CHALLENGING PROSPECTIVE APPROACHES FOR ENRICHING SOIL FERTILITY BY USING BIOFERTILIZER

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ABSTRACT

Soil is a very good medium for all microbial inoculums. Biofertilizer is substances which contain living microorganisms. They have no toxic effect on the soil. The use of biofertilizers is low cost when compared to chemical fertilizers. *Azotobacter* and *phosphobacterium* are the most important microbes of biofertilizers. They play on vital role in solubilizing of nitrogen and phosphorus in soil and reducing the macro nutrition in soil. The biofertilizer which promote soil nutrition are enriched fertility in all aspects. Isolation of *Azotobacter* and *phosphobacteria* from the marine soil sample of Rameshwaram, Ramanadu district. Tamil Nadu, India. The isolated strains of *Azotobacter* sp and *Bacillus megaterium* of *phosphobacterium* with specific medium of Ashby's ager and pikovskaya's agar medium respectively. Preliminary steps of bacteria where isolated from the soil sample and conformation was done using morphological and biochemical test for count reached to 108-109 cells/ml the broth used as inoculants mixed with carrier materials with the formulated microbial production used as biofertilizers. The biofertilizers is naturally produced and does not have any negative effect on the soil crops. The biofertilizers can increase maximum crop yield and control the chemical fertilizers. The biofertilizers of respective plants with the beneficial microorganisms provide protection against drought and some diseases.

INTRODUCTION

The challenging prospective approaches of biofertilizers contains living microorganisms and promotes growth by increasing the availability of nutrients content required by the plant and helps to increase the soil quality with natural microorganisms (Vessey 2003). Some of the important microbes used

in biofertilizers are nitrogen fixing bacteria and phosphate solubilizing microbes which can able to fix the atmospheric nitrogen and solubilizer phosphours in the soil. The microorganisms are extensively used as biofertilizers in agricultural practices. The beneficial microorganisms against the availability of nutrients to the plants. Among the microorganisms, the *Azotobacter* sp and phosphate solubilizing bacteria play major role in the supply of nutrients and promote plant growth activities. These bacteria are present in low population in the natural environment. *Azotobacter* sp naturally fixing atmospheric nitrogen in the rhizosphere. Corresponding, *Azotobacter* strain was maximum nitrogen fixation ability than the other *Azotobacter* sp.

Azotobacter sp are free living aerobic heterotrophic bacteria with unique ability of fixing atmospheric nitrogen. The bacteria are gram negative, non mobile but often mobile by peritrichous flagella. *Azotobacter* sp are highly important for this ability to fix molecular nitrogen, contributing to the productivity of any environment. They are proved experimentally to fix the atmospheric nitrogen per gram of carbohydrate (Becking 1971).

A special group of bacteria *Bacillus megaterium* occurring in sea water is capable of dissolving insoluble inorganic phosphorous in the soluble inorganic phosphorus (Alexander 1978, Chandrasekaran, 1966). Hence the present investigation suggested that the potential uses of *Azotobacter* sp and phosphate solubilizing *Bacillus megaterium* as biofertilizers.

MATERIALS AND METHODS

The isolation and identification of *Azotobacter* sp and *Bacillus megaterium* were analyzed according to the Bergy's manual of systematic bacteriology (William and wilkins 1989). The isolation of *Azotobacter* sp and *Bacillus megaterium* are Asbhy's agar and Pikovskaya's specific medium used respectively. The soil samples were collected from Rameshwaram marine soil. The biofertilizers were isolated and identified by morphological and biochemical characters. The various biochemical test such as gram staining, motility, citrate utilization, catalase, urease, Indole test, utilization of carbon source, methyl red test were anlysed and conformed. The specific medium was standardized for further investigation. The production of mass

inoculums of *Azotobacter* sp and *Bacillus megaterium* potential strain grown on the slants for preservation as per the need culture from slant were transferred to liquid culture broth of selective as well as optimized medium in a rotary shaker for 5 days to prepare started culture. The starter culture inoculums developed transferred to the fermented in batch culture mode with proper maintenance of 27˚C and continuous agitation for 7 days. When cell count reached to 110 cells/ml of broth used as inoculants. The biofertilizers inoculums of handling, packing strong an mixed with carrier materials which containing 10^{-8} population of inoculums was excellent quality product. In the current investigation of carrier mixed with charcoal (R.M.1332) $CaCo_3$ in a different ratio over the carrier in such a way that 40% moisture was maintained. After preparing the inoculums containing sufficient amount and 7 days after the formulated microbial inoculum used as *Azotobacter* sp and *Bacillus megaterium* biofertilizers individually.

RESULTS AND DISCUSSION

Biofertilizer contains living microorganisms and promoting plant growth by increasing the availability of nutrients and also helps to increase the soil quality with natural microbiota. Source of the beneficially microbiota used in biofertilizer are nitrogen fixing bacteria, phosphate solubilizing microbes which are able to fix atmospheric nitrogen and solubilized phosphorus in the soil microorganisms are extensively used as biofertilizers in agricultural practices. Soma prabha (2016) reported that the potential use of *Azotobcter* sp and phosphate solubilizing bacteria *Bacillus megaterium* as biofertilizer by growth and development by seed germination Jayanta Bhaduri *et al.*, (2017) studied that the Isolation and characterization of nitrogen fixing bacteria (*Azotobcter* sp) from Tea field soil of Doors and Darjeeling region of North Bengal India. After microbcteria of soil sample in *Azotobacter* selected media and totally 145 colonies has been thoroughly characterized on the basis of colony color, shape, and diametric of the

colony. Out of them the characteristics features of some (20) selected colony has been summarized. In this study totally (42) colonies isolated from different dilution factors were performed. The 10^{-5} dilution factors has maximum number of colonies recorded when compared with other dilution factors (Table-1, Fig-1, 2).

Dilution factors	Total no.of colonies (CFU/ml)	
	Azotobacter sp	*Phosphobacterium* sp
10^{-3}	9	8
10^{-4}	11	10
10^{-5}	15	12
10^{-6}	7	7
Total	42	37

Table 1: Isolation of Biofertilizers from Rameshwaram Marine soil

The isolated colonies were identified based on the parts of morphological and biochemical characters. The *Azotobacter* sp is a genus of usually motile, oval (or) spherical, thick walled cysts and may produce large quantities of capsular slime. They are free living bacteria. The *Azotobacter* sp biochemically gram staining, catalase, urease, citrate test, indole test, glucose, fructose, sucrose and maltose as a carbon source and methyl red test also characterized for identification. From the results, the *Azotabacter* sp, *Azotobacter chrococcuum* and *Azotobacter vinelandi* were conformed. Whereas phosphobacterium were isolated and performed with different dilutions totally 37 colonies were isolated from Rameshwaram marine soil. In this aspect, the 10^{-5} dilution factors showed maximum colonies (12) isolated when compared with other dilutions. Each and every strain of phosphobacteria, were morphologically and biochemically characterized. Morphologically shape, colour, slime layer, milk white, gram negative with rod shaped, non motile. The bacteria showed positive results for indole production, catalase, methyl red, citrate test and carbon source were analysed. The isolated bacteria were conformed as *Bacillus megaterium*, *Bacillus cereus* and *Bacillus subtilis* recognized (Table-2 and 3).

Table 2: Identification of Biofertilizers from Rameshwaram Marine soil

Biochemical test	***Azotobacter* sp**	***Phosphobacterium* sp**
	Inference	Inference
Gram staining	+	+
Motility	+	+
Catalase test	+	-
Urease test	+	+
Citrate test	+	+
Indole test	+	+
Utilization of carbon source	+	+
A. Glucose	+	+

B. Fructose	+	-
C. Maltose	+	+
A. Sucrose	+	+
Methyl red test	+	+

Table 3: Identification of biofertilizers isolated from marine soil

S. No	Name of the *Azotobacter*	Name of the *Phosphobacterium*
1	*Azotobacter* sp	*Bacillus megaterium*
2	*A. chrococcum*	*B. cereus*
3	*A. vinelandi*	*B. subtilis*

In the supply of nutrients and in the plant growth promoting activities, these bacteria are present in low concentration in the natural environment (Subba Rao, 1997, Lee *et al*., 2005, Zahram and Glick, 1995). The biofertilizers is in long duration, improvement of the soil fertility and sustainable agriculture. The beneficial bacteria used in biofertilizers are N_2 fixing bacteria and phosphate solubilizing bacteria which are able to solubilize phosphorus in the soil (Subba, 1999) biofertilizers are the live (or) contain latent cell of beneficial microorganisms which augment the availability of nutrients to the plants. The beneficial microorganisms are *Rhizobium*, *Azotobacter*, *Azospirillum*, *cyanobacteria*, *phosphobacteria* and mycorrhizae were used. Among these biofertilizers, the *Azotobacter* sp and *Bacillus megaterium* play an major role for agriculture. For the mass production of *Azotobacter* sp and *Bacillus megaterium* were multiplied by liquid broth of selective as well as optimized medium. The quality of inoculums cheeked and mixed with an inert carrier materials which contain sufficient amount of cells. After proper mixing carrier containing inoculants was left the 7 days and above formulated microbial inoculums used as biofertilizers. Umamaheswari and Sudha *et al*., (2018) studied the identification of *Azotobacter* sp and *Bacillus megaterium* with the same methods.

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