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ISOLATION AND MASS PRODUCTION OF BIOFERTILIZER (AZOTOBACTER AND PHOSPHOBACTER)

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Abstract :- ***Biofertilizer is a substance which contains living microorganism, they have no toxic effect on the soil. The use of biofertilizer is low cost when compared to chemical fertilizer. Azotobacteria and phosphobacteria are the most important components of biofertilizer. They play the vital role in solubilising of phosphrous in soil and reducing the macro nutrition in soil.Due to the use of these organism the soil nutrition are enriched and not degraded in anyways.These are the plant growth promoting bacteria which are gram negative and mostly used as biofertilizers. Azotobacter a free living diazotroph has also been reported to produce beneficial effect on crop yield through a variety of mechanisms including biosynthesis of biologically active substances,stimulation of rhizospheric microbes modification of nutrient uptake and ultimately boosting nitrogen fixation.The main aim of this project is the mass production of phosphobacteria and azotobacteria.Here we have used the specific medium of pikovskaya's Media for phosphobacter and Ashby's Agar for the Azobacter for the production.Initially the organism where isolated from the soil sample and conformation was done using the biochemical test.When cell count reached to 108-109 cells/ml,the brouth used as inoculant.For easy handiling,packing,storing and transportation broth is mixed with an inert carrier material which contains sufficient amount of cells.In present study broth is mixed with unsterile soil:Activated charcoal,A.R.(RM 1332):$CaCo_3$ in a ratio of 1:2:1 where as other set prepared by using unsetrile soil:crude caol powder :$CaCo_3$ in same ratio over the carrier in such a way that 40% moisture is maintained.After proper mixing carrier containing inoculant was left for 7 days and above formulated microbial inoculants used as biofertilizer.It is naturaly produced biofertilizer and it does not have any nagative effect on the soil or crops.It increases crop yield by 20-30%,replaces chemical nitrogen and phosphrous by 25%and stimulates plant growth.It can also provide protection against drought and some diseases***

Keywords - Biofertilizer,Azotobacter,Phosphobacter.

INTRODUCTION

Biofertilizer is a substance which contains living micro organisms which ,when applied to the seed ,plant surfaces or soil colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, **(G.S Naganandha,arijitdas,SouravBhattacharta and T.Kalpana)**solubilizing phosphorus , and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides.Bio-fertilizers provide eco-friendly organic agro-input and are more cost-effective than chemical fertilizers.Since a bio-fertilizer is technically living, it can symbiotically associate with plant roots. Involved microorganisms could readily and safely convert complex organic material in simple compounds, so that plants are easily taken up.. It maintains the natural habitat of the soil. It increases crop yield by 20-30%, replaces chemical nitrogen and phosphorus by 25%, and stimulates plant growth. It can also provide protection against drought and some soil-borne diseases**.(Nilabja Ghosh, 2007)**

Azotobacter is a genus of usually motile, oval or spherical bacteria that form thick-walled cysts and may produce large quantities of capsular slime. They are aerobic, free-living soil microbes which play an important role in the nitrogen cycle in nature, binding atmospheric nitrogen, which is inaccessible to plants, and releasing it in the form of ammonium ions into the soil.*Azotobacter* have a full range of enzymes needed to perform the nitrogen fixation : ferredoxin, hydrogenase and an important enzyme nitrogenase**.(B.Juarez,M.V.Martinez Toledo,J.Gonzalez Lopez)**

Phosphobacteria means microbial inoculants capable of phosphate solubilizing nature. Commonly used *Phosphobacteria* is *Bacillusmegaterium*. *Phosphobacteria* is suitable for all crops. Phosphorus besides to nitrogen is one of the most important element in crop production.It makes about 0.2% of the total dry weight of the plants. Phosphobacteria is a plant nutrient that is essential for food synthesis, flower formation, fruit setting and seed setting.When Phosphobacteria is added in soil it produces organic crops and makes it to function well in alkaline soils.Phosphobacteria is recommendable to crops of all categories**.(Hassan Shokri Vahed Parisa Shahinrokhsar and Fatemeh Heydarnezhad,2012)**

MATERIALS AND METHODS:

The identification of Azotobacter and Phosphobacter where done according to the Bergey's Mnaual of systematic bacteriology**(Williams and Wilkins,1989)**. The specific medium used for the isolation of the phosphobacter and azotobacter are Pikovskaya's Media and Asbhy's agar**.(Biofertilizer Dr.Hari Muraleedharan, Dr.S.Sehadri, Dr.K.Perumal December 2010, *In vitro* studies on the**

effect of biofertilizer by G.S Naganandha , arijitdas , SouravBhattacharta and T. Kalpana) .

PROCEDURE:

The soil samples were collected from various fields and serial dilutions were done.The organism were isolated by the analysis of the characteristics according to the Morphological and Biochemical characteristics.The various biochemial tests conducted were citrate utilization, catalase, urease, indole, methyl red,vogues prokauer, H_2S and nitrate reduction test were performed and confirmed.Then using the specific medium Pikovskaya's medium for phosphobacter and Asbhy's agar for Azotobacter were used to grow the organism for the mass production.

MASS PRODUCTION:

For mass production of Azotobacter , bacterial strain is isolated from various regions and grown on slants for preservation as per need culture from slant were transferred to liquid broth of selective as well as optimized medium in the rotary shaker for 4 days to prepare starter culture. Later on the starter cultures is transferred to the fermenter in batch culture mode with proper maintenance of 300C and continuous agitation for 4-9 days. when cell count reached to 108- 109 cells/ml, the broth used as inoculant. For easy handling, packing, storing and transporting broth is mixed with an inert carrier material which contains sufficient amount of cells. In present study broth is mixed with unsterile soil: Activated charcoal, A. R. (RM 1332): CaCo3 in a ratio of 1:2:1where as other set prepared by using unsterile soil: crude coal powder: CaCo3 in same ratio over the carrier in such a way that 40% moisture is maintained. After proper mixing carrier containing inoculant was left for 7days and above formulated microbial inoculants used as biofertilizer.

RESULT AND DISCUSSION:

In the present study of azotobacter and phosphobacter the isolation and mass production were performed .Initially azotobacter were characterised using the observations. The shape was found to be oval or spherical ,the surface was flat with mucoid texture .The organism responded to the gram negative stain with motility and showed positive results for indole production, methylred test, voges proskauer, urease, starch hydrolysis, sugar fermentation(glucose,mannitol)**. (Williams and Wilkins,1989)**

Similarly the morphologial and bochemical characters of phosphobacter were found to be gram negative with rod shaped and non motile characteristics. The organism showed positive results for indole production ,methyl red and catalase test. The organism thus identified were cultivated in mass usinng the specific medias and used as biofertilizer.**(N.Uma Maheswari and S.Sudha).**

It is a natural method without any problems like salinity, alkalinity, soil erosion etc., In vast areas of low input agriculture as in crops like sugarcane these products will be of much use to give sustainability to production. Biofertilizer soaks up and hold these dissolved nutrients substances so that the roots have more time to absorb them. Biofertilizer also adds small amount of zinc, copper, boron and other vital nutrients to the soil and protects water quality**(Biofertilizer a novel tool for agriculture Boraste.A, Vamsi.K.K, Jhadav.A, Gupta.M, Joshi.B, Gupta.N, Gupta.G).**

A bio-fertilizer is technically living, it can symbiotically associate with plant roots. Involved microorganisms could readily and safely convert complex organic material in simple compounds, so that plants are easily taken up. Microorganism function is in long duration, causing improvement of the soil fertility. It maintains the natural habitat of the soil. Biofertilizer inoculation was found to improve plant growth and biomass significantly.The organism has high ability to increase N,P and K as well as other nutrition acquisition for plants.(**Seemaparoha,K.K Chandra and Rakhiyadav).**

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