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Study on Development of Consortia for NPK Bacteria and its Effect on Growth of Selected Crops

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ABSTRACT

A group of beneficial bacteria provides an ecofriendly and economically sound approach to low-input farming systems and for better plant productivity. The present study was conducted during 2018-19 at MGM's Institute of Biosciences and Technology, Aurangabad, Maharashtra to develop consortium of Nitrogen (N), Phosphorus (P) and Potassium (K) bacteria on growth of various crops like Cotton, Maize, Jowar, Cow Pea, Moth Bean, Chili, and Tomato. For development of consortium, seven bacterial strains were taken for nitrogen fixation, solubilization of phosphorus and potassium. Out of seven, three bacterial strains were selected on the basis of positive compatibility test. All these three bacteria were used as *A. chroococcum* for nitrogen fixation, *B. megaterium* for phosphorous solubilization and *F. aurantia* for potassium solubilization. Bacterial consortium was formulated by inoculating three compatible bacterial strains by using emulsifier, dispersant, cell protectant, moisturizer and humectants. Consortium 4 was showed highest population count of *A. chroococcum* (12×10^{10} CFU/ ml), *B. megaterium* (10×10^{10} CFU/ ml) *& F. aurantia* (6×10^{10} CFU/ml) after one month over other consortium. Selected consortia 4 were treated with crops at two different concentrations as 50% and 100% respectively to study the seed germination percentage. Seed treated with consortium 4 showed significant enhancement in seed germination of all crops over control. Instead of giving single NPK bio-formulation, consortium of NPK bacteria is best way to avail NPK nutrients to plant for growth and development.

Key words: *Azatobacter chroococcum, Bacillus megaterium, Frateuria aurantia, NPK consortia, Seed germination*

Introduction

Nitrogen (N), phosphorus (P) and potassium (K) are major essential elements for plant growth and are found in every living plant cell. Nitrogen is an essential component of proteins that build cell material and plant tissue. In addition, it is necessary for the function of other essential biochemical agents, including chlorophyll, many enzymes, and nucleic acids such as DNA, RNA. Major forms of inorganic N in soil are nitrate and ammonium, which plants absorb from roots. (Takuji, 2010). *Azatobacter* is a non-symbiotic bacterium that has the ability for fixation of nitrogen and active colonizes to increasing growth and crop production because of ability to produce growth hormone such as auxin, cytokine and gibberellin (Leveau and Lindow, 2005).

Phosphorus is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to next generation. In the soil, phosphorus is often found in chemical forms that cannot immediately be absorbed by plants. Common source for commercial phosphorus fertilizer is rock phosphate, a calcium phosphate ore found in deposits within the earth. Plants most often absorb phosphorus in the form of phosphate ions

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$H_2PO_4^-$ and sometimes as HPO_4. Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tri-calcium phosphate, di-calcium phosphate, hydroxyapatite, and rock phosphate. Among the bacterial genera with this capacity are *Pseudomonas, Bacillus, Burkholderia, Achromobacter, Agrobacter-ium, Microccocus, Aereobacter, Flavobacterium and Erwinia* (David *et al*., 2014; Istina *et al*., 2015; Chakraborty *et al.,* 2009; Postma *et al*., 2010).

Potassium is important for enzyme activation, stomatal activity, photosynthesis, transport of sugars, water and nutrient transport, protein synthesis (Prajapati and Modi, 2012). Plants absorb potassium only from soil and its availability in soil is dependent upon the K dynamics as well as on total K content. Out of the three forms of potassium found in the soil, soil minerals make up more than 90 to 98 percent of soil potassium and most of it is unavailable for plant uptake. Second non-exchangeable form of potassium makes up approximately 1 to 10 percent of soil potassium and consists predominantly of interlayer K of non-expanded clay minerals such as illite and lattice K in K-feldspars, which contribute significantly to plant uptake. A wide range of bacteria namely *Pseudomonas, Burkholderia, Acidothiobaci-llus ferrooxidans, Bacillus mucilaginosus, Bacillus edaphicus, B. circulans* and *Paenibacillus* sp. has been reported to release potassium in accessible form from potassium-bearing minerals in soils. These potassium solubilizing bacteria (KSB) were found to dissolve potassium, silicon and aluminium from insoluble K-bearing minerals such as micas, illite and orthoclases, by excreting organic acids which either directly dissolved rock K or chelated silicon ions to bring K into the solution (Gore and Navale, 2019).

Bio-fertilizers keep the soil environment rich in all kinds of nutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization (Gore *et al.,* 2020; Nileema and Annasaheb, 2016), release of plant growth regulating substances, increase soil fertility, fertilizer use efficiency and ultimately the yield by 20-30%. However, bio-fertilizers cannot replace chemical fertilizers, but they can reduce the utilization of chemical fertilizers and support sustainable agriculture. Inorganic fertilizers are known for their high cost and their negative environmental effects if managed poorly (Morris, 2007). All these give rise to soil degradation and nutrients imbalance (Ojeniyi, 2000).

Successful formulation of microbial consortium of nitrogen fixer, phosphorus and potassium solubilizing bacteria capable of nitrogen fixation, solubilizing phosphorus and solubilizing potassium mineral (Mica) rapidly in large quantity can conserve our existing resources and avoid environmental pollution hazards caused by the heavy application of chemical fertilizers. Hence, the present investigation on "Development of Consortia of N Fixing, P and K Solubilizing Bacteria for growth of crops" is proposed.

Materials and Methods

Collection of bacteria

The work was carried out during 2018-19 at Mahatma Gandhi Mission's Institute of Biosciences & Technology, Aurangabad, Maharashtra, India. Bacterial strains were collected from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India. Nitrogen fixing strains were *Azotobacter chroococcum* (NCIM No. 5576), *Azotobacter indica* (NCIM No. 2055)*, Rhizobium sp. (NCIM No. 2228).* Phosphate solubilizing strains were *Bacillus megaterium (NCIM No. 5681), Bacillus polymyxa* (NCIM No. 2539) *and Pseudomonas fluorescens* (NCIM No. 2174). *Potassium solubilizing bacteria was Frateuria aurantia (ATCC – 33424). All cultures were maintained in pure form on selective media viz., A. chrococcum (ATCC medium no. 14), A. indica (ATCC medium no. 14), Rhizobium sp .(Locchead's medium), B. megaterium (*Nutrient agar*), B. polymyxa* (Nutrient agar), *P. fluorescens* (Nutrient agar) and *Frateuria aurantia (Manitol Agar, ATCC medium 1).*

Qualitative test of NPK bacteria

Qualitative test of nitrogen fixing bacteria (N bacteria) for nitrogen fixation, phosphate solubilizing bacteria (P bacteria) for phosphate solubilization and potassium solubilizing bacteria (K bacteria) for potassium solubilization was done. In case of nitrogen fixation *Azotobacter* medium (Subba Rao, 1977; Anon., 1992) was used. Ability of phosphate solubilizing bacterial (PSB) strains to solubilize insoluble form of phosphate was tested by spotting 10 µl overnight grown cultures on Pikovaskaya's agar plates and incubating at 28-30 °C for 2-3 days. Ability of potassium solubilizing bacterial (KSB) strains to solubilize insoluble form of Muscovite mica was tested by spotting 10 µl overnight grown cultures on

Aleksandrov's agar plates and incubating at 28-30°C for 2-3 days. Solubilization zone was calculated by diameter of zone of clearance (D)/ diameter of growth (d) (Gore and Navale, 2019).

Compatibility of NPK bacteria

Nine combinations of seven bacterial strains were selected for the test of compatibility. For checking compatibility, three different bacteria were streak on one nutrient agar plate in triangular form in triplicate. When bacteria streak on modified media plate, all three triangles joined to each other. Different modified media used to test compatibility of NPK bacteria.

Development and Population count of consortium of NPK bacteria

Different modified five media were prepared for selected compatible NPK bacteria for consortium. Modified consortium media were incubated on incubator shaker at 120 RPM for 3 days at 28 °C. Population count was taken after 3, 8 days and further monthly.

Effect of consortium on seed germination of different crops

Seeds of different crops such as legumes like Cowpea (*Vigna unguiculata*) and Moth Bean (*Vigna aconitifolia*), vegetables like Chili (*Capsicum anum*) and Tomato (*Solanum lycopersicum*), cereals like Sorghum (*Sorghum bicolor*) and Maize (*Zea mays*), cash crop Cotton (*Gossypium hirsutum*) were collected from different local area of Aurangabad, Maharashtra. All seeds were surface sterilized with 0.1% mercuric chloride. All seeds treated with test consortium media of NPK. Seeds were treated with bio-fertilizer consortium at different concentrations (50% and 100% concentration) for different time interval *viz.*, 1 min, 5 min, 10 min, 15 min & 20 min.

Statistical analysis

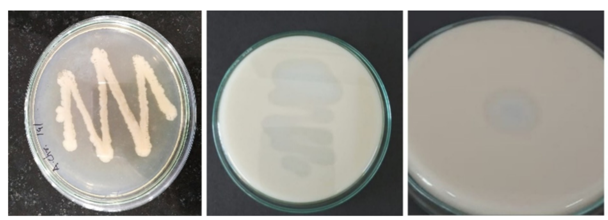
Statistical analysis of data was carried out by using completely randomized design and interpretation of results was carried out in accordance with Panse and Sukhatme (1985).

Results and Discussion

Qualitative test of NPK bacteria

In case of nitrogen fixing bacteria *A. chroococcum* and *A. indica* showed growth on selective media i.e. Jensen's media and *Rhizobium sp*. showed growth on yeast extract manitol agar media. Three bacteria of phosphate solubilization were selected *viz.*, *Bacillus megaterium*, *Bacillus polymyxa* and *Pseudomonas fluorescens*. Out of three bacteria of phosphosphat solubilization bacteria *B. megaterium* was showed highest zone of solubilization on Pikovskaya's agar. Diameter of zone of solubilization by the *B. megaterium* was 15 mm at 48 h. Potassium solubilizing bacteria *F. aurantia* showed zone of solubilization on Aleksandrov's media. Diameter of zone of solubilization by bacteria was 19 mm at 48 h (Fig 1).

Similarly, Sandeep *et al.* (2015) studied *Azotobacter* species on Jensen's medium from different areas of Uttarakhand. Uma and Sathiyavani (2012) screened bacteria for phosphate solubilization on Pikovskaya's medium and studied phosphate solubilizing ability by spot inoculated at the centre of Pikovskaya's plate and incubated at 37 °C. Diameter of zone of solubilization was measured successively after 24 h, upto 7 days. Gore *et al.* (2015) studied potassium solubilization bacteria for potassium solubilization activity by using Aleksandov's media and found *Pseudomonas fluorescens*, *Pseudomonas putida* and *Bacillus megaterium* were efficient potassium solubilizers.



A B C

Fig. 1. Qualitative test of N, P & K bacteria (A) N_2 fixer (*A. Chroococcum*) on Jensen's media, (B) Phosphorussolubilizer (*B. megaterium*) on Pikovskaya's media, (C) potassium solubilizer (*F. aurantia*) on Aleksandrov's media

Compatibility of NPK bacteria

Compatibility test was observed for nine combinations of eight bacterial strains of N, P and K bacteria. Out of nine combinations, one combination of N, P and K bacteria was showed highest compatibility on nutrient agar viz. *A. chroococcum, B. megaterium and F. aurantia.* Inhibition was not observed for this combination. On the basis of compatibility test one combination of N, P and K bacteria, i.e. *A. chroococcum, B. megaterium & F. aurantia* were selected for further study.

This test is similar with the findings of Nileema and Annasaheb (2010) that carried compatibility test for development of consortium of different potassium solubilizing bacteria. Compatibility of the inoculants *Rhizobium* sp., *Bacillus megaterium* and *Pseudomonas fluorescens* tested through cross streak plate assay. The inoculants were found to be compatible with each other and were able to grow simultaneously without any inhibition in growth (Anandaraj and Lemma, 2010).

Development and Population count of consortium of NPK bacteria

Five different consortium i.e. modified media were prepared for development of consortium of N, P & K bacteria (Fig 2). On the basis of their nutrient requirement of different nutrient were selected for development of consortium. For the preparation of suitable medium and to increase the shelf-life of NPK bacteria, base material which contains emulsifier, dispersant, cell protectant, moisturizer and humectants etc. were used in different concentrations (Chandra, 2009).

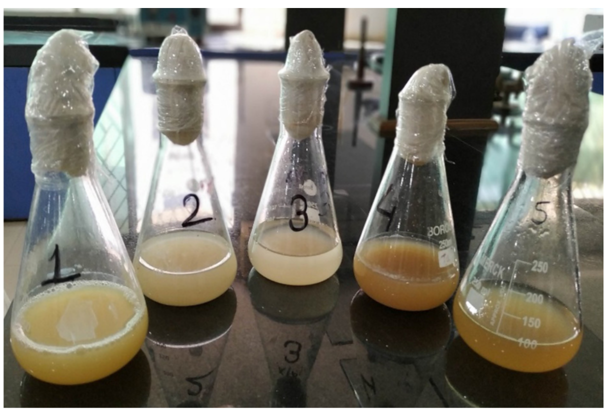


Fig. 2. Consortium of NPK bacterial media

In present study, population count of all developed five consortium were analyzed for population count after 3 days, 8 days and 1 month (Table 1). Consortium 4 was showed highest population count after 1 month as compare to other consortium. In case of consortium 4, population count of *A. chroococcum* (12×10^{10} CFU/ ml), *B. megaterium* (10×10^{10} CFU/ ml) *& F. aurantia* (6×10^{10} CFU/ ml) was observed after one month. On the basis of population count of modified media, media 4 were selected for further study i.e. application to different crop seeds.

In support of these findings, three most efficient *Pseudomonas* sp. were grown on five test media for finding out the most suitable medium for mass multiplication of KSB (potassium solubilizing bacteria) consortium. Population count of three potassium solubilizing bacteria consortium was showed highest population 24.6×10^8 cfu/ml of formulation, at 30 days (Nileema and Annasaheb, 2010).

Effect of consortium on germination percentage of different crops

Application of consortium 4 of N, P and K bacteria showed significant enhancement in seed germination of Cotton, Maize, Sorghum, Moth Beans, Cow Pea, Chili and Tomato. Application of NPK consor-

Table 1. Population count (cfu/ml) of NPK bacteria on different consortia media

		A. Chroococcum	*B. megaterium*	*F. aurantia*
Consortium 1	3 Days	5×10^8	13×10^8	32×10^8
	8 Days	10×10^8	11×10^7	65×10^8
	1 Month	4×10^{10}	9×10^9	12×10^9
Consortium 2	3 Days	33×10^4	50×10^4	10×10^5
	8 Days	1×10^5	1×10^5	1×10^5
	1 Month	1×10^8	1×10^8	1×10^8
Consortium 3	3 Days	3×10^5	5×10^5	7×10^5
	8 Days	2×10^5	3×10^6	1×10^5
	1 Month	1×10^7	10×10^8	8×10^7
Consortium 4	3 Days	3×10^8	7×10^8	2×10^8
	8 Days	2×10^9	3×10^9	2×10^8
	1 Month	12×10^{10}	10×10^{10}	6×10^{10}
Consortium 5	3 Days	8×10^7	30×10^7	21×10^6
	8 Days	1×10^8	1×10^8	2×10^5
	1 Month	45×10^8	37×10^8	28×10^8

Cfu: Colony forming unit; N: Nitrogen; P:Phosphorus; K: Potassium tium in two concentrations 50% and 100% was carried out at different time interval *viz.*, 1 min, 5 min, 10 min, 15 min and 20 min (Fig 3, 4). It was observed that the germination of seeds started on the third day in the case of all crops except Chili and Tomato. Tomato required 4 days and Chili required 5 days to germinate at both concentrations 50% and 100%. But in case of control (non-treated), all crops required 7 days to germinate. In case of 50% application of consortium 4 of N, P & K bacteria, 100% seed germination was recorded in maize (20 min), cowpea (10 min) and mothbean (20 min) at different time intervals but in control lowest seed germination was observed in maize (66.66%) and cowpea (66.66%). In case of Chili, Tomato and sorghum highest 83.33% seed germination was observed in 20 min but in cotton highest seed germination percentage was 66.66% within 1 min application of consortium and in control of cotton lowest 16.66% seed germination was observed. In case of 100% application of consortium 4 of N, P & K bacteria, 100% seed germination was recorded in cotton, sorghum, maize, cowpea and mothbean at different time interval but in control lowest seed germination was observed. In case of chili and tomato highest 83.33% seed germinations was observed at 5 min and 10 min respectively which was highest percentage as compare with control.

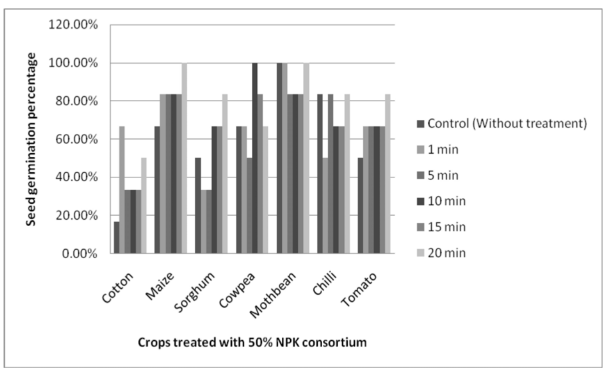


Fig. 3. Effect of NPK bacterial consortium (50%) on seed germination of different crops

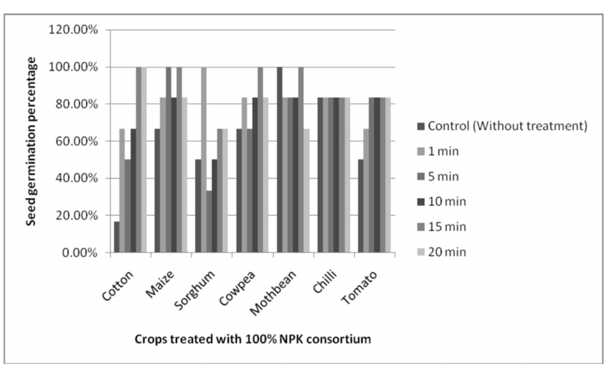


Fig. 4. Effect of NPK bacterial consortium (100%) on seed germination of different crops

Use of plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria as bio-fertilizers, has been suggested as a sustainable solution to improve plant nutrient and production (Vessey, 2003). Raja (2012) studied seed treatment with biofertilizers showed significant enhancement in seed germination of *Jatropha curcas* Seed germination of Jatropha plants under different treatments ranged from 24 % to 80%. Highest seed germination rate of 80% was recorded in T_5 – *Azospirillum* + Phosphate Solubilizing Bacteria (PSB). T_6 – *Azospirillum* + *Azotobacter* + Phosphate Solubilizing Bacteria (PSB) (78%) and T_7 – *Azospirillum* + *Azotobacter* + Phosphate Solubilizing Bacteria (PSB) + AM fungi treatments which was 56 % higher over uninoculated control. *Azotobacter* reported apart from atmospheric nitrogen fixation, it has other properties which are considered to be responsible for their beneficial effect like ability; to produce vitamins and growth substances which enhance seed germination in crops (Sood and Sharma, 2001; Shende *et al.*, 1977). Phosphorus is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available for root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and Phosphate and potassium rocks are a cheaper source of P and K; however, they are less readily available to plants because the minerals are released slowly and their use as a fertilizer. The use of phosphorus and potassium solubilizing bacteria enhances the availability of P and K to plants and increase plant health and yield (Ahmad *et al.*, 2009).

The consortia of Nitrogen fixer *A. Chroococcum*, Phosphorus solubilizer *B. megaterium* and Potassium solubilizer *F. aurantia* is the cheapest and environment friendly way to growth and development of cereals, legumes, vegetables and cash crops like, maize, sorghum, cowpea, moth bean, chili, tomato and cotton. Bio-fertilizer is one way to achieve sustainable productivity and environmental security of crops. Bio-fertilizers will also reduce the use of chemical fertilizers. However, there is need to study this consortium on field trail specific to various regions which will definitely increase the yield of crops and this should be communicated to farmers in relatively short time.

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