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POTASH MOBILIZING BACTERIA

(Frateuria aurentia)

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ABBREVIATIONS

PGPR	-	Plant Growth Promoting Rhizobacteria
KMB	-	Potash Mobilizing Bacteria
KMM	-	Potash Mobilizing Micro-organisms
GYCaA	-	Glucose, Yeast, Calcium Agar
Co_2	-	Carbondioxide gas
SSS	-	Silicate Solubilizing Bacteria
OA	-	Organic acid
K-10	-	Code name of Frateuria aurentia
MPYA	-	Mannitol, Peptone, Yeast, Agar
MPMgA	-	Mannitol, Peptone, Yeast, Magnesium, Agar
GYNiA	-	Glucose, Yeast, Nickle, Agar
$ZnSo_4$	-	Zinc Sulphate
CuSo4	-	Copper Sulphate
Mn	-	Manganese
NH4	-	Ammonium
N	-	Nitrogen
P	-	Phosphorus
ppm	-	Parts per million
VAM	-	Vesicular Arbuscular mycorrhae (VAM)

PREFACE

Micro-organisms in soil play a key role in mineral transformations rendering nutrients available to the plant. Soil biological activity readily mineralize the nutrients from organic matter or from the soil fixed reserve. Beneficial micro-organisms like *Azospirillum, Azotobacter and Rhizobium* that can fix the atmospheric nitrogen, phosphate solubilizing bacteria like *Bacillus megatherium* var. *phosphaticum* and *Pseudomonas* spp. and phosphate mobilizing fungus like *Arbuscular Mycorrhizae* (AM) are being supplied as inoculants to supplement the crop requirements. Plants require almost 20 nutrients to a varying degree and many of them can be solubilized by soil microflora.

Horticultural crops have more potassium requirement which is not supplied sufficiently or organically. But yet crop absorb it from the soil. In soil, potash occurs in different forms but available forms often limits the crop growth. If potassium made available by biological action (or) by physico chemical action like weathering, microbial solubilization / mobilization, the dependency of chemical potash fertilizer could be minimized as well as it serve as new organic input for organic farming for potassium need.

The inoculant for potash mobilization / solubilization isolated and tested are likely to become the newer microbial inoculant that can be of greater use in reducing the cost in chemical potash fertilizers. Though question remains unanswered regarding its mode of action like other biofertilizers, the crop response for the organism in laboratory as well as in field is very much encouraging. Now a days all the biofertilizer industries have included this potash mobilizing biofertilizer as one of the product, interestingly this inoculant is being used by many farmers because of its crop response not only as potash nutrition and also as Plant Growth Promoting Rhizobacteria (PGPR).

In this booklet all the details so far available regarding this inoculant are tried to compile and provide a preliminary documentary to the scientist, organic input manufacturers, farmers. Hopefully, this booklet will provide all information available at present and make the way to review and start new research efforts to make possibilities of tapping this natural reserve to sustain soil fertility and crop productivity.

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1. INTRODUCTION

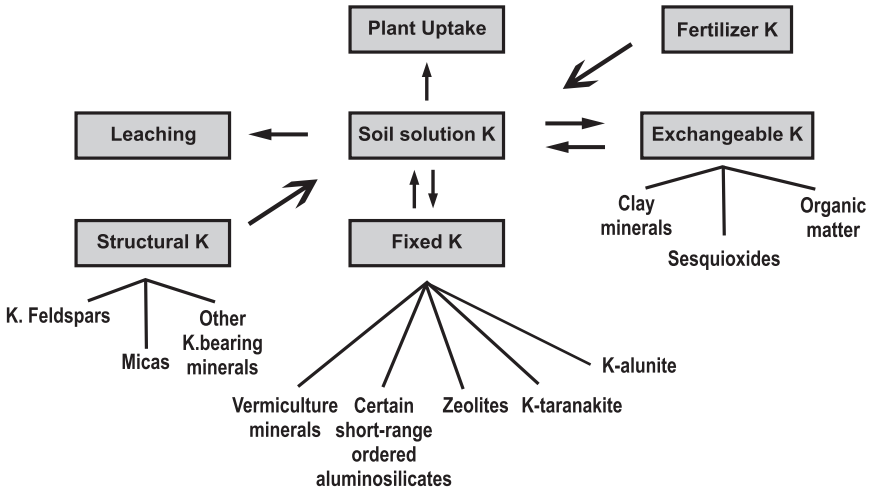
Fertilizer is one of the key inputs for enhancing the crop productivity. Balanced fertilizer application is imperative for sustained productivity. In India, the problem is compounded by imbalanced and indiscriminate fertilizer use, leading to widening of NPK ratio from 5.9:2.4:1 in 1991-92 to 8.5:2.5:1 in 1995-96 and 10:2.9:1 in 1996-97 as against the optimum ratio of 4:2:1. Most of the soils are deficient in nitrogen, but application of nitrogen alone fails to produce sustainable yields over a long period and is found that deleterious effect in phosphorus and potassium deficient soils. The regional disparities in fertilizers use are also limiting the overall agricultural production. There is a big gap between the nutrients applied and those removed by the crops under intensive agriculture. This gap is estimated by 9.8 million tonnes during 1988-89 and 8.8 million tonnes during 1999-2000. India is using about 17.5 million tonnes of plant nutrients (NPK) and is likely to need about 45 million tonnes by 2025.

The results of long term fertilizer experiments conducted over 25 years under intensive cropping on a variety of soils in different agro climatic situations shows considerable decline in crop productivity despite the application of recommended doses of NPK. This is primarily due to emerging deficiencies of micro and secondary nutrients such as Zn and S, that have become limiting factors. Depletion of K from the native soil reserves had necessitated higher rates of K application for sustainable yield increase in rice-rice and maize-wheat cropping systems. The results also show a decline in soil organic carbon due to prolonged use of chemical fertilizers. The production efficiency gone down appreciably. Thus, higher productivity on a sustained basis can be ensured only through integrated nutrient supply system including combined judicious use of chemical fertilizers, FYM, organic residues, green manures and biofertilizers (Yadav 2002).

A study conducted by Murugappan *et. al.* (2002) indicates that potassium is the limiting factor for crop production as nutrient deficiency in the soil series *of* Pechiparai, Madukkar, Ooty, Padugai, Kalathur, Irugur and Palladam in Tamil Nadu, and suggested to add 5.0 to 15 mg K / kg soil fertilizer application schedule.

2. Status and availability of Potassium in soils

Potassium (K) has been recognized as being beneficial to plant growth (Russell 1961). It is usually abundant in soils. Total K contents in soils range between 3000 to 100,000 kg/ha in the upper 0.2m of the soil profile. Of this total K content 98% is bound in the mineral form whereas 2% is in soil solution and exchangeable phases. (Schroeder 1979; Bertsch and Thomas 1985). Dynamic equilibrium reactions exist between the phases of soil K.



(Figure - 1 shows the interrelationships of various forms of soil K. Sparks and Huang,1985).

Certain Short range ordered aluminosilicates

The forms of K in soil in the order of their availability to plants and microbes are

i) Water soluble
ii) Exchangeable
iii) Non-exchangeable

iv) Mineral

The first two forms of 'K' could be availed by plants according to its need, but later two forms involves different mechanism to release into soil solution. Song and Huang (1983) found the sequence of K release from K-bearing minerals by Oxalic and citric acids is in the order of biotite, microcline, muscovite. The dynamic reactions that exist between the four forms of potassium are controlled by a number of factors. Perhaps, the most important is the type and quantity of organic and inorganic colloids present in the soil. Although, more efforts were made to understand the potassium availability and its importance in crop yield. Equal amount of unanswered questions remain (Chandra *et. al.*, 2000).

Release of potassium in soil

Kinetics of K and absorption from solution to exchangeable phase were investigated using modified Freundlich equation on eight soils representing seven soil series, differing in clay content and mineralogy. Potassium absorption with time was evaluated on Ca- saturated soil samples using 50ppm K solution equilibrated on 1.0, 1.5, 3.0, 24.0, 48.0 and 120.0 hrs, Ca-K exchange reaction attained an equilibrium in 3 hrs respectively of the soil type and was instantaneous. This slow rate of K absorption was attributed to diffusion controlled exchange. (Sharma and Mishra, 1991).

Laboratory investigation was made to study the kinetics of non-exchangeable K release in Nagar, and Kanganwal and Badowal soils developed on recent flood plain, old flood plain and sandune respectively in Punjab state differing in K-status, clay, mineralogy and texture. The calculated rate constant and cumulative K release in both NATPB and cation exchange resins followed the order: Kangawal > Nagar > Badowal. Amongst the cation exchange resin extracted relatively higher amount of K. The calculated diffusion co-efficients varied in the range of 0.38×10^{-8} to $8.96 \times 10^{-8} cm^2$ for differention pair system. (Sidhu and Manjaioh, 1999).

Potassium may be released due to replacement by other cations such as Ca from the interlattice positions of micaceous clay

minerals. Release of potassium is associated with the expansion of clay mineral lattices. Exfoliation of illite upon drying especially in presence of $CaCO_3$ is important for supplying K during drying, surface tension forces acting on the weathered edges of micaceous minerals of regions of those minerals which are interstratified with expansible layers and non expansible layers, expose potassium atoms which are replaced by aluminium ion from the lattice. The development of visible cracks in the interlattice positions due to drying, which exposed K. The extent of potassium released by different soils varied considerably depending on genetic character of soils and the numerous factors influencing them. Different crops have got different abilities to use non exchangeable potassium reserves.

Amount of K fixation in different soils vary considerably. There was variation of potash fixation from 2.7 to 60%. In silty loam and clay soils a fixation of 0.38 me K/100g of soil in surface soils and 0.77 me K/100g in subsoil which constituted about 15 and 30% of the added potassium respectively.

Role of K in plants

Potassium plays a vital role in the formation of amino acids and proteins from ammonium ions, which are absorbed by roots from the soil. It is also responsible for the transfer of carbohydrates, proteins etc. from the leaves to the roots. It also plays a vital role in the uptake of other elements particularly nitrogen, phosphorus and calcium, potassium regulates the permeability of the cellular membrane. It increases the hydration of protoplasm. It activates number of enzymes, eg. Alcohol dehydrogenase and its deficiency decreases photosynthesis. Potassium increases the resistance of crops to hot and dry conditions and insect pests and diseases. It increases the stiffness of straw in cereals and therefore the lodging of cereals is reduced. It improves the quality of fruits and grains.

Potassium deficient plants have a stunted and bushy growth. Pale green, older leaves develop chlorisis between veins and light grey to brown, reddish brown or brown colourisation along the leaf apex and the apical margin. The leaf tip and apical margin of the leaf become scorched and necrotic. Thin brown roots

are poorly developed; the small leaflets of the potato are crinkled and curved downwards. The photosynthesis and associated leaf characters of Bidi tobacco as influenced by the levels of potash. Leaf area, chlorophyll content and concentration of N and K increased with the increase of potash level.

With increased cropping intensity and large scale cultivation of high yielding varieties become fairly wide spread and is tending to create a greater need for external supply of this major nutrient. Significant increase in Wheat yield due to addition of K and Mn has been duly envinced particularly with fertilizer responsive improved genotypes.

Role of soil microorganisms in Potassium availability and uptake

Rhizosphere region is the soil adhering to the roots or soil volume well permeated by roots or soil influenced by the roots. The number of organisms in the rhizosphere region is greater than that of the unplanted soil. The root surface secretes significant quantities of organic compounds and the organic acids secreted by microorganisms can influence the transformation and availability of nutrients and also establishment of plants. Thus, the root-zone (Rhizosphere) soil differs physically, chemically and biologically from bulk soil.

Although we concentrate much more approaches towards chemical dynamic, equilibriums inorganic colloids and thermodynamic etc. for making available K to plants in right time but a big gap or very less studies were made on the possibilities or role of organic colloides and rhizosphere microorganisms in the Potassium availability and uptake. A interesting study made by Saraswathy *et. al.,* 2003, to study the influence of root systems on the chemical properties of soil particularly in Potassium contents and its availability indicates the following findings.

1) The available potassium was higher under root zone soil than the unplanted soil (Bulk soil).
2) The available potassium increased with population levels (no

of tillers / m^2) in the case of rice grown under acidic soil. Where as the reverse was with alkali soils.

It indicates the enhanced potassium uptake including luxury consumption and Kuch Enbuch (1987) reported that when potassium content was depleted there was an increase in the potassium flux towards the root mainly through diffusion and the probable reasons for mobilization of potassium in the root zone are the followings.

1) Intense microbial activity
2) Organic acid secretion by root
3) Dissolution effects of organic acids. (Raghu and Mac Rae 1966, Nagarajan *et. al.,* 1970).

From the above said study it is very clear that the microbial population. (Phosphate solubilizers and others) and its activity i.e. organic acid secretions also remarkably contribute the changes of Potassium mobility from the soil towards plant roots. So it is evidence that good microbial population like various biofertilizers and its secretion like plant growth promoting substances also contribute potassium nutrient cycling. Though there was no much study made so far in this directions incorporating beneficial microbes in potassium cycle will lead to much more clear ideas about potassium availability.

Intensive cultivation with mere use of high dose fertilizers without adequate organic manures depletes the micronutrient status of the soil as well. Further, it leads to a decline in organic carbon status of soil resulting in retarded soil biological activity thus minimizing the natural nitrogen fixation or nutrient solubilization or mobilization like phosphorus and micronutrients. Therefore researchers are urged to explore the feasibilities of tapping the natural reserve and reactions of soil to sustain soil fertility and crop productivity by augmenting the biological nitrogen fixation and biodissolution of nutrients in soil. Biofertilizers therefore has become an inevitable and integral part of integrated nutrient management (INM) or integrated plant nutrient supply (IPNS) system.

As per the recent technology it appears as though the entire crop nutrients can be managed through biofertilizers and organic manures if crop residues are properly recycled, which is the goal in organic farming.

The fixed and immobile P has been made available to plants by encouraging soil microbiological activity through addition of green manures, farmyard manure or by inoculation of P solubilizing microorganisms (PSM) and phosphate mobilizers (AM fungi). Incase of nitrogen need of plants nitrogen fixing biofertilizers are capable of fixing atmospheric nitrogen.

In the case of potassium our Indian soils are rich in its content but recent crop studies indicating that vast areas shows the potassium deficiency. Soil exhibits response to potash application indicating a hunger for this nutrient. Primarily, group of soil microbes act upon soil clay minerals like illite, muscovite, microcline, leucite etc. (Contain both Si and K. in them and liberates / solubilize) or / mobilize Si and K, which are known as Silicate Solubilizing Bacteria (SSB). Experiments conducted in rice with SSB have shown the release of potassium. A. Bacillus sp. isolated from the soil of granite crusher yard was found to solubilize silica and potassium and was found to augment rice yield (Anthoni Raj 2000).

Bacillus muciloginosms var. siliceous was found to degrade silicate minerals and liberated potassium from field spar and alumino silicates in invitro cultures. *Bacillus circulars* released silicon and potassium from different silicate minerals. Bacteria are plenty in soil and few of them have the capacity to breakdown silicate minerals to releasing silica. Potassium is also released concurrently when K bearing silicate minerals are attacked. Natural occurrence of Silicate Solubilizing Bacteria (SSB) in rice ecosystem, in agro inputs and in materials of antaropogenic activities is observed.

Biodissolution of plant nutrients will become an integral part of integrated nutrient management in the near future. Soil microorganisms are capable of solubilizing nutrients in soil so that they are readily available to plants, several mechanisms are postulated for dissolution by biological action. They involve :

1) Production of CO_2 by plant roots and during decomposition of organic matter.
2) Production of organic acids (OA) of various types including 2 keto-gluconic acid, humic and fulvic acids.
3) Production of H_2S that determines solubility of P and precipitation of FeS
4) Production of alkalinity
5) Production of polysaccharides
6) Production of specific enzymes eg. Phosphatases
7) Oxidation of S and NO_3 with production of mineral acids

All these decide the solubility of several nutrients in soil. A technology to tap this natural phenomenon to the advantage of crop is alone required to be developed.

3. Potash Mobilizing Bacteria (KMB)

Current interest in the potassium fertility of soil has been changed from simple estimation of exchangeable K to measurement of the rate at which K is supplied from exchangeable fractions. Rate of non exchangeable K release and its mechanism are controlled by nature and amount of clay minerals, besides this exploring the role of microbes present in the soil also started recently. According to preliminary studies and crop response studies gives encouragement in this line. (Chandra *et.al.,* 2005, Chandra *et. al.,* 2000).

An interesting findings was made from Banana rhizosphere by Dr. Krishan Chandra during 1998 and noticed a microbe is predominant and play vital role in help plants in potassium nutrient uptake. Later it was authenticated by Institute of Microbial Technology (IMTECH), Chandigarh as *Frateuria aurentia* and known as Potash Mobilizing Bacteria (KMB). The bacteria belonging to the family Pseudomonaceae, the characterization of the bacteria is as follows :

I. GENERAL TESTS

TESTS	RESULTS (K-10)*
Gram's Reaction	-ve
Shape	Rods
Size	V.shost
Motility	+

*(K-10 is the code name of *Frateuria aurentia* (KMB)

II. PHYSIOLOGICAL TESTS

TESTS	RESULTS (K-10)*
Growth at temperature	
4ºC	-
15ºC	+
42ºC	+
55º C	-
65º C	-
Growth at pH	+
pH 5.0	+
pH 8.0	+
pH 9.0	+
pH 11.0	+
Growth on Nacl(%)	
2.5	+
5.0	+
7.0	+
9.0	-
10.0	-
Growth under anaerobic condition	+

III. BIOCHEMICAL TESTS

TESTS	RESULTS (K-10)*
Growth on Macconkey agar	+
Indole Test	-
Methyl Red Test	-
Voges Proskauer Test	+
Citrate Utilization	+
Csaein hydrolysis	-
Starch hydrolysis	-
Urea hydrolysis	-
Nitrate reduction	-
Nitrite reduction	–
H_2S Production	+
Cytochrome Oxidase test	-
Catalase test	+
Oxidation / fermentation (O/F)	F
Gelatin liquefaction	-
Acid production from carbohydrates	+
Arabinose	-
Dextrose	+
Fructose	+
Galactose	+
Inositol	+
Lactose	+
Maltose	+
Mannitol	+
Melibiose	+
Raffionose	+
Salicin	+
Sorbitol	+
Sucrose	+
Xylose	+
Trehalose	+
Cellobiose	+
Inulin	+
Any special Characteristic(s) Adonitol	-

Source: Institute of Microbial Technology (IMTECH), Chandigarh - 2001

Based on the data and experiments conducted so far, the bacteria having the following salient features.

1) Gram negative bacteria
2) Rod shape and motile
3) Growth from 15°C to 42°C temperature
4) It could grow from pH 3.5 to 11.0
5) It can grow upto 7% of Nacl concentration
6) It is able to grow in anaerobic conditions
7) Shows the positive results from the following tests
   i) Growth on macconkey agar
   ii) Voges Proskauer test
   iii) Citrate utilization
   iv) H_2S production
   iv) Catlase test
8) And it could produce acid from the following carbohydrates Dextrose, Fructose, Galactose, Inositol, lactose, maltose, manitol, melibiose, raffinose, salicin, sorbitol, sucrose, xylose, trehalose, cellobiose and insulin (Source: IMTECH 2001)

Potash Mobilizing Bacteria as Plant Growth Promoting Rhizobacteria (PGPR)

Beneficial free living soil bacteria isolated from the rhizosphere of plants, which have been shown to improve plant health or increase yield are usually referred to as plant growth promoting rhizobacteria or (PGPR) Kloepper *et. al.,*1980. A number of different nitrogen fixing and phosphate solubilizing bacteria may be considered to be PGPR including *Azotobacter, Azospirillum, Rhizobium* other bacterial genera e.g. *Arthrobacter, Bacillus, Burkholderia, Enterobacter, Klebsiella, Pseudomonas, Xanthomonas, Serratia* are also reported as PGPR. According to Chandra *et. al.,* 2005 and field trials. *Frateuria aurentia* also to be considered as PGPR.

Plant growth promoting rhizobacteria have been identified in influencing the growth and yield of many plants. The effects of PGPR on plant growth can be mediated by direct or indirect mechanisms (Glick 1995). The direct effects have been most commonly attributed to the production of plant harmones such as auxins, gibberellins and cytokinins; or by supplying biologically fixed nitrogen. These PGPR also affect growth by indirect mechanisms such as suppression of bacterial, fungal and nematode pathogens by production of sideophores, HCN, ammonia, antibiotics, volatile metabolites etc. by induced systematic resistance and or by competition with the pathogen for nutrients or for colonization space (Glick 1995).

4. Isolation of Potash Mobilizing Bacteria

Potash mobilizing bacteria mobilize potassium from soil. This principle is employed in isolating KMB from soil (Chandra *et. al.,* 1995).

Collect the soil samples from banana rhizosphere, mix well and make into fine particles. Collection of soil sample should be made in the root zone at 5-15 cm depth.

Take 10 gram of soil sample in a measuring cylinder and make upto 100 ml with sterile distilled water and shake well (1:10).

- Take one ml from this and transfer to 9ml of sterile water in tube (1:100).
- make serial dilutions by transferring 1 ml of the suspension to the subsequent tubes to get 1:10,000.
- transfer 1 ml of the desired soil suspension to sterile petriplate.
- Pour the melted and cooled following media in the same petri plate.

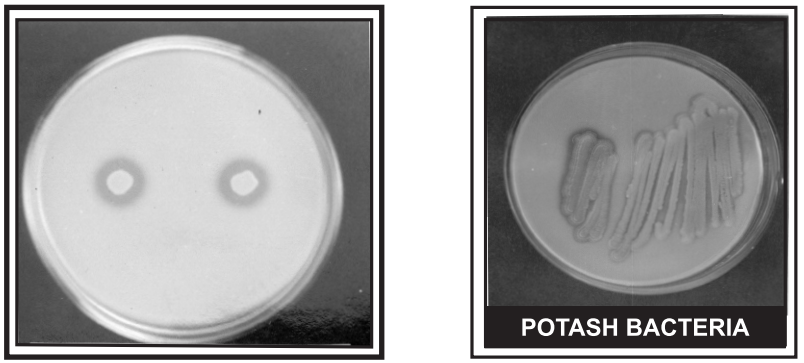
Glucose Yeast Agar Media (GYCaA) for KMB

1. Glucose 20 g / lit.
2. Yeast extract - 3 g / lit.
3. Calcium carbonate 5 g / lit.

4. Agar agar 18.5 g / lit.
5. Distilled water 1000 ml.
   - Rotate the plate gently and allow it to solidify.
   - Incubate inoculated plates in incubator at 28+2^{0}C temperature and observe for the development of colonies after 4 to 5 days.

Testing of potassium release by *F. aurentia*

The isolated bacteria exhibited clear zones. The colonies could be picked up and maintain in nutrient agar slants (Figure - 2). The culture could be inoculated to 50 ml of above said medium containing 5% fixed potassium substance or soil in 100ml Erlenmeyer flasks and the release of potassium could be estimated in flame photometer.



(Figure - 2 Plate showing potassium releasing zone)

Isolation *F. aurentia* by enrichment technique

The potash mobilizing bacteria could also be isolated by enrichment technique.

- Take 100 gms of banana rhizosphere soil and add 5% of vermiculite / Fedsphere.
- 40% moisture content should be maintained and incubate at room temperature for 7 days.

- After incubation period, follow the serial dilution technique upto 10^{-5}. Use spread plate or droplet method for growing the organisms on respective media (GyCaA)

- Incubate the plates at room temperature and examine the colonies showing clear zone of Ca release after 14 days.

Comparing Cell Morphology and Gram Stain Reactions of *Frateuria aurentia*

Make wet mounts of the cultures of *F. aurentia* and examine under the phase-contrast microscope. Compare the motility, size, and shape of the *F. aurentia* (KMB) Place a loopful of sterile distilled water onto a clean, pre-flamed and cooled microscope slide. Flame the loop and transfer a small sample of the bacterial growth from the plate culture to the water on the slide. Mix thoroughly and make a thin smear approximately 1 cm^2 in diameter. For broth cultures, transfer a loopful and make smear directly on the dry slide. Air dry, heat fix, and allow to cool. Flood the smear with diluted carbolfuchsin for 60s. Rinse carefully in a gentle stream of water and blot dry. Locate smears under low power (10X, 25X, or 40X) objective. Apply a drop of cedar wood oil to the smear and observe with the 100X oil immersion objective using bright field illumination.

The carbolfuchsin stain makes the bacteria easily visible (cells appear) pink. Note the characteristic rod shape of the cultured cells of *F. aurentia* and compare the size and shape as mentioned in morphological tests.

Observe the preparation under oil immersion Microscope

The Gram stain procedure separates bacteria into two groups : Gram-positive and Gram-negative organisms. Gram-positive organisms retain the crystal violet stain after treating with iodine and washing with 95% alcohol, and appear dark violet after staining. *F. aurentia* is Gram-negative organisms lose the violet stain after treating with iodine and washing with 95% alcohol but retain the red coloration of the counterstain, safranin. If it also

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Characterizing Growth of *F. aurentia* using a Range of Media

F. aurentia can be described according to their growth in solid and in liquid media. The size, shape, color, and texture of colonies and the ability to alter the pH of the medium are generally stable characteristics useful in defining strains or isolates. Typical colony characteristics, when growth on standard Glucose Calcium Yeast-broth (GYCa) medium, are described above. The luxurious growth apprear on mannitol-peptone medium (MPYA) as -

Manitol-15 g/lit.

Yeast extract - 3g/lit.

Peptone - 2g/ lit.

Agar - 18.5 gm.

Trace elements 1ml/ lit.

Trace elements (Stock solution)

Sodium molybdate - 0.2g

$MnSo_4$ - 0.23g

Boric acid 0.28g

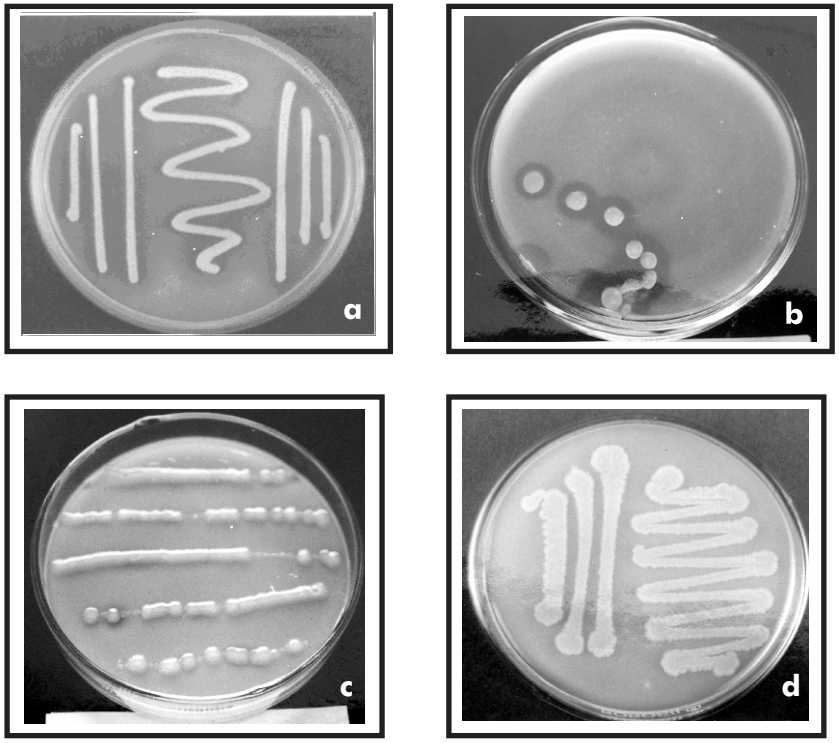
$CuSo_4$ 0.01g

$ZnSo_4$ 0.03g

(Dissolve in 1 litre distilled water)

Shape

Usually discrete, round colonies varying from flat (⌓) to domed (◠) and even conical (△) shape on agar surfaces could be seen. Colonies usually have a smooth margin. When growing surface in the agar, colonies are typically lens shaped in manitol but flat on Glucose media. Colonies will be white-opaque. The opaque colony growth is usually firm with little gum, whereas the less dense colonies are often gummy and soft. Colonies are dull, evenly opaque or translucent, but many colonies develop darker edge of brown marking with age colonies (Figure - 3) turns yellowish on manitol medium.



(Figure 3 shows growth on different media.
a - MPYA.; b - MPMgA; c - GYNiA; d - GYCaA)

Size

When well separated on agar plates, colony size may vary from 1 mm to 2-3 mm as faster-growing strain. In crowded plates, colonies remain smaller and discrete, but coalesce to confluent growth when colonies join. Streak out on plates containing each of the following media. GYCaA, MPYA + bromthymol blue and peptone glucose agar. These indicators and selective media are used as presumptive tests for purity of cultures. Their interpretation is as follows.

Freshly prepared MPYA plates containing bromthymol blue have a pH of 6.8 and are green. *F. aurentia* show an acid reaction, turning the medium yellow.

Heavy growth indicates contamination. The growth and color reactions described here are dependent on the strain metabolism on the standard MPYA media. Reactions may differ when other types of media are used. There are media that are used for special purposes. For instance, if a faster growth rate is desired, the arabinose gluconate (AG) medium may be used (1995d).

Growth Rate

Growth rate generally ranges from 3-5 days as it is fast grower to achieve maximum colony size on agar or growth in liquid medium. Growth rate varies according to the temperature of incubation (optima 25-30^{0}C), origin (Culture), aeration (in liquid culture), and composition of medium. (1995e)

5. Preparation of Mother Culture of *F. aurentia*

F. aurentia are easy to grow in the laboratory. These bacteria are aerobic and also microaerophilic. They require aeration, which may be provided by using a mechanical shaker or by bubbling sterile air through the medium. *F. aurentia* grow best at 25-30^{0}C. The medium must supply energy, a source of N, certain mineral salts, and growth factors. Most commonly, can be used is a yeast extract mannitol mineral salts medium, but if cost or availability is concern, glucose may be substituted. Commercial-scale inoculant production requires the culturing of *F. aurentia* in large volumes of liquid culture media. For cost-effective production, the ingredients for these media must be inexpensive and readily available. Various industrial by-products have been used with acceptable results. Molasses, corn steep liquor, and proteolyzed peanut husk have been used as sources of C and growth factors for multiplication.

Yeast extract is most frequently used as the N source. Successful replacements for yeast extract may be soybean (Glycine max), chickpea (Cicer arietinum), and malt sprout extract, by-product of the cheese industry, can also been used as a N source. Mass culturing *F. aurentia* is done in fermentors in which the growth media are heat sterilized prior to use. Vessels for fermentors vary in size from a few litres to several thousand litres.

Inoculant production starts with a pure slant culture. This culture is used to inoculate Glucose Calcium Yeast-broth (GYCa) in a small flask. The resulting broth culture will serve as inoculum (starter culture) for a greater volume of broth or medium contained in a large flask or a 2-4 liter glass fermentor. The volume of a starter culture should be a minimum of 1% of the broth volume in the fermentor. Generally people use 5% of inoculum but as per the production technology of author it is 1%. Thus, a 1-liter starter culture would be required to inoculate 100 liters of medium in a steel fermentor. Often, a larger inoculum is used to reduce the incubation time needed to obtain 2 x 10^9 cells ml^{-1}. This population level is considered necessary, particularly when using nonsterile carrier material. Aseptic conditions are maintained throughout the production period. The

broth culture is checked frequently for its purity.

Preparation of carrier base *F. aurentia*

The most inoculants are a mixture of the broth culture and a finely milled, neutralized carrier material. The properties of a good carrier material are : (1) nontoxic to inoculants, (2) good moisture absorption capacity, (3) easy to process and free of lump-forming materials, (4) easy to sterilize by autoclaving or gamma-irradiation, (5) available in adequate amounts, (6) inexpensive, (7) good adhesion to seeds, and (8) good pH buffering capacity.

The best analysed and most frequently used carrier material for inoculant production is lignite. Although the Lamphalpat peat (Manipur) provides better protection in the package and in an inculated seed than other carriers. The physical and chemical analysis of well-known Lamphalpat peats are shown in Table - 1. However, physical and chemical analysis of a peat are only partial assessments of its

Table - 1 Characteristics of Lamphalpat (Manipur) Peat

Sedge Peat Contents	Amounts
Total N (%)	1.62
Organic matter (%)	56.00
Ash (500^0) (%)	13.20
Exchangeable K (ppm)	62
N as NH_4 and NO_4 (ppm)	94
Available P (ppm)	12
pH	4.5 5.0
Moisture (%)	7-8

(Suitable as a carrier. Only a test related to growth and survival of F. aurentia can confirm its acceptability)

Lamphalpat peat is not available in India except in Manipur (Table - 1) but commercially yet to be exploited. A wide range of substitutes, e.g., coal, charcoal, bagasse, press mud, vermiculite, polyacrylamide, mineral soils, vegetable oils, and ground plant residues can be used as alternative carriers. Carrier processing, e.g., mining, drying, and milling, are the most capital-intensive aspects of inoculant production. Material with a particle size of 10-40 m (0.001-0.004 mm) is collected for seed coating lignite with a particle size of 150-212m (0.150-0.212mm) is used for the production of soil implant (granular) inoculant. The carriers are neutralized with precipitated calcium carbonate (pH 6.5-7.0). Both sterilized and nonsterilized lignite are used in commercial production systems.

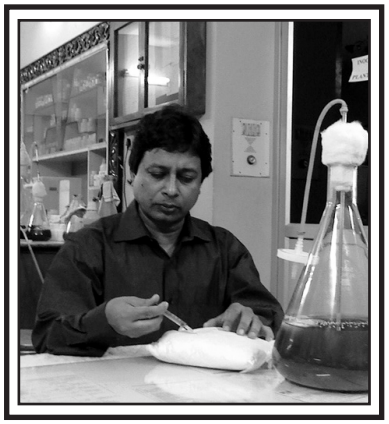
Carrier designated for sterilization is prepackaged in thin-walled polyethylene bags. The sealed bags are then gamma-irradiated at 5.0 Mrads. Alternatively, in India the carriers are autoclaved in partially opened, thin-walled, polypropylene bags for 60 min. at 15 lb/in^2 pressure and 121^{0}C. for pre sterilization but very few manufacturers follow the practices. Gamma-irradiation sterilization is preferred. Facilities are available at Kidwai, Bangalore and Baba Atomic Research Station, Kalpakam, Tamilnadu.

Mixing of Broth Culture into Carriers

Under commercial conditions in India, quality-tested broth cultures are incorporated into vermiculite (best water holding capacity) at the rate of 1 liter per kilogram of vermiculite. After a curing period, the mixture is packaged in thin-gauge (0.03 mm) polyethylene bags. Bags of this specification permit gas exchange and minimizing moisture loss from the inoculant. The expiration date for inoculants based on nonsterile carriers is usually 6 months.

Very few inoculant producers in India produce inoculants with sterilized carriers. In this case, the carrier is first packed and then sterilized by gamma-irradiation or autoclaving. Thin-gauge (0.03 mm) *of* polyethylene bags are used for carriers to be autoclaved are packaged in polypropylene bags of the same

gauge. The *F. aurentia* broth culture is aseptically injected into the packaged carrier with a manually operated motorized syringe / injection system Figure - 4.



(Figure - 4 Injection of broth pre sterilized carrier)

Inoculants based on sterile carriers are usually of higher quality than the nonsterile carrier type. The number of viable cell per gram can be between 10^8-10^9 cells in inoculants produced with sterilized carriers. In nonsterile carriers, the initial number of viable cells tend to be lower by at least one log after curing. The number of cells added to most sterile carriers remain high during shelf life or storage because there are no other microorganisms in the carrier competing with the *F. aurentia*. The quality of such inoculants may still be acceptable after 6-9 months, depending on the temperature during storage.

Although production cost of sterile based is most costly than nonsterile carrier-based inoculants, this is mainly due to the need for sterilization facilities and labour-intensive production operations, using the dilution technique can substantially lower the production cost (Chandra 1994). Here, the broth culture is aseptically diluted with sterile water up to 100 fold before incorporation into the sterile carrier as demonstrated in sterile Lamphalpat peat. The low cell population in the diluted culture will multiply to the same Lamphalpat peat as with undiluted

cultures during the maturing time of 5-7 days (Chandra, 1995) Niftal.

Inoculants are cured for about 2 weeks at 25-30^{0}C to gain maximum numbers in excess of 10^8 and 10^9 cells g^{-1} for nonsterile and sterile carrier-based inoculants, respectively. Thereafter, inoculants are stored in a refrigerated or air-conditioned environment, protected from direct light. Most inoculants are stored at 4^{0}C and tend to survive best at this temperature.

The final moisture content of the carrier inoculant should be 30-40% on a wet-weight basis for inoculants produced with presterilized vermiculite. A lower moisture content (30-35%) is preferred for better *F.aurentia* survival in non sterile carrier.

Inoculants must bear an expiration date and comply with quality standard regarding numbers of cells present. Generally, a minimum of 10^9 viable cells g^{-1} is required for sterile carrier-based inoculants and 10^8 viable cells g^{-1} for nonsterile carrier-based inoculants.

Soil and Seed inoculation

The most common means of introducing *F. aurentia* to the soil is as a seed-applied inoculant. In its simplest (and least satisfactory) form, carrier inoculant is mixed with water to form a slurry and mixed with the seeds. Better results are obtained when the inoculant is coated on the seed with an adhesive. An adhesive increases the amount of inoculant that will adhere to the seed. A good inoculant adhesive must be nontoxic to the inoculants and provide protection during planting and in the soil. Gum Arabic has these properties, but it is expensive to farmers and not readily available at many locations. Other adhesives used successfully include methyl cellulose, sucrose solutions and vegetable oils (Chandra *et. al.,* 1995f). An additional coating of calcium carbonate, rock phosphate, or other pelleting material can enhance the success of inoculation. This is often done when adverse weather conditions prevent immediate sowing of inoculated seeds, as protection against insects in the soil, when the soil is hot and dry or very acidic, or as protection against pesticides (Chandra ,1995b).

Seed coating is not always the best way to inoculate. Some inoculants are designed to be placed into the soil. Soil implant or granular inoculants are designed to be placed into the soil. Soil implant or granular inoculants are generally made from clay granules with a particle size of 0.5 1.5 mm. Other types of granular inoculants are made by spraying a suspension of clay inoculant on an inert substance. Soil inoculants may also be improvised by suspending carrier inoculant in water or by mixing clay inoculant with sand. Soil inoculant is generally placed into the furrow under or alongside the seed.

6. Production of Liquid Form of *F.aurentia*

A small glass fermentor of 2-3 liter capacity can be used to produce broth cultures to be applied as liquid inoculants in field experiments. It is also suitable for producing starter cultures for medium-sized commercial fermentors (Chandra *et.al.,*1995a). A glass fermentor is assembled and then used for small-scale production of broth cultures. The broth cultures are monitored periodically for quality control, including checks for contamination and the progress of growth and cell multiplication.

Preparation of liquid Starter Cultures

Prepare four 50 ml flasks or tubes each containing 25 ml of Glucose yeast calcium agar obtain slant, lyophilized or bead preserved cultures of a strain of K_{10} (Chandra 1995c). Inoculate three flasks with each *F. aurentia* strain and aerate at 25-30^{0}C. These will serve as starter cultures for inoculating the GYCa in the fermentors.

Assembling of Glass Fermentors in Laboratory

Set up fermentors as shown in Figure - 5. The main fermentation vessel is a slightly modified 5 liter Erlenmeyer flask with a sampling port (steel tubing 4 mm i.d.) fitted close to its base. Fill fermentor with 3-3.5 liters of broth. Connect the 0.2 micron filters to prevent the entry of contaminants via the air lines. All rubber stoppers and tubings must be autoclavable.

Insert the large rubber stopper, which holds the air inlet with their respective filter, firmly into the neck of the flask.

Connect the air inlet tube to an aquarium pump. Activate the pump and check the air inlet and outlet for air resistance. Air should flow freely through filter while bubbling through the broth, and simulataneously aerating and agitating the medium. The filter should be packed uniformly, but loosely. Overpacking the air inlet filter can cause resistance to incoming air and lead to poor aeration. Overpacking the outlet filter can lead to poor air escape and pressure buildup in the fermentor (Chandra, 1995a).

(Figure - 5 Glass Assembly Fermentor)

Disconnect the fermentor from the pump and prepare it for autoclaving. Make sure that the stopper which holds the air tubes is still firmly seated. The air-supply system must be well protected to prevent entry of contaminants. Wrap the top of each flask with a wide band of nonabsorbent cotton and secure it with a string. Then add a protective wrapper of newspaper / aluminium foil (Figure - 5). Close the air inlet tube with a clamp at the spot indicated in Figure - 5 to prevent the broth from leaving the flask due to pressure buildup in the flask during autoclaving. Pressure relief during autoclaving occurs through the cotton. The filters should remain connected to the fermentor during autoclaving. To provide a convenient place for them, make an oversized wire ring

to fit snugly around the neck of the fermentor vessel and twist it to obtain an eyelet or loop on each side. Sterilize the assembly for 35 min, if it contains approximately 3.5 liter of broth. Adjust the sterilization time according to the volume of liquid; increase time by 10 min for each additional liter.

After the fermentor has cooled, remove the clamp from the air inlet tubing. Connect the air supply to check for proper aeration once again and for leaks in the system. Various types of air systems have been used to aerate small fermentors, including compressors, compressed air in tanks, aspirators and aquarium pumps. The latter have been very satisfactory for small units and are inexpensive, silent and dependable. Although a pressure relief valve may be desirable, it is not really necessary. Most aquarium pumps generate only low pressure, sufficient however, for several (four) fermentor units that may be connected to one aquarium pump using a manifold (Glass divider three way /plastic good).

Inoculating the Glass Fermentor

If, after autoclaving, the fermentor has been inspected and found to function properly, it is ready for inoculation with the starter culture. If an aquarium pump is used, and more than one fermentor is attached, adjust the air to achieve an equal flow to each fermentor. For other air-supply systems, adjust the air flow 2.3 lits / min. on the bypass, which may be installed between the pump and the air inlet filter.

The glass fermentor is inoculant through the silicon air inlet tubing (at a point just above the main stopper) with a sterilized syringe fitted with an 18 gauge (Figure - 6) needle. Care must be taken that no contaminants are introduced. 20 ml.of the starter culture are removed aseptically from its flask. The air inlet tubing is swabbed with 70% alcohol (or 3% hydrogen peroxide) about 1 in (2.5 cm) above its connection to the glass tube. The needle is inserted downwards into the tubing and the culture is injected. The airstream will facilitate speedy entry and incorporate the starter inoculum into the sterilized GYCa medium. The culture is incubated at 25-30^{0}C under continuous aeration.

(Figure - 6 Injective Glass Assembly Fermentor)

Sampling of liquid starter culture

Aseptically, with a sterile syringe, withdraw culture broth from the fermentor through the sampling tubing attached to the sampling port. Swab the tubing with 70% alcohol or 3% hydrogen peroxide. Insert the needle into the sterilized portion of the tubing and withdraw the desired amount of culture broth. For quality control purposes (such as Gram stain, pH measurements, optical density measurements, the total count, and plate counts), 5-10ml of culture are sufficient and may be withdrawn by using a 5 or 10 ml syringe fitted with a 22 gauge needle.

Testing of liquid starter culture

When the starter cultures have reached the end of their log phase of growth 5 days it is ready to be used for inoculating the fermentor. Take a 10ml sample from fermentor at the end of the growth period and conduct the tests :

pH tests : A contamination problem is usually evident when the pH of the broth decreases toward acidity and unpleasant odor observed.

7. Inoculating liquid starter cultures into Steel Fermentor

The commercial size steel fermentor of 250 liter working capacity requires 2-3.5 liters of starter culture. The glass fermentors described are ideal for this purpose.

Tips for fermentor operation

The body of the fermentor is a pressure vessel with a 250 liter total capacity. It has a working capacity of 200 liters. It is domed at the top and bottom, and held upright by a welded on stainless steel skirt. The top has a centrally positioned oval opening with a snap-type closure, which uses a silicon or tefflon seal. Encircling the opening are the following accessories: a steam pressure gauge, a pressure relief valve, and an aeration system with filters for the intake and exit of sterile air. Inlet and outlet ports for water passage through the built in stainless steel cooling coil are not shown in Figure 7. The inoculation port, thermometer, and the sampling port are positioned on the vessel wall. The fermentor is situated on a steel stand support that houses a 24 KW heater. The air inlet hose is connected to a prefilter, which is attached to a regulated pressurized manifold (not shown in Figure - 7). When in operation, the air manifold is kept at constant pressure.

(Figure - 7 Liquid Steel Fermentor)

Pre-checking Fermentor before Operation

To safeguard against contamination of the growth medium during mass culture, the fermentor must be leak proof. Assuming the fermentor is fully assembled, close all valves and turn on the air compressor for air supply. Now open the air inlet valves slightly (i.e., first the ball valve above the air inlet filter and the ball valve below the air inlet filter). Allow the air pressure to build up to 5lb/in^2, as indicated by the pressure gauge. If the line

pressure is higher than 5 lb/in^2, open the air outflow filter slightly so that 5 lb / in^2 of pressure is maintained. Now check all valves and connections for air leaks by applying soap solution (0.5% liquid soap in water). Using a wash bottle filled with soap solution, apply a small amount of the soap solution to all screw-in connections and valves. Start with the hose connection of the air inlet hose and end with the sampling pot valve at the bottom of the fermentor. Whenever bubbles form after the application of the soap solution, including leak(s), tighten the joints or connections to remove the leak. Once the fermentor has been established to be free of leaks, release the pressure via the air outlet filter.

Remove the air inlet and the air outlet (exit) filters. The air inlet filter has a ball valve on top that connects to the air hose with a snap fitting. The bottom of the filter is attached to the fermentor inlet valve via a union coupling. Loosen the union coupling with an adjustable wrench and remove the air inlet filter unit. Unscrew and remove the air inlet filter cap. Pack the filter with layers of nonabsorbent cotton. The cotton must be packed uniformly and loosely to prevent the air from channeling while under pressure. Screw the filter cap back on and wrap the whole filter unit with aluminium foil. Autoclave the filter unit at 121^{0}C and 15 lb/in for 90 minutes . Store the sterilized air inlet filter unit in a clean environment until needed. The in-line air outlet filter need not be autoclaved. Remove it with a wrench and pack it loosely with fine, high-grade glass wool. Reattach the filter.

Pre-testing liquid culture from Fermentor

A pretest is necessary if the fermentor is used for the first time or if it has not been used for a while. The fermentor is fully assembled, but the air inflow filter is not yet attached. Through the main opening, fill the vessel with 200 liters of water. Close the opening with the snap-type closure. The snap-type closure with the O-ring in place must be immersed in water to obtain a proper seal. Close all valves except the air outlet valve, which should be left open to allow air and steam to escape when the growth medium is boiling. Switch on the plug and bring the growth

medium to boiling under maximum heating. When the boiling point is reached, turn switch off and maintain the boiling. Adjust the air outlet valve to allow steam to escape slowly in order to sterilize the glass wool packed in the outlet filter. After 15 min. close the outlet filter completely to allow pressure when the pressure reaches more than 15 lb/in^2. Allow the relief valve to discharge twice or more. As the pressure rebuilds, reduce the flame and open the air outlet valve slightly to maintain a pressure of 15 lb/in^2 at 121^{0}C for 5 min. Now recheck all joint connections and valves for leaks, evidenced by escaping steam. If necessary, tighten joints to stop leaks.

Install the air inlet filter while the fermentor is still hot. (The installation needs to be done carefully and quickly). Squirt alcohol into the lower half of the union coupling located above the air inflow valve. Ignite it with the flame of a Bunsen burner. Just before the flame extinguishers, quickly unwrap the air inlet filter, bring the union ends together, and secure the filter in place by hand-tightening the coupling screw. Heat the union coupling with a flame for approximately 30 s and use a wrench to further tighten the coupling.

The filter packing should be replaced and the filter unit resterilized after each production run. (Although experienced operators have used a filter for as many as 9 runs before repacking and resterilizing). Turn off the flame and gradually release the tank pressure by opening the air outlet valve. Turn on the water to the cooling coil to cool down the contents of the fermentor. Quick cooling is very important to keep the nutrient value high.

Sterilization of liquid media in steel fermentor

When cool, empty the fermentor with a water pump and hose, and/or by draining it at the sampling port. Using a flashlight to illuminate the interior, inspect the vessel for cleanliness. Make sure the sampling valve is closed and fill the vessel with 190 liters of clean water. In a clean plastic bucket or other suitable container, prepare a concentrate of the growth medium (GYCa) in 10 liters of water. Pour the concentrated growth medium into

the 190 liters of water in the fermentor. Check the pH and adjust to 6.8 if necessary. Close the fermentor opening and all valves except the air outlet valve. Wrap aluminium foil around the inoculation and sampling ports. Switch on the button and bring the medium to a sterilizing pressure and temperature.

After a 50 min sterilization period, turn off the burner and slowly release the pressure by opening the air outlet valve, allowing the steam to escape through the outlet filter. When the pressure reaches 10 lb/in^2, slowly turn on the cooling water. Carefully control the air outlet valve so the pressure decreases slowly. A rapid drop in tank pressure may cause a partial vacuum in the vessel; this should be avoided. Infact, during cooling if vacuum created it may suck contamination from out side. Increase the flow of the cooling water when the tank temperature has reached 90^{0}C. When the temperature has reached 30^{0}C, shut off the cooling water, open the air outlet valve completely, and allow the medium to equilibrate to ambient temperature (overnight). If the growth medium is not completely sterilized, surviving contaminants (e.g., sporeforming bacteria) will grow during this period.

Large scale Mass Culturing *F. aurentia* (KMB)

The next step is to check the sterility of the growth medium. Spray the sampling port with alcohol and thoroughly flame it with a torch. Open the port with a valve tool or adjustable wrench and allow a small amount of broth to flow out without being sampled. Then quickly and aseptically obtain a 50 ml sample in a sterile flask. Perform the following tests :

1. Smell : The medium should have the odor of the Glucose yeast calcium (GYCa) salts medium if GYCa medium used .
2. Clarity : A clear medium indicates the absence of contaminants. However, if the water used is rich in minerals, precipitation may cause turbidity, usually associated with contamination.
3. pH : A near neutral pH (6-7) is expected in a sterile medium.
4. Gram stain : Perform a Gram stain if any turbidity is detected.

If the medium is found free from contamination, inoculate the fermentor. Use the starter culture of glass fermontor *F. aurentia* for inoculating a second steel fermentor.

Sample the contents of the fermentor about 3 days after the starter culture is inoculated. Aseptically remove the broth through the sampling port as previously described in monitor the growth of the culture by total count or optical density (OD) measurement. Perform pH measurements and Gram stain as checks for contamination. The *F. aurentia* population in the culture medium should reach full growth (approximately 1.5-2.5 x 10^{10} cells ml^{-1}) 3 or 6 days after inoculation, depending on the starter count .

Mixing of liquid base materials

The base materials has to be prepared and sterilized separately, The base materials as follows:-

1-3% suspender,1-5% dispersant, 3-8% surfactant, 35-65% water

Mix 40% of broth from steel fermentor aseptically and bottle it through automatic aseptic bottling system. (Figure - 8)

(Figure - 8 Bottling of liquid culture)

8. Physical Features of Liquid KMB

- Solution always brown in colour.
- Smell of H_2S or chocolate.
- pH-4.5 but buffer 6.5-7.0 ensures more than 2 years expiry.

Quality Control

Quality Control begins with selection of the *F. aurentia* strains for inoculant production. Only authenticated, and pure cultures of the strain must be use. Purity is tested by growing the cultures on different test media. Growth reactions must agree with those characteristics known for the culture. Samples of the starter broth culture are tested for purity by Gram stain.

The broth culture must be protected from other microorganisms during mass culture in a fermentor or other culture vessel. Contaminants such as fungi or other bacteria compete with *F. aurentia* and prevent the maximum growth of *F. aurentia* during mass culture. Contaminants usually have fast growth rate, uncharacteristic odor, and cause excessive foaming. The broth culture is checked frequently for any abrupt change in pH (usually lower pH), which indicates contamination. Prior to incorporation with the carrier material (peat, vermiculite, charcoal, etc.), the fully grown culture is once again checked by Gram stain for culture purity.

Before leaving the production plant, batches of inoculants are usually sampled to check their quality. In the case of inoculants based on sterile carriers, viability is tested by the plate count. When non-sterile carrier material is used, inoculant quality is commonly tested by a zone test, as contaminants would interfere with the plate-count methods.

Compatibility with Pesticides and Fertilizers

When seeds are precoated with pesticides or herbicides, they should not be seed inoculated because these chemicals are toxic to *F.aurentia*. Soil inoculation is recommended before sowing so that the pesticides will not harm the inoculant. Most phosphate and calcium carbonate fertilizers do not harm the inoculants.

However, direct mixing with acid superphosphate may seriously affect *F.aurentia* survival (Chandra and Karmakar 1996).

9. KMB Liquid Biofertilizer application methodology and dosage

As like other biofertilizers KMB also could be applied as below :

1. Seed treatment
2. Root dipping
3. Soil application

KMB could be applied individually however, it is recommended along with Nitrogen fixers like *Rhizobium, Azospirillum* and with P-solubilizers will respond more with co-inoculants and soil application is prepared. Table - 2 shows the details of KMB application.

TABLE - 2

Seed Treatment	Mix 3.5 ml of KMB Liquid formulation in sufficient water and coat the seeds (1 kg) well with this solution and shade dry for half an hour before sowing.
Seeding Dipping	Mix 50-100 ml of KMB Liquid formulation in 10-20 litres of water and dip the seeding for 30 minutes before transplanting.
Sett Dipping	Mix 200 ml of KMB Liquid in 25-30 litres of water and dip the Sets required for 1 acre for 30 minutes before transplanting.
Soil Application	250-500 ml of KMB Liquid formulations can be mixed with 100 kg of well decompose farmyard manure. Blend the mixture well and broadcast it over one acre of land before last ploughing or first irrigation. Same quantity of *Azotobacter* for N, PSM for P nutrients could be applied for better results.
Drip Irrigation	500 ml KMB liquid formulation in one acre of land

KMB can be used with any other biofertilizers in combinatiion

10. Crop response studies on Potash Mobilizing Bacteria (KMB)

Effect of Azospirillium and Potash bacteria response on flower plants was studied by Das 2001, revealed very good response.

The effect of Potash mobilizing bacteria - *Frateuria aurentia* (Symbion-K-Liquid formulation) on Brinjal *(Solanum melongena L.)* physio-chemical parameters such as plant height, potash uptake, chlorophyll content and yield were evaluated under field conditions. Different treatments involving potash solubilizing/mobilizing bacteria and Potash fertilizer as stand alone treatment and combination treatments were carried out. (Table - 3)

Irrespective of the treatments, the results were observed to be significantly high in all treatments as compared to the untreated control. Among various treatments, the treatment T2, T3 and T4 which involved Symbion-K coupled respectively with 50%, 75% and 100% of the recommended dose of the Potash recorded a significantly higher yield, plant height, root length, Potassium uptake and chlorophyll content as compared to the stand alone treatment involving only Symbion-K (T1) and only Potash at different proportions viz. 50%, 75% and 100% of the recommended dose.

The difference amongst T2, T3 and T4 is however insignificant. The usage of Potash solubilizing/mobilizing bacteria Frateuria aurentia (Symbion-K) as a potential agent to supplement the potash requirements of Brinjal was also discussed.

TABLE - 3
Effect of Symbion-K application on the yield of the Brinjal (Coimbatore)

Treatments	Product/Acre	Method of Symbion-K application	Potassium uptake (Kg/Ha)	K soil (Kg/ha)	Total chlorophyll (mg g-1 fresh weight)	Plant height (cm)	Fruit Yield (T/Ha)	% Increase over control
T1	1000ml of Symbion-K *(Frateuria aurentia)* (Liquid formulation) alone	Soil application	3.78	122.4	2.52	62	21.14	14.766
T2	1000ml of Symbion-K *(Frateuria aurentia)* (Liquid formulation)+	Soil application	4.12	134.6	2.82	76	28.28	53.528
T3	1000ml of Symbion-K *(Frateuria aurentia)* (Liquid formulation)+	Soil application	4.24	142.4	2.84	78	28.34	53.854
T4	1000ml of Symbion-K *(Frateuria aurentia)* (Liquid formulation)+ 100% of recommended dose of K_2O (30 kgs)	Soil application	4.52	145.8	2.88	81	28.42	54.288
T5	50% of recommended dose of K_20 (15 kgs of K as K_2O alone)	Soil application	3.56	132.6	2.46	68	21.26	15.418

T6	75% of recommended dose of K_2O (23 kgs of K as K_2O alone)	Soil application	3.72	134.8	2.64	68	21.26	15.418
T7	100% of recommended dose of K_2O (30 kgs of K as K_2O alone)	Soil application	3.98	138.2	2.76	78	23.26	26.275
T8	Untreated Control (Minus Symbion-K and K_20 application but with N_2 and P_2O_5 as normally practiced) CD at 5% level	-	2.64	110.6	2.32	56	18.42	0
		-	1.124	12.648	0.822	4.638	3.262	-

(Source : Ramaretuinam and Chandra K. 2006)

To conclude, from the observation made in the present study and from the interferences drawn from the earlier workers, it was proved beyond doubt that the application of Symbion-K *(Frateuria aurentia)*. Potash mobilizer in combination with 50% of the recommended dose of potash fertilization has distinctly shown a significant increase in the growth and yield of Brinjal as compared the treatment with Potash mobilizer alone of Potash alone and untreated control. Thus, the potash mobilizer Symbion-K *(Frateuria aurentia)* offers to be used as a reliable tool to be incorporated into the present day intensive agricultural practices to supplement the Potash requirements of plants.

Response of F.aurentia on Brinjal / Chillies

Uptake of Potash by different plants with the use of potash mobilizing bacteria *(Frateuria aurentia)* was studied by Bismitha *et. al*. Department of Microbiology, O.U.A.T., Bhubaneswar in 2001. (Table - 4 & 9)

Table - 4 Average dry matter yield of brinjal (g/pot) at 45 days of growth.

TREATMENTS	R1	R2	R3	Total	Average
Control (No. Biofertilizer +No. chem. Fertilizer)	24.84	22.38	23.22	70.43	23.50
Potash Mobilizing Bacteria (KMB)	27.63	31.41	28.016	87.05	29.02
100:60:60kg N,P_2O_5, K_2O ha^{1} (General recommended dose)	39.03	40.80	36.913	116.74	48.91
100:60kg N,P_2O_5, ha 1 +KMB	36.53	38.85	39.191	114.57	38.19
100:60:60 kgN, P_2O_5, K_2O ha^{-1} (General recommended dose)+KMB	34.99	36.49	32.741	104.22	34.74
100:60kg N, P_2O_5 ha^{1} +45 kg K_2O +KMB	37.53	33.2	33.37	94.10	31.37
100:60 kg N,P_2O_5 ha_1 +30 kg K_2O +KMB	34.26	36.52	37.903	108.68	36.23
S.E(m)+/-					1.10
C.D.(0.05)					3.40

The results indicating that since the replacement of 60kg K_2O_5/ha by KMB give equivalent results. Hence, 50 % chemical potash can be reduced.

Table - 5 Nutrient uptake of brinjal as affected by different treatments at 45 days of growth

TREATMENTS	Available Nutrients (Kg/ha)		
	N	P	K
Control (No. Biofertilizer+ No. chem. Fertilizer)	0.887	0.229	2.935
Potash Mobilizing Bacteria (KMB)	0.934	0.240	3.076
100:60:60kg N,P_2O_5, K_2O ha 1 (General recommended dose)	1.798	0.249	3.930
100:60kg N,P_2O_5, ha ^{1} +KMB	1.390	0.258	3.762
100:60:60 kgN, P_2O_5, K_2O ha^{-1} (General recommended dose) +KMB	1.36	0.197	3.682
100:60kg N, P_2O_5 ha 1 +45 kg K_2O +KMB	1.097	0.204	4.109
100:60 kg N,P_2O_5 ha ^{1} +30 kg K_2O + KMB	1.369	0.254	4.438

In an experiment for testing or finding equivalance of potash fertilizers to KMB Liquid formulations were conducted and findings reveals that the uptake of K from by the brinjal plants is equal with treatment having full dose of K_2O application. So it is evident from this experiment KMB inoculant in liquid formulations could replace the chemical fertilizers application.

Table - 6 Properties of brinjal rhizosphere at 45 days of growth

TREATMENTS	Available Nutrients (Kg/ha)		
	N	P	K
Control (No. Biofertilizer + No. chem. Fertilizer)	371.2	183.0	89.4
Potash Mobilizing Bacteria (KMB)	476.0	186.5	139.6
100:60:60kg N,P_2O_5, K_2O ha 1(General recommended dose)	422.8	253.0	134.4
100:60kg N,P_2O_5, ha ^{1} +KMB	431.2	232.9	129.0
100:60:60 kgN, P_2O_5, K_2O ha-1(General recommended dose)+KMB	400.4	211.8	155.8
100:60kg N, P_2O_5 ha ^{1} +45 kg K_2O +KMB	408.8	234.6	131.6
100:60 kg N,P_2O_5 ha ^{1} +30 kg K_2O +KMB	470.4	180.3	134.4

Soil reserve of K after the 45 days of brinjal crop shows that highest result (139.6 kg/ha) shows that KMB treated soil. But it is lesser than full dose of chemical fertilizer applied soil.

Table - 7 Effect of biofertilizers on brinjal plant height (cm)

TREATMENTS	Average Plant height(cm)	Average No. of leaves
Control (No. Biofertilizer + No. chem. Fertilizer)	20.5	11
KMB	24.25	13
100:60:60kg N,P_2O_5, K_2O ha^1(General recommended dose)	28.5	15
100:60kg N,P_2O_5, ha^1 +KMB	26.5	16
100:60:60 kgN, P_2O_5, K_2O ha^{-1}(General recommended dose)+KMB	23.75	19
100:60kg N, P_2O_5 ha^1 +45 kg K_2O +KMB	31.18	
100:60 kg N,P_2O_5 ha 1 +30 kg K_2O +KMB	28.25	20

It is evident from the above table that the average plant height (cm) highest in case of treatment having 100kg N 60 Kg P and KMB bacteria.

Table - 8 Average dry matter yield of chilli plants (g/pot) at 45 days growth

TREATMENTS	R1	R2	R3	Total	Average
Control (No. Biofertilizer+No. chem. Fertilizer)	5.96	4.66	6.36	16.99	5.66
KMB	8.97	9.32	8.54	26.84	8.95
100:60:60kg N,P_2O_5, K_2O ha^1 (General recommended dose)	12.2	14.7	12.616	39.50	13.2
100:60kg N,P_2O_5, ha^1 +KMB	12.5	13.3	13.8	39.35	13.1
100:60:60 kg N, P_2O_5, K_2O ha-1 (General recommended dose) + KMB	13.1	11.6	13.6	38.29	12.7
100:60kg N, P_2O_5 ha^1 +45 kg K_2O +KMB	12.9	11.3	11.7	35.80	12.0
100:60 kg N,P_2O_5 ha 1 +30 kg K2 O +KMB	11.87	11.84	11.7	35.45	11.8
S.E(m)+/-					0.56
C.D.(0.05)					1.62

The table indicates that all the treatments were showing significant result over control and it is noted that where there is no potasic fertilizers, KMB release much reserve K_2O.

Table - 9 Nutrient uptake of chilli as affected by different biofertilizer applications at 45 days of growth

TREATMENTS	Available Nutrients (Kg/ha)		
	N	P	K
Control (No. Biofertilizer + No. chem. Fertilizer)	0.190	0.022	0.58
KMB	0.263	0.035	1.17
100:60:60kg N,P_2O_5, K_2O ha 1 (General recommended dose)	0.442	0.063	2.12
100:60kg N,P_2O_5, ha^1 +KMB	0.605	0.071	2.31
100:60:60 kgN, P_2O_5, K_2O ha-1(General recommended dose)+KMB	0.679	0.061	1.90
100:60kg N, P_2O_5 ha^1 +45 kg K_2O +KMB	0.452	0.056	1.84
100:60 kg N,P_2O_5 ha^1 +30 kg K_2O +KMB	0.314	0.051	1.90

All the data shown significant uptake than control and highest K uptake noticed in the treatment received 100 kg N, 60 kg P_2O_5 and KMB inoculant.

Release of potassium in different Orissa soils

The potassium release was observed by Rath *et.al*. in 2002 in different types of soil in Orissa reported in Table - 10.

Table - 10 Performance of Potash Mobilizing Bacteria in different soil conditions at different locations in Orissa State.

Name of place from where soil was collected	Initial 10 days inoculation					20 days inoculation			
Phulbani (Red soil)	6.94	0.04	504.0	7.0	0.09	524.7	6.94	0.07	510.7
Aska (Alluvial soil)	6.71	0.06	73.9	7.48	0.07	140.4	7.33	0.07	120.9
Bhavanipatna (Black soil)	5.50	0.04	208.3	5.53	0.08	215.0	6.66	0.08	275.5
Keonjhar (Black soil)	8.09	0.03	215.0	8.07	0.07	295.7	8.10	0.09	288.9

(Source - M.Rath, N.K.Pradhan, A. Mishra and K.Chandra 2002; Sahoo.2002)

Table indicating that the potash mobilizing bacteria's activity is optimum after 10 days inoculation. 140.4, 295.7 kg/ha in alluvial soil and black soil respectively. Later it shows decrease due to its refixation.

Response of F.aurentia on Yam and Tapioca

The difference treatments was studied by Dr. S. Rath on the Yam and Tapioca Crop (Table - 11).

Table - 11 Effect of Potassium Mobilizer on the growth and yield of Yam and Tapioca.

Crops	Treatment	Treatment	Yield/ha (q)
Yam	C1T1	BF	257
	C1T2	PC	255
	C1T3	BF+PC	248
	C1T4	CF+BF+PC	352
	C1T5	CF	349
	C1T6	CF+PC	343
	C1T7	FYM	245
Tapioca	C2T1	BF	242
	C2T2	PC	246
	C2T3	BF+PC	248
	C2T4	CF+BF+PC	302
	C2T5	CF	283
	C2T6	CF+PC	289
	C2T7	FYM	238

BF Biofertilizer, Azospirillum + Azotobacter

PC Potash culture CF Chemical fertilizer (N:P:K 80:60:100 /ha)

FYM Farm yard manure (1kg per pit common to all)
(Source- Dr. Sabyasachi Rath, ADR, RRTTS, Semiliguda, Koraput, Orrisa).

Potassium uptake in Brinjal

A pot experiment conducted by Nayak indicates results that application of Potash Mobilizers can save 50% chemical potash and additional around 51% uptake of K increased than control pots and 28.5% of biomass increased than control (Table -12).

Table - 12 Potassium uptake by plants & K status in soil and Biomass in Brinjal at different treatments

Treatments	K Uptake (K/ha)	K in soil (Kg/ha)	Biomass generated (g/pot)
Control (No. Biofertilizer +No. Chem. Fertilizer)	2.935	89.4	23.50
Potash Mobilizing Bacteria (KMB)	3.076	139.6	29.02
100:60:60kg $N_1P_2O_5$ K_2O ha^{-1} (General recommended dose)	3.930	134.4	48.91
100:60kg $N_1P_2O_5$ ha^{-1} + KMB	3.762	129.0	38.19
100:60:60kg $N_1P_2O_5$ K_2O ha^{-1} (General recommended dose)	3.682	155.8	34.74
100:60kg $N_1P_2O_5$ ha^{-1} +45 kg K_20 + KMB	4.109	131.6	31.37
100:60kg $N_1P_2O_5$ ha^{-1} +30 kg K_20 + KMB	4.438	134.4	36.23

(Source Nayak. B. 2001 OUAT, Bhubaneshwar)

Frateuria aurentia as a K-mobilizer from ores

The study was conducted by Dash in O.U.A.T with the help of Dr. L.B. Sukla, Regional Research Laboratory, Bhubaneswar recorded use of *"Frateuria aurentia"* is capable of releasing elementary potassium from ores. (Table - 13)

Table - 13 Available K_2O after 20 days incubation with the KMB culture from Manganese 0re.

Ore	% of Inoculum	Available K_2O of dry ore without any incubation (control) (Kg/ha)	Available K_20 of ore inoculated with culture (Kg/ha)
Mn	1%	23.3	27.96
	2%	23.3	27.96
	3%	23.3	37.28
	4%	23.3	46.6
	5%	23.3	60.58
	10%	23.3	55.92
	15%	23.3	55.92
	20%	23.3	46.6

(Source Deepa Murmur 2002; Dash. M.D. 2002)

Response of F.aurentia on Paddy and Okra

The response of Potassium mobilizer on Paddy and Okra was studied by Mrs. S. Biswas, OUAT, Bhubaneshwar.

Table - 14 Soil characterstics before and after inoculation of KMB in Paddy and Okra Rhizosphere

Soil Samples	pH	N (Kg/ha)	P_2O_2 (Kg/ha)	K_2O (Kg/ha)	pH	N (Kg/ha)	P_2O_2 (Kg/ha)	K_2O (Kg/ha)
Paddy Rhizosphere	5.0	190.5	232.9	131.7	7.0	341.6	232.2	241.9
Okra Rhizosphere	5.2	190.5	40.8	108.0	7.2	375.2	392.8	341.2

(Source Biswas. S. 2001)

It is evident from Table - 14 that the availability of K_2O in soil after application of KMB in rhizosphere of paddy increased 83% in the same way in Okra Rhizosphere the availability of K_2O is 215%.

Response of F. aurentia on Paddy

The study was conducted by Biwas *et.al* in 2001 on Paddy Table-15.

Table - 15 Potassium uptake and Biomass of Paddy as affected by different treatments at 45 days harvest

	Treatment	K uptake (g/pot)	Dry weight (g/pot)
T1	Control (No fertilizer + No biofertilizer	0.297	22.235
T2	Potash mobilizing bacteria	0.372	28.238
T3	Potash mobilizing bacteria + Azospirillum	0.569	30.466
T4	Potash mobilizing bacteria + Azospirillum + Phosphorus solubilizing bacteria	0.632	34.175
T5	T_4+ 80 : 40 : 40 kg $N_1P_2O_5$ K_2O ha^{-1} (General recommended dose for rice)	0.527	35.175
T6	T_4 + 56 : 28 : 28 kg $N_1P_2O_5$ K_2O ha^{-1} (30% less than the recommended dose)	0.631	30.502
T7	80 : 40 kg $N_1P_2O_5$,ha^{-1} (Recommended dose) + Potash mobilizing bacteria	0.771	38.559
T8	80 : 40 kg N_1 P_2O_5,ha^{-1} (Recommended dose) + Potash mobilizing bacteria + 30 Kg K_2O ha^{-1} (25% less than recommeded dose)	1.001	50.089

(Source Biswal.S. 2001 Thesis, Orissa University of Agriculture and Technology, Bhubaneswar)

Response of *F.aurentia* on Ground nut

Table - 16 shows that the uptake of K_20 and biomass production due to inoculation of Potash and *Rhizobium* inoculated Groundnut is on far with recommended chemical fertilizers application. So it is evident that the chemical fertilizers could be replaced when we use nitrogenous biofertilizers along with KMB culture.

Table - 16 Potassium and biomass uptake of Groundnut (g/pot) as influenced by different treatments at 45 days of growth

Treatments	K uptake g/pot (g/pot)	Dry weight (pods) g/pot	Dry weight (plant)
Control	0.232	4.915	16.907
Potash mobilizing bacteria	0.270	7.021	17.338
Potash mobilizing bacteria + Rhizobium	0.364	8.584	28.845
20:40:40 kg $N_2P_2O_5$ K_2O ha^{-} (General recommended dose)	0.347	8.359	26.086
20:40:40 kg $N_2P_2O_5$ K_2O ha^{-1}+ (General recommended dose) + Potash mobilizing bacteria	0.516	9.106	31.736
20:40:40 kg $N_2P_2O_5$, K_2O ha^{-1}+ (General recommended dose) + Rhizobium + Potash mobilizing bacteria	0.570	10.079	37.651

(Source Mishra. M. 2001 Thesis, Orissa University of Agriculture and Technology, Bhubaneswar)

11. Co inoculation of *F.aurentia*

Multilocational field trails

In order to maintain sustainable agricultural productivity and soil fertility use of different biological sources for meeting crop nutrient requirements through microbial resources, ministry of Agriculture, Department of Agriculture and cooperation sanctioned a scheme on "National project on development and use of biofertilizers during 7th five year plan so as to promote the use of biofertilizers in Indian agriculture for augumenting crop nutrients through soil microbes. Subsequently same ministry sanctioned "National project on Organic Farming" during 2004 and other organic inputs. On different crops, so as to test the efficacy of biofertilizers and demonstrate their usefulness to the farming community. The Rational Centre of Organic Farming Centres (RCOFs) at Bangalore, Nagpur, Jabalpur, Bhubaneshwar, Hisar and Imphal have been conducting Field demonstrations on biofertilizers and organic manures in various crops. The project conducted large number of field demonstrations using different liquid biofertilizers like *Rhizobium, Azotobacter, Azospirillum,* Phosphate solubilizing microorganisms (PSM), Potash Mobilizing bacteria, BGA, Azolla etc. Crop response studies at the farmers filed at different agro climatic locations have been carried out in order to convince and get confidence of farmers about different organic inputs. The details of the field demonstrations and yield levels presented in the Table - 17.

Table - 17 Effect of Liquid Biofertilizers (Azotobacter, PSM, KSM) on yield of some cultivated legume / non-legume crops (RCOF results)

Location	Crop	(Control) Yield (Q/ha)	(Treated) Yield (Q/ha)	% increase	Year
Deogarh	Wheat	11.2	15.4	37.5	2002
Kaladi, Phulbani	Maize	15.20	15.60	2.63	2001
Gundrigaon, Phulbani	Maize	15.06	15.36	2.00	2000
Katingia, Phulbani	Brinjal	33.12	42.40	28.00	2001
Sundarpali, Phulbani	Paddy	11.00	11.50	4.54	2000
Sasaikuti, Phulbani	Turmeric	22.00	24.00	9.74	2000
Katrajhari, Nayagarh	Paddy	13.60	19.60	44.11	2001
Dhanupara, Bihar	Paddy	18.08	20.50	13.38	2001
Sayabalpur, Bihar	Paddy	8.75	11.50	31.42	2000
Kanitiakatene	Paddy	34.5	40.00	16	2002
Kulagada	Paddy	45.0	55.00	22.2	2000
Karikol	Paddy	42.0	52.00	23.8	2002
Dalaiguda	Paddy	35.00	48.00	37.0	2001
Samantrapur	Paddy	38.00	44.00	15.78	2000
Tangara	G.nut	11	17.50	59.09	2001

Tangara	G.nut	11	17.50	59.09	2001
Sinshour	Paddy	37.00	42.00	13.5	2000
Basudeipur	Paddy	25.87	41.25	59.45	2002
Chipilima	Paddy	51.40	72.80	41.6	2000
Hatipale	Paddy	65.60	90.00	38.46	2000
Kulakaijunga, Jagatsingpur,	Paddy	13.00	15.00	20.0	2000
Jamugaon, Jagatsinghpur	Paddy	13.75	17.25	25.45	2002
Kusapur, Chattrapur	G.nut	13.87	17.40	25.68	2001
R.K. Gram, North Andaman	Paddy	48.40	59.20	22.90	2000
Rampur, Andaman & Nicobar	Paddy	40.8	44.4	8.82	2002
Temple Myo, South Andam	Paddy	28.40	33.20	16.90	2000
Gobardhansole Mayurbhanj	Mung	6.52	7.65	17.33	2000
Chandua, Mayurbhanj	Mung	6/75	7.57	12.14	2001
Subadega, Sundargarh	Paddy	11/46	12.60	9.94	2002
Sundargar, West Benga l	Paddy	10.83	12.27	13.29	2002
Dharmapuri, Tamil nadu	Paddy	09.15	10.83	8	2004

Anaimalai, Tamil Nadu	Paddy	12.25	13.39	9	2004
Harihara, Karnataka	Paddy	17.0	25.0	8.69	2003
Makanahally, Karnataka	Paddy	16.0	20.0	25.0	2003
Seegepalya, Karnataka	Paddy	17.0	21.0	23.5	2003
Gulbarga, Karnataka	G.nut*	6.96	7.10	2.01	2003
Pandavapura, Karnataka	Ragi	20.0	23.5	17.5	2003
Raibag	Maize	17.00	19.86	16.82	2005
Kodagu	Paddy	10.00	11.30	13.00	2005
Tarikere	Paddy	17.00	17.00	0.00	2005
Mardhanahally Hobli	Beetroot	145	159	9.60	2005
Vellore	G. nut	10.50	12.00	14.28	2005
Salem	Cabbage	200	211	5.50	2005
Namakkal	Cabbage	220	260	18.18	2005
Sengottai	Redgram	8.6	9.00	4.65	2005
Omalur	Tomato	191	200	4.71	2005
Kanakapura	Paddy	16.98	18.00	6.00	2005
Bangalore South	Tomato	118	125	5.93	2005

Bangalore South	Bean	50	58	16	2006
Karur	Paddy	21.00	21.80	3.80	2005
Villupuram	Paddy	18.6	20.10	8.06	2005
Amirthi	Paddy	16.20	17.50	8.02	2006
Kalang Kuppam	Paddy	19.00	20.00	5.26	2006
Coimbatore bean	Cluster	46.00	51.00	10.86	2006
Manandhawady	Ginger	13.8	146	5.79	2006

*Rainfed

The demonstration were done at farmers land and different parts of India. All the trails were included potash mobilizing bacterial application and all the biofertilizers used in liquid form. Which is having special formulation techniques formulated by Dr. Krishan Chandra, Regional Director, Bangalore.

The field data indicates that the yield increase has minimum 2% and maximum 59.09% besides all the demonstrations responded well towards potash mobilizing bacteria inoculants.

Advantages of Potash Mobilizing Biofertilizers

1. Reduce cost of potash application by 50-60%
2. Improves resistance of crop plants
3. Suitable to a wide range of soil pH and temperature
4. Suitable to apply for all crops
5. Improves crop growth and yield by 20-30%
6. Compatible with other beneficial microbes in the rhizosphere
7. It encourages early root development
8. Enhances soil health and soil fertility
9. Also secretes growth hormones to increase crop productivity.
10. Benefits the next crop also due to its residual effect.

Effect of Liquid Potassium mobilizer with Vesicular Arbuscular Mycorrhizae (VAM)

A experiment was conducted by Greep *et.al.* (2006) to evaluate the effect of different liquid *Azotobacter, Phosphate* solubilizing organisms. Arbuscular mycorrhae (soil base) and Potassium mobilizing bacteria as individual as well as in combinations along with compost and phosphocompost in capsicum, Hangarian yellow verity shows the better results when compared to the controls. (Plain soil). Further the phospho compost applied field shows pronouncing results when compared to compost applied field in all the parameters studied. In growth parameters Potassium mobilizing bacteria *(Frateuria aurentia)* enriched Phosphocompost shows the highest results next to all the biofertilizers applied together. This may be due to the mobilization of Kas well as release of growth hormones by the K-mobilizer and uptake of potassium also significantly increased in the plants. (Table 18-25).

Data in the Table - 20 shows that compost enriched with *Azotobacter* showed increased nitrogen content than other individual inoculation. Least nitrogen content was observed in control. Plants grown in *Bacillus polymyxa* enriched phosphocompost showed 0.29 per cent of phosphorus while the percentage was significantly increased in combined inoculation with liquid bioinoculants (0.36 percent).

Potassium uptake was found to increase in *Frateuria aurentia* enriched compost (1.50 percent), while there was drastic reduction in control plants (uninoculated).

Calcium content recorded from 30 day old plants treated with compost and phosphocompost varied between 0.011 to 0.021 and 0.014 to 0.028 respectively. Magnesium content in T_5 treated plants showed 0.018 per cent and 0.019 per cent respectively. In T_4 treated plants showed significant reduction in magnesium content than control plants (Table-20). Maximum nitrogen content was recorded in the advanced stages of plant growth and the

maximum was recorded in 60 day old plants (Table-22). Among the treatments studied, higher nitrogen uptake was recorded in T_5 (*Azotobacter,* PSM, AMF, and KMB). In T_5 treated phosphocompost recorded maximum nitrogen content (2.80 per cent) followed by T_1 treated with *Azotobacter*. The lowest value was recorded in T_3 (enriched with AMF) with the value of 1.60 per cent. Similar trend was recorded in nitrogen content of plants treated with enriched compost.

Higher phosphorus content (0.52 per cent) was recorded in T_5 followed by T_2 (0.31 per cent) plants treated with compost enriched with PSM than control.

Significant increase in Potassium content was recorded in T_5 (1.90) and followed by T_4 (1.80) (enriched with KMB). The lowest potassium uptake was recorded in control plants. Marked increase in Calcium and Magnesium content was noticed than control plants.

Field Experiments on Potash Mobilizing Bacteria

The results that were confirmed from poly bag experiment were tested under field condition at Nelamangala in replicated randomized block design with three replications for each treatment. The growth and biomass, nutrient uptake and microbial dynamics were evaluated in Capsicum plant.

The compost enriched with different microbial inoculum viz., *Azotobacter,* PSM, KMB, AMF increased significantly the plant growth of 30 and 60 day old plants than control plants (Table - 22 and 23). There was marked increase in root length in T_1 (*Azotobacter* enriched compost). In T_3 (AMF enriched compost) did not show any appreciable increase in root length in 30 day old plants.

Highest dry weight of shoot and root was recorded in T_5 treatment in 30 day old plants with various combinations of bioinoculants.

The shoot branches were more pronounced in T_5 treatment (14.00). Whereas T_4 showed significant increase in flower and fruit formation (compost enriched with KMB).

The growth and biomass production were much pronounced in phosphocompost enriched plants than compost alone applied treated in 30 day old plants. Highest dry weight (5.62 g) was recorded in T_5 treated plants than other treatments. (Table -22)

The plant height increased with the advancement of crop growth and showed maximum at 60 days (Table -23).

T_4 (liquid KMB enriched compost) treatment exerted marked increase in shoot and root length of 60 day old Capsicum plants than other individual treatment. The lowest plant height was recorded in control plants. *Azotobacter* enriched phosphocompost treatment showed appreciable difference in shoot length of 60 day old plants.

The dry weight of shoot and root are presented in Table-23. Maximum shoot and root dry weight was observed in T_5 (different bioinoculants) and the same trend was reflected in enriched phosphocompost treated plants. It was observed that the treatment T_4 and T_5 recorded the highest (12) branches, and flowers and fruits with the values of 5.00 and 8.00 respectively.

It was observed that there was an increased biomass production in phosphocompost enriched than compost enrichment alone. T_2 (PSM enriched Phosphocompost) showed a highest shoot and root lengths than the control plants. There was considerable increase in dry weight of plants in T_5 (different combination of bioinoculants). The same trend was noticed with regard to number of branches, flowers and fruit formation. (Table - 23)

30 day old capsicum plants treated with enriched compost and phosphocompost showed significant increase in N, P and K content than control plants. Higher nutrient content was recorded

in T_5 treated with combined inoculation of all bioinoculants inoculation. Higher N content was observed in T_5 (1.80) followed by T_1 (1.70), compost and *Azotobacter*.

Phosphorus content varied with different treatments, highest value was recorded in plants treated with T_5(different bioinoculants).

Higher potassium content was recorded in T_5 followed by T_4 plants treated with compost and KMB. With regard to calcium and magnesium content, plants applied with enriched compost and phosphocompost showed increased values than control plants. (Table -24).

The data in Table - 25 represent the variation in N P K content of 60 day old capsicum plants when treated with enriched compost and phosphocompost. Higher N content was recorded in T_1. The recorded values of nitrogen content was 2 per cent. This was followed by the application of compost enriched with combined inoculation of bioinoculants (T_5). The lowest value was recorded in T_2. (Table - 25).

Significant increase in Phosphorus content was recorded in T_5 (different bioinoculants) than other treatments. Capsicum plants treated with Compost KMB (T_4) showed higher potassium content, followed by T_5.The calcium content was recorded with values 0.009-0.028 percent in 60 day old plants. The calcium content was significantly increased with the advancement of plant growth. Enriched phospho compost applied Capsicum plants showed increased nutrient content than control. (Table-25).

Table - 18 Effect of compost and phosphocompost enriched with different liquid bioinoculants in Capsicum (poly bag experiment) 30 day old plants.

Treatments *	Compost						
	Shoot length (cm)	Root length (cm)	Dry weight (g		No. of branches	No. of flowers	No. of fruits
			Shoot	Root			
Control	27.00d ***	33.00e	2.10b	0.50b	2.00b	2.00a	1.00ab
T1	37.00c	39.50d	2.40a	0.80b	2.00b	3.00a	1.00ab
T2	37.00c	47.00b	3.00a	1.10ab	3.00ab	2.00a	0.00b
T3	39.00b	42.00c	3.50a	1.70a	3.00ab	2.00a	2.00a
T4	38.00bc	46.00b	3.80a	1.76a	3.00ab	3.00a	2.00
T5	43.00a	49.00a	3.90a	1.90a	4.00a	3.00a	2.00a

Treatments *	Phosphocompost**						
	Shoot length (cm)	Root length (cm)	Dry weight (g		No. of branches	No. of flowers	No. of fruits
			Shoot	Root			
Control	28.00e	35.00f	2.90b	1.10b	4.00b	4.00a	1.00bc
T1	39.00d	40.00e	3.70b	1.12b	3.00b	1.00b	0.00c
T2	41.00c	49.00b	4.20b	1.40ab	3.00b	3.00a	2.00ab
T3	42.00c	44.00c	3.70b	1.30ab	3.00b	4.00a	2.00ab
T4	46.00b	42.00d	7.70a	2.00ab	3.00b	3.00a	2.00ab
T5	49.00a	52.00a	7.50a	2.70a	6.00a	3.00a	3.00a

* Control only Compost
T1 - Compost + Azoto
T2 - Compost + PSM
T3 - Compost + AMF
T4 - Compost + KMB
T5 - Compost + Azoto + PSM + AMF + KMB
** with the addition of RRP
*** Means in a column followed by same letter(s) are not significant.

Table - 19 Effect of compost and phosphocompost enriched with different liquid biofertilizers in Capsicum in poly bag experiment at 60 days

Treatments *	Compost						
	Shoot length (cm)	Root length (cm)	Dry weight (g		No. of branches	No. of flowers	No. of fruits
			Shoot	Root			
Control	37.50e ***	51.00b	2.72c	0.64a	8.00a	2.00a	4.0a
T1	41.00d	43.00d	3.16bc	1.30a	4.00c	1.00b	5.00a
T2	44.00c	49.00b	3.96ab	1.40a	4.00c	1.00b	5.00a
T3	46.30b	46.80c	3.96ab	1.89a	4.00c	0.00c	5.00a
T4	46.00b	43.00d	4.86ab	1.60a	6.00b	1.00b	4.00a
T5	66.00a	59.00a	4.91a	2.06a	4.00c	2.00a	4.00a

Treatments *	Phosphocompost**						
	Shoot length (cm)	Root length (cm)	Dry weight (g		No. of branches	No. of flowers	No. of fruits
			Shoot	Root			
Control	42.00f	52.50c	3.06b	0.67b	6.00b	3.00c	5.00bc
T1	49.00d	45.20d	3.55ab	1.71ab	8.00a	3.00c	4.00cd
T2	46.50e	56.00b	4.45ab	1.90ab	6.00b	3.00c	3.00d
T3	52.70c	43.20e	3.16b	1.00ab	6.00b	4.00bc	7.00a
T4	61.00b	55.00b	4.98a	2.20a	6.00b	5.00a	4.00cd
T5	70.60a	59.80a	5.09a	2.36a	6.00b	6.00a	5.00bc

* Control only Compost
T1 - Compost + Azoto
T2 - Compost + PSM
T3 - Compost + AMF
T4 - Compost + KMB
T5 - Compost + Azoto + PSM + AMF + KMB
** with the addition of RRP
*** Means in a column followed by same letter(s) are not significant.

Table - 20 Effect of composts and phosphocompost enriched with liquid biofertilizers on uptake of major and minor elements in Capsicum in poly bag experiment at 30 days.

Treatments *	Compost				
	Major Elements (%)			Minor Elements (%)	
	N	P	K	Ca	Mg
Control	1.20d ***	0.13f	0.80d	0.011f	0.010c
T1	1.80b	0.15e	0.98c	0.018c	0.010c
T2	1.30d	0.28b	1.26b	0.016d	0.012b
T3	1.50c	0.23c	1.30b	0.014e	0.013b
T4	1.50c	0.19d	1.50a	0.019bc	0.018a
T5	2.00a	0.30a	1.56a	0.021a	0.018a

Treatments *	Phosphocompost				
	Major Elements (%)			Minor Elements (%)	
	N	P	K	Ca	Mg
Control	1.60c	0.16e	0.82c	0.016c	0.010d
T1	1.80b	0.15e	1.20b	0.019b	0.016c
T2	1.40de	0.29b	1.40a	0.014d	0.017bc
T3	1.30e	0.23c	1.20b	0.016c	0.017bc
T4	1.45d	0.20d	1.50a	0.019b	0.018a
T5	2.20a	0.36a	1.58a	0.028a	0.019a

* Control only Compost
T1 - Compost + Azoto
T2 - Compost + PSM
T3 - Compost + AMF
T4 - Compost + KMB
T5 - Compost + Azoto + PSM + AMF + KMB
** with the addition of RRP
*** Means in a column followed by same letter(s) are not significant.

Table 21 Effect of composts and phosphocompost enriched with liquid biofertilizers on uptake of major and minor elements in Capsicum in poly bag experiment at 60 days

Treatments *	Compost				
	Major Elements (%)			Minor Elements (%)	
	N	P	K	Ca	Mg
Control	1.70c ***	0.16e	0.86e	0.016f	0.012d
T1	2.00b	0.16e	1.13d	0.062c	0.013d
T2	1.70c	0.29b	1.30c	0.081b	0.015c
T3	1.60c	0.25c	1.49b	0.088a	0.016bc
T4	1.70c	0.19d	1.60b	0.019e	0.017b
T5	2.56a	0.36a	1.80a	0.028d	0.019a

Treatments *	Phosphcompost				
	Major Elements (%)			Minor Elements (%)	
	N	P	K	Ca	Mg
Control	1.70c	0.18f	0.92c	0.016e	0.013d
T1	2.56a	0.20e	1.46b	0.017dc	0.013d
T2	1.90b	0.31b	1.42b	0.018d	0.016c
T3	1.60c	0.26d	1.50b	0.026c	0.018b
T4	1.65c	0.29c	1.80b	0.029b	0.018b
T5	2.80a	0.52a	1.90a	0.032a	0.02a

* Control only Compost
T1 - Compost + Azoto
T2 - Compost + PSM
T3 - Compost + AMF
T4 - Compost + KMB
T5 - Compost + Azoto + PSM + AMF + KMB
** with the addition of RRP
*** Means in a column followed by same letter(s) are not significant.

Table 22 Effect of compost and phosphocompost enriched with different liquid biofertilizers in Capsicum under field condition experiment at 30 days

Treatments *	Compost						
	Shoot length (cm)	Root length (cm)	Dry weight (g		No. of branches	No. of flowers	No. of fruits
			Shoot	Root			
Control	22.50d ***	22.50b	1.90b	0.40d	8.00d	3.00a	1.00c
T1	30.00b	30.00a	2.00b	0.40d	8.00d	4.00a	2.00bc
T2	29.00b	17.00d	3.10b	0.80b	6.00e	3.00a	3.00ab
T3	28.00b	15.00e	2.30b	0.40d	10.00c	3.00a	1.00c
T4	25.00c	20.00c	2.70b	0.60c	12.00b	4.00a	4.00a
T5	32.70a	23.50b	5.60a	1.00a	14.00a	3.00a	3.00ab

Treatments *	Phosphocompost**						
	Shoot length (cm)	Root length (cm)	Dry weight (g		No. of branches	No. of flowers	No. of fruits
			Shoot	Root			
Control	38.00ab	23.00b	2.00b	0.50b	10.00b	3.00ab	3.00a
T1	33.00d	30.10a	2.20b	0.60b	6.00c	4.00a	1.00b
T2	32.50d	18.00c	3.00b	0.80b	6.00c	4.00a	2.00ab
T3	36.60b	23.00b	2.80b	0.50b	6.00c	4.00a	3.00a
T4	35.00c	24.50b	2.80b	0.60b	10.00b	2.00b	1.00b
T5	38.20a	24.60b	5.62a	1.60a	12.00a	2.00b	2.00ab

* Control only Compost
T1 - Compost + Azoto
T2 - Compost + PSM
T3 - Compost + AMF
T4 - Compost + KMB
T5 - Compost + Azoto + PSM + AMF + KMB
** with the addition of RRP
*** Means in a column followed by same letter(s) are not significant.

Table - 23 Effect of compost and phosphocompost enriched with different liquid biofertilizers in Capsicum under field conditions experiment at 60 days

Treatments *	Compost						
	Shoot length (cm)	Root length (cm)	Dry weight (g		No. of branches	No. of flowers	No. of fruits
			Shoot	Root			
Control	25.00e ***	17.00d	4.30a	0.80bc	8.00b	3.00bc	5.00b
T1	36.50c	19.00c	4.80a	0.70c	6.00c	3.00bc	3.00c
T2	35.00c	18.00cd	3.00b	0.40d	8.00b	4.00ab	9.00a
T3	29.50d	26.00a	5.80a	0.70c	8.00b	4.00ab	3.00c
T4	38.20b	27.50a	5.20a	0.90a	12.00a	2.00c	5.00b
T5	43.50a	24.00b	5.20a	1.00a	12.00a	5.00a	8.00b

Treatments *	Phosphocompost**						
	Shoot length (cm)	Root length (cm)	Dry weight (g		No. of branches	No. of flowers	No. of fruits
			Shoot	Root			
Control	35.00e	16.00d	4.00b	1.00a	10.00d	2.00c	8.00a
T1	47.00a	19.00c	4.90a	0.90a	14.00c	4.00b	5.00bc
T2	45.50a	24.00b	4.00b	0.70b	16.00c	6.00a	8.00a
T3	37.00d	16.50d	6.00a	1.00a	18.00a	5.00ab	6.00b
T4	39.00c	26.00a	5.30ab	1.20a	8.00e	2.00c	4.00c
T5	41.00b	23.00b	5.80a	1.60a	14.00c	5.00ab	6.00b

* Control only Compost
T1 - Compost + Azoto
T2 - Compost + PSM
T3 - Compost + AMF
T4 - Compost + KMB
T5 - Compost + Azoto + PSM + AMF + KMB
** with the addition of RRP
*** Means in a column followed by same letter(s) are not significant.

Table - 24 Effect of compost and phosphocompost enriched with different liqiud biofertilizers on uptake of major and minor elements in Capsicum under field condition experiment at 30 days

Treatments *	Compost				
	Major Elements (%)			Minor Elements (%)	
	N	P	K	Ca	Mg
Control	1.50b ***	0.15d	0.90d	0.009d	0.011c
T1	1.70a	0.16cd	1.40c	0.018c	0.012c
T2	1.20c	0.26b	1.46c	0.018c	0.016b
T3	1.50b	0.27b	1.50c	0.021b	0.017b
T4	1.60c	0.17c	1.68b	0.026a	0.017b
T5	1.80a	0.29a	1.80a	0.026a	0.019a

Treatments *	Phosphocompost				
	Major Elements (%)			Minor Elements (%)	
	N	P	K	Ca	Mg
Control	1.52c	0.19d	1.00c	0.010d	0.010d
T1	1.80a	0.19d	1.49b	0.020c	0.016bc
T2	1.40c	0.28b	1.46b	0.020c	0.015c
T3	1.70ab	0.23c	1.56b	0.022b	0.016bc
T4	1.60bc	0.19d	1.80a	0.021bc	0.017ab
T5	1.80a	0.32a	1.80a	0.030a	0.018a

* Control only Compost
T1 - Compost + Azoto
T2 - Compost + PSM
T3 - Compost + AMF
T4 - Compost + KMB
T5 - Compost + Azoto + PSM + AMF + KMB
** with the addition of RRP
*** Means in a column followed by same letter(s) are not significant.

Table - 25 Effect of compost and phosphocompost enriched with different liquid biofertilizers on uptake of major and minor elements in Capsicum under field condition experiment at 60 days

Treatments *	Compost				
	Major Elements (%)			Minor Elements (%)	
	N	P	K	Ca	Mg
Control	1.60bc ***	0.15d	1.00e	0.009e	0.007e
T1	2.00a	0.19c	1.26d	0.018d	0.009d
T2	1.50c	0.28b	1.60c	0.019cd	0.018b
T3	1.60bc	0.28b	1.68bc	0.021b	0.016c
T4	1.70b	0.19c	1.84a	0.020bc	0.018b
T5	1.90a	0.30a	1.81a	0.028a	0.020a

Treatments *	Phosphocompost				
	Major Elements (%)			Minor Elements (%)	
	N	P	K	Ca	Mg
Control	1.70b	0.181d	1.20c	0.018e	0.012c
T1	2.01a	0.19d	1.23c	0.023c	0.016b
T2	1.50c	0.29b	1.68b	0.020d	0.017ab
T3	1.68bc	0.26c	1.72a	0.025b	0.018a
T4	1.52c	0.19d	1.83a	0.025b	0.018a
T5	1.86ab	0.36a	1.86a	0.036a	0.018a

* Control only Compost
T1 - Compost + Azoto
T2 - Compost + PSM
T3 - Compost + AMF
T4 - Compost + KMB
T5 - Compost + Azoto + PSM + AMF + KMB
** with the addition of RRP
*** Means in a column followed by same letter(s) are not significant.

Recommended crops

Crop	Soil application
Food crops : Cereals which include Rice, Wheat, Millet	Use 250 ml / acre in sufficient carrier like well decomposed organic matter along with half the recommended dose of potash fertilizer. The blended material can be kept under shade for over night and maintain 50% moisture and applied in rows or during leveling of soil.
Vegetables (Open Cultivation)	Use 750 - 1000 ml / acre. In sufficient well decomposed organic matter along with half the recommended dose of potash fertilizer. Application can be made as band placement / side dress application at the time of planting or during the early stages of the cropping.
Vegetables (Green House condition) : Beans, Black eyed Peas, Cabbage, Cauliflower, Lettuce, Egg plant, Melon, Mustard, Onion, Parsnips, Peas, Pepper, Potato, Radish, Spinach, Squash, Tomato and Turnips	Use 250 - 500 ml / acre in well decomposed organic manure along with half the recomm--ended dose of Potash fertilizer and keep it under shade over night by maintaining 75% moisture. Apply it as a band placement / slide dess application at planting stage or during early stages of the crop.
Roses and other flowering shrubs including Camellia Hibiscus and the like.	Blend 500 ml / acre of the material in well-decomposed organic manure along with half the recommended dose of Potash fertilizer and keep it under shade got over night by maintaining 50% moisture. Apply it as a band placement / side dress application at planting stage or during early stages of the crop. For closely planted crop, 500 ml / acre to be mixed in 500 its of water and apply directly as soil drench ensuring that each plant gets supply from 6 to 12 tea spoons spread over 100 sq. ft. Each teaspoon normally contains 5 ml. Therefore 60 ml of the spray fluid to be applied in 100 sq. ft.

However KMB liquid formulation is recommended for all crops in all agro-climatic zones

Co-inculation

The potash mobilizing biofertilizers can be applied along with *Rhizobium, Azospirilum, Azotobacter, Acetobacter,* PSM etc. Field result, reveals that by applying this K mobilizers, 15-25% yield can be enhanced. Bacteria can be applied for all types of crops at the rate of 200 ml per acre for agriculture crops for perennial crop 400 ml / acre after mixing in 200-500 kgs of matured FYM before sowing or in furrows (Chandra *et.al.*2004). Dr. D.Clarson, 2004 published the details about Potash Mobilizing bacteria (KMB). Table 10 shows the yield increase in yam & Tapioca by potassium mobilizer when it applied along with other Biofertilizers and chemical fertilizers.

Purchase specification of liquid KMB

Parameters	Specification
1. Organisms	*Frateuria aurentia*
2. Microbial count	1 x 10^9 cells per ml.
3. Colour	Dull White coloured solution
4. Physical state	Liquid suspension
5. Odour	Characteristic
6. pH	4.5 $\pm$ 7.5
7. Water solubility	Miscible
8. Storage	Ambient conditions

QULAITY
Liquid Frateuria aurentia

Tests	Result
1. Consistency/Form	- Liquid
2. Source of strain	- R.C.O.F., Bangalore
3. Colour	- Straw
4. pH	- 5.5 (Acidic)
5. Contents	- Bacteria Frateuria aurentia Count 10^9 cfu per ml of the liquid
6. Contamination	- <100 cfu per ml of the liquid
7. Suspended solids	- 0.03%
8. Dissolved solids	- <3.37%
9. Nitrogen	- <0.3%
10. Phosphorus	- <0.005% (<48ppm)
11. Potash	- <0.02%(<119 ppm)
12. Calcium	- <0.2%
13. Sodium	- <0.01% (<64 ppm)
14. Magnesium	- <0.002% (<45 ppm)
15. Iron	- <0.001% (<9.5 ppm)
16. Sugar	- <3.0
17. Total protein	- <1.78%

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