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# STUDIES ON POTENTIAL APPLICATIONS OF BIOINOCULANTS IN AGRICULTURE

**Rahul R. Shelke[1*], Deshpande S.N.[2] and Samrin Inamdar[2]**
[1]Shri Shivaji Mahavidyalaya Barshi. Solapur,Maharastra, India
[2]DBF Dayanand College Solapur, Maharastra, India
Corresponding author Email: rshelke087@gmail.com
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**ABSTRACT**

Microbes in groups - "Microbial Consortia" can do wonders. Microorganisms hold tremendous potential to be used for the betterment of agriculture field. Furthermore, they are more effective when they are combined altogether. Through their individual as well as mutual metabolic activities they would offer additional benefits. We are aiming to isolate some potential microorganisms having different features like siderophore activity, phosphate solubilizing, potash mobilizing and indole-acetic acid producing abilities. Such isolates could be effectively used to develop an effective microbial consortia and formulation that can be used as Bio-fertilizer for the advancement of agriculture field. Biofertilizer (additionally bio-compost) is a substance which contains living microorganisms which, when connected to seeds, plant surfaces, or soil, colonize the rhizosphere or the inside of the plant and advances development by expanding the supply or accessibility of essential supplements to the host plant. Biofertilizers include supplements through the common procedures of nitrogen obsession, solubilizing phosphorus, and animating plant development through the combination of development advancing substances. We prepared the concertia by using two organisms viz- *Bacillus IS1 & Pseudomonas IS2 spp*. On medium designed that containing vegetable waste. This media is very cheap and used as biofertilizer commercially.
***Keywords*** **:** Biofertilizer, consortia, agriculture, multinutrients.

## Introduction

Microorganisms hold tremendous potential to be used for the betterment of agriculture field. Furthermore, they are more effective when they are combined altogether. Through their individual as well as mutual metabolic activities they would offer additional benefits. We are aiming to isolate some potential microorganisms having different features like siderophore activity, phosphate solubilizing, potash mobilizing and indole-acetic acid producing abilities. Such isolates could be effectively used to develop an effective microbial consortia and formulation that can be used as Bio-fertilizer for the advancement of agriculture field. A single formulation with multiple benefits: Indian soils have been used for growing crops year after years without caring much for replenishing. Most importantly our Bio-fertilizer formulation allows much needed "Replenishing of soils". Offers protection to crop from being infected with diseases and thus enhanced production. Present chemical Fertilizers have hazardous effects not only on soil but on environment and human health also. Our formulation– in the form of "Microbial consortia" being Biological in origin won't show such negative impacts.

Nowadays, in India farmers are committing suicide because of agricultural loss or failure. Thus, we need to come up with solutions which will provide multiple benefits to the farmers.

Indian soils have been used for growing crops year after years without caring much for replenishing. This has led to depletion and exhaustion of soils resulting in their low productivity. The average yields of almost all the crops are among the lowest in the world. This is a serious problem which can be solved by using effective and more advanced Biofertilizers.

Smallholder farmers need to optimize their limited available resources to maximize their crop yield, especially in India where there is scarcity of water for irrigation. In such circumstances use of Bio-fertilizers would be an ideal measure as they aid in maximizing crop yield up-to 30% with additional benefits. "Microbial consortia" as biofertilizer formulation offers multiple benefits. They form a mutually beneficial or symbiotic relationship with host plants, protects them from diseases as they grow in the soil. Thus, they enhance the crop yield, boost the amount of organic matter and improve soil texture and structure.

## Materials and Methods

### 1. Isolation of Macro and micronutrient solubilizing bacteria from soil sample:

Isolation of nitrogen fixing bacteria was isolated on specific media that is congo red yeast extract mannitol agar medium. Isolation of phosphate solubilizing bacteria were isolated on specific media that is pikovskayas media.

Isolation of zinc solubilizing bacteria were isolated on specific media that is zinc oxide media. We wrote a culture characteristic after isolation of nitrogen fixing bacteria phosphate solubilizing bacteria and zinc solubilizing bacteria and also performed Gram staning of different isolates. Colonies showing zone of clearance on plate.

**2. Screening of isolates for plant growth promoting properties:**

**(a) Mineral solublization :** The bacterial endophytic isolates will be screened for phosphate solublizing, potash solublizing and zinc mobilizing properties.

**(b) Siderophore production :** Isolates will be checked for the production of siderophore on Blue agar CAS medium.

**(c) Phytoharmone production :** IAA production ability of isolates will be checked by using nutrient broth supplemented with 0.2% of L-tryptophan.

**3. Study of enzymatic activity of isolates:**

The agar diffusion method will be used to detect extracellular hydrolytic enzyme activity of isolates. The isolates will be grown on different media indicating different activity e.g. cellulase activity, amylase activity, lipase activity, pectinase activity.

**4. Evaluation of antibacterial and antifungal activity :**

Well-diffusion method will be used to test the antimicrobial activity of bacterial isolates.

**5. Identification of isolates showing maximum potential :**

Bacterial isolates showing maximum potential will be selected & their molecular identification will be done using biochemical and enzymatic activity.

**6. Pot and field experiment on selected crop plants :**

**(a) Pot preparation :** The soil from the farming fields will be collected, air dried & sieved. A control set of pot will be maintained without any treatment. The culture of different isolates will be added in different pots.

**(b) Seed sowing &harvesting:**The seeds of selected plants will be allowed to grow in each pot. The plants will be regularly monitored till harvest for gradual growth promotion. Plant growth parameters will be measured in an interval of 15 days.

**7. Effect of consortia on growth of selected plants :**

Identified bacterial strains showing high phytostimulent activity will be used for the preparation of consortia. Before the preparation of consortia, compatibility of isolates will be checked. Again the pot experiment will be performed for developed consortia & the results will be recorded.

## Results

Isolate 36 macronutrient and micronutrient producing bacteria, out of 36 isolates, 30 nitogen fixer, 4 Phosphate solubilization (PSB), 4 Potash Solubilization (KSB), 4 Indole acetic acid (IAA), 7 Zinc Solubilization (ZnS), 13 Catalse activity (CAt), 6 Chitinase activity (CAT), 9 Cellulase activity (CLL). 5 promising isolates for used for further study.

**Fig. 1**

**Table 1 :** Efficiency of Isolates

| Bacterial Isolates | Macronutrients | | Micronutrients | | Enzymatic Activity | |
|---|---|---|---|---|---|---|
| | PSB | KSB | IAA | ZnS | CLL | CAT |
| IS1 | ++ | - | +++ | ++ | +++ | - |
| IS2 | - | + | - | ++ | - | - |
| IS3 | + | - | + | + | + | + |
| **IS4** | **++** | **++** | **++** | **+** | **++** | **-** |

Where= - = No Production, += low Production, ++=Moderate Production, +++= Strong production Efficiency of Isolates shows Zone of clearance on Specific media.

| Identification Information | | Analysis Time: | 5.00 hours | Status: | Final |
|---|---|---|---|---|---|
| Selected Organism | | 99% Probability | Pseudomonas aeruginosa | | |
| | | Bionumber: | 0002043001500000 | | |
| ID Analysis Messages | | | | | |

Biochemical Details

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | IARL | - | 7 | dCEL | - | 9 | BGAL | - |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | - | 14 | GGT | + | 15 | OFF | - |
| 17 | BGLU | - | 18 | dMAL | - | 19 | dMAN | - | 20 | dMNE | - | 21 | BXYL | - | 22 | BAlap | + |
| 23 | ProA | + | 26 | LIP | + | 27 | PLE | - | 29 | TyrA | - | 31 | URE | - | 32 | dSOR | - |
| 33 | SAC | - | 34 | dTAG | - | 35 | dTRE | - | 36 | CIT | + | 37 | MNT | - | 39 | 5KG | - |
| 40 | ILATk | + | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | - | 44 | AGAL | - | 45 | PHOS | - |
| 46 | GlyA | - | 47 | ODC | - | 48 | LDC | - | 53 | IHISa | - | 56 | CMT | - | 57 | BGUR | - |
| 58 | O129R | - | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

**Fig. 2 :** VITEK -2 results

**Table :** 2 Biochemical test

| Culture Code | Gram's Staining | Motility | Fermentation of | | | Enzyme Activity | | Indole Production | Methyl Red | Voges-Proskauer | $H_2S$ Production |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Glucose | Sucrose | Lactose | Oxidase | Catalase | | | | |
| IS4 | Gram negative | Motile | + | + | - | - | + | + | - | + | - |

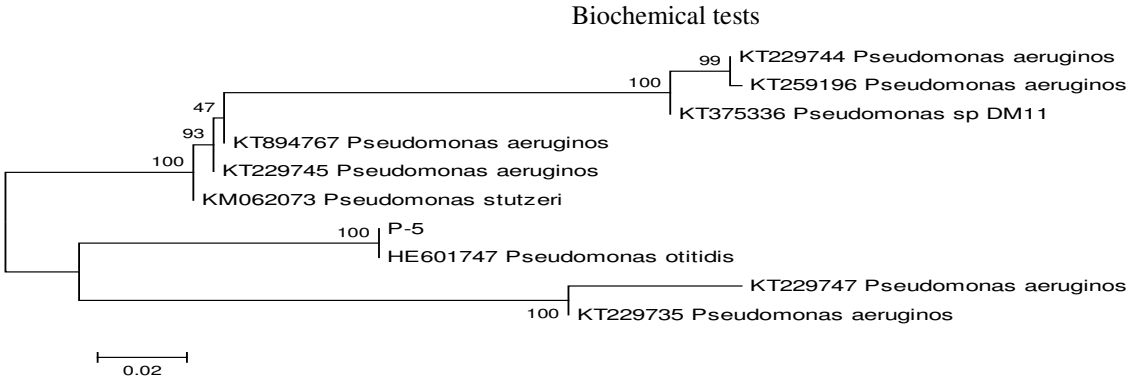


**Fig. 3 :** Phylogenetic analysis

**Two promising isolates *IS1*&*IS4*multiply on following media**

Here we are prepared artificial media 2g corn powder + 1gsoyabean powder + 100 ml distilled water. Stand the mixture for 30 min & then filter. Add 1g yeast extract + 1g Dextrose. Sterilize. Prepared liquid media.

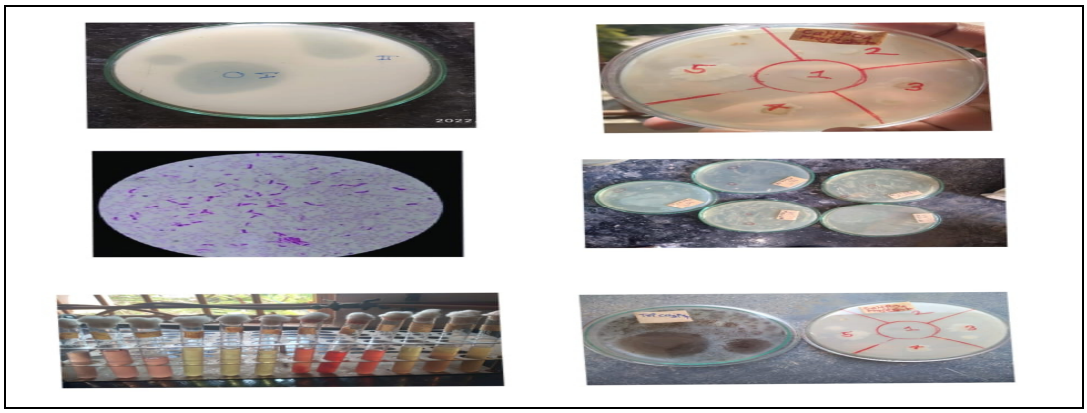

**Fig. 4 :** Isolation, gram staining, biochemical test

| Sr.No. | Parameters | Unit | Sample | F.C.O. Specification 1985 |
|---|---|---|---|---|
| 1 | Base | - | carrier | Carrier based powder/granule/liquid |
| 2 | Viable cell count | Cfu/ml | **$2 \times 10^9$** | Min. $1 \times 10^8$ |
| 3 | Contamination level | Cfu/ml | No contamination | No contamination at $10^5$ dilution |
| 4 | pH | - | 6.8 | 5.0 – 7.5 |
| 5 | Efficiency character | % | 35% | The strain should be have phosphate solubilizing capacity in range of min. 30% when tested by spectrophotometrically |
| | | Mm | 5mm | In terms of zone formation min. 5mm solublization zone in prescribed media having at least 3mm thickness |

**Fig. 5 :** as per FCO (Fertilizer Control Order)
Analysis of Broth after incubation

IS1 & IS4 have capacity to produce multiple nutrient so these two isolate identified with the help of different biochemical test these isolates grow in artificially produced media (**2g corn powder + 1g soybean powder + 100 ml distilled water. Stand the mixture for 30 min & then filter. Add 1g yeast extract + 1g Dextrose)** media sterilized and inoculate promising isolate and incubate broth at $37^0$C at 48 hrs. After incubation 2 x $10^8$ CFU/ml are observed as per FCO minimum 1 x $10^8$ CFU/ml required.

**Quality Checking**

Check viable count in the carrier based inoculants & Bacterial consortia by dilution plate method at the time of manufacturing. The viable cells count in the carrier based inoculants should be maintained as per ISI & F.C.O. specifications.

**Two Promising isolates used in Pot Experiment**

**Table 3 :** Agronomic parameters

| Agronomic parameters | Control plants (Jawar) | Inoculated plants |
|---|---|---|
| | C | Consertia of IS1 & IS4 |
| Root weight (g) | 2.95g | 7.52g |
| Aerial weight (g) | 1.26g | 4.22g |
| Stem diameter (cm) | 0.1cm | 0.3cm |
| Leave's number | 4 | 8 |

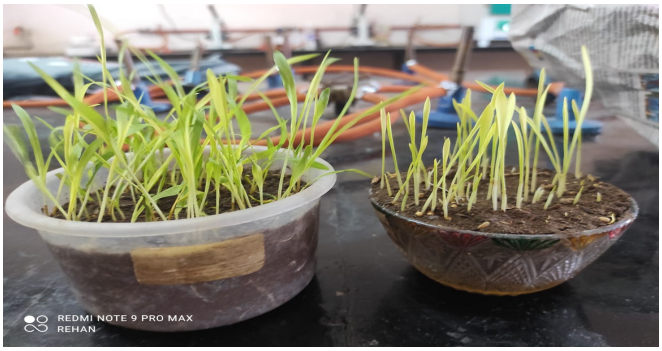


**Photo-1**

## Discussion

Zinc, Nitrogen and phosphate is the key constituent of plants and is very crucial for their development. Zinc deficiency is the most common macronutrient deficiency in crop worldwide and results in substantial losses in crop yields use of zinc fertilizers may not be cost effective in alleviating zinc deficiency and increasing crop yield. Nitrogen deficiency is the most common primary macronutrient deficiency in crops worldwide and results in slow growth and uniform yellowing of older leaves in crop yields. Use of nitrogen fertilizers may not be cost effective in alleviating nitrogen deficiency and increasing crop yield. Bashan & de-Bashan(2005) reported that the single microbial product shows inconsistency in performance. So there is need of co-inoculants or consortia of microbial products.

Phosphates deficiency is the most common macronutrient deficiency in crops worldwide and results in leaves turn dark, dull, blue, green and may become pale in severe deficiency in crop yields. Use of phosphate fertilizers may not be cost effective in alleviating phosphorus deficiency and increasing crop yields.

## Conclusion

Product prepared by us contains the concertia of two organisms viz- *Bacillus Species* (IS1)*& Pseudomonas spp* (IS2). On medium designed that containing vegetable waste. This media is very cheap and used as biofertilizer commercially. The Biofertilizers are ecofriendly, pollution free, unhazardous, and non-toxic to human being. The Biofertilizers increase macronutrients and micronutrients and also increases soil fertility.The product prepared by us contains the isolate which having more efficiency in comparison with today's isolates used in other biofertilizer product.

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