins growth-promoting bacteria. *Nature,* **287**:15-21.

Parvatham, A., K.P. Vijayan and A. Nazar, (1989). Effect of *Azospirillum lipoferum* on sorghum on growth and nutrient uptake of *Pusa sawani* bhendi, *South Indian Horticulture,* **32**: 305-308.

Rodriguez, H., R. Fraga, T. Gonzalez and Y. Bashan, (2006). Genetics of phosphate solubilisation and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil*, 267: 15-29.

Selvakumar, G., S. Reetha and P. Thamizhiniyan,(2012). Response of Biofertilizers on Growth, Yield Attributes and Associated Protein Profiling Changes of Black gram (*Vigna mungo* L. Hepper).*World Applied Sciences Journal,* **16**(10): 1368-1374.

Singh, R.P., S.C. Gupta,(1988). Associative of levels and sources of phosphorus and PSB on effect of *Rhizobium* and phosphate solubilising growth and yield of blackgram (*Vigna mungo* L. Hepper). *Legume Research*, **31**(2): 139-141

Vijla, K. and S. Jebaraj, (2008). Studies on the biofertilizers and phosphorous on NPK contents and improvement of Rhizobium-Green gram symbiosis uptake and grain quality of soybean. *Legume Research*, **32**(2) 126-129.

Wakatsuki, T.,(1995).Metal oxidoreduction by microbial cells. *Journal of Industrial Microbiology*, **14**(2): 169–177.